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TRYPSIN-ACTIVATED COMPLEX OF HUMAN FACTOR B WITH COBRA VENOM FACTOR (CVF), CLEAVING C3 AND C5 AND GENERATING A LYTIC FACTOR FOR UNSENSITIZED GUINEA PIG ERYTHROCYTES. II. PHYSICO-CHEMICAL CHARACTERIZATION OF THE ACTIVATED COMPLEX

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SUMMARY A complex, CVF-B, between cobra venom factor (CVF) and human factor B (B) showed weak, short-lived enzymatic activity against the third component of human complement (C3). Once activated with trypsin, it showed strong, stable activity against C3 and C5. CVF-$\bar{B}$, an activated form of CVF-B complex, was not affected by the trypsin inhibitor, diisopropylfluorophosphate and neuraminidase. Heating at 56 C for 30 min completely destroyed its activity and heating at 50 C for 30 min destroyed approximately half its activity. The activity of CVF-$\bar{B}$ decreased markedly at pH 6.0 but was stable at pH 6.5 to 8.5. CVF-$\bar{B}$ lost 90% of its activity on reduction with 1 mM dithiothreitol, and was completely adsorbed on a cellulose acetate membrane. CVF-$\bar{B}$ was found to be a complex of CVF and glycine-rich γ-glycoprotein, with a molecular weight of 340,000. The CVF-$\bar{B}$ molecule consisted of 4 polypeptide chains, 3 of which were derived from CVF and one from GGG. Hemolytically active CVF-$\bar{B}$ may be formed from 2 molecules with four-polypeptide chains linked by unknown bonds. Human, rat and guinea pig sera could react with CVF-$\bar{B}$ to generate a lytic factor. Human and sheep erythrocytes were not sensitive to the lytic factor generated by CVF-$\bar{B}$, whereas liposomes prepared from their membrane lipids were equally sensitive to the lytic factor.

INTRODUCTION

In the previous paper (Miyama et al., 1975a), we reported that CVF-$\bar{B}$ can be generated in reaction mixture containing isolated human B (B), cobra venom factor (CVF) and trypsin. A purified preparation of CVF-$\bar{B}$ was shown to be a stable enzyme splitting both C3 and C5.

The pattern of CVF-$\bar{B}$ on preparative gel filtration showed that it had a larger molecular weight than the CVF-B complex. Dierich et al. (1974) demonstrated that the C3 cleaving enzyme generated in a system consisting of factor B, CVF and factor D contained factor D in its molecule and that its molecular weight was about 200,000.

This paper reports the physico-chemical properties of CVF-$\bar{B}$ and the possible structure of CVF-$\bar{B}$ is discussed.

MATERIALS AND METHODS

1. *Buffers*

Isotonic veronal-NaCl buffer containing Mg^{++} (Mg^{++} buffer) and 0.01 M ethylenediaminetetraacetic acid (0.01 M EDTA buffer) were prepared as described previously (Miyama et al., 1975a). Gelatin was omitted from the buffers employed for gel filtra tion.

2. *Complement sera, human factor B (B), glycine-rich γ-glycoprotein (GGG) and glycine-rich α-glycoprotein (GAG)*

Fresh human, guinea pig and rabbit sera were ultracentrifuged at 30,000 rpm for 60 min to remove lipid, and then EDTA was added to a concentration of 0.01 M. The human serum used for liposome experiments was dialyzed overnight against cold 0.01 M EDTA buffer to remove endogenous galactose. Individual mouse sera were separated from blood obtained by retro-orbital puncture. Human B and GGG were isolated by the methods of Boenisch et al. (1970a, 1970b). Human GAG was isolated by the method of Miyama et al. (1975b).

3. *Cobra venom factor (CVF)*

Three CVF preparations were employed for formation of the CVF-B complex. CVF preparation #1 and #2 were isolated from venom of the cobra Naja Naja (Ross Allen Reptile Institute, Silver Springs, Fla.) as described by Ballow et al. (1969). The specific activities of these preparations were 330 and 120 units per mg protein, respectively, as determined by the method of Cochrane et al. (1970). CVF preparation #3 (100 units/ml) was purchased from Cordis Laboratories, Miami, Fla.

4. *Erythrocytes and corresponding liposomes*

Human, sheep and guinea pig erythrocytes were obtained from blood stored in 0.02 M EDTA and used after washing with 0.02 M EDTA buffer. They were adjusted spectrophotometrically to concentrations of 1×10^8 cells/ml. When 0.5 ml volumes of these cell suspensions (4×10^8 cells/ml) were lyzed with 7 ml of distilled water, the resulting optical densities at 541 nm were 0.800, 0.320 and 0.775, for human, sheep and guinea pig cells, respectively.

Erythrocytes membranes were isolated by the method of Dodge et al. (1963). One volume of membrane suspension was extracted with 2.5 volumes of methanol and 1.25 volumes of chloroform for 2 hr in a closed flask under nitrogen gas. Insoluble material was removed by centrifugation and the clear supernatant was evaporated to dryness. The dried lipid fractions were re-extracted with a mixture of methanol-chloroform (2:1,v/v). Lipid fraction equivalent to 1×10^{10} erythrocytes was dried in a 20 ml-conical flask. The dried lipid was dispersed in 3 ml of 0.27 M galactose by sonication. Liposome preparations were dialyzed for 96 hr against 0.01 M EDTA buffer to remove untrapped galactose. The ratio of trapped galactose in human, guinea pig and sheep liposomes was 1:3:4, respectively, so the volumes of liposome suspension were adjusted so that equal amounts of galactose were incorporated into the experimental systems.

5. *Antisera*

Anti-B, anti-GGG and anti-GAG antibodies were obtained as described previously (Miyama et al., 1975a, 1975b). Anti-human β1C/β1A antibody was obtained from Boehringwerke A. G., Marburg-Lahn, Germany.

6. *Chemicals*

The following chemicals were used: trypsin (Mochida Pharmaceutical Co., Tokyo); soybean trypsin inhibitor, neuraminidase type V of *Clostridium perfringens* and diisopropylfluorophosphate (DFP) (Sigma Chemical Co.); dithiothreitol (DTT) (Nakarai Chemical Co.); β-galactose dehydrogenase, nicotinamide adenine dinucleotide (NAD), ferritin, catalase, aldolase, bovine serum albumin and egg albumin (Boehringer-Mannheim, New York, N.Y.).

7. *Electrophoresis and Ouchterlony's gel precipitation*

Polyacrylamide gel electrophoresis and SDS-polyacrylamide gel electrophoresis were performed by the methods of Davis (1974) and Weber et al.

(1969). Individual components in polyacrylamide gel were scanned spectrophotometrically at 600 nm using a Densitorol DMU-2 (Toyo Scientific Instrument Co.). Immunoelectrophoresis and Ouchterlony's gel precipitation were performed as described previously (Miyama et al., 1975a).

8. *Gel filtration*

Preparative gel filtration was performed in 2×60 cm column of Sephadex G-200. Molecular weight was determined using a 1.5×90 cm column of Sephadex G-200 calibrated with marker proteins of known molecular weight.

9. *Hemolytic and lipolytic assay*

The hemolytic activity of CVF-$\bar{B}$ was measured as follows. A mixture of 0.2 ml of sample diluted with 0.01 M EDTA buffer, 0.1 ml of human C-EDTA (1:4) and 0.1 ml of unsensitized guinea pig erythrocytes (1×10^8 cells/ml) was incubated at 37 C for 60 min. Then 3 ml of cold 0.01 M EDTA buffer were added and the mixture was centrifuged, and the optical density of the clear supernatant was measured at 413 nm. The hemolytic activity of the nascent CVF-B complex, if present, was assayed in the same way as that of CVF-$\bar{B}$, except that dilution was carried out with Mg^{++} buffer. To detect the CVF-B complex, it was first activated to CVF-$\bar{B}$ by trypsin. For this, 0.02 ml of CVF-B complex was treated with 0.02 ml of trypsin (12.5 μg/ml) in Mg^{++} buffer at 37 C for 7 min. Then 0.02 ml of trypsin inhibitor (37.5 μg/ml) and 0.14 ml of 0.01 M EDTA buffer were added. The hemolytic activity of the reaction mixture was measured in the same way as that of CVF-$\bar{B}$.

The lysis of liposomes was assayed by the method of Knudson et al. (1971). A typical reaction mixture contained an appropriate volume of liposome suspension (0.1 to 0.4 ml) in 1.5 ml of 0.01 M EDTA buffer, containing 20 units of CVF-$\bar{B}$, 0.1 ml of human C-EDTA (1:4), 0.1 ml of NAD (5 mg/ml) and 0.1 ml of β-galactose dehydrogenase (0.23 mg/ml). The reaction mixture was incubated in a cuvette of 1 cm light path at 37 C. Reduction of the NAD was followed spectrophotometrically at 340 nm.

RESULTS

1. *The formation of CVF-$\bar{B}$ from purified CVF-B complex by trypsin*

In the previous paper (Miyama et al., 1975a), we reported that CVF-$\bar{B}$ was generated by trypsin in a mixture containing a limiting amount of CVF and excess B, in which both the CVF-B complex and free B were present. The CVF-B complex was formed from a mixture of equimolar concentrations of CVF and B, and purified by gel filtration on Sephadex G-200. Three CVF preparations were employed for formation of the CVF-B complex.

Two ml of B (3 mg/ml) were incubated with 4,000 units of CVF at 37 C for 15 min in the presence of Mg^{++}, and then the reaction mixture was chromatographed on a column (2× 60 cm) of Sephadex G-200 equilibrated with Mg^{++} buffer. Fractions containing the CVF-B complex were pooled and concentrated to about 3 ml. Two ml of the complex were digested with 0.04 ml of trypsin (2 mg/ml) at 37 C for 7 min in Mg^{++} buffer. Then EDTA was added to a concentration of 0.01 M, and the mixture was subjected to gel filtration on a Sephadex G-200 column equilibrated with 0.01 M EDTA buffer to obtain pure CVF-$\bar{B}$. Figure 1 shows the gel filtration pattern of CVF-$\bar{B}$ prepared from CVF preparation #2. The CVF-B complex prepared from CVF preparation #3 and the corresponding CVF-$\bar{B}$ preparation were not subjected to gel filtration. The activities of the CVF-B complexes and the corresponding CVF-$\bar{B}$'s are shown in Table 1.

The CVF-B complex itself was weakly active only when tested in the nascent state and its activity depended on the CVF preparation used. When the CVF-B complex was diluted and kept in EDTA buffer, its activity to generate a lytic factor from human C-EDTA was lost. On the other hand, all samples of CVF-$\bar{B}$ showed stable activity to generate a lytic factor from human C-EDTA.

The antigenicity of CVF-$\bar{B}$ #2 (Fr. #16, in Fig. 1) and the corresponding CVF-B complex were analyzed on an Ouchterlony's plate against anti-B, anti-GGG and anti-GAG. After precipitation lines had developed, the plate was covered with 2.0 ml of 1.0% agarose containing 5% guinea pig erythrocytes and 0.5 ml of hu-

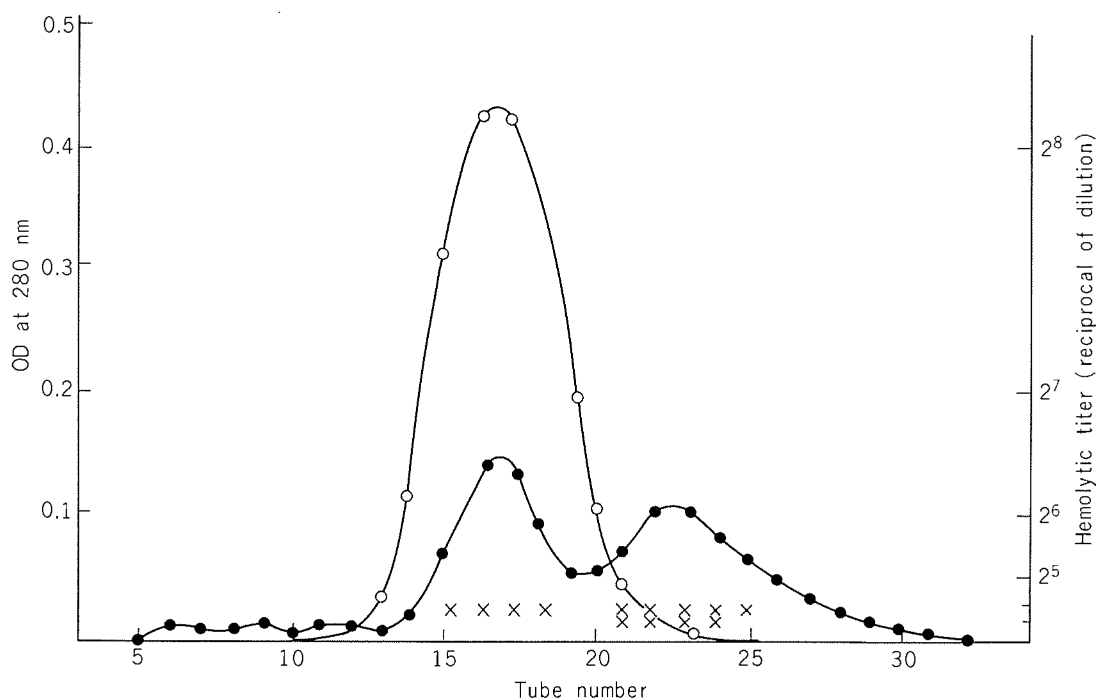
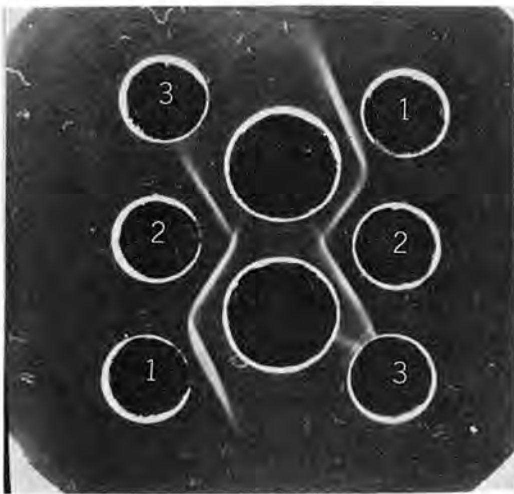


FIGURE 1. *Separation of CVF-$\bar{B}$ from trypsin-activated CVF-B complex. The CVF-B complex #2 was treated with trypsin, and then applied to a column (2 × 60 cm) of Sephadex G-200. Optical density at 280 nm (●), hemolytic activity (○) and reactivity with anti-B (×).*

TABLE 1. *Comparison of hemolytic activities of CVF-B complex and corresponding CVF-$\bar{B}$*

CVF preparations and corresponding CVF-B and CVF-$\bar{B}$	Hemolytic units per ml		Conversion of βIC in serum[b]	Stability in 0.01 M EDTA at 37 C, 60 min
	Before[a]	After		
CVF#1 330 units/mg				
CVF-B complex	0	0	None	
CVF-$\bar{B}$	1,200	1,280	Complete	Stable
CVF#2 120 units/mg				
CVF-B complex	400	50	Partial	Unstable
CVF-$\bar{B}$	6,400	5,500	Complete	Stable
CVF#3 100 units/ml				
CVF-B complex	120	—	None	Unstable
CVF-$\bar{B}$	3,200	—	Complete	Stable

[a] The hemolytic activities of CVF-B and CVF-$\bar{B}$ were assayed before and after gel filtration.
[b] 0.9 ml of C-EDTA (1 : 1) was treated with 0.1 ml of undiluted CVF-B complex or CVF-$\bar{B}$ and analyzed by immunoelectrophoresis against anti-human βIC/βIA.



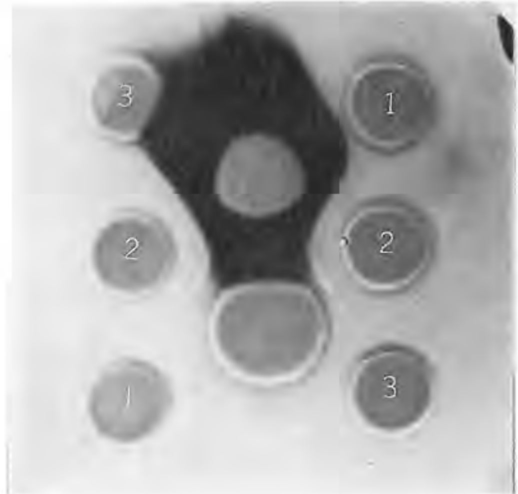


FIGURE 2. *Antigenic and hemolytic analysis of CVF-B̄. CVF-B̄ (upper center well) and CVF-B complex (lower center well) were analyzed against anti-GGG (1), anti-B (2) and anti-GAG (3). When the precipitation lines had developed (Fig. 2A), the plate was covered with 2 ml of 1.0% agarose containing 5% guinea pig erythrocytes and 0.5 ml of human C-EDTA (1: 1) (Fig. 2B).*

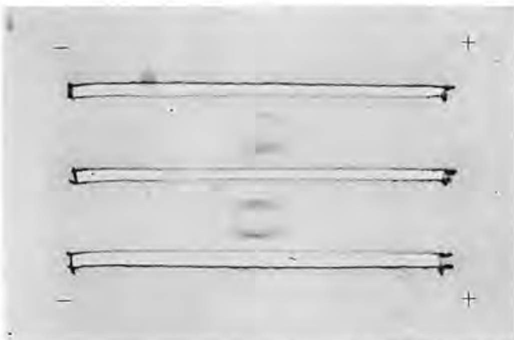


FIGURE 3. *Immunoelectrophoresis of the CVF-B and CVF-B̄ complexes. Upper well, CVF-B̄. Lower well, CVF-B complex. Upper and lower troughs, anti-B. Middle trough, anti-GGG.*

man C-EDTA (1: 1). The precipitation line of CVF-B̄ developed against anti-B completely fused with the precipitation line against anti-GGG, whereas the precipitation lines of CVF-B and CVF-B̄, respectively, against anti-B formed a spur (Fig. 2A). No precipitation line was observed against anti-GAG. The precipitation lines of the CVF-B complex against anti-B and anti-GGG fused. On the other hand, the precipitation lines of isolated B against anti-B and anti-GGG, respectively, formed a spur, as already reported (Miyama et al., 1975b). These results can be explained by supposing that some of the antigenic sites of B are covered in the CVF-B complex. As seen in Fig. 2B, the hemolytic zone produced by CVF-B̄, but not by the CVF-B complex, was clearly bounded by the GGG precipitation line. Although CVF-B complex #2 possessed about 50 units/ml of hemolytic activity after gel filtration, it did not form a hemolytic zone in agarose gel.

The immunoelectrophoretic patterns of CVF-B̄ were compared with those of the CVF-B complex against both anti-B and anti-GGG. Figure 3 shows that CVF-B̄ and the CVF-B complex had the same mobilities and were located in the position of the original B. These observations suggested that CVF-B̄ contained at least a moiety of GGG.

2. *Physico-chemical properties*

The heat stability of CVF-B̄ was determined by heating CVF-B̄ (5 units/ml) in 0.01 M EDTA buffer at 37 C, 50 C and 56 C and then estimating residual CVF-B̄'s activity by meas-

uring the generation of a lytic factor from C-EDTA. Figure 4 shows that CVF-$\bar{B}$ lost half its activity in 240 min at 37 C, 55% in 30 min at 50 C and all its activity in 30 min at 56 C. The pH-stability of CVF-$\bar{B}$ was tested by incubating purified CVF-$\bar{B}$ (100 units/ml) in 0.15 M NaCl at pH 5.5, 6.0, 6.5, 6.8, 7.5, 8.0 and 8.5, at 4 C overnight. Its activity was stable at pH 6.5 to 8.5 and decreased markedly at pH 6.0. Maximal activity was retained at pH 8.0.

The molecular weight of CVF-$\bar{B}$ was determined by gel filtration on Sephadex G-200. As demonstrated in Fig. 5, the molecular weights of the CVF-B complex and CVF-$\bar{B}$ were 240,000 and 340,000, respectively. This value for CVF-$\bar{B}$ was considerably larger than that of the C3 cleaving enzyme reported by Dierich et al. (1974). CVF-$\bar{B}$ forms a single protein band on analytic polyacrylamide gel electrophoresis (Miyama et al., 1975a), so the nature of its complex was studied by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 6, CVF itself was separated to 3 distinct subunits, whereas CVF-$\bar{B}$ was separated to 4 subunits. The inactive product formed by digestion of the CVF-B complex with trypsin (Fr. #22, in Fig. 1) contained 2 polypeptide chains, which were also structural subunits of active CVF-$\bar{B}$. The densities of the individual components in the SDS-polyacrylamide gel were scanned and the ratio of the polypeptide chains in the molecule was calculated. B was a single polypeptide chain and its molecular weight was 76,000. CVF consisted of 3 polypeptide chains, with molecular weights of 61,000, 43,000 and 25,500, respectively. CVF-$\bar{B}$ consisted of 4 polypeptide chains in a molecular ratio of approximately 1 : 1 : 1 : 1. Three of the 4 polypeptide chains of CVF-$\bar{B}$ must be derived from CVF and the other polypeptide chain (mol wt 52,000) seemed to be GGG. These results are summarized in Table 2.

The hemolytic activity of CVF-$\bar{B}$ was tested in the presence of trypsin inhibitor and DFP. CVF-$\bar{B}$ (2 to 2,000 units/ml) was not affected by the trypsin inhibitor at a final concentration of 0.1 mg/ml or by 50 μM DFP. The conversion of β1C and β1F globulins in whole human serum by CVF-$\bar{B}$ could be observed electrophoretically even in the presence of 0.5 mM DFP. Incubation at 37 C for 60 min with neuraminidase at a final concentration of 0.5 mg/ml did not reduce the activity of CVF-$\bar{B}$ (200 units/ml) or alter the electrophoretic mobility of CVF-$\bar{B}$ (3,200 units/ml). However, 90% of the activity of CVF-$\bar{B}$ (640 units/ml) was lost when it was reduced by incubation

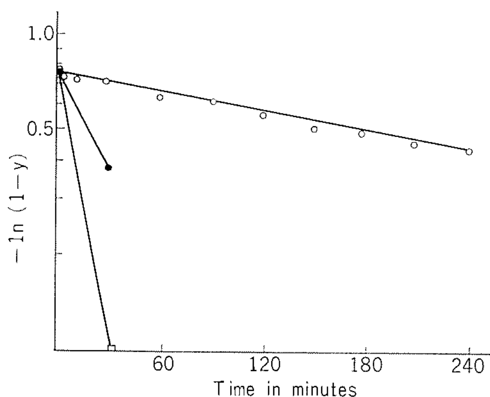


FIGURE 4. *Heat stability of purified CVF-$\bar{B}$. CVF-$\bar{B}$ was heated at 37 C (○), 50 C (●) and 56 C (□) and then its residual activity was assayed.*

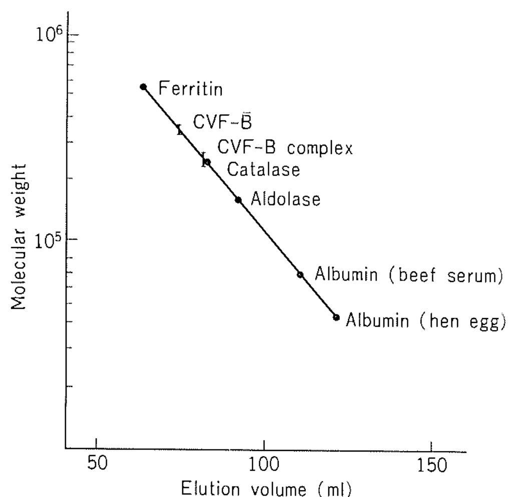


FIGURE 5. *Determination of molecular weight of CVF-$\bar{B}$ on Sephadex G-200.*

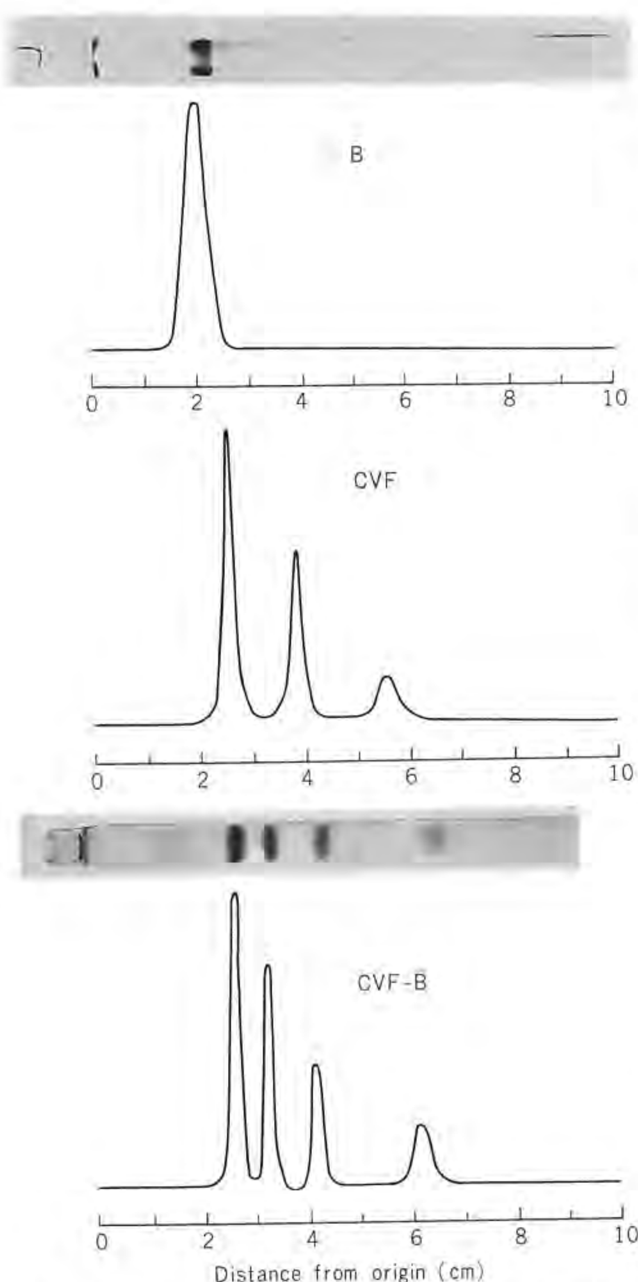


FIGURE 6. *Analysis of the subunit compositions of B, CVF and CVF-B̄ by SDS-polyacrylamide gel electrophoresis. Each factor was applied after treatment with 0.1% SDS and 0.1% 2-mercaptoethanol. Electrophoresis was performed in 10% gel at 8 ma/gel and the gel was stained with 0.02% Coomassie blue. Densitograms of the gels scanned at 600 nm are also shown.*

with 1 mM DTT at 37 C for 60 min and then dialyzed against 0.01 M EDTA buffer without any alkylation procedures.

Another remarkable feature of CVF-B̄ was that it was adsorbed on a cellulose acetate membrane at room temperature. The activity of CVF-B̄ (640 units/ml) was completely lost when it was passed through a millipore membrane filter (Type HA 0.45 μ). These characteristics are listed in Table 3.

3. *Compatibility between CVF-B̄ and C-EDTA prepared from various animal sera*

When mouse serum was used as a source of C3 to C9 instead of human serum, no hemolytic activity of CVF-B̄ could be observed. Therefore the compatibilities of human CVF-B̄ with various kinds of animal sera were measured by testing for the generation of a lytic factor. As shown in Table 4, human serum was highly reactive but mouse sera were incompatible with human CVF-B̄, although mouse (BALB/c) serum contained MuBl antigen which was identical with mouse C5 (Cinader et al., 1964). Although agglutinating antibody against guinea pig erythrocytes is normally present in human serum, preliminary absorption of human serum with guinea pig erythrocytes at 0 C for 2 hr did not affect the generation of a lytic factor by CVF-B̄. The prozone effect seen with human serum is probably due to the inhibitor present in serum.

4. *Lysis of liposomes*

Sheep and guinea pig erythrocytes were equally sensitive to the alternate pathway involving CVF formed when fresh human or guinea pig serum was treated with CVF in the presence of

TABLE 2. *Molecular weights of individual polypeptide chains of B, CVF and CVF-$\bar{B}$*

	Molecular weight	Number of subunits	Mol wt of subunit	Ratio of subunit
B	78,000	1		
CVF	130,000	3	61,000	1.2
			43,000	1.2
			25,000	1.0
CVF-$\bar{B}$	Dimer: 340,000			
	Monomer: 180,000	4	61,000	1.2
			52,000	1.0
			43,000	1.0
			26,500	0.9

TABLE 3. *Physico-chemical characteristics of CVF-$\bar{B}$*

Molecular weight	340,000
Sensitivity to:	
Heating at 37 C	Loss of half activity in 240 min
at 50 C	Loss of 55% of activity in 30 min
at 56 C	Complete loss of activity
H^+ concentration	Stable at pH 6.5 to 8.5
Reduction	Loss of 90% of activity with 1 mM DTT
DFP	No inhibition
Trypsin inhibitor	No inhibition
Neuraminidase	Not altered

TABLE 4. *Compatibility between CVF-$\bar{B}$ and C-EDTA prepared from various animal sera*

	Dilution of Serum					
	1:2	1:4	1:8	1:16	1:32	1:64
Human	94[a]	95	100	89	70	45
Guinea pig	11	9	5	0	0	0
Rabbit			No lysis			
Rat	90	74	40	22	20	20
Mouse (DD)[b]			No lysis			
Mouse (BALB/c)			No lysis			

[a] Numbers indicate percent hemolysis of guinea pig erythrocytes.
[b] Strain DD, genotype: A, B, c, S. MuBl antigen negative. Strain BALB/c, genotype: b, c. MuBl antigen positive.

Mg^{++} (Pickering et al., 1969). The susceptibilities to hemolysis of 0.1 ml volumes of erythrocytes suspensions (1×10^8 cells/ml) of human, sheep and guinea pig were compared by exposing them to 0.2 ml of CVF-$\bar{B}$ (10 units/ml) in the presence of 0.1 ml of human C-EDTA. Human and sheep erythrocytes were both completely resistant to the lytic factor generated by CVF-$\bar{B}$ from human C-EDTA. However, liposomes prepared from lipid frac-

TABLE 5. *Sensitivity of liposomes to CVF-$\bar{B}$ initiated lysis*

Liposomes from	Reactants added to liposomes	Degree of galactose release	
		Exp. 1	Exp. 2
Human	CVF-$\bar{B}$+C-EDTA	0.595	0.925
	C-EDTA	0.341	0.482
	CVF-$\bar{B}$	0	0
Sheep	CVF-$\bar{B}$+C-EDTA	0.600	0.775
	C-EDTA	0.092	0.087
	CVF-$\bar{B}$	0	0
Guinea pig	CVF-$\bar{B}$+C-EDTA	0.595	0.985
	C-EDTA	0.299	0.475
	CVF-$\bar{B}$	0	0

tions of these erythrocytes membranes were found to be equally sensitive to the lytic factor generated from human C-EDTA by CVF-$\bar{B}$ (Table 5). Lysis of liposomes should be compared qualitatively rather than quantitatively, but galactose release from the 3 kinds of liposomes greatly increased when CVF-$\bar{B}$ was added to human C-EDTA.

DISCUSSION

The nature of the C3 cleaving enzyme complex formed in a system containing isolated factor B, CVF and factor D has been examined by Cooper (1973), Hunsicker et al. (1973) and by Dierich et al. (1974). It was demonstrated that the CVF-B complex itself showed labile and weak activity to generate a lytic factor from human C-EDTA. Once activated by trypsin, however, the CVF-B complex acquired strong, stable enzymatic activity against C3 and C5. It was also demonstrated that the GGG moiety of B was incorporated into CVF-$\bar{B}$, and that its reduction by DTT inactivated CVF-$\bar{B}$. It is still uncertain whether trypsin is incorporated into CVF-$\bar{B}$. The resistance of CVF-$\bar{B}$ to the trypsin inhibitor suggested that the CVF-$\bar{B}$ molecule was a dimolecular complex of CVF and GGG. The molecular weights of the CVF-B and CVF-$\bar{B}$ complexes were 240,000 and 340,000, respectively. SDS-polyacrylamide gel electrophoresis showed that CVF-$\bar{B}$ consists of 4 subunits, with molecular weights of 61,000, 52,000, 43,000 and 26,500, respectively. On the other hand, an inactive product obtained by digestion of the CVF-B complex with trypsin, which reacted with anti-GGG, consisted of 2 subunits. The molecular weights of these subunits were 52,000 and 41,000, respectively. The CVF-$\bar{B}$ molecule seemed to consist of 4 subunits in a ratio of 1:1:1:1, giving a total molecular weight of approximately 180,000. From these results, it seems likely that hemolytically active CVF-$\bar{B}$ was formed by combination of 2 four-polypeptide chains. The nature of the interchain bonding is unknown.

Human and sheep erythrocytes were resistant to CVF-$\bar{B}$ initiated lysis, but liposomes prepared from their cell membrane lipids were found qualitatively to be sensitive to CVF-$\bar{B}$. The high susceptibility of guinea pig erythrocytes must be due to special characteristics of their cell surface, like those of erythrocytes of cases of paroxysmal nocturnal hemoglobinuria or glutathione-treated human erythrocytes (Arroyave et al., 1974).

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REFERENCES

Arroyave, C. M., E. H. Vallota, and H. J. Müller-Eberhard. 1974. Lysis of human erythrocytes due to activation of the alternate complement pathway by nephritic factor (C3NeF). J. Immunol. 113: 764–768.

Ballow, M., and C. G. Cochrane. 1969. Two anticomplementary factors in cobra venom: hemolysis of guinea pig erythrocytes by one of them. J. Immunol. 103: 944–952.

Boenisch, T., and C. A. Alper. 1970a. Isolation and properties of a glycine-rich β-glycoprotein of human serum. Biochim. Biophys. Acta 221: 529–535.

Boenisch, T., and C. A. Alper. 1970b. Isolation and properties of a glycine-rich γ-glycoprotein of human serum. Biochim. Biophys. Acta 214: 135–140.

Cinader, B., S. Dubiski, and A. C. Wardlaw. 1964. Distribution, inheritance, and properties of an antigen, MuB1, and its relation to hemolytic complement. J. Exp. Med. 120: 897–924.

Cochrane, C. G., H. J. Müller-Eberhard, and B. S. Aikin. 1970. Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. J. Immunol. 105: 55–69.

Cooper, N. R. 1973. Formation and function of a complex of the C3 proactivator with a protein from cobra venom. J. Exp. Med. 137: 451–460.

Davis, B. J. 1964. Disc electrophoresis-II: method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121: 404–427.

Dierich, M. P., U. Hadding, W. König, M. Limbert, H.-U. Schorlemmer, and D. Bitter-Suermann. 1974. Factor D in the alternate pathway of the complement activation: purification, physicochemical characterization and functional role. Immunochemistry. 111: 527–532.

Dodge, J. T., C. Mitchell, and D. J. Hanahan. 1963. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch. Biochem. Biophys. 100: 119–130.

Hunsicker, L. G., S. Ruddy, and K. F. Austen. 1973. Alternate complement pathway: factors involved in cobra venom factor (CoVF) activation of the third component of complement (C3). J. Immunol. 110: 128–138.

Knudson, K. C., D. H. Bing, and L. Kater. 1971. Quantitative measurement of guinea pig complement with liposomes. J. Immunol. 106: 258–265.

Miyama, A., T. Kato, S. Horai, J. Yokoo, and S. Kashiba. 1975a. Trypsin-activated complex of human B with cobra venom factor (CVF), cleaving C3 and C5 and generating a lytic factor for unsensitized guinea pig erythrocytes. I. Generation of the activated complex. Biken J. 18: 193–204.

Miyama, A., T. Kato, I. Minoda, T. Ueda, R. Kanamiya, E. Aburatani, and S. Kashiba. 1975b. Antigenic conversion of glycine-rich β-glycoprotein activated by alternate pathway of complement. J. Nara Med. Assoc. 26: 44–49.

Pickering, R. J., M. R. Wolfson, R. A. Good, and H. Gewurz. 1969. Passive hemolysis by serum and cobra venom factor: a new mechanism inducing membrane damage by complement. Proc. Natl. Acad. Sci., USA. 62: 521–527.

Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406–4412.