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Physicochemical Properties of Human Fibrinogen-Cationic Detergent Complex

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Abstract

Sedimentation coefficient, $S^0_{20,w}$ of human fibrinogen-stearyltrimethylammonium chloride, FG-STA(Cl), was dependent on pH and the values were found to be 14.3 and 7.2 *s* at pH 5.8 and 3.8, respectively. Circular dichroism spectra of FG-STA(Cl) showed the presence of α-helix (from a double minimum) at between pH 5.8 and 3.8. The molar ellipticity at 222nm were calibrated to be $-10{,}025 \pm 450$ and $-13{,}000 \pm 600$ degree · cm² · decimole^{-1} at pH 5.8 and 3.8, respectively.

It can be suggested that STA-Cl bound to fibrinogen alter the surface character of the protein. Action of STA-Cl is not to denature fibrinogen, but to maintain the conformational structure of the protein.

Introduction

In the previous papers [1),2)], stearyltrimethylammonium chloride, STA-Cl, was found to precipitate fibrinogen by forming fibrinogen-stearyltrimethylammonium complex, FG-STA(Cl), consisting of STA-Cl and fibrinogen in a molar ratio of approximately 160:1. FG-STA(Cl) was still capable of carring out the substrate function, and fibrinogen was reversibly renatured from the complex without loss of the clotting activity [2)]. These facts seem to indicate that cationic detergent, STA-Cl, dose not denature fibrinogen, and thus properties of FG-STA(Cl) have been an interesting subject of further study. In this paper we describe the results of investigation of physicochemical properties of FG-STA(Cl) complex using ultracentrifugation and circular dichroism spectra.

Materials and Methods

Human fibrinogen: Human fibrinogen was isolated and purified according to the method of Laki and Steiner [3)], and further purified with DEAE cellulose column chromatography [4)], Protein concentrations were determined using an extinction coefficient of 13.6 at 280 nm for one per cent solution [5)].

Preparation of FG-STA(Cl): STA-Cl (mol.wt.=347), Kao-Atlas Co., was added to 50 ml of 1 % human fibrinogen (mol.wt.=335,000) solution in 0.35 M NaCl until most of the protein

was precipitated, according to the procedure of previous paper[1]. The glutinous precipitate (FG-STA(Cl)) was washed several times with saline and then suspended in 100 ml of distilled water, and dialyzed against distilled warter. The precipitate gradually dissolved with formation of a colloidal solution of FG-STA(Cl)[2]. The pH of FG-STA(Cl) solution was adjust to be 5.8.

Ultracentrifugation: A Hitachi model UCA-1 ultracentrifuge was used.

Circular dichroism spectra: Circular dichroism measurements were performed on a Jasco Model ORD/UV-5 recording spectropolarimeter with a Model J-15 CD attachiment and a Jasco Model J-40 automatic spectropolarimeter under constant nitrogen flush at 25°C. Experimentals were generally done 2-3times and all CD scans run in duplicate. Cylindrical optical cell with 0.1 cm optical path was used under constant nitrogen flush at 25°C. *d*-10-camphorsulfonic acid (Eastman-Kodak Co.) was employed to calibrated the dichrograph, and the results of the measurements were expressed in a unit of molar ellipticity, $[\theta]_\lambda$ in degree • cm^2 • $decimole^{-1}$.

Incubation of FG-STA(Cl) with thrombin: A mixture of 10ml of 0.03mM FG-STA(Cl) and 10 units of bovine thrombin (Mochida Pharmaceutical Co.) was incubated at pH 5.8.

Renaturation of fibrinogen from FG-STA(Cl): Attempts to prepared renatured fibrinogen from FG-STA(Cl) were carried out using the method described previously[2].

Analysis of peptides: Peptide fractions in each of the incubation mixture were purified as described previously[1][2], and analyzed by cellulose thin layer chromatography using *n*-buthanol: acetic acid: water (4:1:2,v/v).

Clotting activity: Clotting time of fibrinogen and renatured fibrinogen was assayed as follows: sample of 0.9 ml of a 0.06 mM solution of fibrinogen at pH 7.2 was added to one unit of bivine thrombin in 0.1 ml of 0.17 M NaCl solution, and the time required to produce a firm clot.

Results and Discussion

Ultracentrifugation: The schlieren patterns of FG-STA(Cl) in ultracentrifugation were single boundary as seen in Fig. 1, being independent of pH. The values of $S^0_{20,w}$ were obtained by extrapolation of protein concentration to zero, and were found to be 14.3 and 7.2 s at pH 5.8 and 3.8, respectively (Fig. 2). There is a significant difference between the values obtained, and it is interesting to refer to the fact that sedimentation coeffcient of macromolecule is dependent on amount of its net charge[6] It is likely that STA molecules are bound to fibrinogen hydrophobically with their polar groups (quaternary ammonium) outwards, and contribute to the surface net charge of the complex; a strong positive charge could be expected at pH 3.8, and the charge would decrease progressively at pH 5.8 due to neutralization by ionized carboxyl residues of fibrinogen.

The pH-dependence of the value of $S^0_{20,w}$ of FG-STA(Cl), therefore, may be explained from such characteristic charge effect. However, this is not to deny the probability that FG-STA(Cl) may form an oligomer at pH 5.8 because at present nothing is known of the molecular weight of the complex.

Fig. 1 Schlieren patterns of FG-STA(Cl) (A) at pH 5.8 and (B) at pH 3.8, after 40 min at 59850 rpm. Protein concentration is 0.6 %.

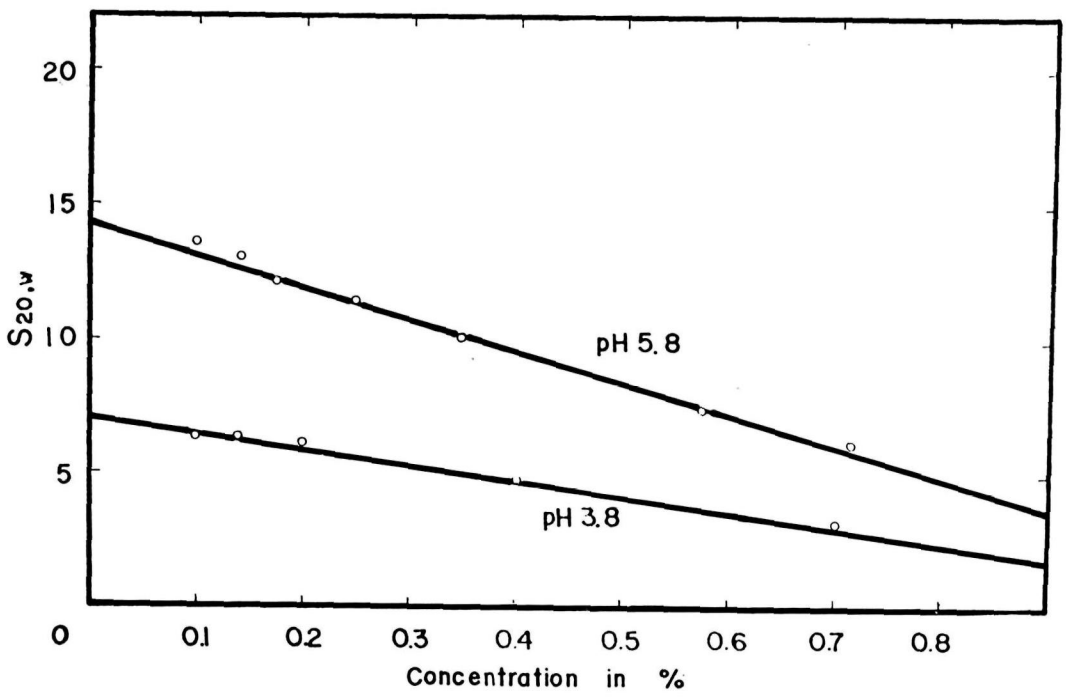

Fig.2 The values of sedimentation coefficient were obtained by extrapolation of FG-STA(Cl) concentration to zero.

Circular dichroism spectra: Circular dichroism spectra of human fibrinogen and FG-STA(Cl) at pH 5.8 and 3.8 is shown in Figs. 3 and 4. The spectra were characterized by bands at 208 and 222 nm(double minimum), 200nm (a crossover point), and 190nm(a positive band), being suggestive of α-helix conformation. As seen in the Fig.4, molar ellipticity, $[\theta]_{222nm}$, of FG-STA(Cl) is observed to be $-10{,}025 \pm 450$ and $-13{,}000 \pm 600$ degree $\cdot$ cm^2 $\cdot$ decimole^{-1} at pH 5.8 and 3.8, respectively. From the data of the circular dichroism spectra, the α-helix contents of FG-STA(Cl) was calibrated to be 26 and 34% at pH 5.8 and 3.8, respectively.

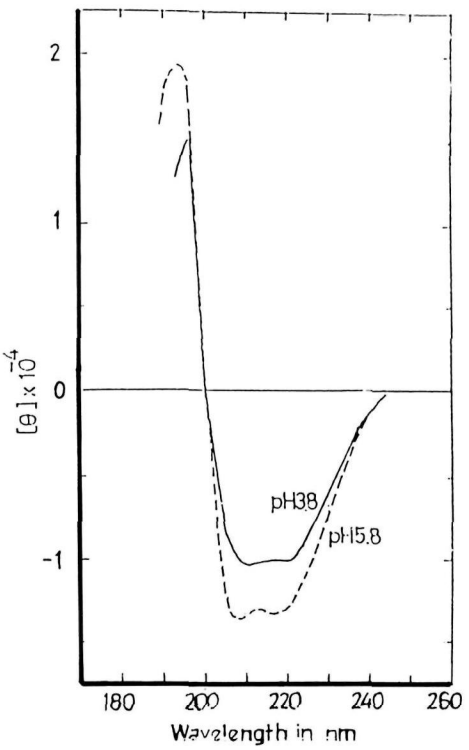

Fig.3 The pH effect on the CD of human fibrinogen.

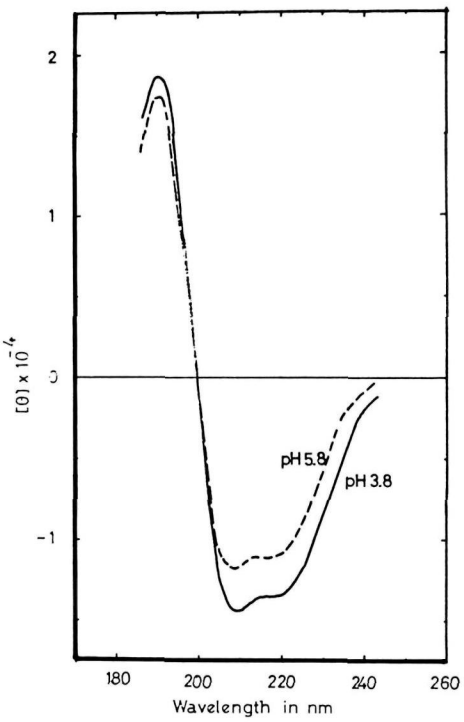

Fig.4 The pH effect on the CD of FG-STA(Cl).

The α-helix content of fibrinogen has been reported to be 30 - 40%[7~10]. It is obvious that STA-Cl interacted with fibrinogen and reduced the α-helix content of the protein. In a prevous study[11], it was observed that circular dichroism spectra of fibrinogen in the presence of sodium dodecylsulfate were very different from both the spectra of FG-STA(Cl) and fibrinogen in shape, and demonstrated the absence of α-helix conformation. This observation supports the fact that sodium dodecylsulfate can denature various proteins. Action of STA-Cl on fibrinogen, however, seems to be completely different from that of dodecyl sulfate, because the spectra of FG-STA(Cl) demonstrate clearly the presence of a typical α-helix conformation. An interesting finding in the present circular dichroism measurements is to increase the α-helix content in acidic medium. As summarized in Table

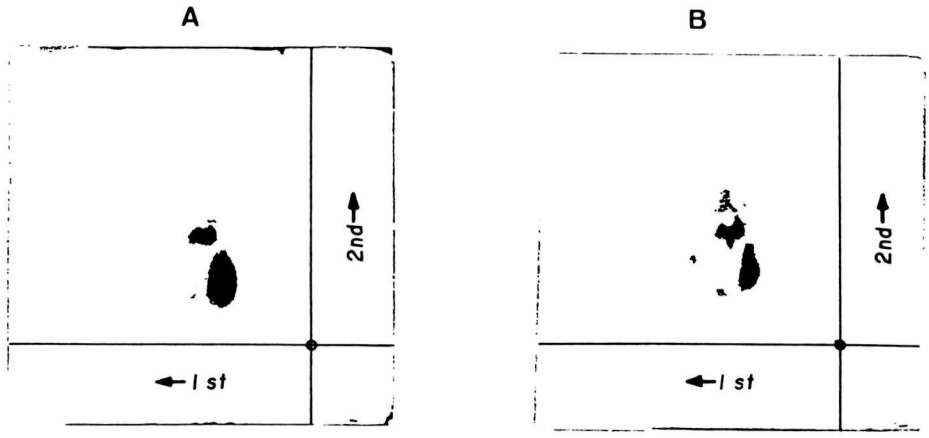

Fig.5 The maps of peptides released from fibrinogen (A) and FG-STA(Cl) (B) under the action of thrombin[2].

I renatured fibrinogen from acid-treated FG-STA(Cl) was less active in clotting. This fact suggests that an induced conformational transition α-helix may occur near the functional site of fibrinogen at pH 3.8, and the transition may remain on the renatured protein irreversibly, resulting in prolongation of the clotting time.

Effects of heating on FG-STA(Cl): Fibrinogen is sensitive to heating and completely coagulates at 48-49°C and becomes insoluble irreversibly. However, FG-STA(Cl) did not coagulate by heating and remained soluble even after heating at 95°C. Effects of heating on the release of fibrinopeptides and the renaturation of fibrinogen from heated FG-STA(Cl) were not shown. Attempts to prepared renatured fibrinogen were unsuccessful, and only insoluble and aggregated materials were obtained through the procedure of extraction of STA-Cl, indicating that fibrinogen was completely denatured by heating.

Table 1

Fibrinogen	Clotting on storage	Clotting time
Intact	After 2 days	60−65 (sec)
Renatured	(−)	45−50(sec)

Properties of renatured fibrinogen. Stability was tested as follows : an aliquot (2ml) of sterile solution containing 0.06 mM fibrinogen and 5 mM $CaCl_2$ at pH 7.2 was stood at 37°C. (−) means that no clotting occurred during the period of observation (15 days). The clotting time was measured as described in the text, and the resulting fibrin clots were incubated at 37°C to estimate fibrinolysis. Fibrinolysis indicates complete lysis after incubation for 15 hr. Intact fibrinogen was prepared by the method of Laki and Steiner[3] .

Figure 5 shows thin layer chromatograms of peptides released from heated FG-STA(Cl). The results demonstrate that thrombin can modify heated FG-STA(Cl) in the same manner as fibrinogen by hydrolyzing the peptide bonds. This suggests that STA-Cl molecules bound to the denatured fibrinogen can help keeping the conformational structure of the protein under the condition in which the limited proteolysis occurs. Circular dichroism measurement of heated FG-STA(Cl) is, therefore, an interesting subject of further study, the result of which will be reported in a coming paper.[11)]

A requirement for the presence of lipid for enzyme activity has been demonstrated in a number of cases, and it can be suggested that the role of lipids in the enzymic reaction is to maintain the structure of a lipoprotein[12)]. It is interesting to note that the role of STA-Cl in heated FG-STA(Cl) seems to be very similar to the function of lipids in natural occurring lipoprotein system, in which apo-lipoprotein often aggregates and has no biological activity without lipids.

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