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Conformation Study on the Interactions of Plasma Proteins with Anionic Lipids and Heparin

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Summary

Effects of anionic lipids and heparin on the conformation of plasma proteins, such as albumin, γ-globulin, and fibrinogen were investigated by evaluating both α-helix and β-structure contents from circular dichroism (CD) spectra. Sodium dodecyl sulfate and sodium dodecylbenzenesulfonate increased the α-helix content of γ-globulin, and β-structure content of fibrinogen accompanied with decreasing α-helix content, while they stepwise disrupted the α-helix of albumin with increasing their concentration. It was concluded that the hydrophobic interaction was predominant for binding albumin, while the electrostatic interaction affected the structural changes of γ-globulin more than hydrophobic interaction.

Introduction

Albumin, whose amino acid sequence was determined by Brown (1), is susceptible of reversible structural changes, e. g., N-F transition (2), N-B transition (3), and N-A isomerism (4) in the different pH range. Since Putnam and Neurath (5) reported conformational change of albumin due to bound anionic surfactants, interactions between them have been studied extensively by means of equilibrium dialysis, viscosity, UV difference spectra, ORD, NMR, and CD. The albumin molecule has been shown to possess specific binding sites to various aromatic drugs by using fluorescence probes (6), derived from Trp-214 and Tyr-411 (7).

Moreover, it was pointed out from surface pressure measurements that the interactions of lecithin with serum albumin (8) depend on the length of alkyl chain of lipids and on the kinds of polar groups of both components. According to Jirgensons (9), a non-helical portion in the native α-chymotrypsinogen could be converted partly into an α-helical conformation by the treatment with anionic surfactants. Thus, the hydrophobic "tail" of the surfactant molecule was presumed to protect the hydrogen bonding of the helix part from competitive interaction with water. In addition, from the investigation on the interactions of anionic surfactants with cationic polypeptides (10, 11) and 15 proteins (12) it was suggested that cationic side chains of amino acid residues in polypeptides and proteins enhanced to propagate helical segments due to the presence of SDS.

Thus, conformational study on the interaction of plasma pro-

teins, such as albumin, γ-globulin, and fibrinogen, with various anionic compounds is important for understanding their specificity and biological functions in blood. The objective of this paper, is to elucidate the interaction of anionic compounds, such as SDS, sodium dodecylbenzenesulfonate (SDBS), lysolecithin, and heparin, with plasma proteins from the conformational change by measuring the CD spectrum.

Materials and Methods

Fatty acid-free bovine serum albumin (Lot. 90F-9315) and bovine serum γ-globulin of 99% electrophoretic purity (Lot. 116c-0147) were purchased from Sigma Chemical Co. Bovine plasma fibrinogen, 95% clottable (Lot. 26) was obtained from Miles Laboratories Inc. The weight average molecular weight, M_w, the average molecular weight per amino acid residue, M_o, the isoelectric points, pI, and the number of ionizable groups of these plasma proteins are listed in TABLE I.

TABLE I. Plasma Proteins Used

Proteins	M_w	M_o	pI	Molar Number of Ionizable Groups per 10^5 g Protein	
				Anionic Group	Cationic Group
Albumin	69,000	118	4.7	194.5	146.7
γ-Globulin	150,000	108	6	102.6	63.4
Fibrinogen	340,000	111	5.5	119.0	124.7

Lysophosphatidylcholine, so-called lysolecithin (Lot.84254), extracted from egg phosphatidylcholine, i. e., lecithin, was purchased from Koch-Light Laboratories Ltd. Heparin-Na (Lot.M6G5365), obtained from Nakarai Chemical Co., had an activity of 152 units/mg, and was found to have 1.5 anionic groups per pyranose unit from conductometry. SDS (Lot. MIT1594) of specially prepared reagent grade and SDBS (Lot. M9E9870) of extra pure reagent grade, purchased from Nakarai Chemical Co., were recrystallized twice from methanol solution.

γ-Globulin and fibrinogen solutions were clarified by filtration through 4.5μm Millipore filter. The concentrations of plasma protein solutions in 0.05M phosphate buffer at pH 7.4 were determined by the optical density measurement with a Hitachi Spectrophotometer Model EPS-3T. The numerical values of the specific extinction coefficients used for 0.1% solutions of albumin at 279 nm, γ-globulin at 280 nm, and fibrinogen at 280 nm per cm path length were 0.667 (13), 1.12 (14), and 1.506 (15), respectively.

The CD spectra were measured at 25±0.5°C using a JASCO J-20 CD/ORD Spectropolarimeter equipped with a quartz cell of 1 mm path length. Spectra were recorded in a range of wavelength from 250 to 200 nm. According to Greenfield and Fasman (16), the structures of proteins were classified to three fundamental conformations, α-helix, β-structure, and random coil on the basis of CD spectrum analysis of poly-L-lysine conformation. In order to investigate structural changes in plasma proteins, contributions from α-helix, β-structure, and random coil are represented by eq (1), introduced by Chen et al. (17).

$$[\theta] = f_\alpha [\theta]_\alpha^\infty (1 - k/\tilde{n}) + f_\beta [\theta]_\beta + f_R [\theta]_R \quad (1)$$

where f is the number fraction of amino acid residues, subscripts α, β, and R mean α-helix, β-structure, and random coil forms, respectively, $\tilde{n}$ is the average number of residues per helical segment of the protein molecule, and k is a wavelength-dependent constant. The numerical values of $[\theta]_\alpha^\infty$ and k, and of $[\theta]_\beta$ and $[\theta]_R$ were cited from the paper reported by Chen et al. (17), and $\tilde{n}$=10 was used for albumin and fibrinogen in obeydance with them because of enough content of α-helical structure in the proteins. In the case of γ-globulin, however, $\tilde{n}$=6 was adopted owing to its minor α-helix content (18). Values of f_α, f_β, and f_R were computed using all possible pairs of wavelengths at 1 nm interval between 210 and 240 nm of CD spectra. Mathematical solutions which gave positive values for f_α, f_β, and f_R were averaged to give the mean values for the fraction of each forms.

Results and Discussion

CD spectra of albumin, γ-globulin, and fibrinogen in the absence of anionic compounds are shown in Fig. 1. Contents of α-helix, β-structure, and random coil structure in plasma proteins were computed by analyzing CD spectra in accordance with Chen et al. (17), and are listed in TABLE II. The values of α-helix and β-structure contents of albumin (19, 20), and that of α-helix content of fibrinogen are in good agreement with our data (21).

The effects of anionic compounds (A) on the conformational changes of plasma proteins (P) were investigated from CD spectra by changing mixing ratio. The effects of four compounds on the conformational changes of albumin are shown in Fig. 2, where H, β, and the subscript o denote respectively the content of α-helix structure, that of β-structure, and the respective values in the absence of anionic compounds. The β-structure in albumin seems not to be affected in the presence of anionic compounds, as obvious from Fig. 2 (b), while the α-helix content of albumin largely decreases by binding anionic compounds in the following order: SDBS > SDS > lysolecithin >

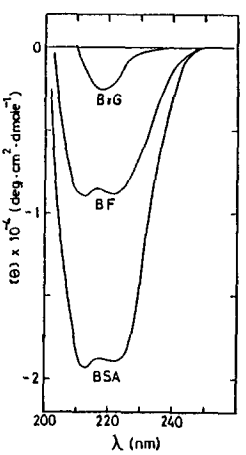

Fig. 1 CD spectra of albumin (BSA), γ-globulin (BG), and fibrinogen (BF) in concentration range of 0.048 to 0.075 g/dl.

TABLE II. Contents of α-Helix, β-Structure, and Random Coil

Protein	α-Helix (%)	β-Structure (%)	Random Coil (%)
Albumin	67	20	13
γ-Globulin	6	29	65
Fibrinogen	31	15	54

heparin. The benzene ring of SDBS seems to strengthen the binding with albumin by hydrophobic interaction, as pointed by Decker and Foster (22).

As shown in Fig. 2 (a), an eminent conformational change of albumin takes place abruptly at mixing ratios of 0.050, 0.155, 0.405, and 0.640, which correspond to 12, 40, 160, and 420, respectively in molar ratio of SDS/albumin. The former two numerals agree well with those obtained from the binding isotherm for albumin and SDS. In addition, our data on large molar ratio of SDS/albumin correspond to the results that the helical content of albumin was nearly constant until more than a 100-fold molar excess of SDS had been added (23,24). On the other hand, Tanford (25) reported some loss of helical content in albumin when SDS bound to high affinity sites of albumin molecules. As can be seen from Fig. 2 (a), the α-helical conformation of albumin has certainly disrupted in accordance with the binding isotherm.

Heparin, however, does not affect the conformation of albumin at all. Heparin was observed not to form complex with albumin in neutral pH region, but to form soluble complex in low pH region (26). The affinity of various residues to albumin was pointed out to be in the following order; sulfate > sulfonate > carboxyl > hydroxyl > alkane (26). Hydrophobic interactions of anionic compounds, carrying hydrophobic long chains, with non-polar region of albumin molecules is presumed to be necessary for disruption of α-helix structure of albumin. So, heparin possessing no bulky non-polar portions could not disrupt the α-helix structure of albumin despite of the presence of sulfate groups in heparin. In addition, 4 % disruption of α-helix structure in albumin by lysolecithicn even in excess amount may be attributed to more hydrophilic properties, as observed in the surfactants of shorter alkyl chains (less than 8) (24). Such properties, however, may be preferable functions of albumin as a carrier protein in vivo.

The effects of four anionic compounds on the conformation of γ-globulin are shown in Fig. 3. The measured absolute values of ellipticity at 217 nm increased by the addition of SDS, SDBS and heparin. Although the estimation of β-structure content in γ-globulin may include enlarged errors because of small absolute values of ellipticity for β-structure in comparison with ones for α-helix structure, the β-structure in γ-globulin seems to be slightly disrupted by the addition of a small amount of SDS and SDBS at the mixing ratio where α-helix content first increases, as shown in Fig. 3.

On the other hand, a dramatical change of α-helical content amounting to about the twice value is seen in the presence of SDS. Such noticeable effects of surfactants have been observed in the case of proteins rich in β-structure, e. g., between concanavalin A and SDS (28), between β-lactoglobulin and SDS (29), and between β-lactoglobulin and phosphatidylcholines (30). The structural changes in γ-globulin at higher SDS concentration may be attributed to the random coil-to-α-helix conversion, because the content of β-structure is not largely changed at that mixing ratio. This conversion may be interpreted by such interactions in which hydrogen bondings are formed among amino acid residues in random coil conformation of γ-globulin in contact with water, whereby the

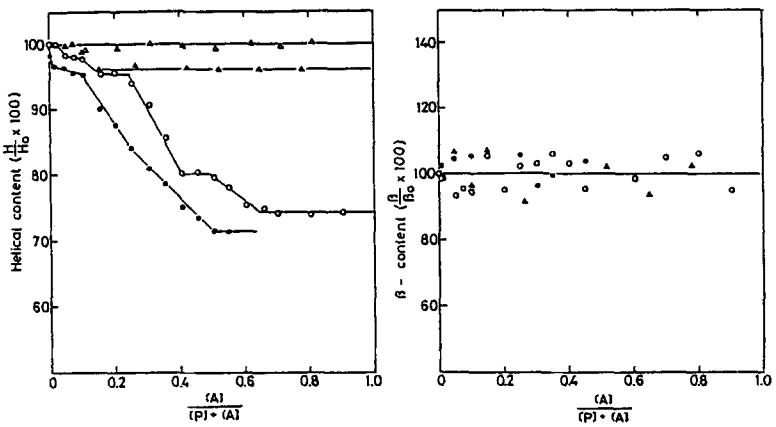

Fig. 2 Effects of SDS (○), SDBS (●), lysolecithin (▲), and heparin (△) on relative contents of α-helix and β-structure of albumin in concentration range of 0.063 to 0.072 g/dl.

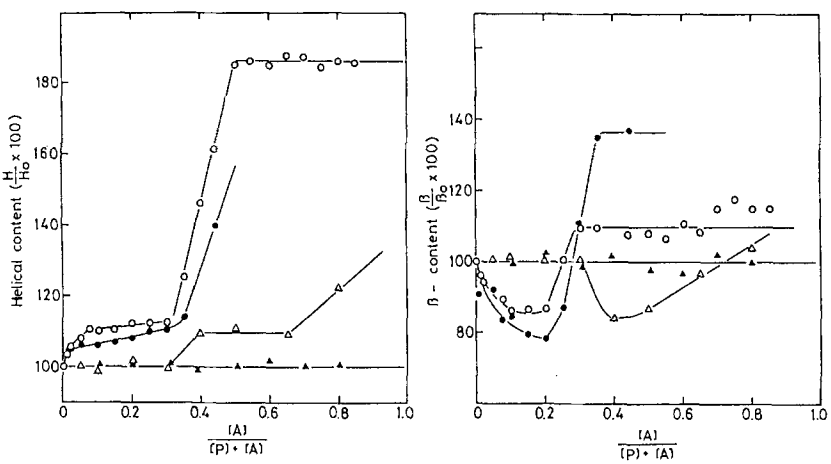

Fig. 3 Effects of SDS (○), SDBS (●), lysolecithin (▲), and heparin (△) on the α-helix and β-structure contents of γ-globulin in concentration range of 0.073 to 0.075 g/dl.

random coil regions are shielded from water molecules by the effect of bound SDS.

The effects of four anionic compounds on the fibrinogen conformation are shown in Fig. 4, which indicates that the effects are in the following order: SDBS > SDS > lysolecithin > heparin. By the addition of SDS and SDBS, the decrease of α-helix structure and the increase of β-structure content take place simultaneously in the conformation of fibrinogen. This observation may be taken to suggest that β-structure is formed at the disrupted region of α-helix structure.

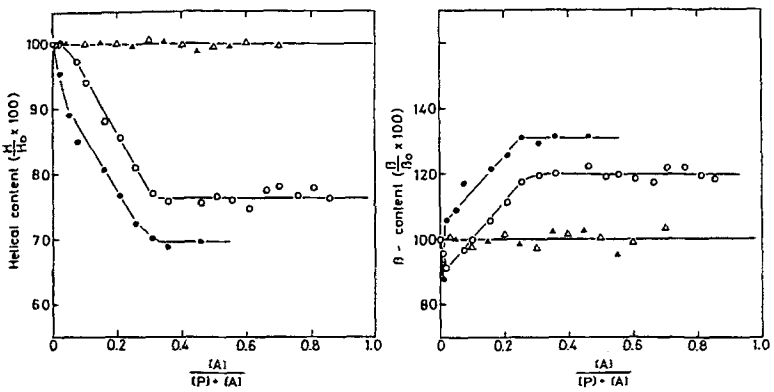

Fig. 4 Effects of SDS (○), SDBS (●), lysolecithin (▲), and heparin (△) on the α-helix and β-structure contents for fibrinogen in concentration range of 0.048 to 0.057 g/dl.

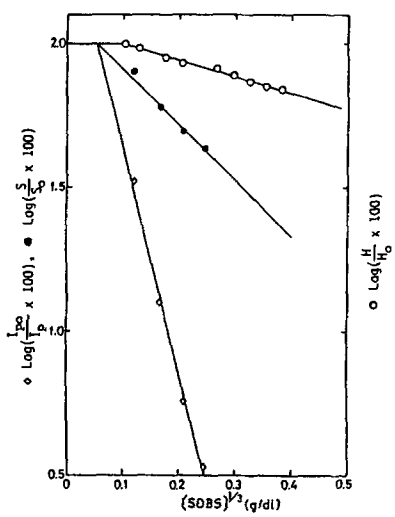

Fig. 5 Logarithms of the relative helical content H (○), the relative S (●), and the relative I_p (◇) plotted against $[SDBS]^{1/3}$ for SDBS and fibrinogen systems. Data on H and I_p are obtained from our previous paper (31) for 0.03 g/dl fibrinogen solution.

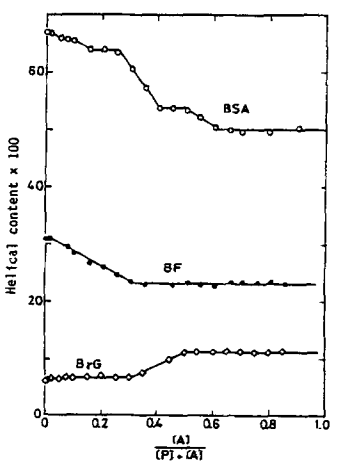

Fig. 6 Effect of SDS on the α-helix contents of BSA (○), globulin (◇), and fibrinogen (●).

From the results of Fig. 4 as well as those of our previous paper (Figs. 3 and 4) (31) for the system of SDBS and fibrinogen, Fig. 5 is depicted in order to elucidate the effects of the conformational change of fibrinogen on the biological functions. For

the initial reaction of fibrinogen to fibrin conversion catalyzed by thrombin, the reaction mechanism was analyzed on the basis of both terms of the slope, S, in turbidity-time curves, relating to polymerization rate, and of the induction period, I_p, for polymerization. The result that the logarithms of two relative terms, S/S_o and I_{po}/I_p, are proportional to $[SDBS]^{1/3}$ suggests that SDBS molecules react with fibrinogen molecules in obedience with a mode expressed by the Freundlich-type adsorption isotherm (31).

Although the change of α-helical content is very closely correlated with the retardation effect on the fibrinogen-fibrin conversion, it is worthwhile to consider a reaction mixture at SDBS concentration of 0.001 g/dl. In such a mixture of 2.87×10^{-5}M SDBS, ca. 10^{-6} M fibrinogen, and ca. 2×10^{-8}M thrombin, SDBS molecules give almost no effect on the conformation of fibrinogen molecules, as observed from Fig. 5. The addition of thrombin to a fibrinogen solution in adsorption equilibrium with SDBS of ca. 20 times in molar amount leads to a remarkable effect on I_p in comparison with on S. Such unbalanced effects on I_p and S were neither observed in systems of acidic polysaccharides, e. g., heparin, nor could be explained by our theoretical analysis. However, these facts may imply that SDBS should participate in the binding with the site of fibrinogen susceptible of hydrolysis by thrombin.

Finally conformational changes of plasma proteins by the addition of SDS are compared in Fig. 6. Conformations of plasma proteins are stable against SDS in the order of γ-globulin > fibrinogen > albumin, i. e., albumin is concluded to be most deformable among three proteins. It is interesting that this result coincides with the order of conformational stability of plasma proteins adsorbed on charged copolypeptide membranes reported by Soderquist and Walton (21).

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