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TWO DIFFERENT NADH DEHYDROGENASES IN RESPIRATION OF *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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TWO DIFFERENT NADH DEHYDROGENASES IN RESPIRATION OF RHODOSPIRILLUM RUBRUM CHROMATOPHORES

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SUMMARY

In Rhodospirillum rubrum chromatophores, the oxidation of NADH by molecular oxygen was mostly exhibited by two different respiratory systems, one being composed of NADH:hemeprotein oxidoreductase (enzyme-H), flavin, cytochrome B and ubiquinone-10, and the other of NADH:quinone oxidoreductase (enzyme-Q) and flavin. Contributions of these two systems to the respiration were nearly equal. ATP formation was coupled with both systems. Added cytochrome c_2 was reduced by the system involving enzyme-H, whereas 2.6-dichlorophenol indophenol (DCPI) by the system involving enzyme-Q. Since the rate of oxidation was depressed to half when chromatophores were incubated either with the antiserum against enzyme-H (antiserum-H) or with the antiserum against enzyme-Q (antiserum-Q), it is probable that the molecules of enzyme-H and enzyme-Q were bound on the surface of chromatophore membrane in such a manner that the antibodies would combine with the respective enzymes. The system involving enzyme-Q was able to reduce bound quinone at a relatively slow rate. In intact chromatophores, carbon monoxide did not combine with the bound cytochrome B as well as the bound cytochrome cc', although these hemeproteins became to be combined with carbon monoxide when chromatophore membrane was impaired. Conceivably, the main sites responsible for the reduction of molecular oxygen were the quinone in the system involving enzyme-H, and the flavin in the system

involving enzyme-Q.

It was found by Geller (1,2) and by Geller and Lipmann (3) that chromatophores from the photosynthetic bacteria, R. rubrum, are able to catalyze the oxidation of NADH by molecular oxygen, which is capable of coupling the formation of ATP from ADP and Pi (1-3). Yamashita et al. (4) found that, in chromatophores, there were two electron transport systems different in optimum pH, and they suggested the presence of two kinds of NADH dehydrogenases. Later, Horio et al. (5) succeeded in purification of NADH:hemeprotein oxidoreductase (NADH:hemeprotein oxidoreductase, EC 1.6.99.3) and NADH:quinone oxidoreductase (NADH:quinone oxidoreductase, EC 1.6.99.2) from extracts of the light-grown cells. Both purified enzymes were free of flavin ; thus, they were inactive unless flavin was added. They found also that purified enzyme-H, if added to chromatophores, without addition of soluble flavin, could catalyze reductions by NADH of the cytochrome B (6) and the quinones, which were bound with chromatophores. Using antibodies against pure preparations of enzyme-H and enzyme-Q, it was previously demonstrated that chromatophore membrane possesses both enzymes, and that the bound enzyme-H is functional in the reduction of bound cytochrome B by NADH, but not the bound cytochrome cc', and suggested that bound enzyme-H catalyzes the electron transfer from NADH to the photosynthetic, cyclic electron transport system.

The present paper deals with immunological studies on functions of enzyme-H and enzyme-Q in the oxidation of NADH by molecular oxygen in chromatophores.

MATERIALS AND METHODS

The blue-green mutant strain (G-9) of R. rubrum was used. The cells were grown in the light, and chromatophores were prepared therefrom, according to the method described previously (6). Chromatophores thus prepared were washed with approximately 100 volumes of water, and then lyophilized (lyophilized chromatophores). Lyophilized chromatophores were suspended in isooctane (ml/36 nmoles of bacteriochlorophyll). The resulting suspension was homogenized, and stirred gently for one hr in an ice-water bath, followed by centrifugation (7). The resulting precipitate and supernatant are called "extracted chromatophores" and "isooctane extract", respectively. Higuti in our laboratory found that approximately 90% of the amounts of the bound quinones (ubiquinone-10 and rhodoquinone) (7) were extracted by the procedure described above (personal communication). Half the amount of the resulting extracted chromatophores was suspended in half the volume of the resulting isooctane extract. The suspension thus prepared was placed under vacuum in order to remove the isooctane. The resulting dried material is called "reconstructed chromatophores". Lyophilized, extracted and reconstructed chromatophores were separately suspended in such volumes of 0.1 M Tris-HCl buffer (pH 8.0) that the resulting suspensions would show $A_{873\ nm} = 50$. These suspensions were used for activity assay.

Enzyme-H and enzyme-Q were purified by the method previously described (5). Crystalline cytochrome c_2 was purified from light-grown cells of R. rubrum according to the method of Bartsch et al. (8). Antiserum against enzyme-H (antiserum-H) and antiserum against enzyme-Q (antiserum-Q) were prepared by the method described in the preceding paper (9).

The activities of chromatophores in darkness for cytochrome c_2 reduction by

NADH and for 2.6-dichlorophenol indophenol (DCPI) reduction by NADH were measured by the method described previously (9,10). The activity in darkness for NADH-O_2 reduction was spectrophotometrically measured at room temperature (24°C), in which the rate of NADH oxidation was estimated on the basis of the decrease in absorbance at 340 nm. The molar extinction of NADH at 340 nm was taken as 6.2×10^3 (11). The reaction mixture was composed of 20 mM Tris-HCl buffer (pH 6.9 or pH 8.0), 2 mM NADH and a chromatophore preparation (22 nmoles of bacteriochlorophyll) in 0.3 ml. In some cases were used the chromatophores that had been incubated with normal serum or antiserum at pH 6.9 or pH 8.0. The method for incubation was previously described in detail (9,10). The activity of chromatophores in darkness for oxidative ATP formation was measured according to the method of Yamashita et al. (4). The standard components of the reaction mixture were 40 mM Tris-HCl buffer (pH 6.9 or 8.0), 6.7 mM $MgCl_2$, 0.67 mM ATP, 0.09 unit of hexokinase, 67 mM glucose, 6.7 mM [^{32}P]Pi (5.0×10^6 cpm) and 6.7 mM NADH in 1.0 ml. The reactions were started by adding 0.2 ml of chromatophore preparation (230 nmoles of bacteriochlorophyll) and carried out at 30°C for 10 min, and stopped by adding 0.5 ml of 30% perchloric acid, previously cooled. The resulting solution were centrifuged, and the supernatants thus obtained were subjected to assay. The amount of ATP formed was measured according to the method of Avron et al. (12).

Difference spectra of chromatophore-bound cytochrome B and cytochrome cc' were measured as follows. To the reference cuvette were added 2.4 ml of a chromatophore suspension ($A_{873\ nm}$ = 20) in 0.1 M Tris-HCl buffer (pH 8.0) and 0.3 ml of 1.0 M ascorbate (pH 7.0). Instead of ascorbate, 0.3 ml of 10 mM NADH or 0.1 M succinate was added to the sample cuvette. Liquid paraffin was laid over the reaction mixture ; thus, anaerobic conditions were attained by the oxidation of substrate.

It is known that such a concentration of ascorbate reduces the bound cytochrome $\underline{c}_2$ completely, but barely the bound cytochrome cc' or cytochrome B, whereas succinate reduces the bound cytochrome $\underline{c}_2$ and cytochrome cc' (6). With the same sample and reference cuvettes, the difference spectrum was measured repeatedly. When the reduction of bound cytochrome B or bound cytochrome cc' reached the steady state, carbon monoxide was bubbled into the sample cuvette for 5 min at room temperature (20-24°C), followed by measurement of the difference spectrum. These difference spectra were automatically and repeatedly measured by a Cary model-17 spectrophotometer supervised by a Varian Spectrophotosystem-100 (Fig. 1). At the same time, the

Fig. 1

difference spectra were stored in the core memory of the computer. The stored data were converted into the difference spectra and recorded by a Varian Aerograph G-2500 recorder. The lock-in amplifier used was model 126, Princeton Applied Research Corporation, Princeton, New Jersey.

The uptake of molecular oxygen in chromatophores was measured by the apparatus shown in Fig. 2. To the vessel were added 0.6 ml of a chromatophore suspension

Fig. 2

($A_{873\ nm}$ = 75) and 3.8 ml of 0.1 M Tris-HCl buffer (pH 6.9 or pH 8.0). The reaction mixture was in contact with the air through the narrow channel of the removable plug ; thus, this device was suitable for measurement of oxygen uptake at a slow rate. Care was taken that the stirring bar would rotate at a constant speed.

A magnetic stirrer, model 4815 (Cole-Parmer Instrument of Equipment Co.,Chicago) was used. After the rate of oxygen uptake reached the steady state, 100 µl of 0.2 M NADH was added through the channel of the removable plug with the use of a micro-syringe equipped with a long, slender needle. The oxygen uptake was measured by a Beckman Fieldlab oxygen analyzer, and the resulting signals were recorded by a Varian Aerograph G-2500 recorder through the lock-in amplifier mentioned above.

NADH was the product of Oriental Yeast Co., Ltd., Osaka. [^{32}P]Pi was purchased from Radiochemical Centre, Amersham, and further purified according to the method of Suelter et al. (13).

RESULTS

Effect of extraction of quinones from chromatophores on NADH-cytochrome c_2 reduction and NADH-DCPI reduction.

It was demonstrated by Okayama et al. (7) that when quinones were extracted from chromatophores, the photosynthetic ATP formation was depressed, and that when the extracted quinones were added back, it was restored to the original level. Of the extracted quinones, ubiquinone-10 and rhodoquinone, only the former was effective.

The activities for NADH-cytochrome c_2 reduction and NADH-DCPI reduction varied from one chromatophore preparation to another. In addition, these activities remaining after lyophilization of chromatophores varied to significant extents. It was found that by extraction of quinones from chromatophores, the remaining activity for NADH-DCPI reduction was no longer influenced, whereas approximately 90% of the remaining activity for NADH-cytochrome c_2 reduction was further decreased (Table I).

Table I

When the extracted quinones were added back, the activity for NADH-cytochrome $\underline{c}_2$ reduction was restored to 30%. The restoration was reproducible in repeated experiments, although the restored activity was far below the activity before extraction. The inhibition of the electron transport system, antimycin A, inhibited half the activities with non-treated, lyophilized and reconstructed chromatophores, but barely the activity with isooctane-extracted chromatophores, at a concentration of 5 μg/ml, at which the photosynthetic ATP formation could be completely inhibited (4). These results suggest that quinones were involved in the system for NADH-cytochrome $\underline{c}_2$ reduction, but not the system for NADH-DCPI reduction.

Effect of antiserum-H and antiserum-Q on NADH-DCPI reduction in chromatophores.

With non-treated, lyophilized, isoctane-extracted and reconstructed chromatophores, 30-50% of the activity for NADH-DCPI reduction was depressed by antiserum-Q (Table II). However, the activity was barely influenced by normal serum and

Table II

antiserum-H. These results indicate that in chromatophores, bound enzyme-Q, but not bound enzyme-H, was functional in NADH-DCPI reduction, although both purified enzyme-H and enzyme-Q were able to catalyze NADH-DCPI reduction with addition of flavin (5).

Effect of antiserum-H and antiserum-Q on NADH-O_2 reduction and on ATP formation in chromatophores.

It was found that 40 to 60% of the activities for NADH-O_2 reduction and ATP formation coupled with the reduction were inhibited either by antiserum-H or by antiserum-Q ; the measurement of decrease in amount of NADH and the measurement of consumption

of molecular oxygen gave essentially the same results (Table III). This suggests

Table III

that in chromatophores, approximately half the activity for oxygen uptake by NADH was brought about by the system involving enzyme-H, the remainder by the system involving enzyme-Q. The fact that the activities for NADH-O_2 reduction and coupled ATP formation were significantly stimulated by normal serum, suggests the possibility that some molecules present in the preparation of normal serum could combine with chromatophore membrane. When both enzyme-H and enzyme-Q were simultaneously added, the activities for NADH-O_2 reduction and coupled ATP formation were inhibited to a higher extent than when any one of these two antisera was added. The extent of the inhibition by the simultaneous addition of the two antisera was partially additive. This may be explained as follows. Besides the formation of immunological complex with bound enzyme-H and enzyme-Q, some of the molecules contained in preparations of antiserum-H and antiserum-Q (antibody or other proteins) adhere non-immunologically to the surface of chromatophore membrane, so that the sdhereing molecules will prevent other molecules of antibody from their formation of the immunological complex with the bound enzyme.

Contrary to the expectation by Yamashita et al. (4), the activities for NADH-O_2 reduction and coupled ATP formation measured at pH 6.9 and those measured

at pH 8.0 were inhibited to almost the same extent either by antiserum-H or by antiserum-Q. Values for P/2e were essentially the same (0.5 to 0.7) for all the reactions, which were carried out at pH 6.9 and 8.0, and in the presence and absence of the antisera. These results indicate that the respiration by the system involving enzyme-H and the respiration by system involving enzyme-Q were coupled with ATP formation in almost the same efficiency. Yamashita et al. (4) used chromatophores from the wild type strain of R. rubrum, with which the activity for NADH oxidation at pH 6.9 was stimulated in the presence of the energy-trapping system (glucose + hexokinase) to a remarkably higher extent than that at pH 8.0. However, chromatophores from the blue-green mutant strain were used in the present study, in which the activities at pH 6.9 and pH 8.0 were stimulated only slightly by the addition of the energy-trapping system. There may be differences in respiratory system between chromatophores from the wild type strain and the mutant strain.

Effect of extraction of quinones from chromatophores on NADH-O_2 reduction.

The activity for NADH-O_2 reduction was depressed, when chromatophores were lyophilized. The depression by lyophilization varied to significant extent from one treatment to another. In the case shown in Table IV, approximately two-thirds

Table IV

the activity was depressed. When purified enzyme-H and enzyme-Q were added simultaneously to lyophilized chromatophores, the depressed activity was restored to the level before lyophilization. This suggests that, by lyophilization, the system involving enzyme-H and the system involving enzyme-Q were not influenced except the inactivation of bound enzyme-H and bound enzyme-Q. The restoration by

addition of enzyme-H was higher in extent than that by addition of enzyme-Q. When lyophilized chromatophores were sujected to isooctane extraction, the activity was further depressed. The depressed activity was significantly restored when the extract was added to the extracted chromatophores, in a good accordance with Yamamoto et al.(14), who found that the ubiquinone-10 present in the extract was the effective substance. When purified enzyme-H was added to the reconstructed chromatophores, the activity was increased significantly, but not when purified enzyme-Q was added. The activity increased by addition of enzyme-H was remarkably higher in reconstructed chromatophores than in isooctane-extracted chromatophores. Together with the findings by Yamamoto et al. (14), this suggests that bound ubiquinone-10 was contained as a redox-component in the respiratory system involving enzyme-H. Different from the case with lyophilized chromatophores, the restoration of the activity by the simultaneous addition of enzyme-H and enzyme-Q was incomplete in the case with reconstructed chromatophores ; approximately 70% of the activity was restored. In reconstructed chromatophores, the activity by addition of enzyme-H was approximately one-third as high in rate as that in lyophilized chromatophores. It is doubtless that in the system involving enzyme-H, some components other than the bound enzyme-H were also inactivated by isooctane-extraction and reconstruction. Obviously, the system involving enzyme-Q was also impaired by isooctane-extraction and reconstruction. In reconstructed chromatophores, the activity increased by addition of enzyme-Q was significantly low. However, the increased activity was usually higher in reconstructed chromatophores than in isooctane-extracted chromatophores. It seems likely that a part of the activity by the system involving enzyme-Q was brought about through the oxidation-reduction of the bound quinone.

Effect of carbon monoxide on absorption spectra of chromatophore-bound cytochrome B and cytochrome cc'.

It is known that in chromatophores, there are cytochrome c_2, cytochrome cc' and cytochrome B bound with the membrane (6). In the presence of an appropriate concentration of ascorbate, all the cytochrome c_2 is reduced, whereas the other cytochromes remain in the oxidized form. In addition, Kakuno et al. (6) have demonstrated that bound cytochrome B, but not bound cytochrome cc', is reduced by NADH, whereas bound cytochrome cc', but not bound cytochrome B, is reduced by addition of succinate, in most chromatophore preparations. The reduced-minus-oxidized difference spectra of bound cytochrome B and that of bound cytochrome cc' resemble each other, showing the α-peak at 561 nm and the γ-peak at 430 nm. It was found in the present study that the increase in absorbance at 430 nm due to the reduction of bound cytochrome B by NADH and that due to the reduction of bound cytochrome cc' by succinate were in agreement with the finding by Kakuno et al. (6) (Figs. 3,4 and Table V). Carbon monoxide was bubbled under anaerobic conditions into suspensions of

Fig. 3

Fig. 4

Table V

the chromatophores in the sample cuvette, the bound cytochrome B of which had been

reduced by NADH to the steady state. In most chromatophore preparations, it was observed that the α- and γ-peaks of cytochrome B were lowered in absorbance, while a new peak or shoulder was formed at 421 nm (Fig. 4). It seemes likely that the CO-bound cytochrome B had the γ-peak at 421 nm. The ratio in absorbancy of the newly-formed peak (or shoulder) at 421 nm to the remaining peak at 430 nm varied to great extent from one chromatophore preparation to another. With some chromatophore preparations, two-headed γ-peaks were observable. The two difference spectra, "NADH"-minus-"ascorbate" spectrum (A) and "NADH + CO"-minus-"ascorbate" (B), were stored in the core memory of the computer, which was directly connected to the recording spectrophotometer through a on-line system. Difference spectra (C) obtained by the following equation were then brought out from the computer.

$$(B \times 10) - (A \times n) = (C \times 10)$$

where n = 1,2,3, -----,10. The difference spectrum at n = 6 (C_6) still has a shoulder around 430 nm, whereas the difference spectrum at n = 8 (C_8) has a trough at 430 nm. The difference spectrum at n = 7 (C_7) shows a symmetric peak at 421 nm, which does show neither shoulder nor trough, and appears to approximate the difference spectrum, "NADH + CO"-minus-"ascorbate", of the CO-bound cytochrome. The value, n = 7, indicates that approximately 30% of the peak at 430 nm was converted into the peak at 421 nm. The absorbance of the latter peak was almost equal to the lowered absorbance of the former peak (Table V), suggesting that the molar extinction coefficients for the two peaks were similar. Repeated measurements of the difference spectra, A and B, showed that the α-peak at 551 nm was merely lowered in the presence of carbon monoxide. However, detailed changes around the α- and β-peaks have not yet been measured with accuracy. In most chromatophore preparations, only a shoulder was observed around 421 nm. In some chromatophore preparations, carbon

monoxide did not influence the difference spectrum, Effect of carbon monoxide on the bound cytochrome cc' reduced by succinate was essentially the same as that on the bound cytochrome B reduced by NADH. On the other hand, various activities such as photosynthetic and oxidative ATP formations or respirations were examined with several chromatophore preparations. The results indicate that the rates of NADH-O_2 reduction and succinate-O_2 reduction were completely independent of the amounts of the cytochromes capable of binding with carbon monoxide. To the contrary, they suggest that higher the activities of chromatophores (more "intact" the chromatophores), less in amount the CO-binding cytochromes. It may be worth the notice that the ratio in amount of the CO-binding cytochrome B to the total cytochrome B was similar to the ratio in amount of the CO-binding cytochrome cc' to the total cytochrome cc', in the several chromatophore preparations tested (Table V).

DISCUSSION

Soluble enzyme-H, if flavin is added, reacts either with soluble cytochrome c_2 or with DCPI (5). However, chromatophore-bound enzyme-H is functional in the reduction of cytochrome c_2, but not in that of DCPI (9). It was previously reported that when soluble enzyme-H is added to some chromatophore preparations, which are low in content of bound enzyme-H, the activity for reduction of bound cytochrome B is restored to the level for "intact" chromatophore preparations (5). It is conceivable, therefore, that enzyme-H, whether bound or added, is able to utilize as the prosthetic group, the flavin, which has been bound with chromatophore membrane, and that the flavin has been so baried in the membrane as to react neither with DCPI nor with soluble cytochrome c_2. It was found that the rate of oxygen uptake in the presence of

NADH by lyophilized chromatophores was depressed to approximately 35% when bound quinones were extracted, and that the depressed rate was restored to approximately 80% when the extract was added to the extracted chromatophores (Table IV), in accordance with the finding by Yamamoto et al.(14), who used ubiquinone-10 instead of the extract. It was previously reported that in chromatophores, antimycin A inhibits the reduction of soluble cytochrome c_2 to 20-50%, but not the reduction of bound cytochrome B, bound quinones, or the oxygen uptake by NADH (1,14). These results suggest that at least a part of the activity for oxygen uptake is shown by the system, bound or added enzyme-H ⟶ bound flavin (probably, FAD) ⟶ bound cytochrome B ⟶ bound quinone (ubiquinone-10)(Fig. 5).

Fig. 5

In most of the chromatophore preparations tested, carbon monoxide could combine with a part of the bound cytochrome B in the reduced form (Fig. 4). The total amount of bound cytochrome B is approximately 7 mmoles/mole bacteriochlorophyll, remarkably constant among several chromatophore preparations. However, the amount of the cytochrome B capable of binding with carbon monoxide varied to a significant extent from one chromatophore preparation to another ; the maximum extent was approximately 35% (Table V). In some chromatophore preparations, which were highly active in oxygen uptake by NADH and other activities involving photosynthetic and oxidative ATP formations, the bound cytochrome B did not react with carbon monoxide at all. It seems rational to conclude, therefore, that in "intact" chromatophores, carbon monoxide could not get in the heme moiety of the bound cytochrome B, and that when chromatophore membrane was impaired, the bound cytochrome B became so

naked as to be able to combine with carbon monoxide. It is probable that in "intact" chromatophores, the oxygen uptake due to the system involving enzyme-H was exhibited by the autooxidizability of the bound quinone. It was reported in the preceding papers (9, 10) that approximately half the activities of soluble enzyme-H and enzyme-Q are inhibited by antiserum-H and antiserum-Q, respectively. It was found in the present study that approximately half the activity for oxygen uptake by NADH in chromatophores was inhibited either by antiserum-H or by antiserum-Q (Table III). It is probable, therefore, that in chromatophores, approximately half the activity for oxygen uptake by NADH was exhibited by the system involving enzyme-H, whereas the remainder by the system involving enzyme-Q. Obviously, the system involving enzyme-Q for reduction of DCPI contained neither bound cytochrome B nor bound quinone, because the reductions of the cytochrome and quinone were not influenced by antiserum-Q (10) and by extraction of bound quinones (Table I & II). The system may be as follows : Bound or added enzyme-Q ⟶ bound flavin (Fig. 5). Perhaps, the bound flavin was the site, which was able to react with molecular oxygen as well as with DCPI. The oxygen uptake by the system involving enzyme-Q was slightly influenced by extraction-addition of quinones (see the difference between the values for "+enzyme-Q in isooctane-extracted and reconstructed chromatophores" in Table IV). It seems likely, therefore, that, in non-treated chromatophores, the contribution of the oxygen uptake by the quinone-dependent system involving enzyme-Q (dotted line in Fig. 5) to the total oxygen uptake was so slow that the depression of the oxygen uptake by antiserum-Q would be hardly detectable. It may be definite that both the oxygen uptake by the system involving enzyme-H and by the system involving enzyme-Q were coupled with ATP formation in similar efficiencies (Table III). In addition, it was found that bound cytochrome cc'

did not combine with carbon monoxide if chromatophores were "intact". It seems likely that in cells, the respiration is exhibited by the autooxidizability of the soluble cytochrome cc' present in the cytoplasmic fluid (16,17,18), besides the respiration by the membrane-bound respiratory systems described above.

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TABLE I

Effect of isooctane extraction on NADH-cytochrome c_2 reduction and NADH-DCPI reduction with variously treated chromatophores.

For activity assay of NADH-cytochrome c_2 reduction, the standard components of the reaction mixture were 0.1 ml of 10 mM NADH, 0.1 ml of 0.32 mM cytochrome c_2, 50 µl of a chromatophore suspension (36 nmoles of bacteriochlorophyll), 50 µl of 100 µg/ml antimycin A (in methanol), 0.1 M Tris-HCl buffer (pH 8.0) and water to make the total volume 1.00 ml. The reactions were started by adding NADH and continued at 24°C for 5 min. The reduction of cytochrome c_2 was monitored by measuring the absorbance change at 550 nm. For activity assay of NADH-DCPI reduction, 30 µl of 10 mM DCPI was added to the reaction mixture instead of cytochrome c_2, and the absorbance change at 600 nm was measured. The other experimental conditions were described in the text. The NADH-DCPI reduction was not influenced by antimycin A (5 µg/ml). BChl, bacteriochlorophyll.

Chromatophores	NADH-cytochrome c_2 reduction (mole of cytochrome c_2 reduced/mole BChl·min)		NADH-DCPI reduction (mole of DCPI reduced/mole BChl·min)
	No addition	+Antimycin A	
Non-treated	0.198	0.072	0.160
Lyophilized	0.101	0.042	0.090
Isooctane-extracted	0.012	0.010	0.088
Reconstructed	0.030	0.016	0.089

TABLE II

Effects of normal serum, antiserum-H and antiserum-Q on NADH-DCPI reduction with variously treated chromatophores.

A chromatophore suspension (0.10 μmole of bacteriochlorophyll and 9.3 mg of protein per ml) was incubated in the presence and absence of normal serum, antiserum-H or antiserum-Q. The other experimental conditions were the same as those for Table I.

Chromatophores	NADH-DCPI reduction (mole of DCPI reduced/mole BChl·min)					
	No addition	Normal serum		Antiserum-H		Antiserum-Q	
			(%)		(%)		(%)
Non-treated	0.44	0.41	(100)	0.44	(107)	0.28	(68)
Lyophilized	0.37	0.35	(100)	0.37	(106)	0.23	(66)
Isooctane-extracted	0.35	0.37	(100)	0.35	(95)	0.24	(65)
Reconstructed	0.34	0.37	(100)	0.34	(92)	0.19	(51)

TABLE III

Effects of normal serum, antiserum-H and antiserum-Q on NADH-O_2 reduction and ATP formation in chromatophores.

Chromatophores (9.3 mg as protein) were incubated with or without normal serum, antiserum-H or antiserum-Q (25 mg as protein) in 1.3 ml of 0.1 M Tris-HCl buffer (pH 8.0) ; the ratio in mg protein of the serum to the chromatophores was 2.7. The incubated chromatophore suspension was used for the activity assay. The oxidation of NADH by molecular oxygen, the uptake of molecular oxygen and the ATP formation were measured at pH 6.9 and pH 8.0. The other experimental conditions were described in the text.

Additions	NADH-O_2 reduction (mole of NADH oxidized or O_2 consumed/mole BChl·min)				ATP formation (mole of ATP formed/mole BChl·min)			
	pH 6.9		pH 8.0		pH 6.9		pH 8.0	
	NADH	O_2	NADH	O_2	ATP	P/2e	ATP	P/2e
No addition	0.24	0.12	0.20	0.090	0.12	0.50-0.50	0.10	0.50-0.56
Normal serum	0.30 (100%)	0.14 (100%)	0.26 (100%)	0.11 (100%)	0.16 (100%)	0.53-0.57	0.15 (100%)	0.58-0.68
Antiserum-H	0.16 (53%)	0.077 (55%)	0.14 (54%)	0.052 (47%)	0.097 (61%)	0.61-0.63	0.079 (53%)	0.56-0.76
Antiserum-Q	0.18 (60%)	0.076 (54%)	0.14 (54%)	0.054 (49%)	0.099 (62%)	0.55-0.65	0.077 (51%)	0.55-0.71
Antiserum-H + antiserum-Q	0.13 (43%)	0.056 (40%)	0.11 (42%)	0.046 (42%)	0.078 (49%)	0.60-0.70	0.064 (43%)	0.58-0.70

TABLE IV

Effects of additions of soluble enzyme-H and enzyme-Q to variously treated chromatophores on NADH-O_2 reduction.

The standard components of the reaction mixture were treated or non-treated chromatophores (25 nmoles of bacteriochlorophyll) and 0.1 M Tris-HCl buffer (pH 6.9 or pH 8.0) to make the total volume 0.3 ml. In some cases, a purified preparation of enzyme-H (75 μg) or/and a purified preparation of enzyme-Q (160 μg) were added to the reaction mixture as indicated. The other experimental conditions were described in the text.

Kinds of chromatophores / Additions	NADH-O_2 reduction (mmoles of NADH oxidized/mole BChl·min)			
	pH 6.9		pH 8.0	
	Net	Increased	Net	Increased
Non-treated chromatophores	276		257	
Lyophilized chromatophores	96	(0)	90	(0)
+enzyme-H	225	129	231	141
+enzyme-Q	141	45	148	58
+enzyme-H + enzyme-Q	283	187	276	186
Isooctane-extracted chromatophores	32	(0)	32	(0)
+enzyme-H	51	19	51	19
+enzyme-Q	32	0	32	0
+enzyme-H + enzyme-Q	60	28	62	30
Reconstructed chromatophores	77	(0)	74	(0)
+enzyme-H	122	45	135	61
+enzyme-Q	84	7	84	10
+enzyme-H + enzyme-Q	186	109	186	120

TABLE V

Changes by carbon monoxide of absorbances at 430 nm and 420 nm of chromatophore-bound cytochrome cc' and cytochrome B.

The experimental conditions were the same as those for Fig. 3. Because the base lines for the difference spectra were generally not horizontal, straight lines were drawn between positions at 410 nm and 460 nm, and between positions at 405 nm and 450 nm for the peak at 430 nm and the peak at 421 nm, respectively, and the absorbances at 430 nm and 421 nm relative to these respective base lines were used.

Reductants added to sample cuvettes	Difference absorbance at 430 nm ("+reductant"-minus-"+ascorbate")		Changes by CO of difference absorbance			
			At 430 nm		At 421 nm	
		(%)		(%)		(%)
Dithionite	0.276	(100)	-0.099	-36	0.094	34
Succinate	0.077	(100)	-0.018	-23	0.021	27
NADH	0.183	(100)	-0.060	-33	0.059	32
Succinate + NADH	0.264	(100)	-0.084	-32	0.090	34

Fig. 1. Block diagram of apparatus for measurement of various difference spectra.

Fig. 1.

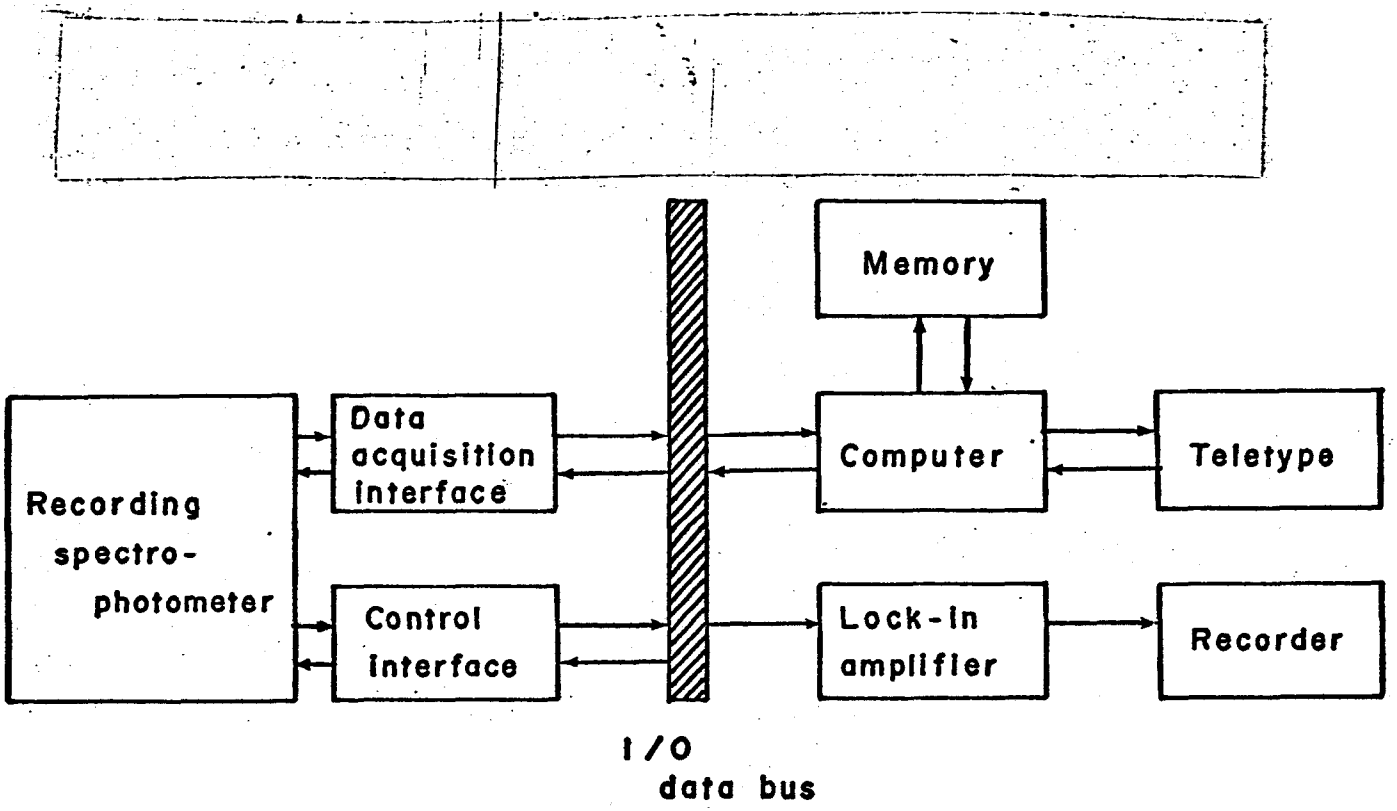

Memory
Recording spectro-photometer
Data acquisition interface
Control interface
Computer
Teletype
Lock-in amplifier
Recorder
I/O data bus

Fig. 2. Diagram of apparatus for measurement of oxygen uptake.

Fig. 2.

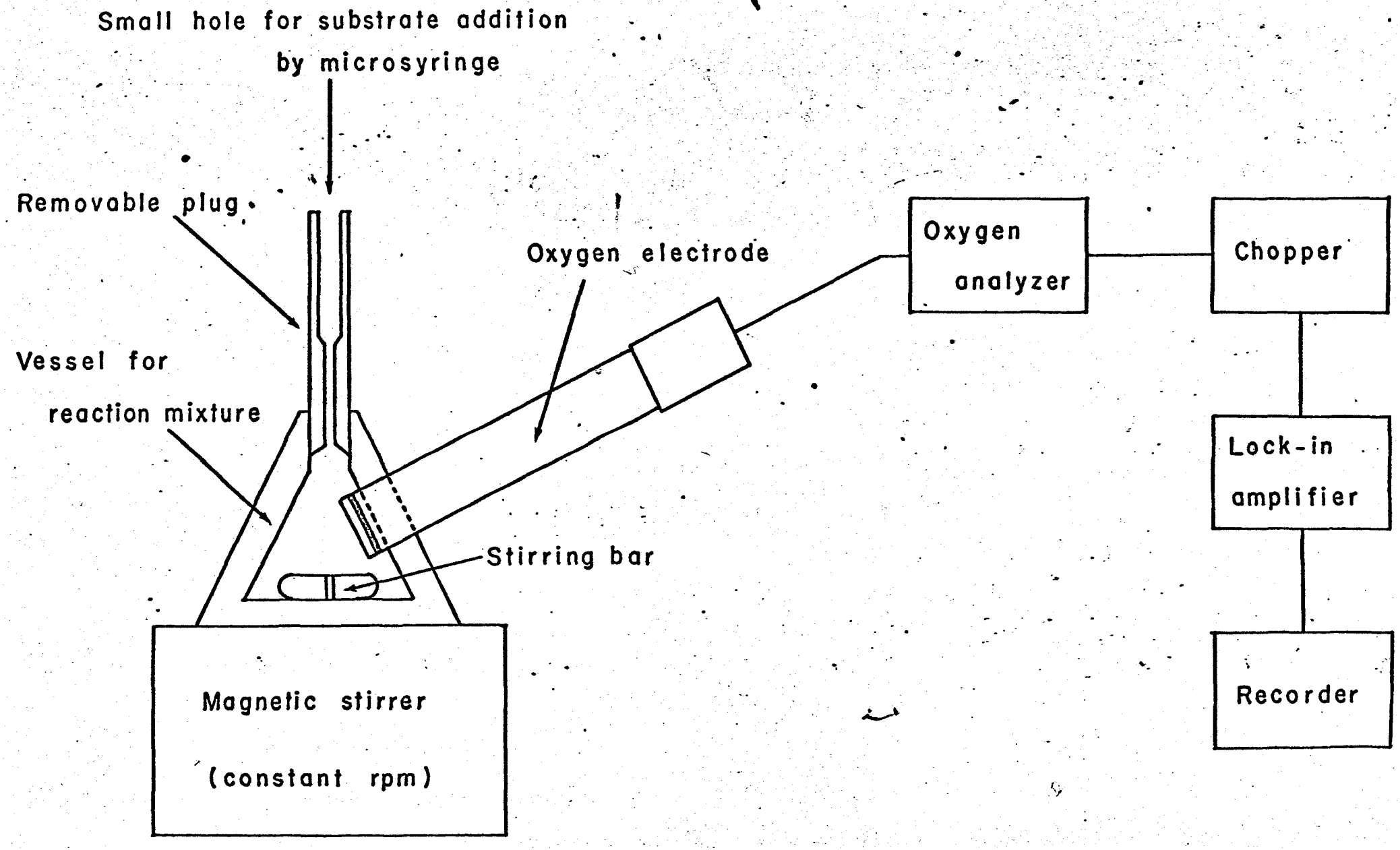

Small hole for substrate addition
by microsyringe
Removable plug
Vessel for
reaction mixture
Oxygen electrode
Stirring bar
Magnetic stirrer
(constant rpm)
Oxygen
analyzer
Chopper
Lock-in
amplifier
Recorder

Fig. 3. Reductions of chromatophore-bound cytochrome cc' and cytochrome B in presence and absence of carbon monoxide, and their difference spectra.
The standard components of the reaction mixture were 2.4 ml of a chromatophore suspension ($A_{873\ nm}$ = 20) in 0.1 M Tris-HCl buffer (pH 8.0) and an appropriate volume of water to make the total volume 3.0 ml. To the reference cuvette was added 0.3 ml of 1.0 M ascorbate (pH 7.0). To the sample cuvette was added 0.3 ml of 0.1 M succinate (I), 0.3 ml of 10 mM NADH (II), 0.3 ml each of 0.1 M succinate and 100 mM NADH (III), or a trace amount of solid dithionite (IV).
After the reduction of cytochromes reached the steady state, carbon monoxide was bubbled in the sample cuvette for 5 min at room temperature, during the period of which the absorbance change was completed. I', II', III' and IV' are the difference spectra newly formed from I, II, III and IV by bubbling carbon monoxide in I, II, III and IV, respectively. The other experimental conditions were described in the text.

Fig. 3.

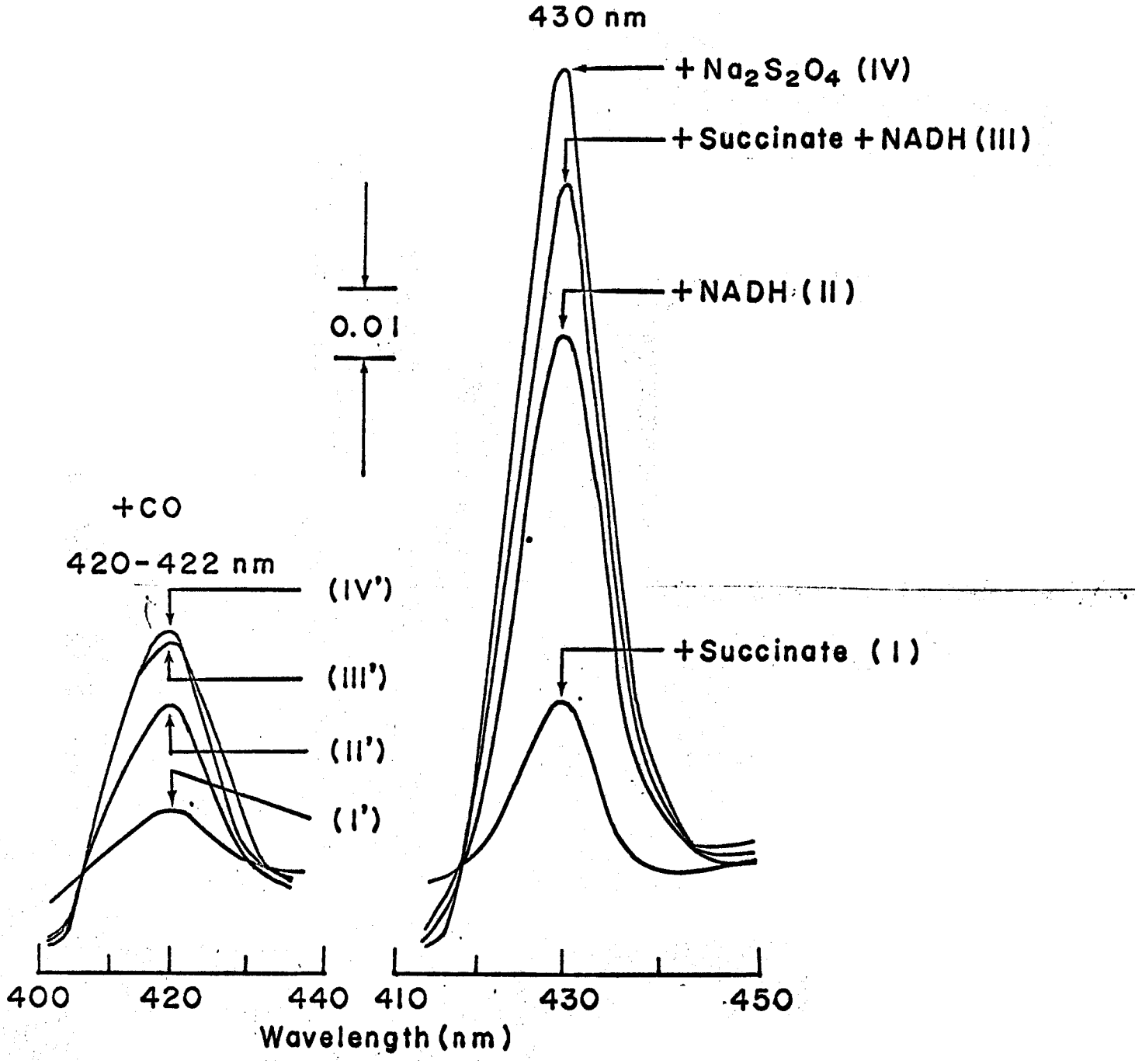

430 nm
+ $Na_2S_2O_4$ (IV)
+ Succinate + NADH (III)
+ NADH (II)
+ Succinate (I)
0.01
+CO
420-422 nm
(IV')
(III')
(II')
(I')
400
420
440
410
430
450
Wavelength (nm)

Fig. 4. Difference spectra of chromatophore-bound cytochrome B in the presence and absence of carbon monoxide.

Both the sample and reference cuvettes contained 2.4 ml of a chromatophore suspension ($A_{873\ nm}$ = 20) in 0.1 M Tris-HCl buffer (pH 8.0) and 0.3 ml of 0.1 M Tris-HCl buffer (pH 8.0). To the reference and sample cuvettes were added 0.3 ml of 1.0 M ascorbate (pH 7.0) and 0.3 ml of 10 mM NADH, respectively. A, "+NADH"-minus-"+ascorbate" ; B, "+NADH + CO"-minus-"+ascorbate" ; C_6, n = 6 ; C_7, n = 8 ; C_8, n = 8. The other experimental conditions were described in the text.

Fig. 4.

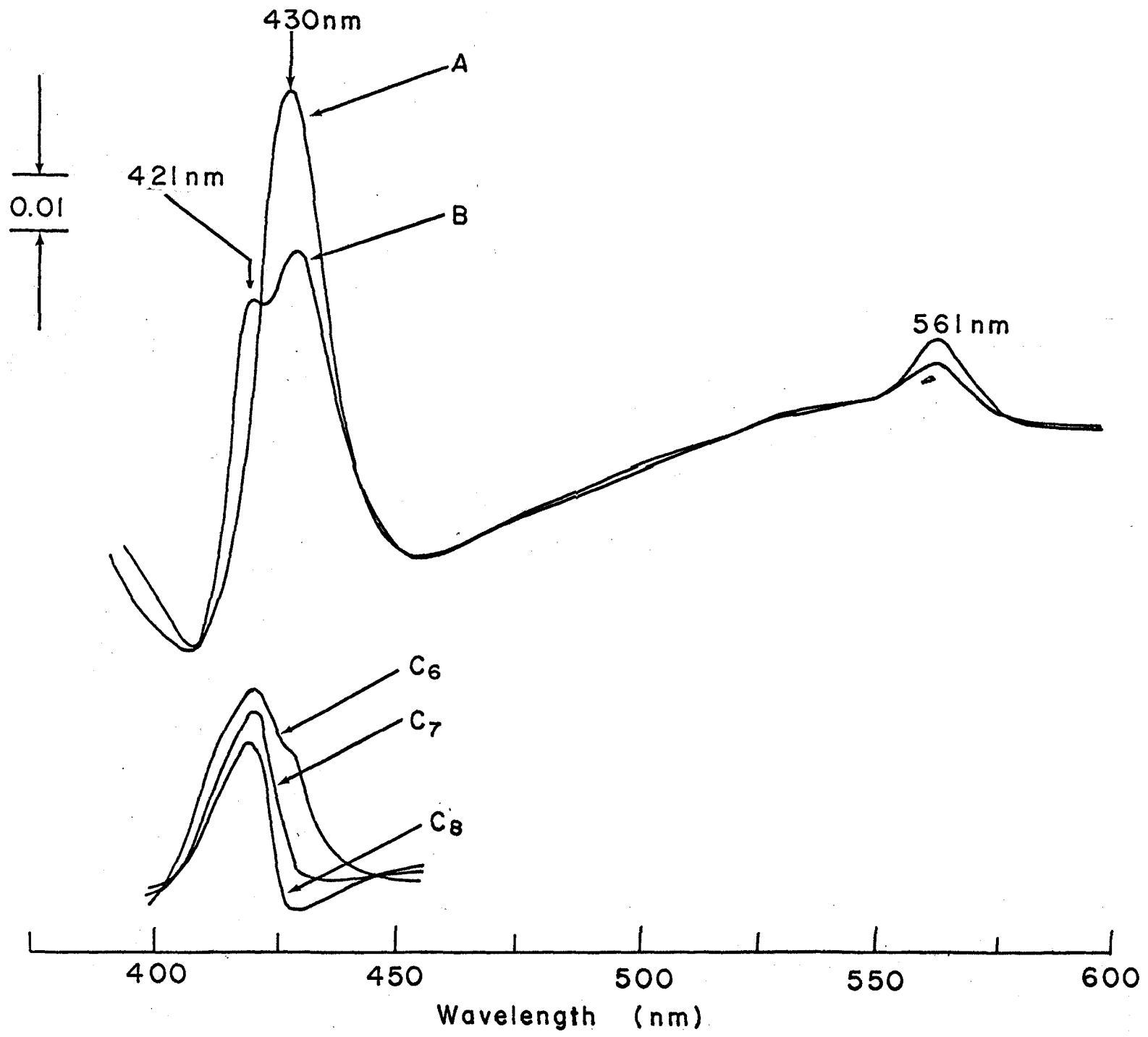
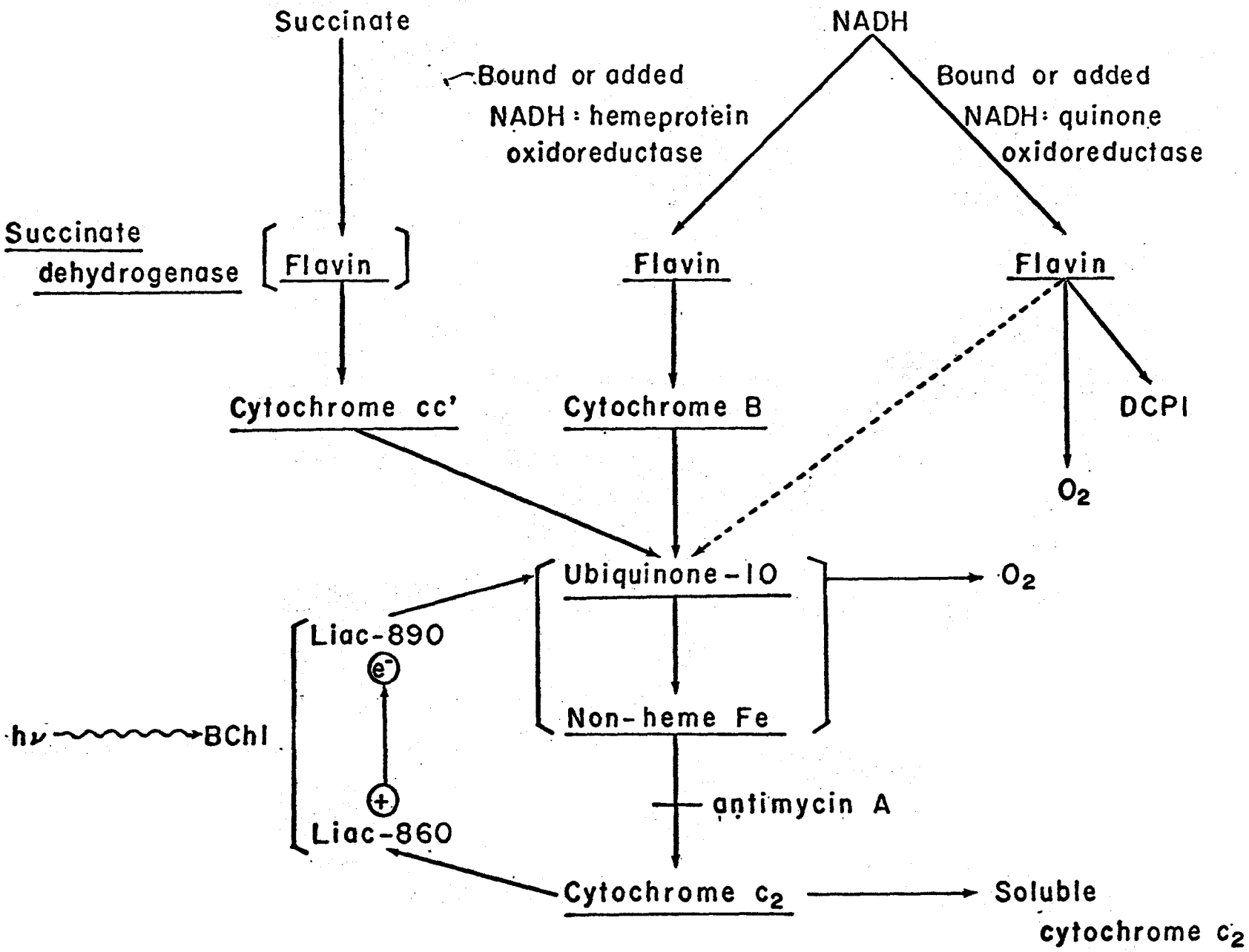

430nm
A
421nm
B
0.01
561nm
C6
C7
C8
400
450
500
550
600
Wavelength (nm)

Fig. 5. Scheme for respiratory systems bound with chromatophore membrane.

Fig. 5.


Succinate
NADH
Bound or added
NADH : hemeprotein
oxidoreductase
Bound or added
NADH: quinone
oxidoreductase
Succinate
dehydrogenase
Flavin
Flavin
Flavin
Cytochrome cc'
Cytochrome B
DCPI
O_2
Ubiquinone-10
O_2
Liac-890
e^-
$h\nu$
BChl
Non-heme Fe
antimycin A
Liac-860
Cytochrome c_2
Soluble
cytochrome c_2