博士学位申請論文

1980　2-7

西　望

FUNCTIONAL AND STRUCTURAL FEATURES OF CHROMATOPHORE MEMBRANE FROM *RHODOSPIRILLUM RUBRUM*

by Nozomu NISHI

SUMMARY

1. The light-induced proton movement in *Rhodospirillum rubrum* chromatophores reflected in the light-induced pH change and in the light-induced absorbance changes of pH indicators was investigated. The light-induced pH change of chromatophore suspensions from *Rhodospirillum rubrum* was stimulated significantly and similarly by KCl, NaCl, LiCl, RbCl, CsCl, $MgCl_2$, $MnCl_2$, and $CaCl_2$. In the dark, the pH of chromatophore suspensions decreased immediately and markedly on adding these salts. The light-induced pH change stimulated by KCl plus valinomycin was inhibited by LiCl and NaCl, but not by RbCl.

The optimum pH values for light-induced pH change and photosynthetic ATP formation were around 5 and 8, respectively. The amount of chromatophore-bound ubiquinone-10 reduced in the light was independent of pH from 5 to 9. At pH 8, the number of protons incorporated into chromatophores in the light was one-

half of the number of ubiquinone-10 molecules reduced in the light.

2. Among several pH indicators tested, bromothymol blue (BTB) and neutral red (NR) showed absorbance changes on illumination of chromatophores. Although the pH change indicated by the absorbance change was opposite to the light-induced pH change of the medium, the effect of KCl on the absorbance changes of BTB and NR, and the effect of valinomycin on that of NR, but not on that of BTB, were similar to those on the light-induced pH change. The light-induced absorbance change of BTB was significantly inhibited by NR, whereas that of NR was hardly influenced by BTB.

Oligomycin stimulated the light-induced absorbance change of BTB under either non-phosphorylating or phosphorylating conditions. On the other hand, that of NR under phosphorylating conditions was 50% of that under non-phosphorylating conditions, and was increased by oligomycin.

3. The membrane of Rhodospirillum rubrum chromatophores was disintegrated with mild detergents (cholate and deoxycholate) in order to study the spatial arrangement of the functional proteins in the photochemical apparatus and the electron transport system in the membrane. The components solubilized from the membrane by a mixture of cholate and deoxycholate (C-DOC) were separated into four fractions by molecular-sieve chromatography in the presence of C-DOC; they were designated as F1, F2, F3, and F4 in the order of elution. The fractions were further purified by repeated molecular-sieve chromatography in the presence of C-DOC

until each fraction was chromatographically homogeneous.
4. F1 appeared to be conjugated forms of F2.

The purified F2 was composed of a rigid complex having a weight of 7×10^5 daltons, containing approximately 10 different kinds of protein species with molecular weights of 3.8×10^4, 3.6×10^4, 3.5×10^4, 2.8×10^4, 2.7×10^4, 2.6×10^4, 1.3×10^4, 1.2×10^4, 1.1×10^4, and 1.0×10^4. The complex contained 33 bacteriochlorophylls, 4 iron atoms and 90 phosphates, but no cytochrome, ubiquinone or phospholipid. It showed the same reaction center activity as chromatophores, indicating that the complex was a unit of the photochemical apparatus (photoreaction unit). Each chromatophore of average size was estimated to possess about 24 photoreaction units.

The purified F3 showed an absorbance spectrum characteristic of reaction centers, and contained 3.4 bacteriochlorophylls, 2.0 bacteriopheophytins and 1.9 acid-labile iron atoms, but no cytochrome or ubiquinone (C-DOC reaction center). It had a weight of 1.2×10^5 daltons, and the main components were 4 protein species with molecular weights of 2.8×10^4, 2.7×10^4 2.6×10^4 and 1.0×10^4.

The purified F4 showed a molecular weight of about 11,000, and contained one mole of ubiquinone-10 per mole (ubiquinone-10 protein).
5. The reaction center activity of C-DOC reaction centers was stimulated by ubiquinone-10 protein. In addition, the reaction

center oxidizesd reduced cytochrome $\underline{c}_2$ in the light, provided that ubiquinone-10 protein was present (photo-oxidase activity). 6. Chromatophores and the purified F2 were enzymatically labeled with ^{125}I. In the chromatophore membrane, polypeptides with molecular weights of higher than 2.5×10^4 were preferentially labeled and the major polypeptide species with molecular weights of around 1×10^4 were hardly labeled. On the other hand most polypeptide species including polypeptides with molecular weights of around 1×10^4 in the purified F2 were accessible to enzymic iodination.

When chromatophores were treated with proteases, most polypeptides accessible to iodination were digested, but hardly the unlabeled polypeptides. And the polypeptides resistant to protease treatments were still inaccessible to iodination after protease treatments.

INTRODUCTION

The cells of the photosynthetic bacterium, Rhodospirillum rubrum, if grown phototrophically, contain hundreds of membranous, tubular constructions called chromatophores (1-5). In preparations, chromatophores are closed vesicles with an average diameter of approximately 600 A; thus, the volume of one chromatophore is estimated to be only $1/10^4 - 1/10^6$ of the volume of one mitochondrion

or chloroplast (2). It is well known that, in spite of this small size, chromatophores are furnished with light-driven electron transport system and can catalyze photosynthetic ATP-formation. Chromatophore suspensions also show a reversible pH change on illumination which is similar to those of chloroplast suspensions (6, 7).

The electron transfer sequence for oxidation-reduction components bound to the chromatophore membrane is reasonably well understood, but the spatial arrangement of the components in the membrane is not (8-13).

In photosynthetic membranes, the components of photochemical system, electron transport system and phosphorylating system are supposed to exist consisting a functional complex called photosynthetic unit. In fact, photosystem I and photosystem II particles have been solubilized from chloroplasts with detergents.

In the present paper, features of chromatophore membrane are studied on two sides, namely functional side and structural side. The functional part of this paper deals with light-induced and salt-induced pH changes and light-induced absorbance changes of bromothymol blue and neutral red in suspensions of chromatophores. The light-induced translocations of protons and other cations are discussed in terms of ion-exchange properties of the chromatophore membrane and the oxidation-reduction of membrane-bound ubiquinone-10.

Many kinds of detergents have been used for the solubilization

of proteins from biomembranes. Among them, cholate and deoxycholate are known to be relatively mild and to be advantageous for reconstitution studies with the solubilized proteins because of their high critical micellar concentrations and low aggregation numbers (14-17).

The structural part deals with solubilization of chromatophore membrane with a mixture of cholate and deoxycholate. The solubilized components were separated and purified by repeated molecular-sieve chromatography. The purified components thus obtained were photoreaction units, reaction centers and ubiquinone-10 protein, and they were characterized. The vectorial arrangement of polypeptides in chromatophore membrane is discussed in consideration of the results from protease digestion and enzymic iodination.

MATERIALS AND METHODS

Cell Culture and Preparation of Chromatophores

The carotenoid-less blue-green mutant strain (G-9) of *R. rubrum* was used in most cases, and the wild-type strain in a few cases, as indicated. The cells were incubated at 30°C for a day in dark in order to complete anaerobisis, and then grown for 4 days under continuous illumination from tungsten lamps. The grown cells were collected and washed with 0.1 M Tris-HCl

buffer. The washed cells were suspended in 0.1 M Tris-HCl buffer (pH 8.0), and disrupted by sonication (10 kHz) at 0-10°C for 4 min. Chromatophores were centrifugally collected, washed with 0.1 M Tris-HCl buffer (pH 8.0), and suspended in the buffer (18). For experiments on light-induced pH change and light-induced absorbance change of pH indicators, washed cells were disrupted by grinding with aluminum oxide powder and suspended in 0.1 M glycylglycine-NaOH buffer (pH 8.0) containing 10% sucrose. Chromatophores were centrifugally collected, washed with 0.1 M or 1 mM glycylglycine-NaOH buffer (pH 8.0) containing 10% sucrose and suspended in the buffer.

Measurement of pH change

The light-induced pH change of chromatophore suspensions was monitored at 25°C using a Radiometer GK 2302C glass electrode in association with a Hitachi-Horiba F-7 pH meter, and recorded on a Hitachi 056 recorder. Chromatophore suspensions (4 ml) were placed in a 6-ml cylindrical glass container (1.4 cm in diameter and 4 cm in height) and stirred continuously by means of a magnetic stirring bar. The actual change of hydroxy ion concentrations was determined by titration with 1/50 N NaOH and HCl. The standard reaction mixture contained 1 mM glycylglycine, 10% sucrose, and chromatophores (A_{783nm} = 25-50). Illumination was provided from a 100-W tungsten lamp through a water layer 5 cm thick (approximately 3.7×10^3 foot-candles on the surface).

The salt-induced pH change of chromatophore suspensions in the dark was measured in the same manner as the light-induced pH

change, except that various salts were added; appropriate volumes of 1.66 M salt solutions in 1 mM glycylglycine-NaOH buffer containing 10% sucrose (pH 8.0) were added to the reaction mixtures so that the final volume of the reaction mixture would be 4 ml. Actual changes in proton concentration caused by the addition of salts were estimated by titrating the pH decreases of chromatophore suspensions with 1/50 N NaOH. In the cases of divalent cation salts, which are slightly acidic, changes in proton concentration were estimated as described above, and the values thus estimated were corrected for the changes of the reaction mixture without chromatophores.

Values for changes in the number of protons or hydroxy ions per chromatophore were based on the estimated bacteriochlorophyll content of chromatophores. Each chromatophore of average size contains approximately 790 molecules of bacteriochlorophyll (14).

The acid and base titrations of chromatophores were carried out in the same manner, except that the reaction mixture comprised 1 mM glycylglycine-NaOH buffer, 10% sucrose, 0.33 M KCl, and chromatophores (A_{873nm} = 50), and titration was performed with 1 N NaOH and HCl in the dark.

Optical spectroscopy

Absorbances were measured at 25°C with a Cary model 17 spectrophotometer. Difference spectra were measured with a Union High-Sense SM-401 spectrophotometer equipped with an SM-405 spectral data processor with modifications, and recorded

with a National 6421A X-Y recorder.

Light-induced absorbance change of pH indicators were measured at 25°C with the apparatus used for difference spectra. Actinic light (880nm) was obtained with a Bausch and Lomb monochromator fitted with a V-R69 cut off filter (Vacuum Optics Corporation of Japan, Tokyo) and a 12-V halogen lamp. The sample cuvette having four transparent sides (1 x 1 x 4 cm) was cross-illuminated (approximately 5.75×10^4 erg/s·cm^2 on the surface of the cuvette) with the aid of a glassfiber scope. The standard reaction mixture contained 0.1 M glycylglycine, 10% sucrose, 20 μM bromothymol blue or neutral red, and chromatophores ($A_{873nm} = 1$). The volume of the reaction mixture was 3 ml and the pH was 8.0. The light-induced absorbance changes of bromothymol blue and neutral red were measured at 615nm and 525nm,. respectively. In some cases, the light-induced absorbance change of neutral red was measured at 498nm, the isobestic point for the spectral change of bromothymol blue, whereas that of bromothymol blue was measured at 615nm and the value thus obtained was normalized with the value for the light-induced absorbance change of neutral red at this wavelength. The light-induced absorbance changes of other pH indicators were measured at their absorbance peaks at alkaline or acidic pH. In all cases, the values for light-induced absorbance changes of pH indicators were corrected for the light-induced absorbance changes of chromatophores alone.

Light-induced absorbance change of reaction centers were

measured in the same manner as light-induced absorbance change of pH indicators, except that 590-nm actinic light was obtained with a Bausch and Lomb monochromator fitted with a V-R56 cut-off filter (Vacuum Optics Corporation of Japan, Tokyo). A V-R69 cut-off filter was inserted in the light-path between the cuvettes and the photomultiplier, and the absorbance changes between 750nm and 950nm or at 865nm were measured. The standard reaction mixture contained 0.05 M Tris-HCl buffer (pH 8.0), 0.1% cholate, 0.3% deoxycholate and C-DOC reaction centers (see below) in a total volume of 2.0 ml. In some cases, ubiquinone-10 protein (see below) and cytochrome c_2 were added.

Photo-oxidations of reduced cytochrome c_2 were measured in the same manner as light-induced absorbance changes of pH indicators, except that 800-nm actinic light was obtained with a Bausch and Lomb monochromator fitted with a CF-B cold filter (Vacuum Optics Corporation of Japan, Tokyo). A V-R69 cut-off filter was inserted in the light-path between the cuvettes and the photomultiplier, and the absorbance changes at 550nm were measured. The standard reaction mixture contained 0.05 M Tris-HCl (pH 8.0), 0.1% cholate, 0.3% deoxycholate, C-DOC reaction centers, ubiquinone-10 protein and cytochrome c_2 in a total volume of 2.0 ml.

Photo-reductions of ubiquinone-10 were measured in the same manner as light-induced absorbance changes of pH indicators, except that the absorbance change at 275nm was measured; a

UV-33S filter (Vacuum Optics Corporation of Japan, Tokyo) was used. In some cases 590-nm actinic-light was used.

Activity assay of ATP-formation

Activities for ATP formation in the light by chromatophores were measured by the method described previously (18, 19). The standard reaction mixture was composed of 0.3 M glycylglycine-NaOH buffer (pH 8.0), 4% sucrose, 6 mM $MgCl_2$, 6 mM ADP, 6 mM [^{32}P]Pi (approximately 1 x 10^6 cpm), 60 mM ascorbate and chromatophore suspension (A_{873nm} = 5) in a total volume of 1.50 ml. The reaction was started by adding the chromatophores, carried out at 30°C for 4 min in the light (approximately 2,000 foot-candles), and stopped by adding 0.50 ml of 30% trichloroacetic acid. The amount of ATP thus formed was estimated by measuring the radioactivity of [^{32}P]Pi incorporated into the organic phosphate fraction according to the method of Nielsen and Lehninger (20) as modified by Avron (21).

Analytical procedures

The extraction of ubiquinone-10 was carried out as described previously (8, 22), except that isooctane containing 0.1% methanol was used (23). An aliquot of the extract was reduced by adding solid $NaBH_4$. The non-treated minus reduced difference spectra were measured 10 min after reduction. The value of $-(\Delta A_{275nm} - \Delta A_{300nm})$ was used as an index of the ubiquinone-10 content (24). The cytochrome contents of samples were estimated in the same manner as those of ubiquinone-10, except that the samples were

reduced by adding $Na_2S_2O_4$. Reduced minus oxidized difference spectra were measured 3 min after reduction. The value of ΔA_{428nm} was used as an index of the cytochrome content.

Bacteriochlorophyll and bacteriopheophytin were estimated from the absorbance in acetone-methanol (7:2) as follows. The extinction coefficient of bacteriopheophytin in the acetone-methanol was calculated from the absorbance spectra of bacteriochlorophyll and bacteriopheophytin in ether (25), assuming that the ratio of the extinction coefficient in acetone-methanol to that in ether of bacteriopheophytin was the same as for bacteriochlorophyll.

The contents of protein were determined by the method of Folin and Ciocalteu (26) as modified by Lowry et al. (27). Bovine serum albumin was used as a standard.

The contents of phosphorus were determined by wet-ashing in $HClO_4$ with H_2O_2 (28), according to the method of Fiske and Subbarow (29).

The contents of total iron were determined with an SAS-721 atomic absorption spectrophotometer (Daini Seikosha Co. Ltd., Tokyo). The contents of non-heme iron were colorimetrically determined with sulfonated bathophenanthroline (30).

Purification of cytochrome c_2

Cytochrome c_2 was highly purified from cells grown in the light, as described previously (31, 32).

Thin-layer chromatography of phospholipids

Phospholipids were extracted from lyophylized samples with chloroform-methanol (2:1), concentrated, charged on chromatoplates (Merck, TLC plate silica gel 60) previously heated at 110°C for 30 min, and developed with chloroform-acetone-methanol-acetic acid-water (100:40:30:20:12) (33). The spots of phospholipids were detected with the Dittmer-Lester reagent (34).

SDS-Polyacrylamide Gel electrophoresis

Polyacrylamide disk gel electrophoresis in the presence of sodium dodecylsulfate (SDS) was carried out according to the method of Weber and Osborn (35) with minor modifications, using a KPI electrophoresis apparatus, model E-IE 7-100 (Koike Precision Instruments, Kanagawa). Gels (4.5 x 80 mm) were prepared with 12.5% polyacrylamide, 0.33% N,N'-methylene-bisacrylamide and 0.05 M Tris-HCl containing 0.1% SDS (pH 8.0). Electrophoresis was carried out at 4 mA per gel column at 25°C for 4 h. Protein samples were dissolved in 0.01 M Tris-HCl buffer containing 1% SDS and 1% 2-mercaptoethanol (pH 8.0), then incubated at 95°C for 2 min. The resulting solution was applied to the top of the gels. After electrophoresis, the gels were stained for 3 h in staining solution (0.5% Coomassie brilliant blue R-250 in 46% methanol and 12% trichloroacetic acid). Destaining was carried out by washing the gels several times with solutions containing 7.5% acetic acid and 5% methanol.

SDS-polyacrylamide concentration-gradient slab gel electrophoresis was carried out according to the method of

Laemmli (36), using a KPI electrophoresis apparatus, model E-IE 17-30TR (Koike Precision Instruments, Kanagawa). A polyacrylamide gel slab (150 x 270 x 2 mm) was prepared between two glass plates. The separating gel slab (24 cm high) was composed of 0.375 M Tris-HCl buffer (pH 8.0), 0.1% SDS, acrylamide having a concentration gradient from 10% to 20%, and N,N'-methylene-bisacrylamide having a concentration gradient from 0.27% to 0.53%. On top of the separating gel slab was the stacking gel slab (3 cm high), which was composed of 0.125 M Tris-HCl buffer (pH 6.8), 0.1% SDS, 4% acrylamide and 0.2% N,N'-methylene-bisacrylamide. Samples were dissolved in 0.063 M Tris-HCl buffer containing 2% SDS and 5% 2-mercaptoethanol (pH 6.8), then incubated at 95°C for 2 min. The resulting solutions were applied to wells previously formed in the stacking gel slab. Electrophoresis was carried out at 20 mA per gel slab and at 25°C for 18 h. The methods for staining, drying and destaining the gel slabs, and the molecular weight markers used, were as described previously (37).

Densitograms were obtained with a Shimadzu CS-910 dual-wavelength TLC scanner.

Treatment of chromatophore membrane with mixture of cholate and deoxycholate

A chromatophore suspension (A_{873nm} = 200) was diluted with an equal volume of 0.1 M Tris-HCl buffer containing 2% cholate and 4% deoxycholate (pH 8.0), stirred overnight, and sonicated

at 10 kHz for 3 min. The sonicated suspension was centrifuged for 1 h at 100,000 x g. The resulting supernatant was collected. The precipitate was resuspended in 0.1 M Tris-HCl buffer containing 1% cholate and 2% deoxycholate (pH 8.0), sonicated at 10 kHz for 3 min, and centrifuged for 1 h at 100,000 x g. The resulting supernatant was combined with the first supernatant, and subjected to ammonium sulfate fractionation. The precipitate in 30%-saturated ammonium sulfate solution was collected, suspended in 0.05 M Tris-HCl buffer containing 0.1% cholate and 0,3% deoxycholate (pH 8.0), and dialyzed against the buffer containing the detergents. The dialyzed solution was designated as cholate-deoxycholate (C-DOC) soluble fraction. All the procedures described above were carried out at 4°C and in the dark as far as possible.

Enzymic iodination

Iodination of chromatophore membrane and purified F2 was catalyzed by lactoperoxidase upon addition of hydrogen peroxide. The reaction mixture was composed of 0.01 M sodium phosphate buffer (pH 7.0), 1.3×10^{-6} M lactoperoxidase, 1×10^{-7} M [^{125}I]KI (0.5 m Ci/ml) and chromatophores (A_{873nm} = 100) or purified F2 (A_{865nm} = 13) in a total volume of 0.5 ml. The reaction was initiated by the addition of 8 µl of 5×10^{-4} M hydrogen peroxide, and carried out at 25°C. The same amount of hydrogen peroxide was added three more times to the reaction mixture at an interval of 10 min. The sequential additions were employed

to maintain a low concentration of hydrogen peroxide (38, 39). The reaction was stopped by dilution with 8.5 ml of chilled 0.01 M sodium phosphate buffer (pH 7.0). Iodinated samples were centrifugally washed two times and dialyzed against the same buffer. And the dialyzed samples were applied to SDS-polyacrylamide concentration-gradient slab gel electrophoresis.

Detection of radioactivity in slab gel

Radioactivity was detected by autoradiogram. An X-ray film (25.4 x 30.5 cm) was exposed to a dried slab gel containing iodinated polypeptides for an appropriate length of time, and the optical density of the developed film was measured by a densitometer.

Protease treatment

Chromatophores were treated with proteases as follows. The reaction mixture was composed of 5 vol of chromatophore suspension (A_{873nm} = 240) in 0.1 M Tris-HCl buffer (pH 8.0) and 1 vol of a protease solution (16.5 mg/ml of trypsin or subtilisin BPN') in 0.1 M Tris-HCl buffer (pH 8.0). The reaction mixture was incubated for 1 h at 25°C. 1 vol of the protease solution was added two more times to the reaction mixture at an interval of 1 h. Chromatophores thus treated were centrifugally washed three times with 0.01 M sodium phosphate buffer (pH 7.0) and finally suspended in the same buffer.

Reagents used

Antimycin A was a commercial preparation from Kyowa Hakko Kogyo Co., Tokyo, valinomycin was from Calbiochem, San Diego,

California, ADP from Oriental Yeast Co., Osaka, and trypsin-TPCK from Warthington Biochemical Co., Freehold, New Jersey. Cholic acid, deoxycholic acid, oligomycin, lactoperoxidase, subtilisin BPN' and the proteins used as molecular weight markers (thyroglobulin, ferritin, γ-globulin, bovine serum albumin, ovalbumin, α-chymotrypsinogen A and insulin B chain) in molecular-sieve chromatography and in SDS-polyacrylamide gel electrophoresis were purchased from Sigma Chemicals Co., St. Louis. Cholic acid and deoxycholic acid were recrystallized. Among the pH indicators used, bromothymol blue, bromophenol red, bromocresol purple, and 2,4-dinitrophenol were obtained from E. Merck, Darmstadt, and bromophenol blue from Wako Pure Chemical Industries Ltd., Osaka. Other pH indicators were obtained from BDH Chemicals Ltd., Poole. Carrier-free [^{125}I] iodine was obtained from The Radiochemical Centre, Amersham, Bucks., UK., at a concentration of 100 m Ci/ml.

RESULTS

I. FUNCTIONAL PART

Light-induced pH change of chromatophore suspensions and effects of metal salts on it

Stedingk and Baltscheffsky (6) found that when chromatophores were suspended in a weakly buffered solution and the pH of the suspension was measured with a pH electrode, the pH of the

suspension became more alkaline on illumination, and was restored to the original level on cessation of the illumination. The rise and fall of pH suggests that protons were incorporated into the chromatophores in the light and liberated therefrom in the dark. In addition, they reported that the light-induced pH change was significantly stimulated by KCl and NaCl. It was found in the present study that, in addition to these two alkali metal salts, LiCl, RbCl, and CsCl also stimulated the light-induced pH change (Fig. 1). The stimulative effects of these five alkali metal

Fig. 1

salts on the light-induced pH change were nearly the same in rate and extent; they were most pronounced around 0.33 M and decreased at higher concentrations. Among the divalent cation salts tested, $CaCl_2$, $MgCl_2$, and $MnCl_2$ stimulated the light-induced pH change; the maximum stimulations were observed at about 0.1 M, and the extents of change were almost the same as for the monovalent cation salts described above (Fig. 2). However, $NiCl_2$, $CoCl_2$, and $SrCl_2$

Fig. 2

showed slight stimulating effects at 0.1 M.

Rapid pH decrease of chromatophore suspensions on addition of inorganic salts in dark

When KCl was added in the dark to chromatophores suspended in a weakly buffered solution, the pH of the suspension decreased rapidly and markedly (Fig. 3). Under the experimental conditions

Fig. 3

used, the pH decrease reached approximately 0.6 pH unit. A similar phenomenon was seen when each of the mono- and divalent cation salts was added to chromatophore suspensions in the dark (Table I). It is noteworthy that the pH decrease of chromatophore

Table I

suspensions caused by adding $MgCl_2$ and $CaCl_2$ exceeded one pH unit. It can be calculated that approximately two to three thousand protons were liberated from each chromatophore of average size on adding various kinds of inorganic salts at 0.33 M. These numbers are seven or more times higher than the maximum numbers of protons incorporated in each chromatophore of average size on illumination.

Effect of valinomycin on light-induced pH change of chromatophore suspensions

Stedingk and Baltscheffsky (6) reported that in the presence of KCl, but not in the presence of NaCl, the light-induced pH changes of chromatophore suspensions were significantly stimulated in rate by valinomycin. Their findings were confirmed and extended

in the present study. In the presence of 0.1 M KCl, valinomycin stimulated the initial rate of light-induce pH change two-fold, and the time required for the change to reach the steady state was significantly shorter in the presence than in the absence of the anitibiotic. In the presence of valinomycin, the optimum concentration of KCl for the light-induced pH change shifted from 0.33 M to 0.1 M, and at 0.1 M , the extent of the light-induced pH change was stimulated to approximately twice the maximum extent in the absence of the antibiotic (Fig. 4).

Fig. 4

It is known that Rb^+ and Cs^+ can form complexes with valinomycin in essentially the same manner as K^+ (40). It was found that the light-induced pH change of chromatophore suspensions containing RbCl or CsCl was stimulated in the presence of valinomycin in almost the same manner as by KCl (data not shown). In addition, the stimulative effect of valinomycin plus 0.033 M KCl on the light-induced pH change was significantly suppressed by the co-presence of NaCl and LiCl, but not RbCl (Fig. 5). In the presence

Fig. 5

of 0.033 M KCl, a concentration far lower than that required for the maximum stimulation of the light-induced pH change (0.33 M) (see Fig. 1), the stimulative effect of valinomycin on the light-

induced pH change was gradually suppressed with increasing concentration of either NaCl or LiCl. On the other hand, RbCl, which is able to form a complex with valinomycin, did not suppress the stimulative effect of the antibiotic, while at 0.1 M, it stimulated the light-induced pH change.

Effects of pH on light-induced pH change of chromatophore suspensions and on photoreduction of ubiquinone-10 bound to chromatophores

The optimum pH for photosynthetic ATP formation with chromatophores was around pH 8; the activity at pH 8 was approximately 4 times that at pH 5 (Fig. 6). On the other hand, the light-

Fig. 6

induced pH change of chromatophore suspensions was maximum in initial rate and extent at around pH 5, and at pH 8 it was approximately one-third of that at pH 5. In the pH range tested, the light-induced pH change was strongly inhibited in the presence of antimycin A, well known as a potent inhibitor of the electron transport system between ubiquinone-10 and the non-heme iron protein (POC-275 mV) (13). The number of protons incorporated in each chromatophore of average size in the light, if estimated on the basis of the light-induced pH change, was as low as 20 at pH 8-9 when antimycin A was present. On the other hand, the photoreduction of ubiquinone-10 bound to chromatophores increased by one-half in the presence of antimycin A, the amount of photoreduced

ubiquinone-10 being independent of pH from 5 to 9. Approximately 35 molecules of ubiquinone-10 were photoreduced in the presence of the antibiotic. If 2 protons were incorporated into each ubiquinone-10 molecule on reduction, the number of protons incorporated into each chromatophore of average size in the light would have been approximatly 70 at pH 8-9. This suggests the possibility that, at least at pH 8-9, in the presence of antimycin A approximately 70% of the photoreduced ubiquinone-10 molecules were reduced by electrons, but not by hydrogen atoms (electron and proton pairs).

Light-induced absorbance changes of bromothymol blue and neutral red in chromatophores

It is known that some pH indicators change their absorbance on oxidation of substrates in mitochondria and on illumination in chloroplasts and chromatophores (41-48). In the present study, nineteen kinds of pH indicator from quinaldine red (pK_a = 2.3) to alizarin yellow G (pK_a = 10.9) were tested to determine whether their absorbances changed on illumination in highly buffered chromatophore suspensions (Table II). Among the pH

Table II

indicators tested, bromothymol blue (pK_a = 7.3) and neutral red (pK_a = 6.7) appreciably changed their absorbances on illumination at pH 8, but not at pH 5.5. It has been reported that the activity

of photosynthetic ATP formation was hardly influenced by 25 μM neutral red, whereas it was approximately 50% inhibited by 25 μM bromothymol blue (50). This concentration (25 μM) was used in most cases in the present study. The absorbance of bromothymol blue at 615 nm decreased when the light was switched on, and recovered when it was switched off, whereas the absorbance of neutral red at 525 nm increased when it was on and recovered when it was off (Fig. 7). These light-induced absorbance changes

Fig. 7

of both pH indicators indicate that the pH became more acidic; this pH change was opposite to the light-induced pH change observed with a pH electrode. This discrepancy may be accounted for if the light-induced absorbance change of the pH indicators reflects the pH change of the inner space of chromatophore vesicles, the pH change of the intra-chromatophore membrane space or the pH change of the surface of the chromatophore membrane on illumination.

The effects of buffer concentration on the light-induced absorbance changes of bromothymol blue and neutral red in chromatophore were measured at pH 8.0 (Fig. 8). The extent of the light-induced

Fig. 8

absorbance increase of neutral red increased with increasing concentration of glycylglycine-NaOH buffer, reached a mximum at 0.1 M (as glycylglycine), and fell at higher concentrations. The extent of the light-induced absorbance decrease of bromothymol blue at 615 nm also increased with increasing concentration of the buffer up to 0.1 M. The light-induced absorbance changes of neutral red and bromothymol blue were significantly stimulated by adding 0.1 M KCl when the concentration of the buffer was 1 mM, but not when it was 0.1 M. This suggests that the stimulation of the light-induced absorbance changes with increasing buffer concentration was largely due to the inorganic cation present in the buffer, although it was previously suggested that it might be due to neutralization of the light-induced pH change of the outer space of chromatophore vesicles by the higher buffer concentration (48).

Effects of KCl, valinomycin, and other reagents on light-induced absorbance changes of bromothymol blue and neutral red

The effects of KCl and valinomycin on the light-induced absorbance change of neutral red in chromatophores were examined in a reaction mixture containing 1 mM glycylglycine-NaOH buffer (Fig. 9). The initial rate and extent of the

Fig. 9

absorbance change increased with increasing concentration of

KCl, reached a maximum at 0.33 M, and decreased at higher concentrations. Valinomycin shifted the optimum concentration of KCl down to 0.1 M, and the initial rate and extent of the absorbance change increased significantly. These stimulative effects of KCl and valinomycin on the light-induced absorbance change of neutral red in chromatophores resembled the stimulative effects of both drugs on the light-induced pH change of chromatophore suspensions (see Fig. 4). The light-induced absorbance change of bromothymol blue was influenced by KCl in the same manner as that of neutral red (Fig. 9). However, the light-induced absorbance change of bromothymol blue was significantly inhibited by valinomycin in the presence and absence of KCl, different from the case with neutral red.

The light-induced absorbance change of bromothymol blue in chromatophores was depressed when neutral red was also added (Fig. 10). In the presence of 15 μM or higher concentrations

Fig. 10

of neutral red, the light-induced absorbance change of 25 μM bromothymol blue was depressed by one-half, but not further. On the other hand, the absorbance change of neutral red was scarcely influenced by adding bromothymol blue. Lineweaver-Burk plots of the absorbance changes of neutral red and

bromothymol blue showed that the K_m values were 14 $\mu\underline{\underline{M}}$ for neutral red and 22 $\mu\underline{\underline{M}}$ for bromothymol blue, and that at infinitely high concentration of neutral red or bromothymol blue, 42 mmol of the former dye and 32 mmol of the latter dye could be protonated per mol of bacteriochlorophyll (Fig. 11). These values correspond

Fig. 11

to 33 molecules of neutral red and 25 molecules of bromothymol blue protonated in each chromatophore of average size.

Two alkaline reagents, 2-amino-2-methyl-1,3-propanediol and tris(hydroxymethyl)aminomethane, were found to inhibit the light-induced absorbance change of neutral red significantly, but that of bromothymol blue was not much influenced (Table III).

Table III

In addition, the light-induced absorbance change of neutral red, but not that of bromothymol blue, was significantly lower when it was measured in a reaction mixture in which photosynthetic ATP formation was taking place (phosphorylating reaction mixture) than when it was measured in a reaction mixture in which photosynthetic ATP formation was not taking place (non-phosphorylating reaction mixture). Oligomycin stimulated the absorbance change of bromothymol blue to significant extent under both phosphorylating and non-phosphorylating conditions.

However, the antibiotic restored the absorbance change of neutral red under phosphorylating conditions to the level observed under non-phosphorylating conditions.

Acid-base titration curve with chromatophore suspensions

The titration curve with chromatophore suspensions, measured with NaOH and HCl, indicated that the chromatophores possessed many ionizable groups (Fig. 12). The ionizable groups can be

Fig. 12

classified roughly into three kinds; their apparent pK_a values were 9.1-9.4, 4.1-4.3, and around 3.2. The numbers of ionizable groups having apparent pK_a values of 9.1-9.4, 4.1-4.3, and around 3.2 which could be titrated in each chromatophore of average size were estimated to be 3.9×10^3, 1.2×10^4, and 8.3×10^3, respectively Earlier, Kakuno et al. (14) reported that approximately 3.1×10^3 atoms of organic solvent-soluble phosphorus were present in each chromatophore. This value coincides well with the number (8.3×10^3) of ionizable groups having a pK_a value of approximately 3.2, if one assumes that the groups represent phospholipids.

II. STRUCTURAL PART

Molecular-sieve chromatography of C-DOC soluble fraction of chromatophore membrane

The C-DOC soluble fraction described in "Materials and

Methods" was subjected to molecular-sieve chromatography on a Sepharose 6B column (Fig. 13). The elution profile in terms of

Fig. 13

A_{280nm} showed two sharp peaks at fractions No. 19 and No. 32, a shoulder around fraction No. 40 and a broad peak at fraction Nos. 52-60. The fractions centered at the peaks and the shoulder were pooled separately. The resulting four fractions were designated as F1, F2, F3, and F4 in the order of elution. Almost all the amount of bacteriochlorophyll (A_{873nm}) solubilized from chromatophores appeared in F1 and F2. F1 was concluded to be mostly conjugated forms of F2, since F1 and F2 resembled each other as regards the content of bacteriochlorophyll per protein and the profile in SDS-polyacrylamide gel electrophoresis. The peak of A_{280nm} for F2 coincided with the peak of ligh-induced absorbance change at 865nm ($-\Delta A_{865nm}$). However, the peak of $-\Delta A_{865nm}$ showed a shoulder, where the ratio of $-\Delta A_{865nm}/A_{873nm}$ (reaction center activity/bacteriochlorophyll concentration) has a peak (F3). The ratio is 0.13 at the peak of F3, whereas it is approximately 0.007 and 0.02 at the peaks of F1 and F2, respectively. This suggests that reaction centers bound to F1 were in part dissociated, and collected in F3. The reaction centers thus obtained are designated as C-DOC reaction centers. In addition, F2 and F3 contained ubiquinone-10 ($-\Delta A_{275nm}$) and

cytochromes (ΔA_{428nm}), respectively, whereas F4 was richest in ubiquinone-10.

Purification of photoreaction units from F2 and their characterization

F2 was subjected to three more successive molecular-sieve chromatographies on a Sepharose 6B column (5 x 90 cm) with 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate. Figure 14 shows the elution profile of the third chromatography.

Fig. 14

Fraction Nos. 30 to 40 were collected as purified F2. The purified F2 (photoreaction units) was nearly homogeneous with respect to the concentration ratio of bacteriochlorophyll to protein in the last chromatography, and had a molecular weight of 7×10^5 (Fig. 15). Its absorbance spectrum was similar to that

Fig. 15

of chromatophores, except that the main peak due to bacteriochlorophyll was at 865nm, which is 8nm shorter than with chromatophores (Fig. 16). The millimolar extinction coefficient

Fig. 16

of the peak at 865nm was estimated to be 104 $mM^{-1}\ cm^{-1}$; this

value is somewhat lower than the value at 873nm with chromatophores in the absence of detergents (140 mM^{-1} cm^{-1}) (25). The purified F2 contained 33 molecules of bacteriochlorophyll, 4 atoms of iron and 90 phosphate groups in each protein complex (Table IV).

Table IV

In SDS-polyacrylamide concentration-gradient slab gel electrophoresis, the purified F2 was separated into approximately 10 kinds of major protein species, significantly fewer than were obtained with chromatophores (Fig. 17). The apparent molecular weights of

Fig. 17

the protein species were 3.8×10^4, 3.6×10^4, 3.5×10^4, 2.8×10^4, 2.7×10^4, 2.6×10^4, 1.3×10^4, 1.2×10^4, 1.1×10^4 and 1.0×10^4.

Extracts of chromatophores and the purified F2 with mixtures of chloroform and methanol (2:1) were subjected to thin-layer chromatography. Phosphatidyl choline, phosphatidyl glycerol, cardiolipin and phosphatidyl ethanolamine were detected in chromatophores, in agreement with the results of Haverkate et al. (51), but not in the purified F2 (Fig. 18).

Fig. 18

The purified F2 obtained from the wild-type cells contained carotenoids in addition to components obtained from the blue-green mutant cells, indicating that carotenoids were also bound to same of the protein components.

Purification of reaction centers from F3 and their characterization

F3 was subjected to three more successive molecular-sieve chromatographies on an Ultrogel AcA 22 column (5 x 60 cm), using 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0). During the course of the repeated chromatographies, the cytochromes and ubiquinone-10 originally present in F3 were gradually dissociated and removed. Figure 19

Fig. 19

shows the elution profile of the third chromatography. Fraction Nos. 50 to 60 were collected as purified F3. The purified F3 (C-DOC reaction centers) was nearly homogeneous in terms of the concentration ratio of the reaction center activity to bacteriochlorophyll in the last chromatography, and had a molecular weight of approximately 1.2×10^5 (Fig. 20). This

Fig. 20

value is in agreement with those for reaction centers purified from R. rubrum and Rhodopseudomonas spheroides with the aid

of N,N-dimethyllaurylamine oxide (LDAO) (52-54). The absorbance spectrum of purified F3 is shown in Fig. 21. When ferricyanide

Fig. 21

was added, the peak at 865nm was bleached, whereas the peak at 802nm was shifted to 799nm, in the same way as with LDAO reaction centers. The purity indices (A_{280nm}/A_{802nm}) were 1.22 for LDAO reaction centers (53) and 2.31 for purified F3. The profile of purified F3 in SDS-polyacrylamide concentration-gradient slab gel electrophoresis showed 4 major peaks with molecular weights of 2.8×10^4, 2.7×10^4, 2.6×10^4, and 1.0×10^4 (Fig. 17). The extent of contamination of the peaks with molecular weights of 3.8×10^4, 3.6×10^4, and 3.5×10^4 varied from one experiment to another. In one case, the contamination peaks were comparable in height to the peaks with molecular weights of $2.8\text{-}2.6 \times 10^4$.

The other components present in purified F3 are summarized in Table V.

Table V

When purified F3 was illuminated with 590-nm actinic light, the peak at 865nm was bleached to the extent of only 20% of that induced by ferricyanide.

Purification of ubiquinone-10 protein from F4 and its characterization

F4 was subjected to three more successive molecular-sieve chromatographies on a Sephadex G-75 column (2.5 x 90 cm) with 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0). The fraction obtained in the last chromatography contained ubiquinone-10, and showed a single protein band on SDS-polyacrylamide disk gel electrophoresis. The protein bound with ubiquinone-10 was designated as ubiquinone-10 protein. Its molecular weight was estimated to be 1.1×10^4 by molecular-sieve chromatography on a Sephadex G-75 column (Fig. 22) and 1.4×10^4 by SDS-polyacrylamide disk gel

Fig. 22

electrophoresis, indicating that each molecule of ubiquinone-10 protein was composed of a single peptide chain. Ubiquinone-10 protein showed an absorbance spectrum having a sharp peak at 275nm due to the apo-protein and ubiquinone-10. When ubiquinone-10 was reduced with $NaBH_4$, the peak was shifted to 280nm, and the absorbance fell (Fig. 23). The minor peaks at 680nm

Fig. 23

and 760nm may be due to contaminating bacteriopheophytin. However, the origin of the large shoulder at 300-450nm is not

known. It was estimated that 0.8 mole of ubiquinone-10 was bound to 11,000 g of protein, indicating that the ubiquinone-10 protein contained one molecule of the quinone per molecule.

Effects of ubiquinone-10 protein and cytochrome c_2 on C-DOC reaction center activities

The extent of the light-induced absorbance change at 865nm of C-DOC reaction centers (purified F3) was significantly increased when ubiquinone-10 protein was added (Fig. 24). The

Fig. 24

extent of the increase reached a maximum when the molar ratio of ubiquinone-10 protein/reaction center was increased to 10. However, the rate of change was appreciably slower than the rate observed without addition of the quinone protein (Fig. 25). In

Fig. 25

addition, the change in the presence of the quinone protein was hardly restored when the light was switched off. In the presence of ubiquinone-10 protein at a molar ratio of 5, the effect of reduced cytochrome c_2 on the light-induced absorbance change at 865nm of C-DOC reaction centers was examined (Fig. 26).

Fig. 26

The extent of the change increased with increasing concentration of reduced cytochrome $\underline{c}_2$, and reached a maximum when the molar ratio of reduced cytochrome $\underline{c}_2$/reaction center was 2. However, it was significantly inhibited at higher ratios; almost complete inhibition was attained at a ratio of 40.

C-DOC reaction centers, when illuminated with 800nm actinic light, showed activity for the oxidation of reduced cytochrome $\underline{c}_2$, provided that ubiquinone-10 protein was present (Fig. 12). A substrate amount of the cytochrome was oxidized, although reduction of the quinone protein was not observable. This indicates that C-DOC reaction centers catalyze the reduction of oxidized ubiquinone-10 protein by reduced cytochrome $\underline{c}_2$, and that the quinone protein thus reduced is oxidized by molecular oxygen.

Determination of exposed proteins on chromatophore membrane by lactoperoxidase-catalyzed iodination and protease digestion

Enzymic iodination and protease digestion have been employed to determine the vectorial arrangement of proteins on biomembranes because of their specificity for exposed proteins when they are applied to the membranes (38,39,55-60).

Chromatophores and the purified F2 were labeled with ^{125}I by means of lactoperoxidase and the labeled polypeptides were analyzed by SDS-polyacrylamide gel electrophoresis and subsequent autoradiography (Fig. 27). Polypeptides with molecular weights

Fig. 27

more than 25,000 were preferentially labeled in chromatophores, but the major polypeptide species with lower molecular weights (designated as group-L in Fig. 27) were hardly labeled. In case of the purified F2, not only the polypeptides labeled in chromatophores but the polypeptides in group-L were also significantly labeled. These results indicate that the polypeptides in group-L are at least partially accessible to lactoperoxidase in the purified F2, whereas they are almost completely hidden from the enzyme in chromatophore membrane.

Chromatophore membrane was as much as possible digested by trypsin or subtilisin BPN', and then subjected to enzymic iodination. Polypeptide composition and distribution of radioactivity were analyzed by SDS-polyacrylamide gel electrophoresis and subsequent autoradiography (Fig. 28). Almost all the peaks

Fig. 28

with molecular weights higher than 25,000 present in chromatophores were eliminated after trypsin- or subtilisin BPN'-treatment, and new peaks with molecular weights around 20,000-10,000 were formed. The major peaks in group-L were somewhat decreased by subtilisin BPN' digestion, but hardly by trypsin digestion. When the protease-treated chromatophores were iodinated, radioactivity was mostly found in group-L region which was different from the case of intact chromatophores. However,

the main peaks of radioactivity did not correspond to the protein peaks in the trypsin-treated chromatophores. In addition, these peaks of radioactivity in group-L region in trypsin- or subtilisin BPN'-treated chromatophores were also formed when protease digestions were performed after iodination. Therefore, it is supposed that the polypeptides in group-L remained inaccessible to iodination even after protease digestion, whereas polypeptides with higher molecular weights were fragmented by protease treatments so that some of the resulting fragments would be bound to the surface of chromatophore membrane.

DISCUSSION

I. FUNCTIONAL PART

It is known that pH changes occur in weakly buffered suspensions of mitochondria and chloroplasts when they oxidize substrates and are illuminated, respectively (61,62). It is currently considered that the "active transprot" of H^+ from or to the inner aqueous phase of the vesicular organelles is responsible for the pH changes. Kobayashi and Nishimura (63-65) found that whole cells of photosynthetic bacteria grown in the light also show such a pH change on illumination; the light-induced pH change is readily dinimished if the cells are sonicated.

It was found in the present study that at pH 8 and in the steady state in the light, the number of H^+ ions incorporated in each chromatophore was one-half of the number of chromatophore-bound ubiquinone-10 molecules reduced. Earlier, Kakuno et al. (66) stated that ubiquinone-10 is a two-hydrogen carrier in the state in which the electron-transfer system is functioning, and that ubiquinone-10 is reduced by electrons from the electron-transfer system and protons from water. Therefore, it seems likely that under the conditions described above, the light-induced pH change of the medium was largely, if not completely, brought about by the reduction of ubiquinone-10, each molecule of which was reduced by two electron and proton pairs.

The minimum volume of one vesicle, in which one each of H^+ and OH^+ are allowed to exist "all the time", is calculated to be approximately 10^{10} A^3. The average volume of each of the chromatophores prepared from R. rubrum is approximately 10^8 A^3, i.e., one-hundredth of the minimum volume (2). According to the chemiosmotic coupling hypothesis for oxidative and photosynthetic phosphorylations (67-70), the total potential difference of the protons across the membrane (the protonmotive force, Δp) consists of the sum of the electric potential difference ($\Delta\psi$) and the pH difference across the membrane (ΔpH). The average volume of one chromatophore indicates that ΔpH across the membrane is not obtainable, unless the total volume of all the chromatophores present in the reaction mixture is used

in the calculation of ΔpH. Earlier, Hosoi et al. (19,50) and Oku et al. (71) suggested that an intramolecular localization of H^+ provides the motive force for the formation of ATP from ADP and P_i.

Stedingk and Baltscheffsky (6) found that the pH of a chromatophore suspension in a weakly buffered solution, if measured with a pH electrode, rose on illumination. In the present study, it was found that the light-induced pH change was significantly stimulated by various inorganic salts, LiCl, NaCl, KCl, RbCl, CsCl, $MgCl_2$, $MnCl_2$, and $CaCl_2$, to almost the same extent, although the optimum concentrations were different for the mono- and divalent cation salts (Figs. 1 and 2). This phenomenon is not in harmony with the active transport of various inorganic cations by mitochondria, chloroplasts and whole cells of R. rubrum. In addition, the light-induced pH change of chromatophore suspensions was optimum at around pH 5, at which the photosynthetic ATP and PP_i formation activities (optimum at pH 8) are low. This suggests that most of the light-induced pH change in the presence of the inorganic salts had no relation to the coupling between photosynthetic electron transport and phosphorylation.

The acid-base titration curve with chromatophore suspensions indicates that there is a large number of acidic groups in the chromatophore membrane (Fig. 12). The acidic groups having an apparent pK_a of 4.1-4.3 may be mostly the carboxyl residues of

proteins and those having an apparent pK_a of around 3.2 may be mostly the phosphoryl groups of phospholipids. In the neutral pH range, the carboxyl residues could exist in the dissociated form, have negative charges and combine electrostatically with protons in the same manner as cation exchangers. In fact, when various kinds of mono- and di-valent inorganic cation salts were added to chromatophore suspensions at neutral pH in the dark, the inorganic cations were adsorbed on the chromatophore membrane, liberating protons by means of cation exchange (Table I). Together with the findings that the light-induced pH change occurred to an appreciable extent only if inorganic cation salts were present and was optimum at around pH 5, near the apparent pK_a (4.1-4.3) of the acidic groups of proteins, this suggests that the light-induced pH change in the presence of inorganic salts was brought about by the acidification of the surface of chromatophore vesicles resulting from photosynthetic electron and proton transport (72-78). This phenomenon is shown schematically in Fig. 29. The stimulative effect of valinomycin in the

Fig. 29

presence of K^+ or Rb^+ on the light-induced pH change of chromatophore suspensions (Figs. 4 and 5) may be brought about by stimulation by the antibiotic of the rate and amount of liberation of K^+ or Rb^+ from the chromatophore membrane. The

inhibitory effects of NaCl and LiCl on the stimulation of the light-induced pH change by K^+ plus valinomycin (Fig. 5) suggests that these cations could be adsorbed on common anionic residues of the chromatophore membrane.

In chromatophore suspensions, the light-induced absorbance changes of bromothymol blue and neutral red were similar to the light-induced pH change, as regards the effects of KCl (Fig. 8). The absorbance changes of both dyes were stimulated by high concentrations of glycylglycine-NaOH buffer. This suggests that both dyes bound with the chromatophore membrane and/or near the surface of the membrane were protonated to higher extents when the chromatophores were illuminated. In low concentrations of the buffer, their absorbance changes were enhanced to the highest extent by 0.33 M monovalent inorganic cation salts. However, the light-induced absorbance changes of bromothymol blue and neutral red were different (Fig. 9 and Table III). These redults indicate that parts of bromothymol blue and neutral red were bound with and/or accessible to different loci in the chromatophore membrane. The K_m value for bromothymol blue was appreciably higher than that for neutral red (Fig. 11). In addition, neutral red inhibited one-half of the absorbance change of bromothymol blue, whereas bromothymol blue hardly influenced the absorbance change of neutral red (Fig. 10). Although the pK_a values of bromothymol blue and neutral red are similar, the electrostatic charges of both dyes are opposite, and the

former dye is more hydrophobic than the latter. This suggests that bromothymol blue is more accessible to loci carrying positive charges, while neutral red is more accessible to those carrying negative charges, and that the former dye can more easily bind to hydrophobic loci than the latter. This speculation is supported by the findings that 2-amino-2-methyl-1,3-propanediol and tris(hydroxymethyl)aminomethane inhibited the absorbance change of neutral red, but that of bromothymol blue was hardly influenced (Table III). It seems likely that, of the loci in the chromatophore membrane which could bind with bromothymol blue, only one-half was common with loci which could bind with neutral red, and that the common loci could bind with neutral red significantly more easily than with bromothymol blue. The common loci may have a hydrophobic nature and a negative charge.

Hosoi et al. (50) found that bromothymol blue, but not neutral red, inhibited photosynthetic ATP formation significantly at a concentration of 25 μM. They (19) suggested that several inhibitory pH indicators, including bromothymol blue, compete with p_i for the "energized" sites of the coupling enzyme in chromatophore membrane. At present, it seems reasonable to speculate that the loci in the chromatophore membrane which can bind with bromothymol blue, but not with neutral red, are present very near the coupling enzyme and are sensitive to oligomycin. On the other hand, the loci carrying negative charges,

which can bind with neutral red, may reflect the coupling mechanism between photosynthetic electron transport and phosphorylation.

II. STRUCTURAL PART

The concept that the photochemical apparatus and the electron transport systems exist as complexes composed of proteins and phospholipids in the membranes of chloroplasts and chromatophores is currently accepted (77-82). Each of these complexes is called a photosynthetic unit. In fact, photosystem I and photosystem II have been purified from chloroplasts, and shown to have 400 and 600 chlorophylls in each unit, respectively (83-86). By measuring the photosynthetic ATP formation in response to a flashing light, Nishimura (87) deduced that in photosynthetic bacteria, the photosynthetic unit contains 20-40 bacteriochlorophylls. However, this bacterial photosynthetic unit has not been isolated.

In the present study, particles of 7×10^5 daltons (purified F2) were solubilized from R. rubrum chromatophores by a mixture of cholate and deoxycholate. They contained about 10 different kinds of protein species, but not phospholipids, indicating that these particles were complexes of proteins. These protein complexes exhibited the same level of reaction center activity as chromatophores. In addition, they contained 33

bacteriochlorophylls, in good accord with the report by Nishimura (87). Recently, Kataoka and Ueki have found that purified F2 shows the same X-ray diffraction pattern as chromatophores, possibly involving a six-fold rotational symmetry (to be published). Their findings indicate that the protein complex has the same spatial arrangement as in the chromatophore membrane. The fact that the protein complex did not possess oxidation-reduction components such as cytochromes or ubiquinone-10 suggests that it is the photoreaction unit. There is a possibility that the photosynthetic unit is constructed when the oxidation-reduction components are properly attached to the photoreaction unit.

The reaction centers solubilized by mixtures of cholate and deoxycholate (C-DOC reaction center) (purified F3) consisted of 4 kinds of protein species. The protein species with molecular weights of 2.8×10^4, 2.7×10^4, and 2.6×10^4 in C-DOC reaction centers were the same, within experimental error, as those in LDAO reaction centers (53), whereas the protein species with a molecular weight of 1.0×10^4 in C-DOC reaction centers was not observed in LDAO reaction centers. The other components present in the purified F3 are bacteriochlorophyll, bacteriopheophytin and acid-labile iron (Table V). The contents of these components in purified F3 are practically the same as those of LDAO reaction centers; the experimentally obtained content of bacteriochlorophyll in purified F3 was somewhat lower

than that in LDAO reaction centers reported by van der Rest and Gingras (88). The weight of C-DOC reaction centers suggests that each C-DOC reaction center complex is composed of one LDAO reaction center complex and approximately 4 of the protein species of 1×10^4 daltons. It is possible that the linkages of the photoreaction unit to ubiquinone-10 protein and cytochrome $\underline{c}_2$ in the photosynthetic unit are so weak that mixtures of cholate and deoxycholate dissociate the quinone protein and cytochrome $\underline{c}_2$ during the course of repeated molecular-sieve chromatography. A preliminary study indicated that there were proteins capable of specifically binding cytochrome $\underline{c}_2$ and cytochrome $\underline{c}'$; the cytochrome $\underline{c}_2$-binding protein and the cytochrome $\underline{c}'$-binding protein were solubilized by cholate alone in the states bound with the appropriate cytochromes. The cytochrome $\underline{c}_2$-binding protein was probably dissociated from the reaction centers by C-DOC and by LDAO.

C-DOC reaction centers showed photo-oxidase activity (89-91). Ubiquinone-10 protein was essential for C-DOC reaction centers to oxidize ferro-cytochrome $\underline{c}_2$ in the light. This indicates that C-DOC reaction centers catalyze the electron transfer from cytochrome $\underline{c}_2$ to ubiquinone-10 protein, utilizing light energy. Erabi *et al.* (12) found that the quinone ring of ubiquinone-10 protrudes outside the chromatophore membrane, whereas Prince *et al.* (92) reported that cytochrome $\underline{c}_2$ is bound to the inside surface of the membrane. This indicates that the

photo-energized reaction center pumps electrons from the inside to the outside of the chromatophore membrane.

Earlier, Okayama et al. (9) observed light-induced absorbance changes at 860nm and 890nm (Liac-860 and Liac-890) in chromatophores, and deduced that Liac-860 ($E_{m,7}$ = +0.45 v) and Liac-890 ($E_{m,7}$ = -0.17 v) (12) are the electron-accepting and donating sites to the electron transport system, respectively. The component that exhibits Liac-860 presumably corresponds to C-DOC and LDAO reaction centers, although both reaction centers have a peak at 865nm (P-865). Liac-890 has not been observed with C-DOC and LDAO reaction centers, suggesting that the preparations of both reaction centers lack the Liac-890 component.

Trypsin and subtilisin BPN' catalyze the hydrolysis of peptide bonds with different specificities, and lactoperoxidase catalyzes iodination of tyrosine and histidine residues of proteins. Because both reactions occur by means of the usual enzyme-substrate complex and these enzymes have relatively high molecular weights, especially lactoperoxidase has a molecular weight of 77,500 (93), these reactions have been employed to determine the exposed proteins on biomembranes. When chromatophores were subjected to these treatments, it was found that almost all the polypeptides with molecular weights of higher than 25,000 were both accessible to enzymic iodination and sensitive to protease treatments, whereas, the polypeptides in group-L were practically inaccessible to either the iodination or the

digestion. The polypeptides in group-L were still inaccessible to the iodination after hydrolysis of the polypeptides with higher molecular weights. The results indicate that the polypeptides in group-L, which are main components of the photoreaction unit (purified F2), are buried in the lipid-bilayer structure, and that the polypeptides with molecular weights of higher than 25,000 exist partly protruding their peptide chains from the outer surface of chromatophore membrane into the medium. The latter polypeptides were digested by proteases added to the medium. However, after protease treatments followed by washing, some part of their fragments remained on the surface of chromatophore membrane so that the remaining fragments could be iodinated.

REFERENCES

1. Lascelles,J. (1959) Biochem.J. 72, 508-518
2. Oda,T.& Horio,T. (1964) Exptl.Cell Research 34, 414-417
3. Hickman,D.D.& Frenckel,A.W. (1965) J.Cell Biol. 25, 279-291
4. Yamaguchi,J.,Hasebe,E.& Higuchi,M. (1965) J.Electron Microscopy 13, 44-45
5. Holt,S.C.& Marr,A.G. (1965) J.Bacteriol. 89, 1402-1412
6. von Stedingk,L.-V.& Baltscheffsky,H. (1966) Arch.Biochem. Biophys. 117, 400-404
7. Hackson,J.B.,Crofts,A.R.& von Stedingk,L.-V. (1968) Eur. J.Biochem. 6, 41-54
8. Okayama,S.,Yamamoto,N.,Nishikawa,K.& Horio,T. (1968) J.Biol. Chem. 243, 2995-2999
9. Okayama,S.,Kakuno,T.& Horio,T. (1970) J.Biochem. 68, 19-29
10. Kakuno,T.,Hosoi,K.,Higuti,T.& Horio,T. (1973) J.Biochem. 74, 1193-1203
11. Higuti,T.,Erabi,T.,Kakuno,T.& Horio,T. (1975) J.Biochem. 78, 51-56
12. Erabi,T.,Higuti,T.,Kakuno,T.,Yamashita,J.,Tanaka,M.& Horio,T. (1975) J.Biochem. 78, 795-801
13. Erabi,T.,Higuti,T.,Sakata,K.,Kakuno,T.,Yamashita,J.,Tanaka,M. & Horio,T. (1976) J.Biochem. 79, 497-503
14. Kakuno,T.,Bartsch,R.G.,Nishikawa,K.& Horio,T. (1971) J.Biochem. 70, 79-94

15. Kagawa,Y. (1972) Biochim.Biophys.Acta 265, 297-338

16. Ito,A.& Sato,R. (1968) J.Biol.Chem. 243, 4922-4923

17. Helenius,A.& Simons,K. (1975) Biochim.Biophys.Acta 415, 29-79

18. Horio,T.,Nishikawa,K.,Katusmata,M.& Yamashita,J. (1965) Biochim.Biophys.Acta 94, 371-382

19. Hosoi,K.,Yoshimura,S.,Soe,G.,Kakuno,T.& Horio,T. (1973) J.Biochem. 74, 1275-1278

20. Nielsen,S.O.& Lehninger,A.L. (1955) J.Biol.Chem. 215, 555-570

21. Avron,M. (1960) Biochim.Biophys.Acta 40, 257-272

22. Yamamoto,N.,Hatakeyama,H.,Nishikawa,K.& Horio,T. (1970) J.Biochem. 67, 587-598

23. Cogdell,R.J.,Brune,D.C.& Clayton,R.K. (1974) FEBS Lett. 45, 344-347

24. Morton,R.A. (1965) in Biochemistry of Quinones (Morton,R.A.,ed.) Academic Press, New York

25. Clayton,R.K. (1963) in Bacterial Photosynthesis (Gest,H., San Pietro,A.& Vernon,L.P.,eds.) pp.495-500, The Antioch Press, Yellow Springs, Ohio

26. Folin,O.& Ciocalteu,V. (1927) J.Biol.Chem. 73, 629

27. Lowry,O.H.,Rosenbrough,N.J.,Farr,A.L.& Randall,R.T. (1951) J.Biok.Chem. 193, 265-275

28. Allen,R.J.L. (1940) Biochem.J. 34, 858

29. Fiske,C.H.& Subbarow,Y. (1925) J.Biol.Chem. 66, 375

30. Miller,R.W.& Massey,V. (1965) J.Biol.Chem. 240, 1453-1465

31. Horio,T.& Kamen,M.D. (1961) Biochim.Biophys.Acta 48, 266-286

32. Bartsch,R.G. (1963) in Bacterial Photosynthesis (Gest,H., San Pietro,A.& Vernon,L.P.,eds.) pp.475-494, The Antoich Press, Yellow Springs, Ohio

33. Yamamoto,A.& Adachi,S. (1972) Rinshokagaku (in Japanese) 1, 154

34. Dittmer,J.D.& Lester,R.L. (1964) J.Lipid Res. 5, 126

35. Weber,K.,Pringle,J.R.& Osborn,M. (1972) in Methods in Enzymology (Colowick,S.P.& Kaplan,N.O.,eds.) 26, pp.3-27, Academic Press, New York

36. Laemmli,U.K. (1970) Nature 227, 680-685

37. Miyazaki,K.,Hagiwara,H.,Nagao,Y.,Matuo,Y.& Horio,T. (1978) J.Biochem. 84, 135-143

38. Morrison,M. (1974) in Methods in Enzymology (Fleischer,S.& Packer,L.,eds.) 32, pp.103-109, Academic Press, New York

39. Phillips,D.R.& Morrison,M. (1971) Biochemistry 10, 1766-1771

40. Mueller,P.& Rudin,D.O. (1967) Biochem.Biophys.Res.Commun. 26, 398-404

41. Chance,B.& Mela,L. (1966) J.Biol.Chem. 241, 4588-4599

42. Chance,B.& Mela,L. (1967) J.Biol.Chem. 242, 830-844

43. Mitchell,P.,Moyle,J.& Smith,L. (1968) Eur.J.Biochem. 4, 9-19

44. Nishimura,M.,Kadota,K.& Chance,B. (1966) Arch. Biochem. Biophys. 117, 158-166

45. Lynn,W.S. (1968) J.Biol.Chem. 243,1060-1064

46. Pick,U.& Avron,M. (1976) FEBS Lett. 65, 348-353

47. Cost,K.& Frenkel,A.W. (1967) Biochemistry 6, 663-667

48. Jackson,J.B.& Crofts,A.R. (1969) Eur.J.Biochem. 10, 226-237

49. BDH Laboratory Chemicals (1975/6)

50. Hosoi,K.,Soe,G.,Kakuno,T.& Horio,T. (1975) J.Biochem. 78, 1331-1346

51. Harverkate,F.,Teulings,F.A.G.& van Deenen,L.L.M. (1965) Proc.Koninkl.Ned.Akad.Wetensch. Ser.B68, 154

52. Nöel,H.,van der Rest,M.& Gingras,G. (1972) Biochim.Biophys. Acta 275, 219-230

53. Okamura,M.Y.,Steiner,L.A.& Feher,G. (1974) Biochemistry 13, 1394-1402

54. Steiner,L.A.,Okamura,M.Y.,Lopes,A.D.,Moskowitz,E.& Feher,G. (1974) Biochemistry 13, 1403-1410

55. Walach,D.F.H. (1972) Biochim.Biophys.Acta 265, 61-83

56. Ito,A.& Sato,R. (1969) J.Cell Biol. 40, 179-189

57. Yamanaka,N.& Deamer,D.W. (1976) Biochim.Biophys.Acta 426, 132-147

58. Oelze,J. (1978) Biochim.Biophys.Acta 509, 450-461

59. Marchalonis,J.J.,Cone,R.E.& Santer,V. (1971) Biochem.J. 124, 921-927

60. Zürrer,H.,Snozzi,M.,Hanselmann,K.& Bachofen,R. (1979) Biochim. Biophys.Acta 460, 273-279

61. Mitchell,P.& Moyle,T. (1965) Nature 208, 147-151

62. Neumann,J.& Jagendorf,A.T. (1964) Arch.Biochem.Biophys. 107, 109-119

63. Kobayashi,Y.& Nishimura,M. (1973) J.Biochem. 74, 1217-1226

64. Kobayashi,Y.& Nishimura,M. (1973) J.Biochem. 74, 1227-1232

65. Kobayashi,Y.& Nishimura,M. (1973) J.Biochem. 74, 1233-1238

66. Kakuno,T.,Hosoi,K.,Higuti,T.& Horio,T. (1973) J.Biochem. 74, 1193-1203

67. Mitchell,P. (1961) Nature 191, 144-148

68. Mitchell,P. (1966) in Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation Glynn Research Ltd., Bodmin, Cornwell

69. Mitchell,P. (1966) Biol.Rev. 41, 445

70. Mitchell,P. (1972) in Mitochondria/Biomembranes, FEBS Symp. 28, held at Amsterdam pp.353-370

71. Oku,T.,Hosoi,K.,Kakuno,T.& Horio,T. (1974) J.Biochem. 76, 233-235

72. Horio,T.& Kamen,M.D. (1962) Biochemistry 1, 144-153

73. Horio,T.,von Stedingk,L.-V.& Baltscheffsky,H. (1966) Acta Chem.Scand. 20, 1-10

74. Horiuti,Y.,Nishikawa,K.& Horio,T. (1968) J.Biochem. 64, 577-587

75. Yamamoto,N.,Yoshimura,S.,Higuti,T.,Nishikawa,K.& Horio,T. (1972) J.Biochem. 72, 1397-1406

76. Baltscheffsky,H.& Baltscheffsky,M. (1974) Ann.Rev.Biochem. 43, 871-897

77. Kok,B. (1956) Biochim.Biophys.Acta 21, 245-258

78. Thomas,J.B.,Blaauw,O.H.& Duysens,L.M.N. (1953) Biochim. Biophys.Acta 10, 230-240

79. Becker,M.J.,Gross,J.A.& Schefner,A.M. (1962) Biochim.Biophys. Acta 64, 579-581

80. Park,R.B.& Pon,N.G. (1963) J.Mol.Biol. 6, 105-114

81. Park,R.B.& Biggins,J. (1964) Science 144, 1009-1011

82. Gross,J.A.,Becker,M.J.& Schefner,A.M. (1964) Nature 203, 1263-1265

83. Wessels,J.S.C. (1963) Proc.Roy.Soc.London B 157, 345

84. Ogawa,T.,Obata,F.& Shibata,K. (1966) Biochim.Biophys.Acta 112, 223-234

85. Huzisige,H.,Usiyama,H.,Kikuti,T.& Azi,T. (1969) Plant & Cell Physiol. 10, 441-455

86. Ohki,R.& Takamiya,A. (1970) Biochim.Biophys.Acta 197, 240-249

87. Nishimura,M. (1970) Biochim.Biophys.Acta 197, 69-77

88. van der Rest,M.& Gingras,G. (1974) J.Biol.Chem. 249, 6446-6453

89. Horio,T.& Yamashita,J. (1964) Biochim.Biophys.Acta 88, 237-250

90. Chaney,B.T.H.& Reed, D.W. (1970) Biochim.Biophys.Acta 216, 373-383

91. Prince,R.C.,Codgell,R.J.& Crofts,A.R. (1974) Biochim.Biophys. Acta 347, 1-13

92. Prince,R.C.,Baccarini-Melandri,A.,Hauska,G.A.,Melandri,B.A.& Crofts,A.R. (1975) Biochim.Biophys.Acta 387, 212-227

93. Rombants,W.A.,Schroeder,W.A.& Morrison,M. (1967) Biochemistry 6, 2965-2977

94. Straley,S.C.,Parson,W.W.,Mauzerall,D.C.& Clayton,R.K. (1973) Biochim.Biophys.Acta 305, 597-609

TABLE I. Salt-induced changes in dark and light-induced pH change of chromatophore suspensions. The initial pH of the chromatophore suspension before addition of inorganic salts in the dark was between pH 7.5 and 7.7. In the dark, various inorganic salts were added to the reaction mixtures, and the pH increases were measured ("Liberated in dark"). The resulting reaction mixtures were then illuminated and the pH increases were measured ("Incorporated in light"). Other experimental conditions were the same as for Fig.3.

Salts	Concentrations (mM)	Number of H^+/chromatophore	
		Liberated in dark	Incorporated in light
KCl	10	1,110	130
	33	1,110	170
	100	1,340	190
	330	1,670	230
NaCl	330	1,650	220
LiCl	330	2,230	210
RbCl	330	1,650	250
CsCl	330	1,470	160
$MgCl_2$	100	—	230
	330	2,710	100
$CaCl_2$	100	—	210
	330	2,890	80

TABLE II. Light-induced absorbance changes of various pH indicators in chromatophores. The buffers for pH 8.0 and 5.5 were 0.3 M glycylglycine-NaOH buffer containing 10% sucrose and 0.3 M 3,3-dimethylglutaric acid-NaOH buffer containing 10% sucrose, respectively.The pKa values of pH indicators were taken from the catalog of BDH Laboratory Chemicals (49) and from Hosoi et al. (50).

pH indicator	pKa	Concentration (μM)	mmol of pH indicator protonated/mol BChl	
			pH 8.0	pH 5.5
Qinaldin red	2.3	50	0.5	0.0[a]
Methyl orange	3.7	20	0.1	0.2
Bromophenol blue	3.8	17	0.1	0.0[a]
2,4-Dinitrophenol	3.9	50	0.0[a]	0.0
Ethyl orange	4.1	50	0.0	0.1
Bromocresol green	4.6	20	0.2	0.3
Gallein	5.1	50	0.0	0.0
Resazurin	5.6	50	0.0	0.2
Bromophenol red	6.0	17	0.0[a]	0.0
Bromocresol purple	6.0	17	0.0	0.0
4-Nitrophenol	6.0	150	0.2	0.0[a]
Neutral red	6.7	30	27.3	0.0[a]
Bromothymol blue	7.3	30	19.1	0.2
3-Nitrophenol	7.6	100	0.0	0.0
Phenol red	7.6	17	0.1	0.1
α-Naphtholphthalein	8.0	50	0.0	0.3
o-Cresolphthalein	9.6	17	0.0	0.0
Thymolphthalein	9.9	17	0.0[a]	0.0[a]
Alizalin yellow G	10.9	50	0.0[a]	0.0[a]

a Values from -0.1 to -0.4 were obtained

TABLE III. Effects of various reagents on light-induced absorbance changes of bromothymol blue and neutral red in chromatophores. Non-phosphorylating reaction mixtures comprised chromatophores (A_{873nm}= 1), 0.1 M glycylglycine-NaOH buffer (pH 8.0), and additives as indicated. Phosphorylating reaction mixtures comprised chromatophores, 0.1 M glycylglycine-NaOHbuffer (pH 8.0), 67 mM ascorbic acid, 6.7 mM ADP, 6.7 mM $MgCl_2$, and 6.7 mM sodium phosphate. In some cases, oligomycine was added. In this experiment, the concentrations of bromothymol blue and neutral red were 10 µM, a level at which both dyes had virtually no effect on photosynthetic ATP formation.

Reaction mixtures	Light-induced absorbance change (%) of	
	Bromothymol blue	Neutral red
Non-phosphorylating:		
No addition	(100)[a]	(100)[b]
+0.1 M 2-Amino-2-methyl-1,3-propanediol	79	17
+0.1 M Tris(hydroxymethyl)aminomethane	84	50
+0.1 M 3,3-Dimethylglutaric acid	84	86
+Oligomycine(7 µg/ml)	239	129
Phosphorylating:		
No addition	105	55
+Oligomycine(7 µg/ml)	341	94

a 21 mmol of bromothymol blue protonated/mol bacteriochlorophyll. b 35 mmol of neutral red protonated/mol bacteriochlorophyll.

TABLE IV. Comparison of components and activities of chromatophores and purified F2 (photoreaction units). Reaction center activities were measured in 0.05 M Tris-HCl buffer (pH 8.0) containing 0.3 % deoxycholate and 0.1 % cholate at 860 nm and 865 nm with chromatophores and purified F2, respectively, at which the preparations showed peaks in the light-induced absorbance change. In the absence of detergents, chromatophores showed a ratio of reaction center activity to 7.1 μM bacteriochlorophyll of 0.02.

Components and activity	Chromatophores	Purified F2 (photoreaction units)
Ubiquinone-10 (molecules)	308	<0.3
Phosphate, total (molecules)	5,000	90
Iron, total (atoms)	280	4
Reaction center activity/7.1 μM bacteriochlorophyll	0.01	0.01
Bacteriochlorophyll (molecules)	790	33
Weight in daltons:		
Total	2.5×10^7	7×10^5
Protein + bacteriochlorophyll	2.2×10^7	7×10^5
Phospholipid	0.3×10^7	0
Photoreaction units (number/chromatophore)	24	

TABLE V. Components associated with C-DOC reaction centers. The extinction coefficient at 802 nm of C-DOC reaction centers was taken as 2.88×10^5 $\underline{M}^{-1} \cdot cm^{-1}$ (94).

Components	Molecules or atoms in each C-DOC reaction center
Bacteriochlorophyll	3.4
Bacteriopheophytin	2.0
Acid-labile iron	1.9
Cytochromes, c-type	0.14
Ubiquinone-10	0.0

Fig. 1. Effects of monovalent inorganic cations on light-induced pH change of chromatophore suspensions. The reaction mixture contained various kinds of monovalent inorganic salt, 1 mM glycylglycine, 10% sucrose and chromatophores (A_{873nm}= 25), and its pH was adjusted to approximately 6 with HCl. On adding the salts, the initial rate of the light-induced pH change to a more alkaline value was stimulated, and the pH change reached an equilibrium state within a shorter time. ΔpH is expressed as the number of OH^- ions liberated/chromatophore.

Fig. 1

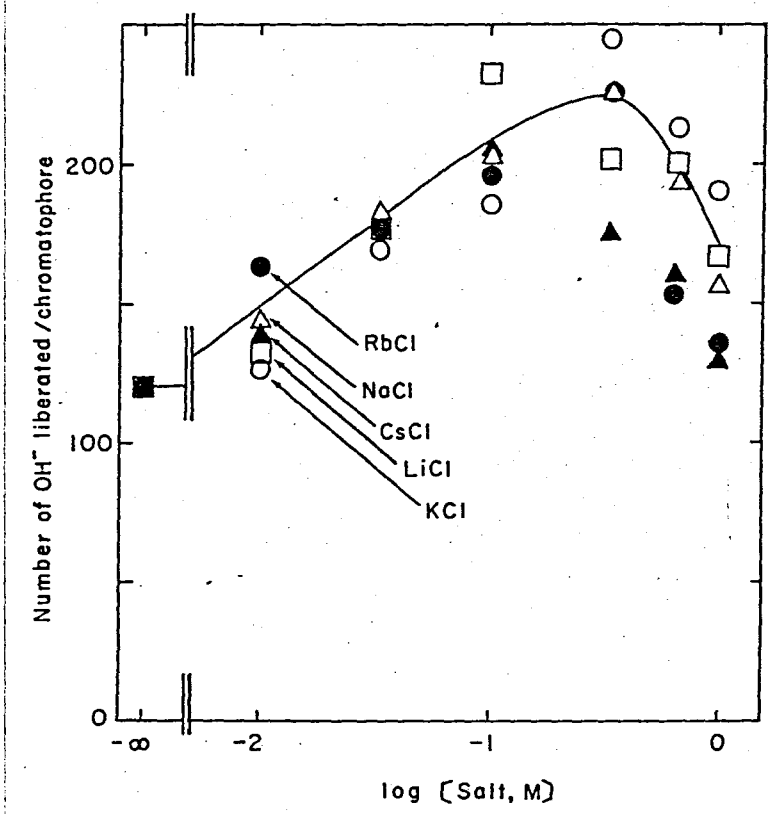

Number of OH⁻ liberated/chromatophore
200
100
0
-∞
-2
-1
0
log (Salt, M)
RbCl
NaCl
CsCl
LiCl
KCl

Fig. 2. Effects of divalent inorganic cation salts on light-induced pH change of chromatophore suspensions. The experimental conditions were the same as for Fig. 1, except that divalent inorganic cation salts were used.

Fig. 2

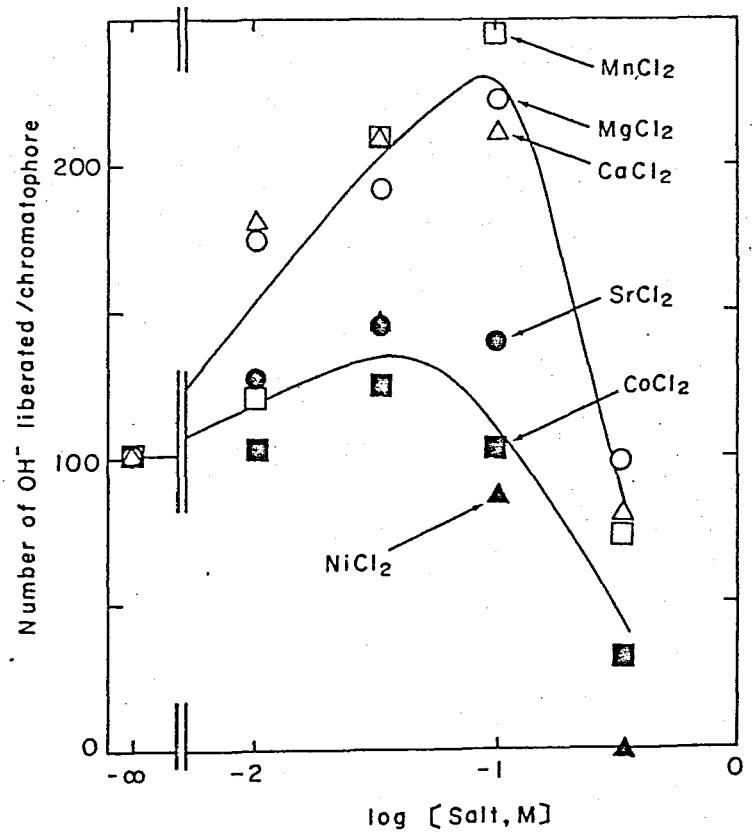

MnCl2
MgCl2
CaCl2
SrCl2
CoCl2
NiCl2
200
100
0
-∞
-2
-1
0
Number of OH⁻ liberated /chromatophore
log [Salt, M]

Fig. 3. Salt-induced changes in dark and light-induced pH changes of chromatophore suspensions. The components of the reaction mixtures were as follows. In 3.2 ml, (A) 1 m$\underline{\underline{M}}$ glycylglycine, 10% sucrose and chromatophores (A_{873nm}= 31), and (B) 1 m$\underline{\underline{M}}$ glycylglycine and 10% sucrose. At "+KCl," 0.8 ml of 1 m$\underline{\underline{M}}$ glycylglycine-NaOH buffer (pH 8.0) containing 10% sucrose and 1.66 $\underline{\underline{M}}$ KCl was pipetted into the reaction mixture in the dark. ON and OFF represent "light-on" and "light-off," respectively. The other experimental conditions are described in the text.

Fig. 3

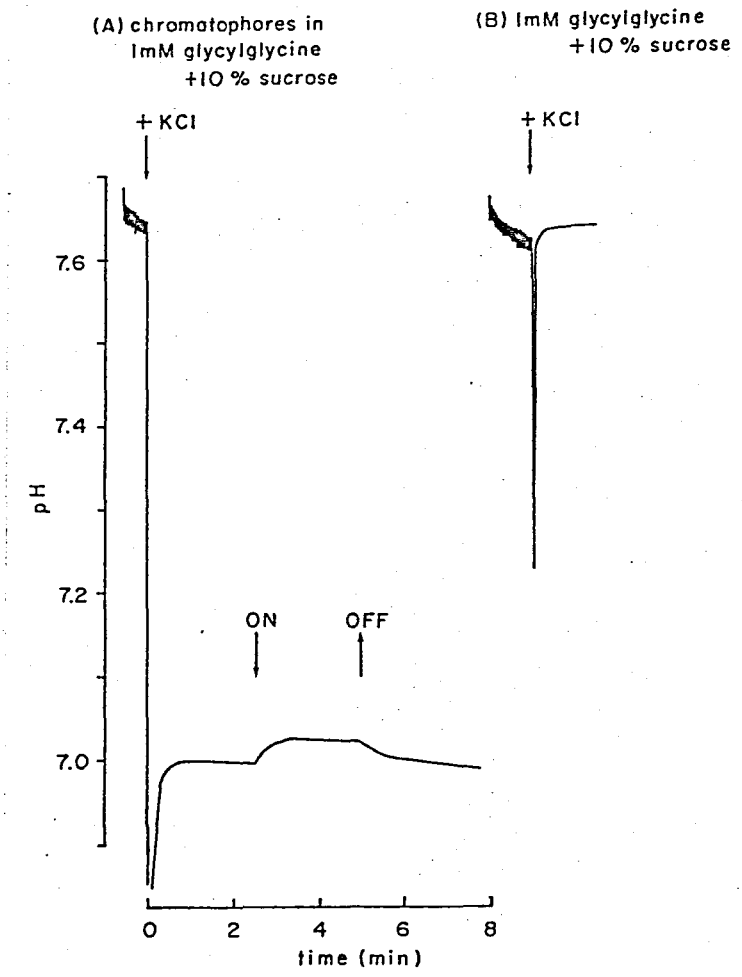

(A) chromatophores in
1mM glycylglycine
+10 % sucrose
+ KCl
(B) 1mM glycylglycine
+10 % sucrose
+ KCl
7.6
7.4
7.2
7.0
pH
ON
OFF
0
2
4
6
8
time (min)

Fig. 4. Effect of KCl concentration on light-induced pH change of chromatophore suspensions in presence and absence of valinomycin. In some cases, valinomycin (2 µg/ml) was added to the reaction mixture a few minutes before illumination. Other experimental conditions were the same as for Fig. 1. ○, No antibiotics; ●, +valinomycin.

Fig. 4

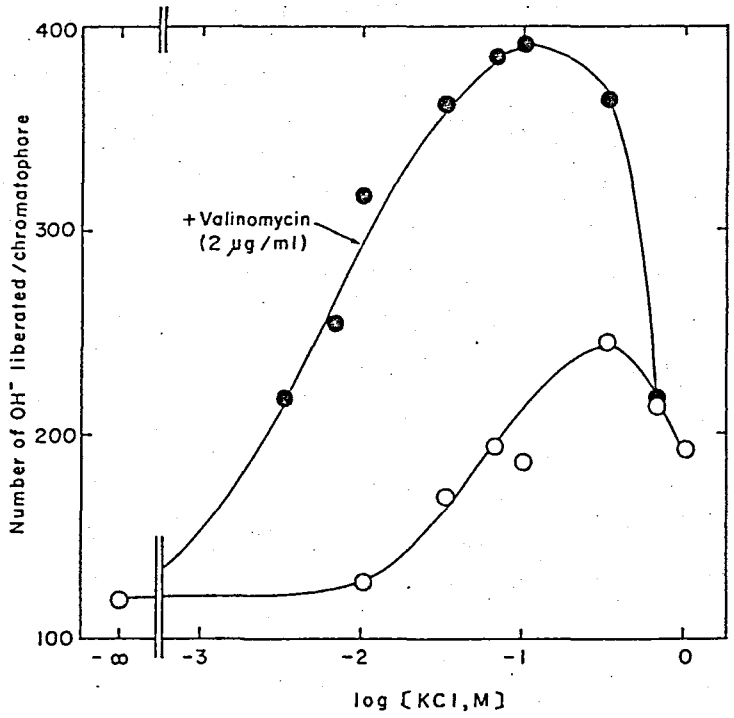

400
300
200
100
+Valinomycin
(2 μg/ml)
Number of OH⁻ liberated /chromatophore
- ∞
-3
-2
-1
0
log [KCl, M]

Fig. 5. Inhibitory effects of Li^+ and Na^+ on stimulation of light-induced pH change of chromatophore suspensions by valinomycin plus K^+. The standard reaction mixture contained 1 mM glycylglycine, 10% sucrose, 33 mM KCl, and chromatophores (A_{873nm}= 25). ○ & ●, NaCl; □ & ■, LiCl; △ & ▲, RbCl. Open symbols, in the absence of valinomycin; closed symbols, in the presence of the antibiotic.

Fig. 5

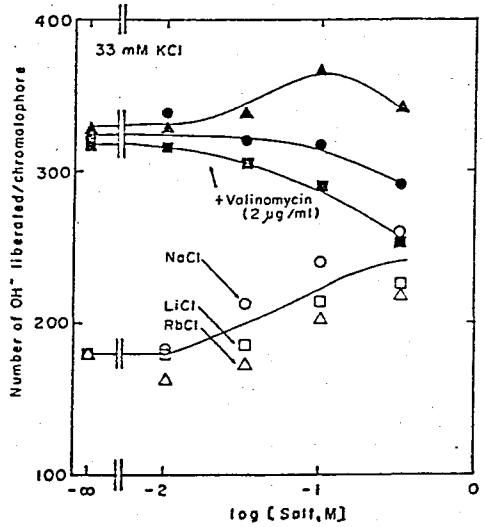

400
300
200
100
33 mM KCl
+Valinomycin
(2 μg/ml)
NaCl
LiCl
RbCl
-∞
-2
-1
0
log [Salt, M]
Number of OH⁻ liberated/chromatophore

Fig. 6. Effects of pH on light-induced pH change of chromatophore suspensions and photoreduction of ubiquinone-10 bound to chromatophores. The standard reaction mixture for determining the light-induced pH change of chromatophore suspensions contained 1 mM glycylglycine, 10% sucrose and chromatophores (A_{873nm}= 25). The pH of the reaction mixture was adjusted with HCl or NaOH as indicated, before illumination. The other experimental conditions are described in the text. The photoreduction of ubiquinone-10 (UQ) was not influenced by KCl.

Fig. 6

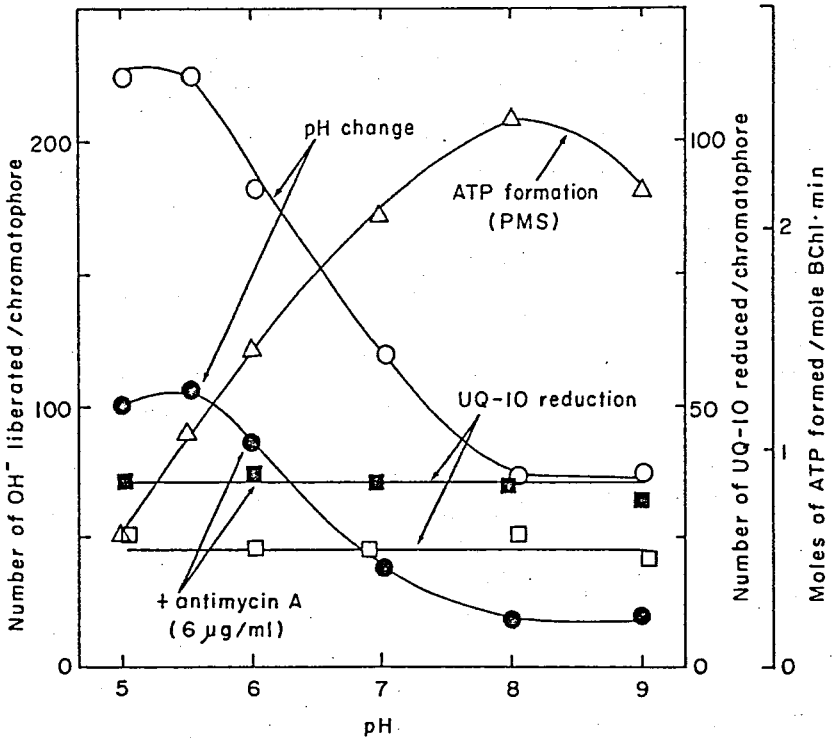

pH change
ATP formation
(PMS)
UQ-10 reduction
+ antimycin A
(6 μg/ml)
Number of OH⁻ liberated /chromatophore
Number of UQ-10 reduced /chromatophore
Moles of ATP formed /mole BChl·min
pH
200
100
0
100
50
0
2
1
0
5
6
7
8
9

Fig. 7. Light-induced absorbance changes of neutral red and bromothymol blue in chromatophores. The buffer used was 0.1 M glycylglycine-NaOH buffer (pH 8.0) containing 10% sucrose. The concentrations of neutral red (NR) and bromothymol blue (BTB) were always 20 μM.

Fig. 7

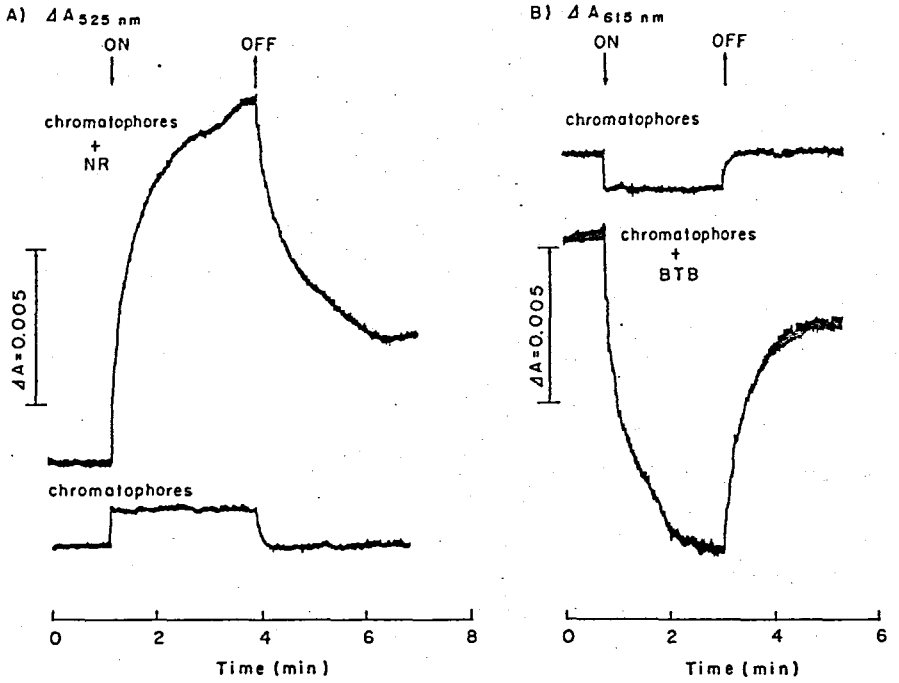

A) ΔA525 nm
ON
OFF
chromatophores
+
NR
ΔA=0.005
chromatophores
0
2
4
6
8
Time (min)
B) ΔA615 nm
ON
OFF
chromatophores
chromatophores
+
BTB
ΔA=0.005
0
2
4
6
Time (min)

Fig. 8. Effects of buffer concentration on light-induced absorbance changes of neutral red and bromothymol blue in chromatophores. ○ & △, Without other salts; ● & ▲, with 0.1 M KCl.

Fig. 8

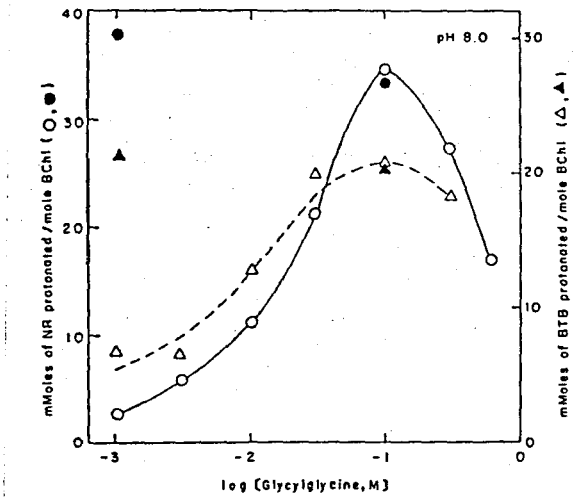

pH 8.0
mMoles of NR protonated / mole BChl (○, ●)
mMoles of BTB protonated / mole BChl (△, ▲)
log (Glycylglycine, M)
40
30
20
10
0
-3
-2
-1
0

Fig. 9. Effects of KCl concentration on light-induced absorbance changes of neutral red and bromothymol blue in presence and absence of valinomycin in chromatophores. ○ & △, No antibiotics; ● & ▲, +valinomycin.

Fig. 9

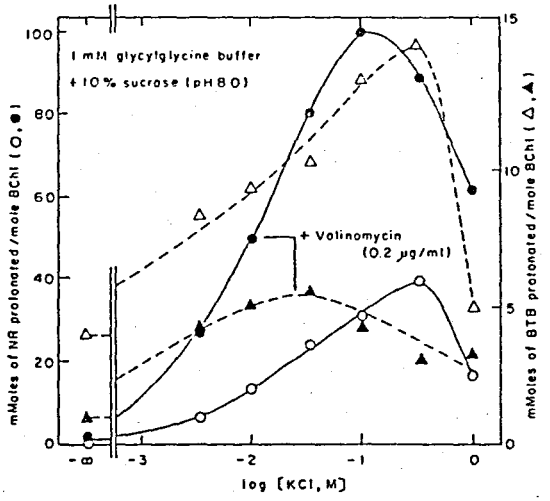

1 mM glycylglycine buffer
+ 10% sucrose (pH 8.0)
+ Valinomycin
(0.2 μg/ml)
mMoles of NR protonated / mole BChl (○, ●)
mMoles of BTB protonated / mole BChl (△, ▲)
log [KCl, M]
100
80
60
40
20
0
15
10
5
0
-∞
-3
-2
-1
0

Fig. 10. Effects of bromothymol blue and neutral red concentrations on light-induced absorbance changes of latter and former dyes, respectively, in chromatophores. The reaction mixture (3 ml) comprised 0.1 M glycylglycine-NaOH buffer (pH 8.0), 10% sucrose, chromatophores (A_{873nm}= 1), 25 μM neutral red (NR) (○) or bromothymol blue (BTB) (□), and the indicated concentration of BTB (○) or NR (□). The absorbance changes of NR and BTB were measured at 498 nm and 615 nm, respectively. The other experimental conditions are described in the text.

Fig. 10

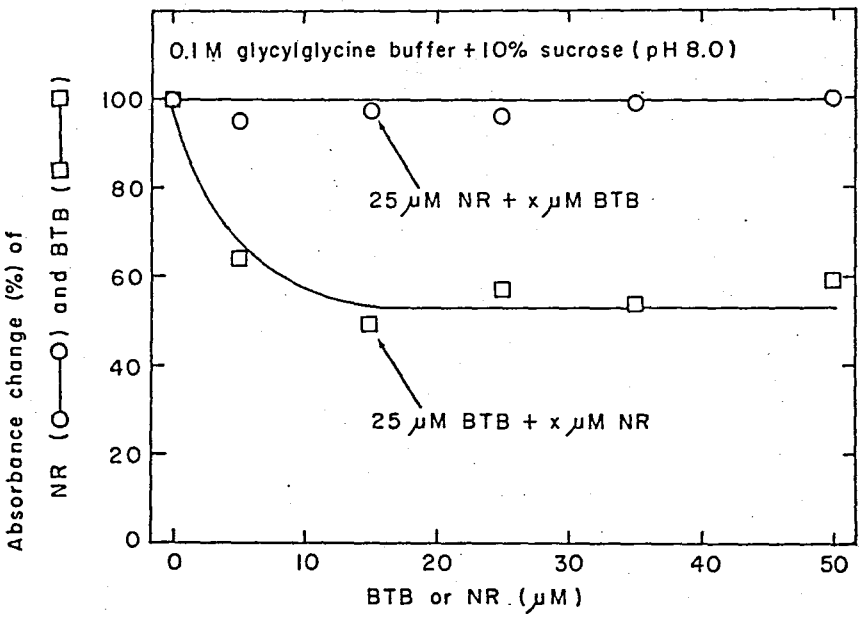

0.1 M glycylglycine buffer + 10% sucrose (pH 8.0)
25 μM NR + x μM BTB
25 μM BTB + x μM NR
Absorbance change (%) of
NR (O—O) and BTB (□—□)
BTB or NR (μM)
0
20
40
60
80
100
0
10
20
30
40
50

Fig. 11. Lineweaver-Burk plot for light-induced absorbance changes of neutral red and bromothymol blue in chromatophores.

Fig. 11

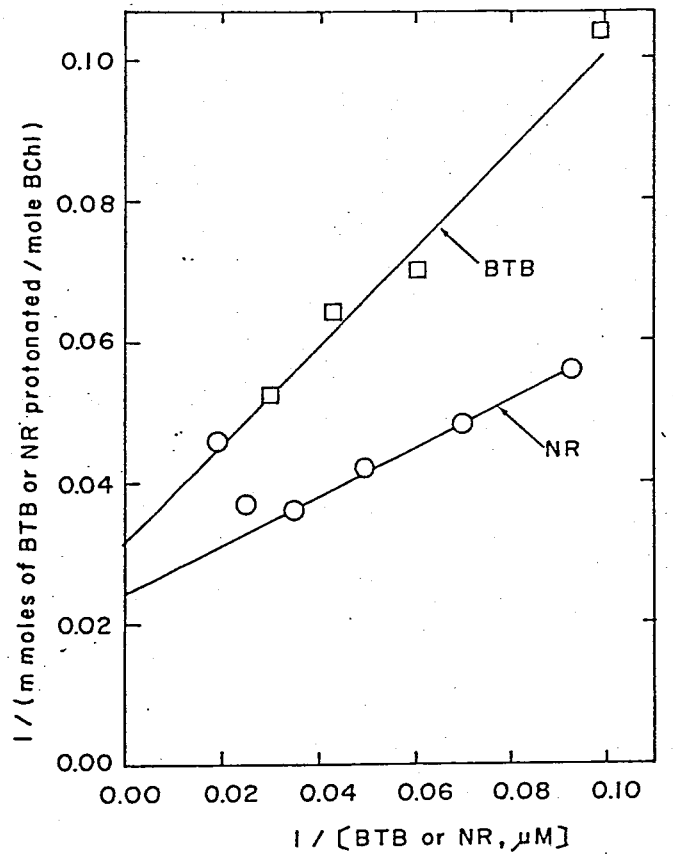

0.10
0.08
0.06
0.04
0.02
0.00
0.00
0.02
0.04
0.06
0.08
0.10
BTB
NR
1 / (m moles of BTB or NR protonated / mole BChl)
1 / [BTB or NR, μM]

Fig. 12. Acid-base titration curves of chromatophores in presence of 0.33 M KCl. Titration was performed in the dark with NaOH or HCl, with stirring. The other experimental conditions are described in the text.

Fig. 12

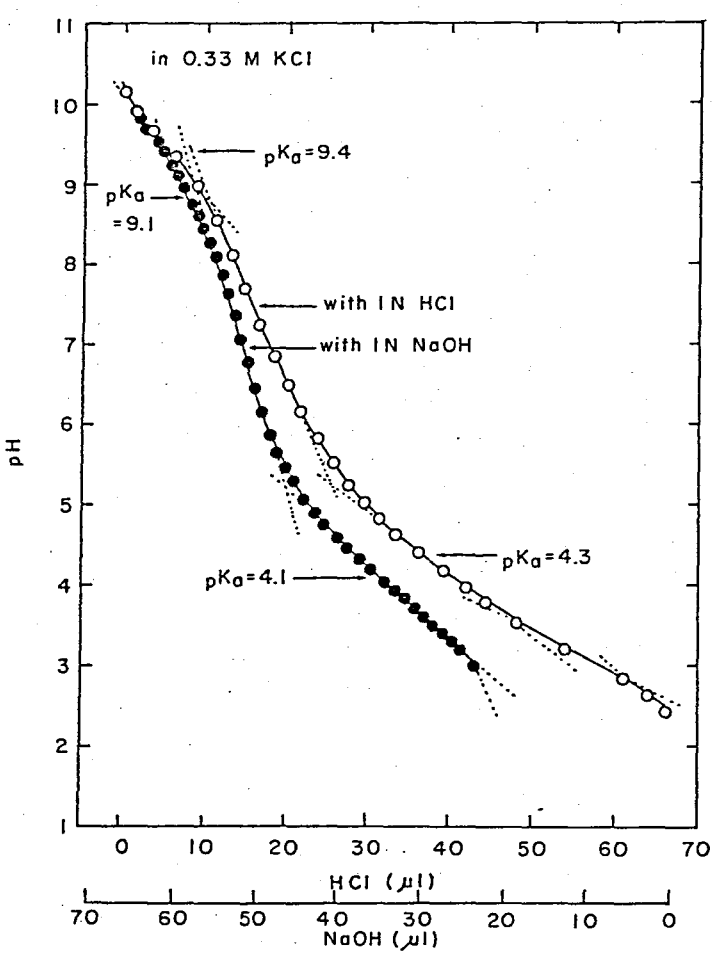

in 0.33 M KCl
pKa=9.4
pKa
=9.1
with 1N HCl
with 1N NaOH
pKa=4.3
pKa=4.1
pH
11
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
HCl (μl)
70
60
50
40
30
20
10
0
NaOH (μl)

Fig. 13. Molecular-sieve chromatography of C-DOC soluble fraction on Sepharose 6B column. The C-DOC soluble fraction (A_{873nm}= 60) (30 ml) was charged on a Sepharose 6B column (5 x 90 cm) and eluted with 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0), collecting 25-ml fractions. Other experimental conditions are described in the text.

Fig. 13

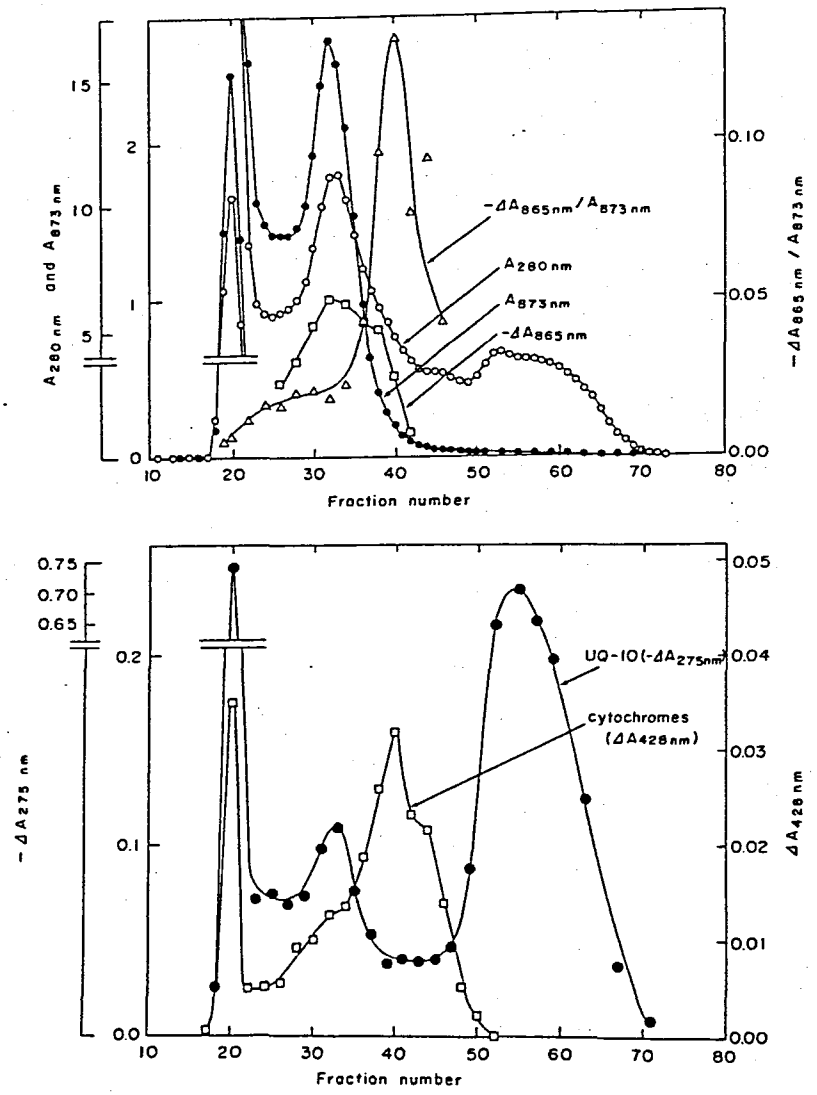

$A_{280\,nm}$ and $A_{873\,nm}$
$-\Delta A_{865nm}/A_{873\,nm}$
$A_{280\,nm}$
$A_{873\,nm}$
$-\Delta A_{865\,nm}$
$-\Delta A_{865\,nm}$ / $A_{873\,nm}$
Fraction number
$-\Delta A_{275\,nm}$
UQ-10($-\Delta A_{275nm}$)
cytochromes ($\Delta A_{428\,nm}$)
$\Delta A_{428\,nm}$
Fraction number

Fig. 14. Third molecular-sieve chromatography of F2 on Sepherose 6B column. The experimental conditions were the same as for Fig.13. The absorbance peak of chromatophores shifted from 873 nm to 865 nm on treatment with C-DOC.

Fig. 14

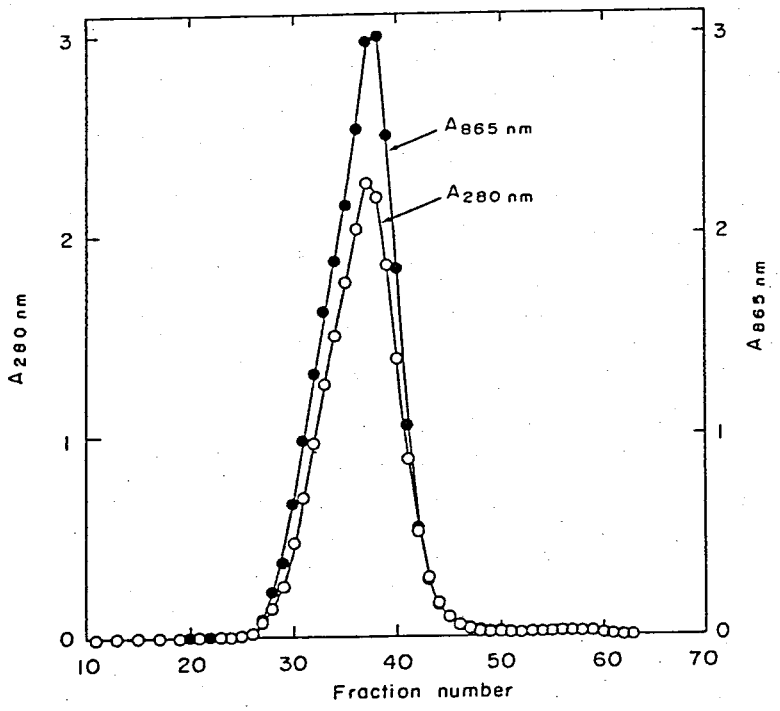

A865 nm
A280 nm
A280 nm
A865 nm
Fraction number
0
1
2
3
10
20
30
40
50
60
70

Fig. 15. Estimation of particle weight of purified F2 by molecular-sieve chromatography on Sepharose 6B column. The experimental conditions were the same as for Fig.14, except for the use of various molecular weight markers.

Fig. 15

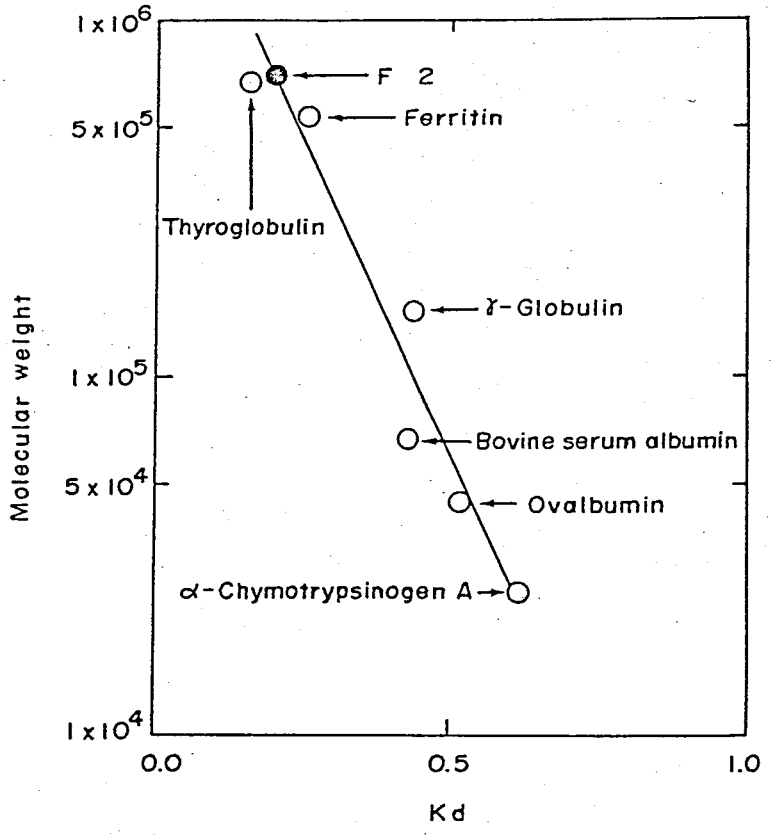

1x10^6
5x10^5
1x10^5
5x10^4
1x10^4
Molecular weight
F 2
Ferritin
Thyroglobulin
γ-Globulin
Bovine serum albumin
Ovalbumin
α-Chymotrypsinogen A
0.0
0.5
1.0
Kd

Fig. 16. Absorbance spectra of chromatophores and photoreaction units. A preparation of purified F2 (photoreaction units) was dissolved in 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0); chromatophores were suspended in 0.1 M Tris-HCl buffer (pH 8.0).

Fig. 16

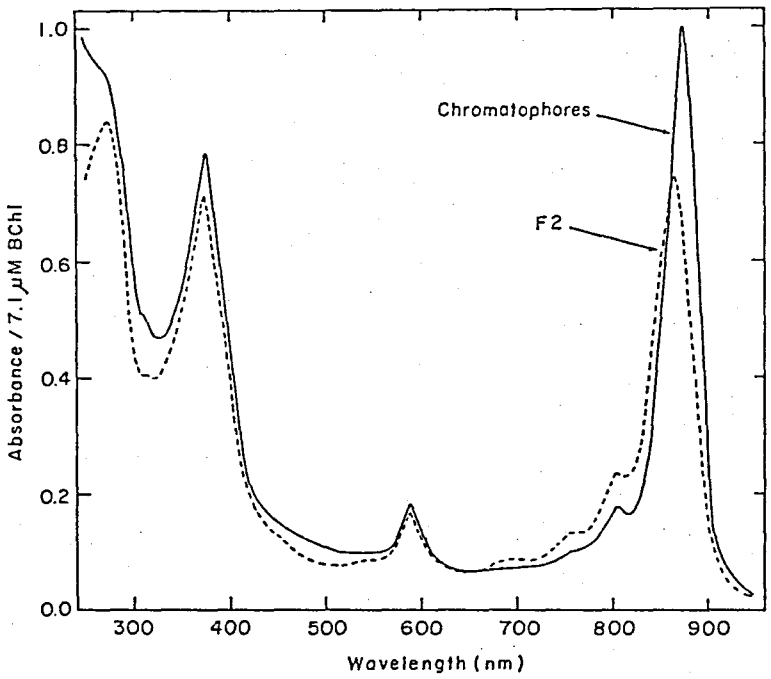

Chromatophores
F2
Absorbance / 7.1 μM BChl
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800
900

Fig. 17. SDS-polyacrylamide concentration-gradient slab gel electrophoresis of chromatophores, photoreaction units and C-DOC reaction centers. The stained gel slab was dried under a vacuum to give a thin film, and scanned at 670 nm. F2, photoreaction units; F3, C-DOC reaction centers. Other experimental conditions are described in the text.

Fig. 17

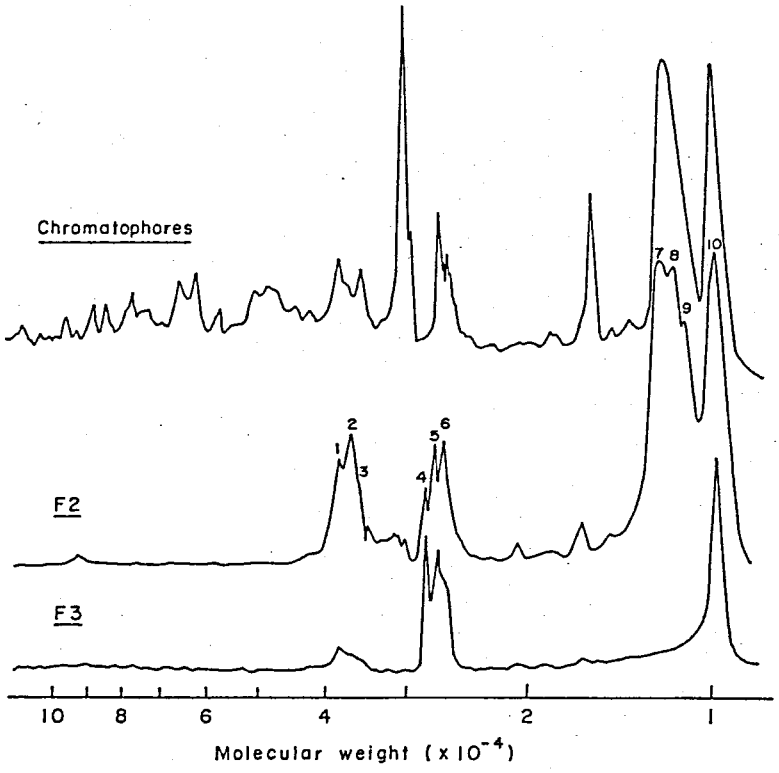

Chromatophores
F2
F3
1
2
3
4
5
6
7
8
9
10
10
8
6
4
2
1
Molecular weight (x 10⁻⁴)

Fig. 18. Thin-layer chromatography of extracts from chromatophores and photoreaction units with mixture of chloroform and methanol (2:1). F2, a preparation of photoreaction unit. Experimental conditions are described in the text.

Fig. 18

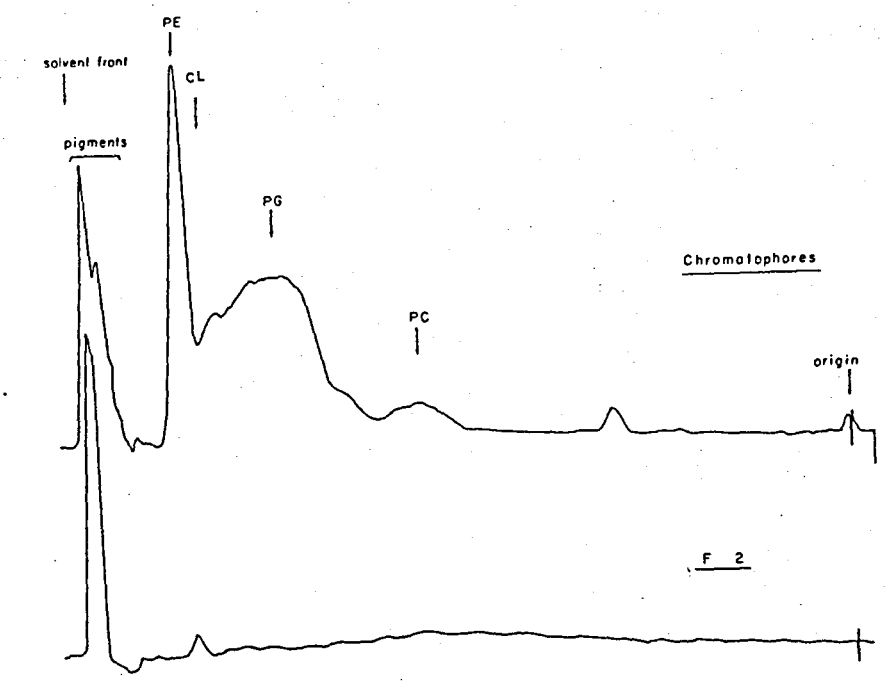

PE
solvent front
CL
pigments
PG
Chromatophores
PC
origin
F 2

Fig. 19. Third molecular-sieve chromatography of F3 on Ultrogel AcA 22 column. A concentrated solution of F3 was charged on an Ultrogel AcA 22 column (5 x 60 cm), and the charged column was developed with 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0). The resulting eluate was divided into 14-ml fractions.

Fig. 19

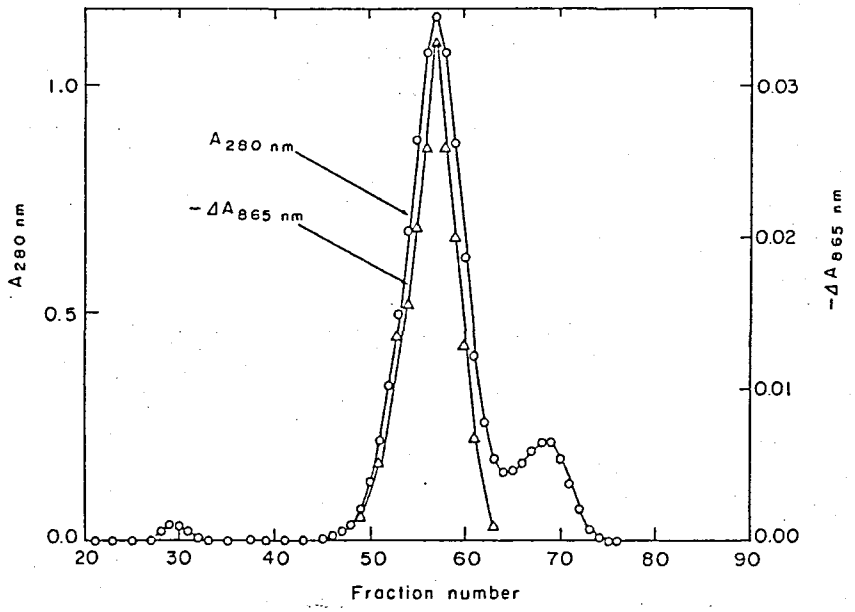

1.0
0.5
0.0
0.03
0.02
0.01
0.00
A280 nm
−ΔA865 nm
20
30
40
50
60
70
80
90
Fraction number

Fig. 20. Estimation of particle weight of C-DOC reaction centers by molecular-sieve chromatography on Ultrogel AcA 22 column. The experimental conditions are described in the text. ○, Marker proteins as indicated; ●, C-DOC reaction centers.

Fig. 20

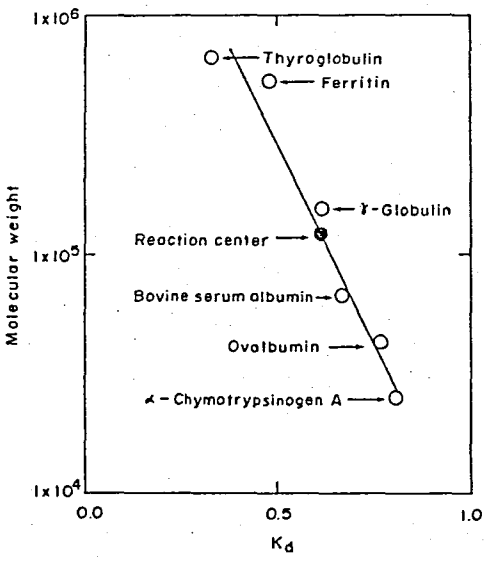

1x10^6
1x10^5
1x10^4
Molecular weight
Thyroglobulin
Ferritin
γ-Globulin
Reaction center
Bovine serum albumin
Ovalbumin
α-Chymotrypsinogen A
0.0
0.5
1.0
Kd

Fig. 21. Absorbance spectra of C-DOC reaction centers. The absorbance spectra of a sample of C-DOC reaction centers in 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0) were measured with and without the addition of potassium ferricyanide.

Fig. 21

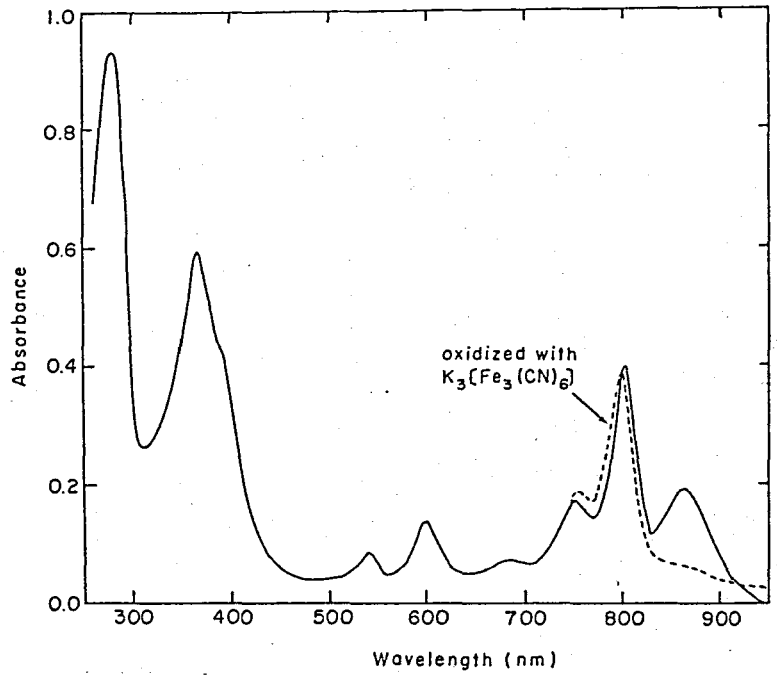

1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
oxidized with
$K_3[Fe_3(CN)_6]$
300
400
500
600
700
800
900
Wavelength (nm)

Fig. 22. Estimation of molecular weight of ubiquinone-10 protein by molecular-sieve chromatography on Sephadex G-75 column. The experimental conditions are described in the text. ○, Marker proteins as indicated; ●, ubiquinone-10 protein.

Fig. 22

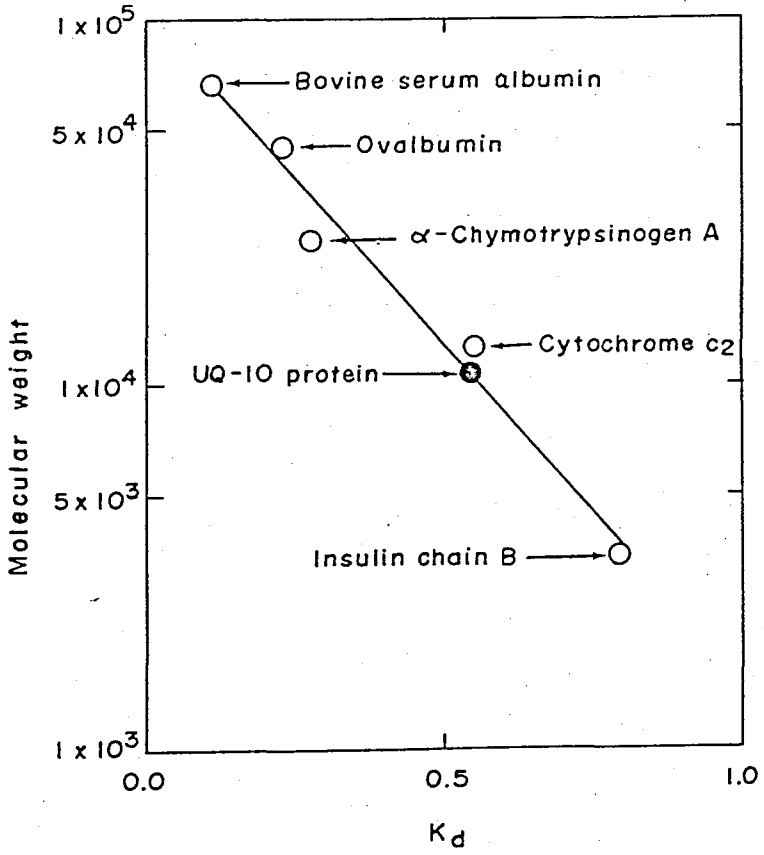

1 x 10^5
5 x 10^4
1 x 10^4
5 x 10^3
1 x 10^3
Molecular weight
Bovine serum albumin
Ovalbumin
α-Chymotrypsinogen A
Cytochrome c2
UQ-10 protein
Insulin chain B
0.0
0.5
1.0
Kd

Fig. 23. Absorbance spectra of ubiquinone-10 protein. A preparation of ubiquinone-10 protein was dissolved in 0.05 M Tris-HCl buffer containing 0.1% cholate and 0.3% deoxycholate (pH 8.0), and its absorbance spectra were measured with and without addition of $NaBH_4$.

Fig. 23

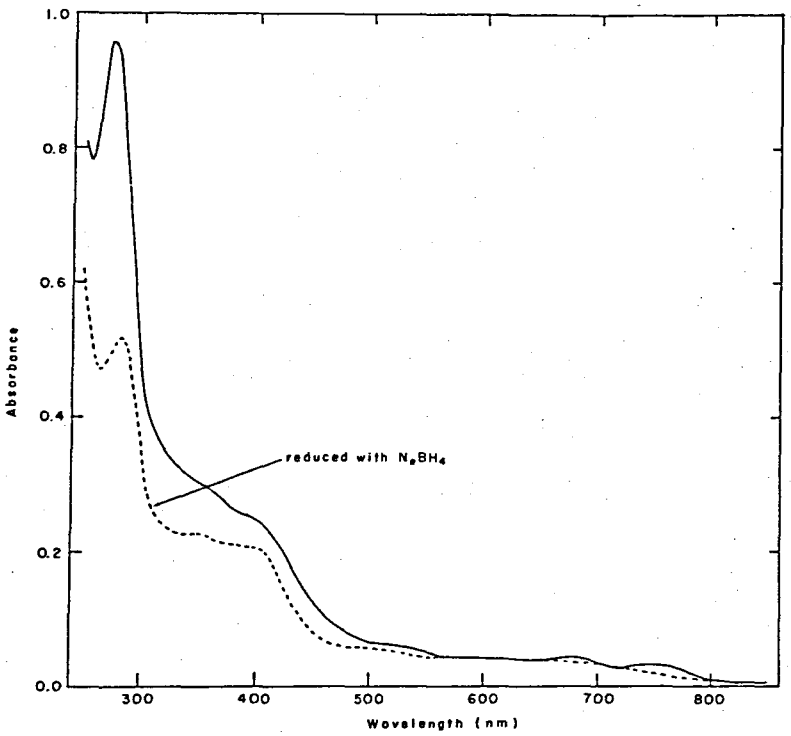

1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
reduced with N_aBH_4
300
400
500
600
700
800
Wavelength (nm)

Fig. 24. Effect of ubiquinone-10 protein on light-induced absorbance change of C-DOC reaction centers. The concentration of reaction centers was 0.44 μM. ○, Total change at both the fast and the slow phases; ●, change at the fast phase.

Fig. 24

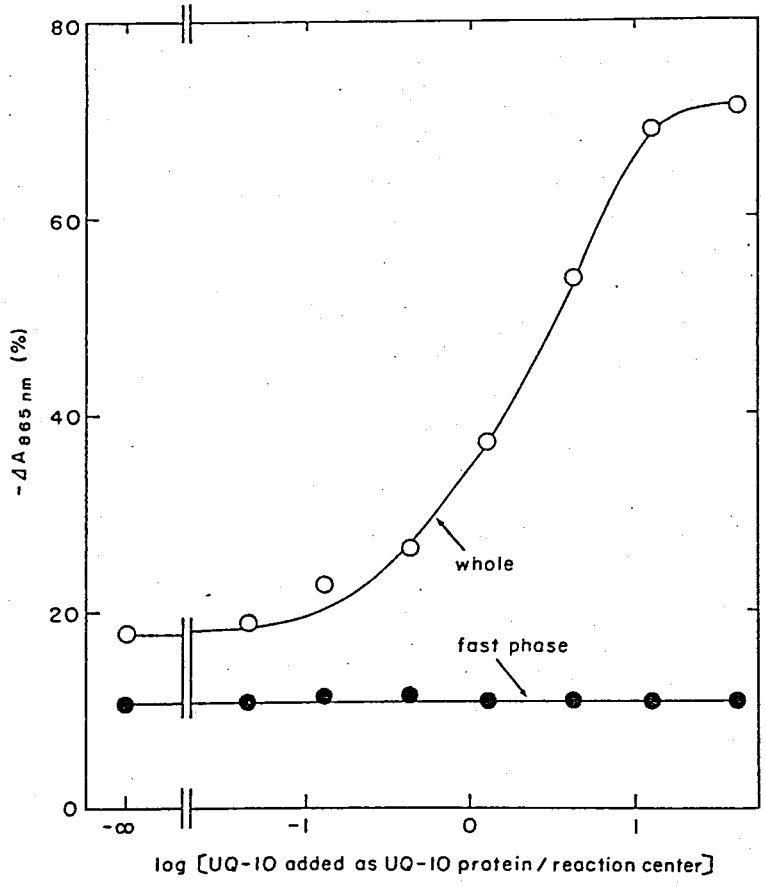

80
60
40
20
0
$-\Delta A_{865\,nm}$ (%)
whole
fast phase
-∞
-1
0
1
log [UQ-10 added as UQ-10 protein / reaction center]

Fig. 25. Kinetics of light-induced absorbance change of C-DOC reaction centers and of photo-oxidation of reduced cytochrome $\underline{c}_2$ in presence of C-DOC reaction centers. A1 and A2 show the kinetics of the light-induced absorbance change at 865 nm of C-DOC reaction centers; the concentrations of C-DOC reaction centers and ubiquinone-10 were 0.44 $\mu\underline{\underline{M}}$ and 5.8 $\mu\underline{\underline{M}}$, respectively. B1 and B2 show the kinetics of the photo-oxidation at 550 nm of reduced cytochrome $\underline{c}_2$; the concentrations of C-DOC reaction centers, reduced cytochrome $\underline{c}_2$ and ubiquinone-10 were 0.44 $\mu\underline{\underline{M}}$, 4.4 $\mu\underline{\underline{M}}$ and 1.3 $\mu\underline{\underline{M}}$, respectively. ON, light on; OFF, light off. Other experimental conditions are described in the text.

Fig. 25

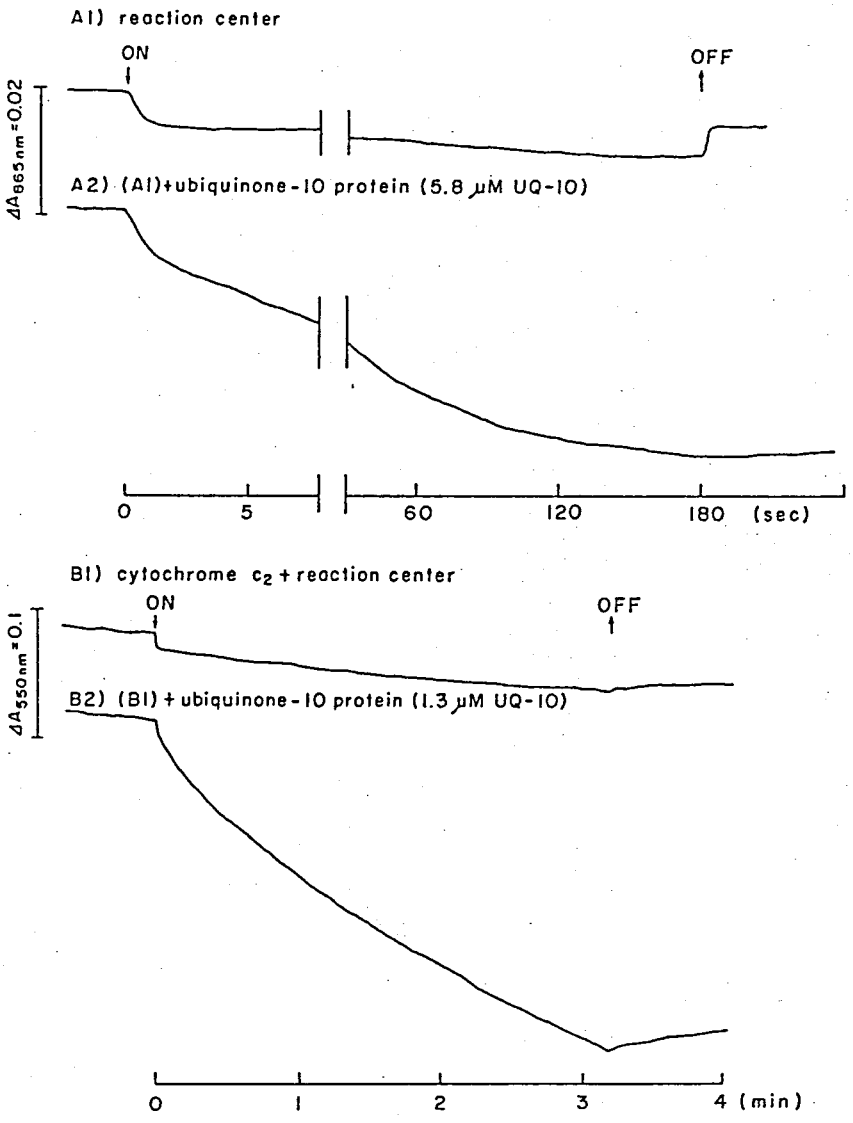

A1) reaction center
ON
OFF
ΔA865nm=0.02
A2) (A1)+ubiquinone-10 protein (5.8 μM UQ-10)
0
5
60
120
180 (sec)
B1) cytochrome c2 + reaction center
ON
OFF
ΔA550nm=0.1
B2) (B1) + ubiquinone-10 protein (1.3 μM UQ-10)
0
1
2
3
4 (min)

Fig. 26. Effect of reduced cytochrome $\underline{c}_2$ on light-induced absorbance change of C-DOC reaction centers. The concentrations of C-DOC reaction centers and ubiquinone-10 protein were 0.44 μ$\underline{\underline{M}}$ and 2.2 μ$\underline{\underline{M}}$. ○, Total change at both the fast and slow phases; ●, change at the fast phase.

Fig. 26

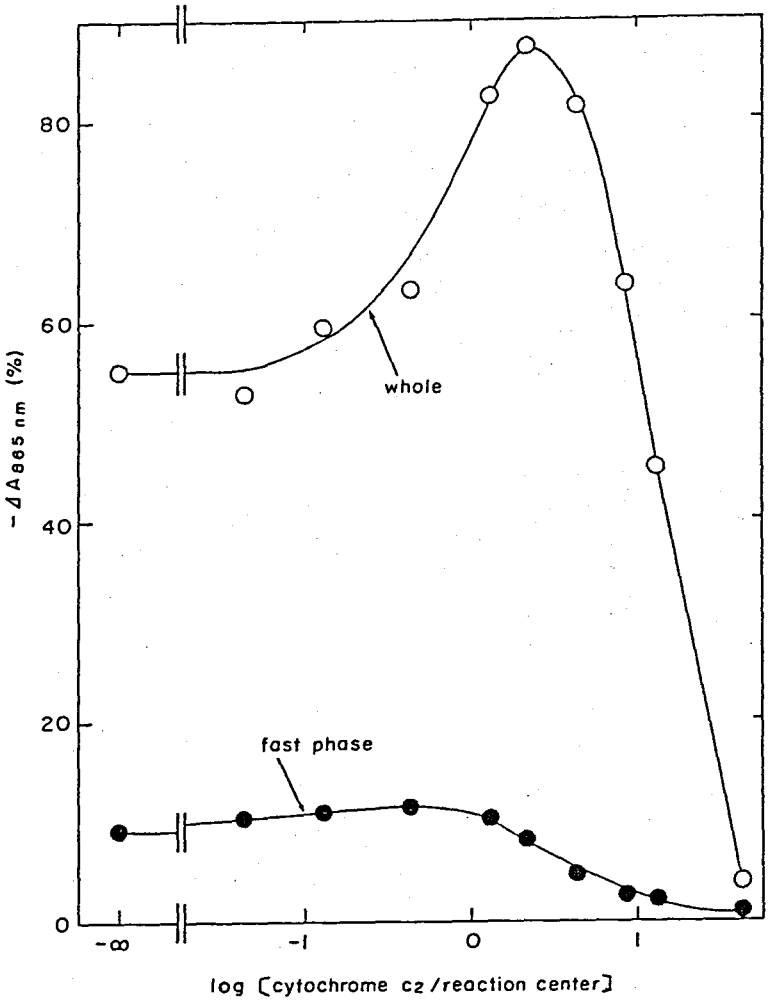

80
60
40
20
0
$-\Delta A_{865\,nm}$ (%)
whole
fast phase
$-\infty$
-1
0
1
log [cytochrome c_2 / reaction center]

Fig. 27. SDS-polyacrylamide concentration-gradient slab gel electrophoresis of iodinated chromatophores and iodinated photoreaction units, and their autoradiograms. The stained gel slab was dried under a vacuum to give a thin film, and the dried gel was exposed to X-ray film. F2, photoreaction units. Other experimental conditions are discribed in the text.

Fig. 27

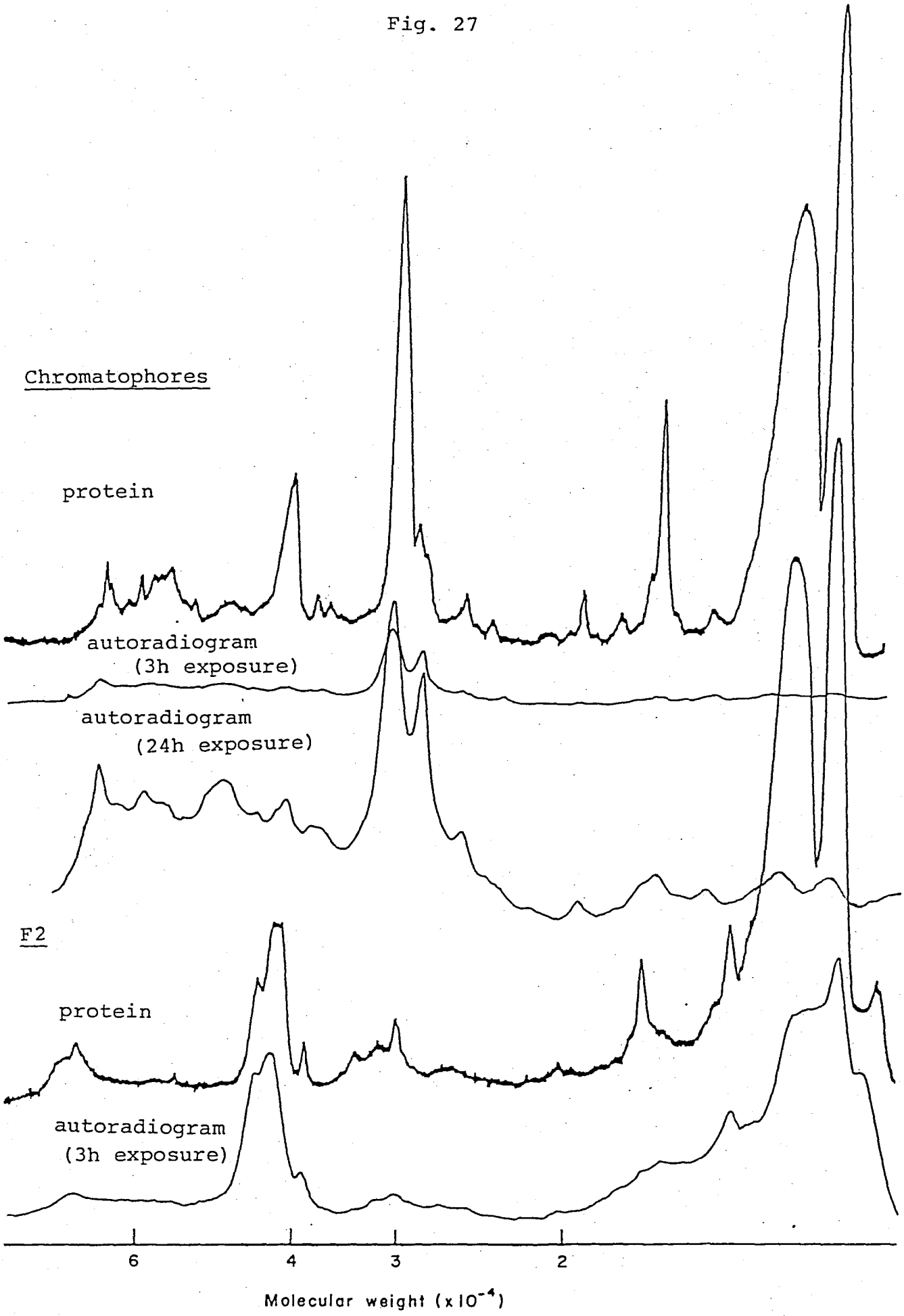

Chromatophores
protein
autoradiogram
(3h exposure)
autoradiogram
(24h exposure)
F2
protein
autoradiogram
(3h exposure)
6
4
3
2
Molecular weight (x10⁻⁴)

Fig. 28. SDS-polyacrylamide concentration-gradient slab gel electrophoresis of iodonated chromatophores, trypsin-treated then iodinated chromatophores and subtilisin BPN'-treated then iodinated chromatophores, and their autoradiograms. The experimental conditions were the same as for Fig. 27.

Fig. 28

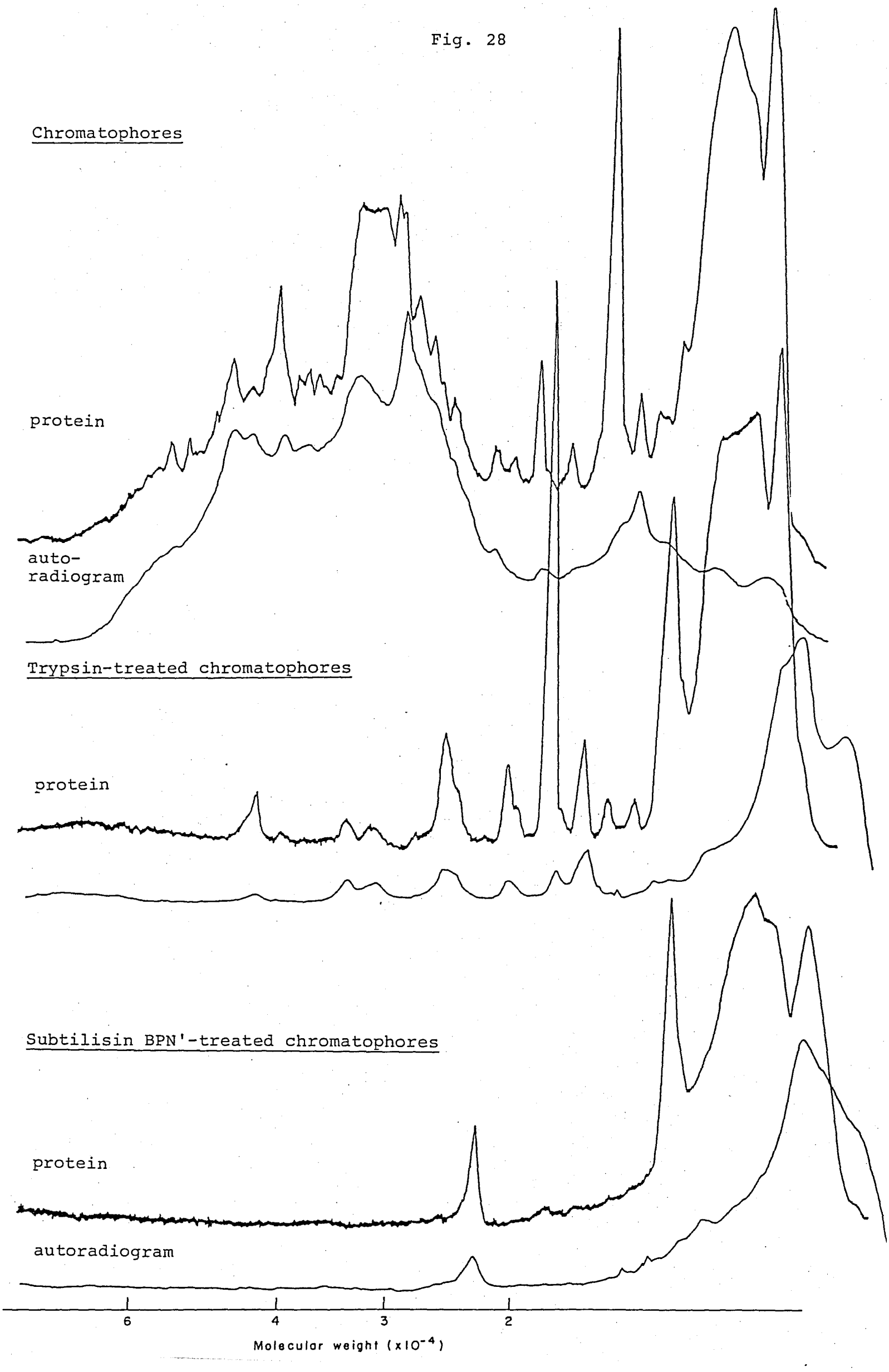
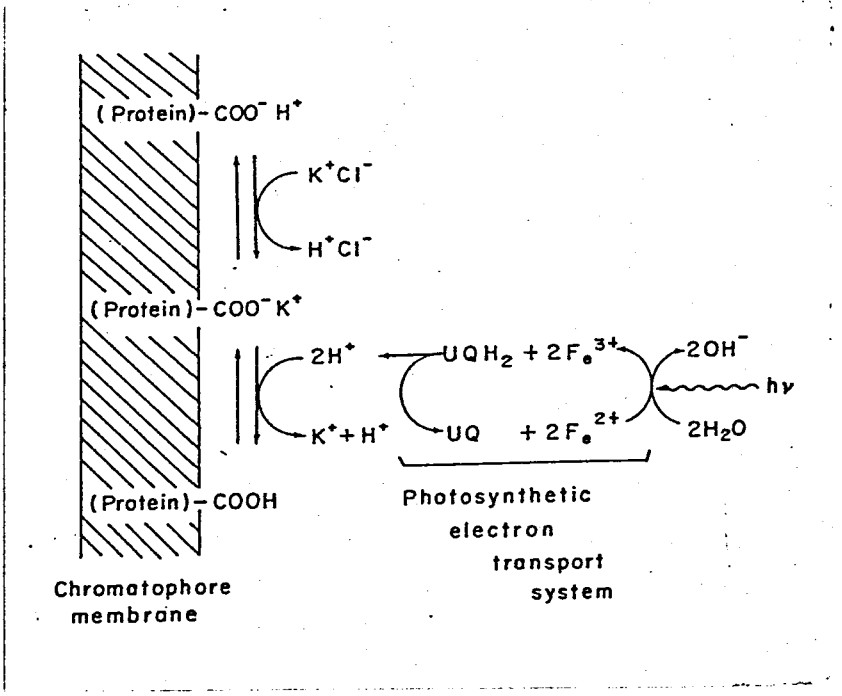

Chromatophores
protein
auto-
radiogram
Trypsin-treated chromatophores
protein
Subtilisin BPN'-treated chromatophores
protein
autoradiogram
6
4
3
2
Molecular weight (x10⁻⁴)

Fig. 29. Schematic mechanism for salt-induced changes in dark and light-induced pH changes of chromatophore suspensions. In the scheme, carboxyl groups in membrane proteins are regarded as anion-exchange groups present in the chromatophore membrane.

Fig. 29


(Protein)-COO⁻ H⁺
K⁺Cl⁻
H⁺Cl⁻
(Protein)-COO⁻K⁺
2H⁺
K⁺+H⁺
UQH₂ + 2Fe³⁺
UQ + 2Fe²⁺
2OH⁻
hν
2H₂O
(Protein)-COOH
Photosynthetic electron transport system
Chromatophore membrane