Light-scattering Studies of the Interaction of Sodium Chondroitin Sulfate C with Bovine Serum Albumin[1)]

Masayuki NAKAGAKI and Yoh SANO

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto

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Angular light-scattering investigations were carried out in 0.01 M sodium chloride solutions at 25°C for various mixed solutions of bovine serum albumin (BSA) and chondroitin sulfate (Chs). The experimental values of molecular weight and of the molecular dimensions showed an entirely different tendency from the calculated values, and we may assume that there is no interaction between them. It has, therefore, been concluded that Chs interacts with BSA strongly and that a complex is formed between them. For the soluble complex thus formed, the number of the bound BSA molecules with one Chs molecule was expressed by a Langmuir-type equation as a function of the amount of excess BSA existing free in the solution. When the latter was sufficiently large, the number of bound BSA molecules per Chs molecule approached 20.6.

A Zimm plot of the complex was drawn, and the molecular weight, the radius of gyration, and the second virial coefficient of the complex were determined. It has been concluded from these results that the complex molecule has a long-like shape; the length was about 30% longer, but the diameter was about 6 times thicker, than the Chs molecule at the maximum binding of BSA.

Chondroitin sulfate, one of natural acid mucopolysaccharides composes the primary part of the cartilage, together with collagen-like protein, and is present in the skin or in other connective tissues as mucoproteins, playing important physiological functions. Since the acid mucopolysaccharides are anionic polyelectrolytes, their protein complexes are bound primarily by polar linkages. The interaction between chondroitin sulfate and bovine serum albumin has been studied by quite a few workers.[2-11)] It seems, however, that is studies reported thus far have been little connected with the complexes themselves. Soluble complexes formed between chemically-different macromolecular species have recently come under investigation, but the angular light scattering of solutions containing the soluble complexes[12-14)] has not yet been studied very extensively. In the present paper, the interaction between bovine serum albumin and chondroitin sulfate will be discussed on the basis of the experimental results obtained by the light-scattering technique and the binding coefficient, *i.e.*, the binding ratio of protein to chondroitin sulfate; the molecular shape and size of the soluble complex will also be reported.

Experimental

Materials and Solvents. The sodium chondroitin sulfate (hereafter called Chs) used in this work was of the C type; it was extracted so carefully that it contained no electrolyte except, sometimes, sodium chloride. This sample is the same as that investigated by Nakagaki and Ikeda[15)] by the light-scattering technique in various ionic strengths. For the purpose of the experiments, a stock solution of about 1% was prepared, kept in a refrigerator at 4°C, and used after dilution if necessary. The stock solution was made up a fresh and kept for less than three days because of the denaturation of the sample. Bovine serum albumin (hereafter called BSA) was that made by Armour Co., Ltd., (Fraction V, No. G5717, D. 1029). Much as in the case of Chs, a stock solution of about 1% was prepared and diluted if necessary. The reduced scattering intensity of this sample did not change when it was left as long as 30 hr at 25°C. The water used here was distilled, refined by the use of ion-exchange resin, and distilled again with an all-glass still. Concentrations were corrected for the water content in the sample. The water content necessary for this correction was determined by drying in a vacuum over P_2O_5 at 60°C for two days.

Refractive Index Increment. The refractive index increment (dn/dc) was measured by means of a Shimadzu electrophotometric differential refractometer at 25°C. Measurements were carried out using unpolarized light from a mercury arc (100 V, 85 W), with a spectrum filter whose wavelength was 4360 Å. The instrument constant was obtained by using a standard solution of potassium chloride, whose concentration was 0.01483 g/m*l*; the value of (dn/dc) of 0.1344 was taken from the literature.[16)] The potassium chloride used was a reagent-grade commercial product dried in a vacuum at about 200°C for more than six hours. The refractive index increments obtained were 0.1535 for Chs and 0.1935 for BSA. The value for Chs agreed with that reported by Nakagaki and Ikeda[15)] for the same sample. The value for BSA agreed well with

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11) S. M. Partrige, "Chem. Physiol. Mucopolysaccharides, Proc. Symp. Milar." (1965) p. 51.

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16) A. Kruis, *Z. Phys. Chem.*, **34B**, 13 (1936).

those of the references.[13,17,18] For mixed solutions of Chs and BSA, the value of the refractive increment obtained by direct determination for each mixture was used for the calculation of the apparent molecular weight.

Light-scattering measurements. The apparatus used in this investigation was a Shimadzu electrophotometric light-scattering photometer, PG-21 type. All the measurements were performed with unpolarized light with a wavelength of 4360 Å in a vacuum and at 25°C, the temperature being kept constant by circulating thermostatted water. The apparatus constant was calibrated on the basis of the value, $R_{90°}=48.5\times10^{-6}$, for the reduced scattering intensity of dust-free benzene at a 90° angle. The benzene was a commercial product of a reagent grade. It was dried with Drynap (an alloy of sodium metal and lead; weight ratio, 1 : 10; specific gravity, 6.4; mp, 368°C),[20-22] distilled twice by means of an all-glass still, and filtered directly into the cylindrical glass cell for light-scattering measurements through a Cella Filter (Mitter, Sartorius Co., Ltd.). The correction of the illuminated volume was done by using a fluorescein solution which had been optically clarified with Membrane Filter No. 15 (Sartorius Co., Ltd.) under pressure. The solutions and the solvent for the light-scattering measurements were optically clarified as well with the same mebrance filter.

All the light-scattering measurements were done over the range of scattering angles between 35°—135° for solutions of BSA, Chs, and their mixtures in various mixing ratios, γ, (gBSA/gChs), in a 0.01 M sodium chloride solutions as a solvent.

$$\gamma = c_{BSA}/c_{Chs} \tag{1}$$

where c_{BSA} and c_{Chs} are concentrations (g/ml) of BSA and Chs respectively. After the solutions had been made up by the dilution of stock solutions, these solutions were kept at 4°C in the refrigerator until the light-scattering experiments were carried out the next day. Dust-free solution were obtained in the way described above. In order to avoid errors due to the temperature difference and due to the existence of tiny bubbles, the scattering intensities were read about an hour after setting.

Density measurements. All the measurements were done with a picnometer of about 35 ml at 25°C. The water was used directly after distillation.

Results

Light Scattering. The results of the light-scattering measurements were analyzed according to the well-known equation:

$$\frac{Kc}{R_\theta} = \frac{K'\phi^2 c}{R_\theta} = \frac{1}{M}\left\{1+\left(\frac{16\pi^2 n_0^2}{3\lambda_0^2}\right)R_g^2\sin^2\left(\frac{\theta}{2}\right)\right\} + 2A_2c \tag{2}$$

where R_θ is the reduced scattering intensity of the solution in an excess over the solvent at the scattering angle, $\theta°$, c is the weight concentration of the polymer, (g/ml), M is the weight-average molecular weight of the polymer, and K' is the optical constant given by the following equation:

$$K = K'\phi^2, \quad K' = 2\pi^2 n_0^2/\lambda_0^4 N_A, \quad \phi = dn/dc \tag{3}$$

where ϕ is the refractive index increment of the solution, λ_0 is the wavelength in a vacuum (the value used in this experiment being 4360 Å), n_0 is the refractive index of the solvent, N_A is Avogadro's number, R_g^2 is the mean square radius of gyration, and A_2 is the second virial coefficient of the system.

In order to obtain the values of the weight-average molecular weight, M, the mean square radius of gyration, R_g^2, and the second virial coefficient, A_2, according to Eq. (2), the experimental data were plotted according to the so-called Zimm plot method, taking Kc/R_θ and $\sin^2(\theta/2)+100c$ on the ordinate and the abscissa respectively.

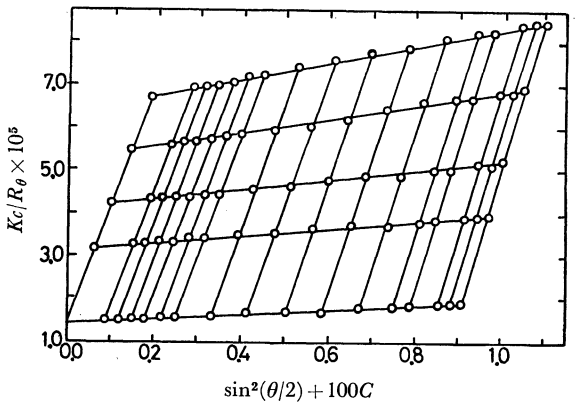

Fig. 1. Zimm plot for sodium chondroitin sulfate C (Chs) in 0.01 M NaCl at 25°C, pH 5.56.

Figure 1 shows the Zimm plot of Chs in 0.01 M sodium chloride (pH=5.56). The apparent weight-average molecular weight thus obtained was 70900. The values of the apparent mean square radius of gyration and the apparent second virial coefficient, as well as the molecular weight, agreed well with those obtained independently by Nakagaki and Ikeda[15] for the same sample. Figure 2 shows the Zimm plot of BSA in 0.01 M sodium chloride (pH=5.00). The molecular weight obtained from this was 71900; this value was in good agreement with the value found in the lite-

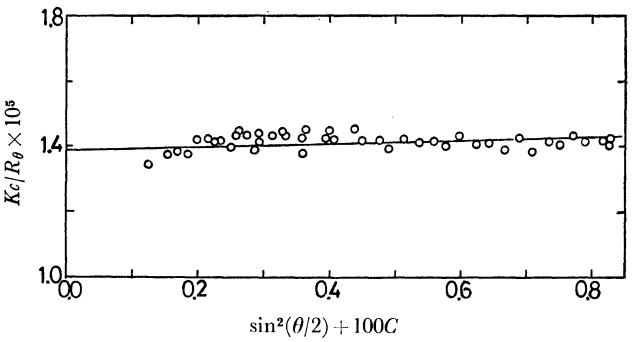

Fig. 2. Zimm plot for bovine serum albumin (BSA) in 0.01 M NaCl at 25°C, pH 5.40

17) G. E. Perlmann and L. G. Longsworth, *J. Amer. Chem. Soc.*, **70**, 2719 (1948).
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rature.[23] In the case of the mixed solutions of BSA and Chs, the scattering intensity was measured and the data were tentatively analyzed, at first, according to Eq. (2) by assuming them to be solutions of one solute whose weight concentration was equal to the sum of the two components. The apparent values of the molecular weight, the radius of gyration, and the second virial coefficient thus obtained were further examined theoretically as two-solute systems. All the experiments were carried out at concentrations between 3.5×10^{-3} and 5×10^{-4} g/m*l* in the total of the two solutes. Within this concentration range, the light-scattering data could be extrapolated without any difficulty.

Figure 3 (a), (b), and (c) are examples of the Zimm plot for the mixing ratios of $\gamma=12.9$, 24.4, and 101

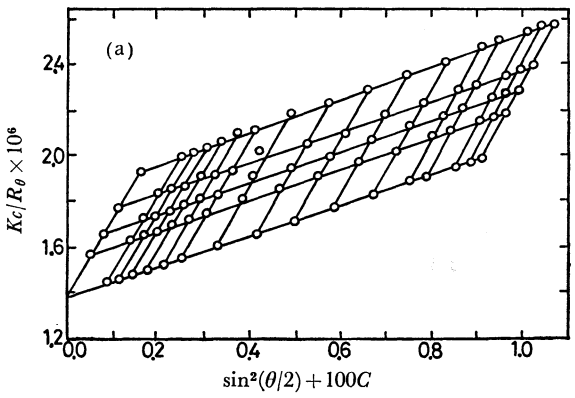

Fig. 3(a)

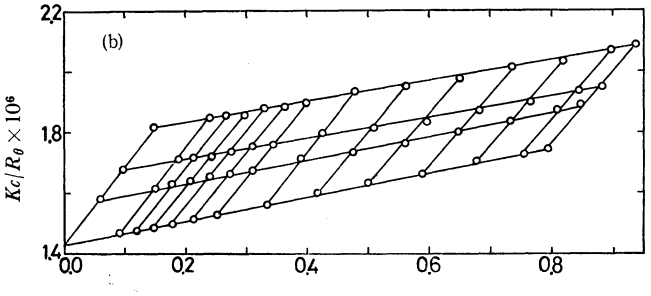

Fig. 3(b)

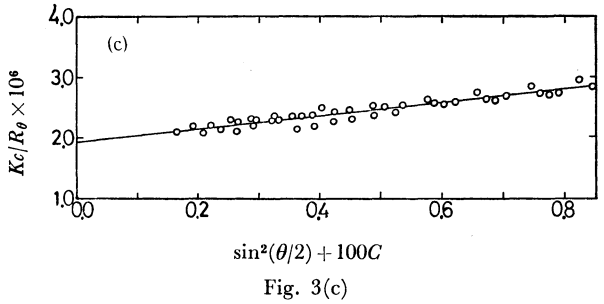

Fig. 3(c)

Fig. 3. Zimm plots for Chs-BSA mixture. (a), $\gamma=12.9$ gBSA/gChs, pH 5.62; (b), $\gamma=24.4$ gBSA/gChs, pH 5.83; (c), $\gamma=101$ gBSA/gChs, pH 5.56. All measurements were carried out at 25°C, in 0.01 M NaCl.

23) J. T. Edsall, H. Edelhoch, R. Lontie, and P. R. Morrison, *J. Amer. Chem. Soc.*, **72**, 464 (1950).

respectively. As is shown in Fig. 3, the Zimm plot changed its shape and became narrower with an increase in the mixing ratio, γ. The Zimm plot at low γ-values was similar to that of Chs, while the Zimm plot at γ-values higher than 35.2 was similar to that of BSA.

The apparent values of the weight-average molecular weight, the second virial coefficient, and the radius of gyration, M_{app}, R_{gapp}, and A_{2app} respectively, obtained from these Zimm plots according to Eq. (2) are plotted against the mixing ratio, γ, in Fig. 4 for the region of rather low mixing ratios. The dotted curves are the calculated ones, which will be explained later.

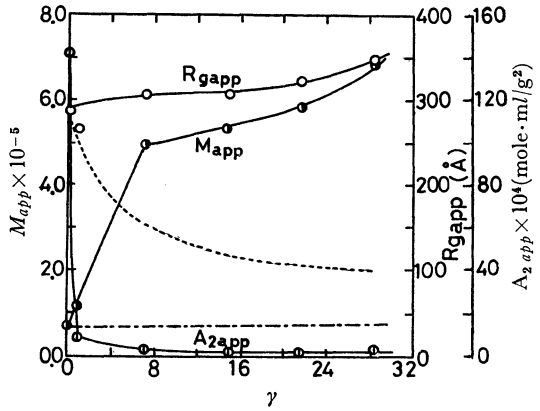

Fig. 4. Apparent molecular weight, M_{app}(◑), apparent radius of gyration, R_{gapp}(○), and apparent second virial coefficient, $A_{2\,app}$(ⓘ), obtained from BSA-Chs mixture of various mixing ratio, γ (gBSA/gChs)
◑,ⓘ,○: experimental values
------ theoretical curve of molecular weight for Chs-BSA system caluclated by equation (10)
------- theoretical curve of radius of gyration for Chs-BSA system calculated by equation (11)

It may be seen from this figure that the apparent molecular weight increases appreciably with the mixing ratios. It can further be seen from Fig. 4 that the apparent second virial coefficient decreases remarkably with the increase in the mixing ratio. In the region of the higher mixing ratios, that is, in the region of the higher BSA content, the apparent molecular weight has its maximum, as is shown in Fig. 5, where the mole fraction of BSA, X_{BSA}, is on the abscissa in place of the mixing ratio, γ. The mole fraction of BSA in the total solute, X_{BSA}, is related to by Eq. (4):

$$X_{BSA}=\frac{\gamma M_{Chs}}{M_{BSA}+\gamma M_{Chs}} \tag{4}$$

where M_{BSA} and M_{Chs} are the weight-average molecular weights for BSA and Chs respectively.

Density. The measurements of the density for the mixed solutions were carried out with a picnometer at 25°C. The values of the partial specific volume, V_{app}, of the mixed solute were calculated, they are plotted against the mole fraction of BSA in Fig. 6. It can be seen that the apparent partial specific volume increases linearly with the mole fraction of BSA. The value of the partial specific volume of Chs was 0.4448 m*l*/g. This value agrees well with the value obtained independently by Nakagaki and Ikeda.[15]

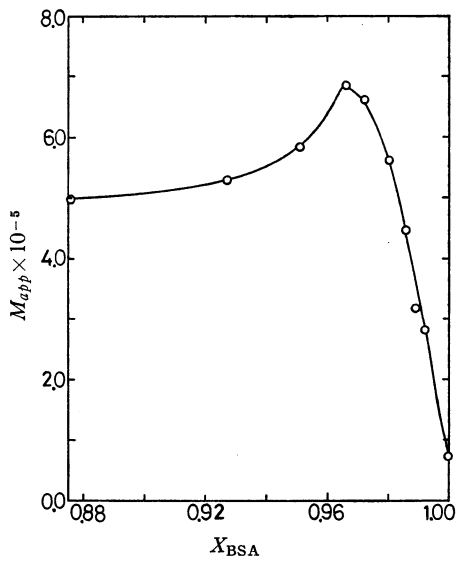

Fig. 5. Apparent molecular weight, M_{app}, plotted against mole fraction of BSA, X_{BSA}.

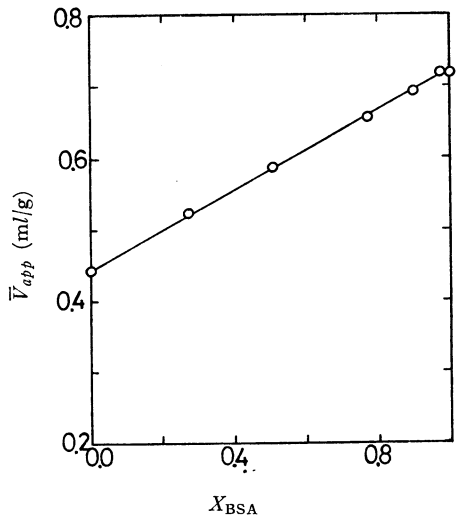

Fig. 6. Apparent partial specific volume, $\bar{V}_{app}$, (ml/g), plotted against mole fraction of BSA, X_{BSA}.

On the other hand, the partial specific volume of the native BSA was 0.7165 ml/g. This value agrees reasonably well with those values listed in the literature.[24-26]

Discussion

Binding of BSA on Chs Molecules. For the light scattering of a solution containing z macromolecular components, the following equation has been derived by Zimm,[27] by Stockmayer,[28,29] and by Blum and Morales[30,31] according to the theory of the fluctuation of the chemical potentials;

$$\frac{R_\theta}{K'c_t} = \sum_{i=1}^{i=z} M_i P_i(\theta) w_i \phi_i^2 - 2c_t \sum_{i=1}^{i=z}\sum_{j=1}^{j=z} A_2^{(ij)} M_i M_j P_i(\theta) P_j(\theta) w_i w_j \phi_i \phi_j \quad (5)$$

where R_θ is the reduced scattering intensity of the solution containing z macromolecular components and where $P_i(\theta)$ is the interference factor of the i-th macromolecular component and is approximately given by the following equation:

$$P_i(\theta) = 1 - \frac{16\pi^2 n_0^2}{3\lambda_0^2} Rg_i^2 \sin^2\left(\frac{\theta}{2}\right) \quad (6)$$

$A_2^{(ij)}$ is the second virial coefficient describing the interaction between the i-th and j-th components, w_i is the weight fraction of the i-th component in the total solute, and c_t is the total weight concentration (g/ml) for the mixed solution. Here,

$$c_t = \sum_{i=1}^{i=z} c_i \quad (7)$$

and

$$w_i = c_i/c_t \quad (8)$$

where c_i is the weight concentration of the ith macromolecular component.

Since the second term of Eq. (5) is smaller than the first term, Eq. (5) can be written as follows by using Eq. (6), in which the second term is again assumed to be smaller than the first:

$$\frac{K'c_t}{R_\theta} = \frac{1}{\sum_{i=1}^{i=z} M_i w_i \phi_i^2}\left\{1 + \frac{16\pi^2 n_0^2}{3\lambda_0^2}\left(\frac{\sum_{i=1}^{i=z} M_i w_i \phi_i^2 Rg_i^2}{\sum_{i=1}^{i=z} M_i w_i \phi_i^2}\right)\sin^2\left(\frac{\theta}{2}\right)\right\} + 2c_t \frac{\sum_{i=1}^{i=z}\sum_{j=1}^{j=z} A_2^{(ij)} M_i M_j P_i(\theta) P_j(\theta) w_i w_j \phi_i \phi_j}{\left(\sum_{i=1}^{i=z} M_i P_i(\theta) w_i \phi_i^2\right)} \quad (9)$$

By comparing Eq. (9) with Eq. (2), the following equations can be obtained for a mixed solution of two macromolecular solutes described by the subscripts 1 and 2:

$$M_{app} = (M_1 w_1 \phi_1^2 + M_2 w_2 \phi_2^2)/\phi^2 \quad (10)$$

$$R^2_{gapp} = (R_{g1}^2 M_1 w_1 \phi_1^2 + R_{g2}^2 M_2 w_2 \phi_2^2)/M_{app}\phi^2 \quad (11)$$

Here, M and R_g in Eq. (2) are replaced by M_{app} and R_{gapp} respectively. The results calculated according to these equations, assuming no interaction between Chs and BSA, that is, by setting 1=Chs and 2=BSA, are shown in Fig. 4 by dotted curves. It may be seen that the experimental values show an entirely different tendency from the calculated curves. This means that the chondroitin sulfate molecules interact with the BSA molecules strongly and that a complex is formed between them.

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25) C. Tanford, K. Kawahara, and S. Lapanje, *J. Amer. Chem. Soc.*, **89**(**4**), 729 (1967).
26) A. Ullman, M. E. Goldberg, D. Perrin, and J. Monod, *Biochemistry*, **7**(**1**), 261 (1968).
27) B. H. Zimm, *J. Chem. Phys.*, **16**, 1093 (1948).
28) W. H. Stockmayer, *ibid.*, **18**, 58 (1950).
29) J. G. Kirkwood and R. J. Goldberg, *ibid.*, **18**, 54 (1950).
30) J. J. Blum and M. F. Morales, *J. Chem. Phys.*, **20**, 1822 (1952).
31) J. J. Blum and M. F. Morales, *Arch. Biochem. Biophys.*, **43**, 208 (1952).

From Figs. 4 and 5, it can be seen that, with an increase in the mixing ratio, the apparent molecular weight and the apparent radius of gyration increase, while the apparent second virial coefficient decreases remarkably. These results suggest the formation of a complex between BSA and Chs by the following chemical equation:

$$(\text{Chs}) + \delta_m(\text{BSA}) \rightarrow (\text{Chs})\cdot(\text{BSA})_{\delta_m}$$

If the affinity between Chs and BSA is strong, the complex is formed to as great an extent as one of these components is mostly used up. In this instance, the concentration of the component is negligibly small, so that the system can be considered to be a solution of two macromolecular solutes for which Eqs. (10) and (11) are applicable. By using these equations, the possibility that either Chs and BSA remains in excess after the complex formation has been examined. To explain the experimental data satisfactorily, it has been concluded that a part of the BSA is left free in the solution and that many BSA molecules are bound on one Chs molecule ($\delta_m > 1$). Equations (10) and (11) can be used, in this case also, by setting 1=complex (to be designated by the subscript m) and 2=free BSA (to be designated by the superscript f). The following equation:

$$M_{app} = [w_{\text{Chs}}M_{\text{Chs}}(\phi_{\text{Chs}} + \phi_{\text{BSA}}\delta)^2 + (w_{\text{BSA}} - w_{\text{Chs}}\delta)M_{\text{BSA}}\phi_{\text{BSA}}{}^2]/\phi^2 \quad (12)$$

can be deriven from Eq. (10) by using the following obvious relations:

$$M_m = M_{\text{Chs}}(1+\delta) = M_{\text{Chs}} + \delta_m M_{\text{BSA}} \quad (13a)$$

$$w_m = w_{\text{Chs}}(1+\delta) \quad (13b)$$

$$w_{\text{BSA}}{}^f = w_{\text{BSA}} - \delta w_{\text{Chs}} \quad (13c)$$

$$\delta = \delta_m M_{\text{BSA}}/M_{\text{Chs}} \quad (13d)$$

together with the Geiduschek-Doty equation:[12]

$$\phi_m = (\phi_{\text{Chs}} + \delta\phi_{\text{BSA}})/(1+\delta) \quad (14)$$

The weight-binding coefficient, δ, can be obtained according to Eq. (12) by using the experimental values of M_{app} and of all other quantities. As a result, it has been concluded that the value of δ increases with the mixing ratio, γ. The molar binding coefficient, δ_m, thus obtained is shown in Fig. 7 as a function of the molar ratio of free BSA, $\gamma_m{}^f$, relative to the molar concentration of Chs.

$$\gamma_m{}^f = (M_{\text{Chs}}/M_{\text{BSA}})\gamma - \delta_m \quad (15)$$

An equation of the same form as Langmuir's adsorption isotherm is often used to describe the amount of binding of a low-molecular-weight substance to macromolecules as a function of the equilibrium concestration of the latter.[32] A similar equation can be expected to be used as well in the case of complex formation between two macromolecular substances. In our case, the number of molecules, δ_m, of BSA bound on one molecule of Chs may be given by the following equation:

$$\delta_m = \delta_m{}^\infty \frac{k\,\gamma_m{}^f}{1 + k\,\gamma_m{}^f} \quad (16)$$

where the constant, k, indicates the strength of adsorption and where $\delta_m{}^\infty$ is the saturated molar binding coefficient.

On the basis of the linear relationship between $\gamma_m{}^f/\delta_m$ *vs.* $\gamma_m{}^f$, which is expected from Eq. (16) and which is shown in Fig. 7 by a dotted line, the parameter values, $\delta_m{}^\infty = 20.6$ and $k = 0.255$, were obtained. The solid curve in Fig. 7 shows the values calculated from Eq. (16) by using these parameter values. From this, it can be seen that the agreement between experimental and calculated values is good and that the maximum number of BSA molecules to be bound to a Chs molecule is about 21.

Since a Chs molecule consists of 145 disaccharide units, according to the observed value of its molecular weight, it may be concluded that, at saturation, one BSA molecule is bound to about seven disaccharide residues.

Zimm Plot of the Complex Molecules. Since it has been concluded in the preceding section that a complex and excess (free) BSA exist in the mixed solution of Chs and BSA, the next step is to obtain the reduced scattering intensity of the complex, $R_{\theta m}$, by subtracting the contribution of free BSA from the total reduced scattering intensity, and to attempt to draw a Zimm plot of the complex. The reduced scattering intensity of the complex may be calculated from the experimental data by the following equation, because the second virial coefficient of BSA is almost zero, as is shown in Fig. 2:

$$R_{\theta m} = R_\theta - K' c_{\text{BSA}}{}^f M_{\text{BSA}} \phi_{\text{BSA}}{}^2 P_{\text{BSA}}(\theta) \quad (17)$$

The Zimm plot may be analyzed according to the following equation, which has the same form as Eq. (2):

$$\frac{K'\phi_m{}^2 c_m}{R_{\theta m}} = \frac{1}{M_m}\left\{1 + \left(\frac{16\pi^2 n_0{}^2}{3\lambda_0{}^2}\right) R_{\theta m}{}^2 \sin^2\left(\frac{\theta}{2}\right)\right\} + 2A_{2m}c_m \quad (18)$$

Here, ϕ_m has been given by Eq. (14). Finally, it

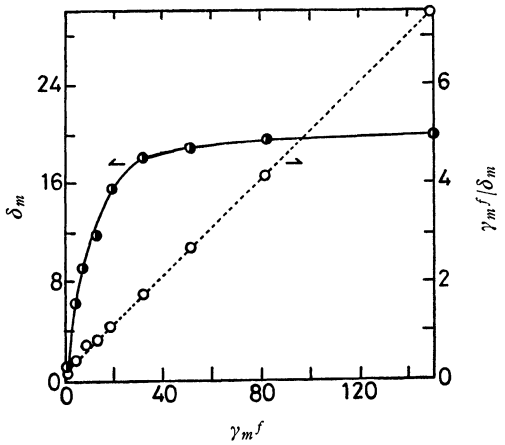

Fig. 7. Molar binding coefficient, δ_m, plotted against molar ratio of free BSA, $\gamma_m{}^f$ (BSA/Chs). The broken line is Langmuir plot of equation 16, where $\gamma_m{}^f/\delta_m$ is plotted against $\gamma_m{}^f$.

32) M. Nakagaki, N. Koga, and H. Terada, *Nippon Yakugaku Zasshi*, **83**(6), 586 (1963); *ibid.*, **84**(6), 516 (1967); *ibid.*, **86**(6), 447 (1966); M. Nakagaki and H. Terada *ibid.*, **87**(7), 817 (1967); *ibid.*, **87**(8), 980 (1967); *ibid.*, **87**(9), 1089 (1967).

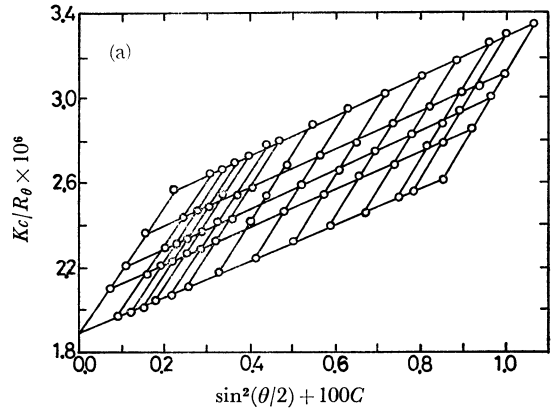

Fig. 8(a)

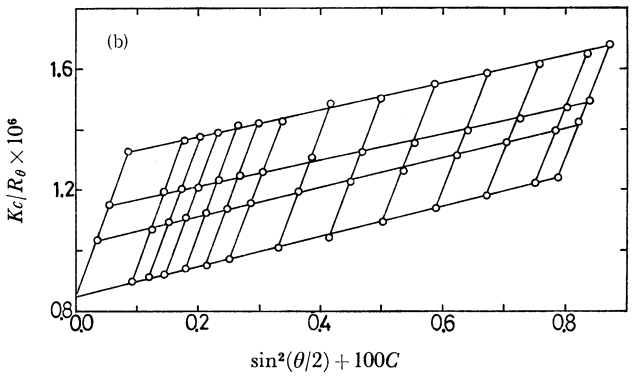

Fig. 8(b)

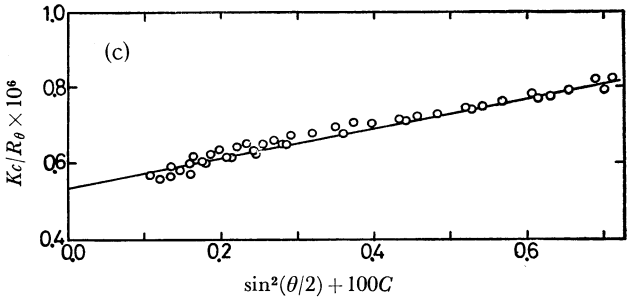

Fig. 8(c)

Fig. 8. Zimm plots for BSA-Chs complex molecule calculated from Fig. 3. Curves (a), (b), and (c) correspond to curves (a), (b) and (c) in Fig. 3.

should be mentioned that the A_{2m} term in Eq. (18) is not the second virial coefficient of the complex itself, but contains the contribution due to the interaction between the complex and the free BSA.

Examples of the results are shown in Fig. 8, which corresponds to Fig. 3. The molecular weight, M_m; the radius of gyration, R_{gm}, and the second virial coefficient, A_{2m}, for the complex are plotted against the molar binding coefficient, δ_m, in Fig. 8. It can be seen that M_m increases remarkably and linearly with δ_m but that R_{gm} increases only slightly, while A_{2m} decreases rapidly and approaches the value of BSA alone ($A_{2BSA}=0$).

It is natural that the molecular weight of the complex, M_m, increases linearly with δ_m, as is to be expected from Eq. (13a). The molecular weight of the bound BSA, calculated from the inclination of the straight line of Fig. 9, is 72000, which is in good agreement with the molecular weight of native BSA, M=71900, already cited.

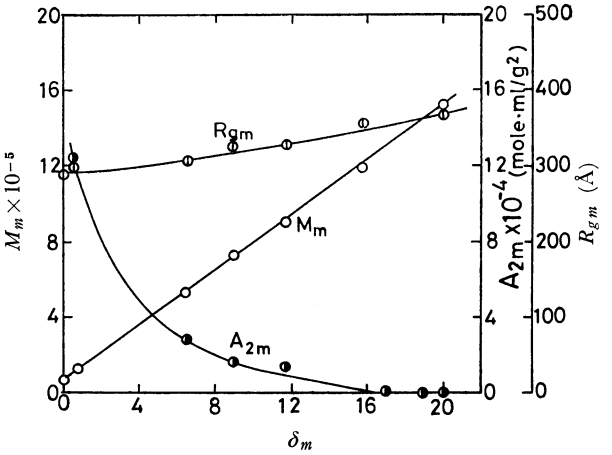

Fig. 9. Molecular weight, M_m(○), radius of gyration, R_{gm} (ⓘ) and second virial coefficient, A_{2m}(◑) for BSA-Chs complex molecule against molar binding coeffient, δ_m, in 0.01 M NaCl at 25°C.

On the other hand, R_{gm} value for the complex is almost equal to R_{gapp} for the mixed solutes, as is clear from a comparison between Figs. 4 and 9, because R_g obtained from the light-scattering measurements is z-average and the radius of gyration of the free BSA is very small. Moreover, the length of the complex molecules seems to be nearly equal to that of Chs itself, because the value of R_g does not increase very much with δ_m. Finally, the second virial coefficient for the complex decreases rapidly with δ_m. This indicates that the electric charges of the Chs are shielded by the binding of BSA molecules. Therefore, it can be concluded that the complex molecule has as its core the chondroitin sulfate molecule, with its surface covered with BSA molecules.

Shape and Size of the Complex Molecule. The shape and size of the complex molecule will be discussed. The chondroitin sulfate is an unbranched linear anionic polyelectrolyte and is stretched extensively by the electrostatic repulsive force if the polyions have a low ionic strength. It is, however, known that the polyions gradually contract with the increase in the ionic strength because of the formation of ionic pairs or because of electrostatic shielding effect of the ionic atmosphere. Nakagaki and Ikeda[15] found, from their viscosity and the light-scattering investigations, that the polyion of chondroitin sulfate is a long rod-like shape which is, at an infinite ionic strength, about 800 Å in long and about 10 Å in diameter. The shape of the complex molecule may be assumed to be almost the same as that of chondroitin sulfate, because the radius of gyration does not change very much. Therefore, the complex molecule is assumed to be a rod-like molecule.

The radius of gyration, R_{gm}, and the volume, V_m, of a rod-like molecule are expressed as follows:

$$R_{gm}^2 = L_m^2/12 + D_m^2/8 \quad (19)$$

$$V_m = \pi(D_m/2)^2 L_m \quad (20)$$

According to these equations, the diameter, D_m, and the length, L_m, of the complex can be calculated, ignoring the solvation, from the value of the radius of gyration and the value of the partial specific volume of the complex.

The partial specific volume of the complex, $\overline{V}_m$, is calculated from Eq. (21), which is based on an additivity rule, as is generally assumed:

$$\overline{V}_{app} = \overline{V}_m w_m + \overline{V}_{BSA} w_{BSA}{}^f \quad (21)$$

Further, the value of the molecular volume of the complex, $M_m\overline{V}_m/N_A$, can be calculated from the data already shown in Fig. 6 according to Eq. (22). The results are plotted against the molar binding coefficient, δ_m, in Fig. 10. This figure shows that the volume of the Chs molecule does not change with the binding of BSA and also that the volume of a bound BSA is 0.862×10^5 (Å^3/molecule), which is 0.7212 ml/g in the partial specific volume. The partial specific volume of the native BSA, on the other hand, is 0.7165 ml/g. There is a reasonably good agreement between these values and those listed in the literature.[24-26] As has been mentioned above, the length and the diameter of the complex molecule can, therefore, be calculated, according to Eqs. (19) and (20), from the experimental values of $\overline{V}_m$ and

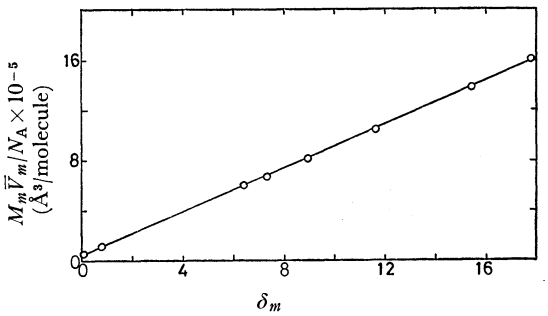

Fig. 10. Partial molar volume per complex molecule, $M_m\overline{V}_m/N_A$, (Å^3/molecule) plotted against molar binding coefficient, δ_m.

R_{gm} if the density in the complex molecule is assumed to be homogeneous. The results obtained are shown in Table 1. It may be seen that the length increases about 20 percent as compared with the Chs molecule, while the diameter increases about five times, upon the binding of BSA.

In contradistinction to the assumption of a homogenous density in complex molecules, if the molecule has a structure in which the core of the Chs molecule with a density of ρ_{Chs} is surrounded by BSA molecules with a density ρ_{BSA}, and if the complex molecule as a whole is assumed to be a rigid prolate spheroid with major axis a and minor axis b, the radius of gyration of the complex will be given by:

$$R_{gm}^2 = \frac{\left(\frac{a^2+2b^2}{5}\right)\rho_{BSA}\left(\frac{4}{3}\pi ab^2\right)+(\rho_{Chs}-\rho_{BSA})\frac{\pi}{4}D_{Chs}^2L_{Chs}\left(\frac{L_{Chs}^2}{12}+\frac{D_{Chs}^2}{8}\right)}{\rho_{BSA}\left(\frac{4}{3}\pi ab^2\right)+(\rho_{Chs}-\rho_{BSA})\frac{\pi}{4}D_{Chs}^2L_{Chs}} \quad (22)$$

$$V_m = \frac{4}{3}\pi ab^2 \quad (23)$$

According to Eqs. (22) and (23), the length of the two axes, $2a$ and $2b$, can be calculated from the experimental values of the radius of gyration and the partial specific volume by assuming that the Chs core has the same length, L_{Chs}, and the same diameter, D_{Chs}, as free Chs molecules. The results thus obtained are shown in Table 1.

If the complex molecule is assumed to be a rigid rod which has a Chs core, much like the structure of the prolate spheroid considered above, the length, L', and the diameter, D', of the complex molecule can be calculated in the same way:

$$R_{gm}^2 = \frac{\left(\frac{L'^2}{12}+\frac{D'^2}{8}\right)\rho_{BSA}\left(\frac{\pi}{4}D'^2L'\right)+(\rho_{Chs}-\rho_{BSA})\frac{\pi}{4}D_{Chs}^2L_{Chs}\left(\frac{L_{Chs}^2}{12}+\frac{D_{Chs}^2}{8}\right)}{\rho_{BSA}\left(\frac{\pi}{4}D'^2L'\right)+(\rho_{Chs}-\rho_{BSA})\frac{\pi}{4}D_{Chs}^2L_{Chs}} \quad (24)$$

Table 1. Molecular dimensions of the complex molecule between chondroitin sulfate C and bovine serum albumin at various molar binding coefficient

δ_m	0	0.707	6.404	8.895	11.628	15.455	20.360
(rigid rod-like molecule of a uniform density)							
L_m	998	1039	1068	1130	1126	1227	1278
D_m	8.0	12.2	27.1	32.0	36.7	36.7	46.1
(a prolate ellipsoid of revolution which is considered for a different density between Chs and BSA)							
$2a$		959.2	978.2	1036	1032	1125	1162
$2b$		15.0	18.6	38.6	44.0	48.2	55.2
(rod-like molecule which is considered for a different density between Chs and BSA)							
L'		1180	1096	1152	1143	1241	1279
D'		11.0	26.3	29.9	34.1	37.5	42.9

These results are also shown in Table 1.

The results shown in Table 1 are in fairly good agreement with each other, irrespective of the model. It seems, at any rate, that the complex molecule has a long, deformed shape. In these models, the prolate spheroid is the most suitable to explain the results of viscosity measurements, as will be published elsewhere.

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