A Viscometric Study for Adsorption of Bovine Serum Albumin onto the Inner Surface of Glass Capillary

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Received 7 July 2006; accepted 11 July 2007
DOI 10.1002/app.27269
Published online 25 October 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Viscosities of aqueous solutions of bovine serum albumin (BSA) at different temperatures were carefully measured in a common glass capillary Ubbelohde viscometer in the concentration range from dilute down to extremely dilute concentration regions. The adsorption effect occurred in viscosity measurements were theoretically analyzed and discussed. The theory based on the Langmuir isotherms could adequately describe the existing data. The influence of temperature on the adsorption of BSA solution was discussed and a simple adsorption model was proposed. The conformational change of BSA macromolecules happened both in the bulk solution and on the inner surface of glass capillary. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 1850–1856, 2008

Key words: BSA; viscosity; adsorption; conformational change

INTRODUCTION

The adsorption of protein is of great interest for many reasons, such as the use of proteins to stabilize colloids, prevention of the biofouling of plant and equipment, the functioning of cell membranes, and so on.[1] Numerous adsorption models have been proposed for the adsorption of protein to solid surfaces such as the simple Langmuir adsorption models[2] and the cluster models.[3] To comprehensively and quantitatively understand the adsorption process, many researchers have tried to study the adsorption kinetics. For example, Cottin[4] developed a model incorporating an apparent kinetic constant constructed as a combination of the adsorption constant and the rate constant; Lundström[5] believed that there existed multiple conformational states and he had discussed the competitive adsorption at the interface; and Lee and Bothwell[6] set up a mechanistic approach to modeling single protein adsorption at solid–water interfaces.

The protein adsorption at solid–liquid interfaces has been investigated by various techniques such as ellipsometry,[7] fluorescence spectroscopy,[8] radiotracers,[9] surface-enhanced raman spectroscopy,[10] attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic flow cell method,[11,12] and atomic force microscopy (AFM).[13,14] Viscosity measurement is one of the most convenient means for characterizing polymers. For example, the intrinsic viscosity of polymer bears the size of molecules in the solution. Singh[15] reported a survismeter that can measure surface tension, viscosity, and friccohesity data of polymer solution of concentration down to 0.0005%. In concentrated solutions, since the adsorption on the wall of the container could be neglected compared to the bulk solution, it has little effects on the viscosity.[16] However, in extremely dilute solutions ($C < 10^{-2}$ g/cm^3),[17] the actual concentration of solution is inevitably lessened due to the adsorption of solute onto the inner wall of the container, and then the inner radius of viscometer capillary reduces for the same reason, therefore the measured viscosity appears deviation. For instance, a graph of reduced viscosity plotted against concentration shows an upward curve as the concentration dips below a certain level.[18,19] The abnormal phenomena of viscosity in extremely dilute concentration region had attracted the notice of many investigators since early 1950s and different explanations had been proposed.[20] The hypothesis of adsorption is one of the most probable reasons for the existence of viscosity abnormality, but it is not widely accepted till a quantitative explanation was raised and proved by Cheng and his coworkers through great works on neutral polymer solutions.[21–26]

Few documents are involved in the studying of adsorption of protein by viscometric means.[27–32] The purpose of this article is to study the adsorption of bovine serum albumin (BSA) on the inner surface of glass capillary by measuring the viscosity of BSA solution from dilute to extremely dilute concentration region at different temperatures. Through an iterative fitting procedure to treat the reduced viscosity

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data according to the viscosity equations raised by Cheng and his coworkers, some adsorption parameters were obtained and analyzed, and a simple model was proposed to simulate the adsorption process for BSA at different temperatures. The results provided evidence of the existence of conformational changes both on the surface and in the solution.

THEORETICAL

Viscosity of extremely dilute solution

In extremely dilute solutions, the density of solution is almost equal to the density of pure solvent, so the experimental relative viscosity of a polymer solution is usually calculated according to[18]:

$$\eta_{r,\mathrm{exp}} = \frac{t(C)}{t_0} \tag{1}$$

Owing to the existence of interfacial interactions between flowing solution and capillary wall surface, the relationship between the true and the experimental relative viscosity has been proposed by Cheng and his coworkers on basis of Langmuir adsorption isotherm[21–26] as

$$\eta_{r,\mathrm{exp}} = \eta_{r,\mathrm{true}} \cdot \left(1 + \frac{kC}{C_a + C}\right) \tag{2}$$

where the constant k denotes the maximum fractional change of flow time of pure solvent due to the variation of the surface properties of viscometer wall in the course of determining polymer solution viscosity,[21,24,25] and C_a is a characteristic concentration at which the half of the inner surface of the viscometer is covered by the adsorbed polymer molecules. A positive k with $C_a > 0$ means the polymer will anchor on the capillary wall surface and results an up-bending reduced viscosity versus concentration plot. A negative k with $C_a = 0$ indicates the flow mode of the solution been converted from viscous to slipping and results a down-bending reduced viscosity versus concentration plot.[23] In the case of polymer adsorption, an effective adsorbed layer thickness b_{eff} could be deduced from parameter k in the light of Poiseuille law[22,23] as

$$b_{\mathrm{eff}} = R \cdot \left(1 - \frac{1}{(1+k)^{1/4}}\right) \tag{3}$$

where R is the radius of the capillary.

On the basis of the assumptions that Einstein viscosity law is valid for nonassociable dilute polymer solution

$$\eta_r = 1 + [\eta]C \tag{4}$$

And any deviation from it is due to macromolecular self-association or cluster formation. The true relative viscosity of polymer solution may be represented by

$$\eta_{r,\mathrm{true}} = 1 + [\eta]C + 6K_m[\eta]C^2 \tag{5}$$

K_m is apparent self-association constant. It is related to the size and interactions between polymer chains in solution and numerically correlates with the Huggins coefficient k_H and intrinsic viscosity $[\eta]$ as

$$K_m = \frac{k_H \cdot [\eta]}{6} \tag{6}$$

Combining eqs. (2) and (5), the experimental relative viscosity of polymer solution should be expressed as

$$\eta_{r,\mathrm{exp}} = (1 + [\eta]C + 6K_m[\eta]C^2)\left(1 + \frac{kC}{C_a + C}\right) \tag{7}$$

Consequently, the experimental reduced viscosity will be

$$\left(\frac{\eta_{\mathrm{sp}}}{C}\right)_{\mathrm{exp}} = \frac{(1 + [\eta]C + 6K_m[\eta]C^2)(1 + \frac{kC}{C_a+C}) - 1}{C} \tag{8}$$

Placing V mL polymer solution with initial concentration C_0 into a glass viscometer, the equilibrium concentration C of the solution should be diminished due to solute adsorption onto the wall surface of the viscometer. Denoting the maximum amount of polymer adsorbed per unit area on glass by G_{max} in unit g/cm^2, according to Langmuir adsorption isotherm the fractional coverage of the glass surface in contact with solution due to adsorption should be expressed as

$$\theta = \frac{G(C)}{G_{\mathrm{max}}} = \frac{b \cdot C}{1 + b \cdot C} = \frac{C}{C_a + C} \tag{9}$$

where $G(C)$ is the specific solute adsorption amount at equilibrium concentration C and the characteristic concentration $C_a = 1/b$ have the same physical meaning cited above. Therefore, the absolute solute adsorption amount W_a(C) at equilibrium concentration C is

$$W_a(C) = \frac{S \cdot G_{\mathrm{max}} \cdot C}{C_a + C} \tag{10}$$

where S is the contacting area of solution with the viscometer. Owing to the solute amount W in initial solution is

$$W = C_0 \cdot V \tag{11}$$

Then the concentration C at adsorption equilibrium becomes

$$C = \frac{[W - W_a(C)]}{V} = C_0\left[1 - \left(\frac{S \cdot G_{\max}}{V}\right) \cdot \left(\frac{C}{C_0 \cdot (C_a + C)}\right)\right] \quad (12)$$

Defining

$$A = \frac{S \cdot G_{\max}}{V} \quad (13)$$

In one viscometer, the variance of parameter A could represent the change of adsorption amount. Ignoring the small difference between C and C_0 for the correction term in the bracket of Eq. (12), we have the expression of the actual equilibrium concentration as

$$C = C_0 \cdot \left(1 - \frac{A}{(C_a + C_0)}\right) \quad (14)$$

The fractional reduction in concentration therefore is

$$\frac{(C_0 - C)}{C_0} = \frac{A}{(C_a + C_0)} \quad (15)$$

which is determined by the relative magnitudes of C_a and A, and in turn both of them are depending on the ability and capacity of adsorption on glass surfaces.

Inserting the equilibrium concentration of Eq. (14) into Eq. (8) instead of the initial concentration originally presented in the equation, we have the final expression of the experimental reduced viscosity as:

$$\frac{\eta_{sp}}{C_0} = \frac{\left[1 + [\eta] \cdot C_0 \cdot (1 - \frac{A}{C_a + C_0}) + 6 \cdot K_m \cdot [\eta] \cdot [C_0(1 - \frac{A}{C_a + C_0})]^2\right] \cdot \left[1 + k \cdot \frac{C_0\left(1 - \frac{A}{C_a + C_0}\right)}{C_a + C_0\left(1 - \frac{A}{C_a + C_0}\right)}\right] - 1}{C_0} \quad (16)$$

The experimental reduced viscosities are generated from two different sources. The first source is the true relative viscosity of polymer solution itself as described by the first bracket of the numerator of Eq. (16) or briefly by Eq. (5). The second source is raised from the interfacial effect during measuring viscosity as described in the second bracket of the numerator. By an iterative fitting procedure to treat the reduced viscosity data according to Eq. (16), the parameters K_m, C_a, k, A, and $[\eta]$ could be obtained simultaneously.

Novel interpretation of the viscosity of dilute protein solution

As was first demonstrated by Fuoss[33,34] early in 1948 in one of the first exploration of synthetic polyelectrolyte solution viscosity, the effect of charging a polymer chain complicates interpretation of viscosity experiments considerably. The typical viscometrical behavior for polyelectrolyte in salt-free polar solvents is that the reduced viscosity, $\eta_{sp/C}$, increases remarkably with decreasing polymer concentration. This observed specific behavior is not well understood in terms of the structure of the solution and the essential factors causing the behavior.[35,36] The conventional explanation is that the intramolecular repulsion between fixed ions on the same chain causes the expansion of the chain, leading to polyelectrolyte behavior.[35,36] However, solutions of ionomers that have a small number of ionic groups per chain and even solution of halato-telechelic ionomer[37–39] showed characteristic polyelectrolyte behavior similar to that of typical salt-free polyelectrolyte in aqueous solution. It may be noticed that the so-called viscometrical polyelectrolyte behavior is phenomenologically very similar to the viscometrical behavior of neutral polymer solution in extremely dilute concentration region. The later abnormal phenomena could be explained by the reduction of radius of viscometer capillary due to solute adsorption.[21–25]

Most proteins are polyampholytes. This characteristic makes them intrinsically surface-active molecules. The abnormalities of viscosity for dilute protein aqueous solution, as is shown in this article, has been ascribed to the interfacial adsorption in our point and could be quantitatively analyzed by the viscosity equations mentioned above for neutral polymer solution. Therefore, through the analysis for viscosity data, we may knew about the adsorption actions happened on the inner surface of glass capillary during viscosity measuring, while such adsorption conditions are difficult to be tested by common instrumental analytical techniques.

EXPERIMENTAL

Viscometers

A conventional Ubbelohde type viscometer made of borosil glass with a capillary of 0.20 mm inner diameter was used to measure the viscosity of the BSA solution. The glass capillary is smooth and uniform. For

TABLE I
Efflux Times of Pure Solvent (Water) and the Results of Linear Regression for $\eta_r - c$ Lines

Temperature (°C)	T_0 (s)	Intercept	Slope	Corr. coef
15	500.33	1.005	6.66	0.99
25	390.97	1.002	7.34	0.99
35	317.38	1.004	5.82	0.99
45	264.22	1.006	5.89	0.99

cleaning the glass viscometer, first to soak it in a chromic acid mixture for a day, then to rinse the viscometer repeatedly with deionized distilled water, and finally to rinse it with boiled deionized distilled water under ultrasonic vibrations. At last, the clean glass viscometer was sent to a common oven to dry at 125°C.

Viscosity measurements

An aqueous stock solution of the BSA (Sigma) was freshly prepared by weighing and then dissolving in distilled water at room temperature. The viscosity measurements were performed at 15–45°C, respectively. According to the sequence from low to high concentration, a known weight of pure solvent (deionized distilled water) was first transferred into the viscometer and its efflux time was measured. The constancy of the solvent efflux time serves as a criterion for judging the cleanness of the viscometer and the consistency of the experimental conditions. The efflux times of water in the viscometer (t_0) are listed in Table I.

After the efflux time of the solvent was measured, a definite amount of stock sample solution with a known weight concentration was added into the viscometer by weighing and then a syringe was used to produce air purge to mix the solution well in the viscometer. After about half an hour the efflux time of the solution was measured. This operation was repeated successively until the relative viscosity reaches a predetermined point. To examine whether the solution was mixed well, it can be judged by the constancy of the flow time of the measured solution. The ratio of the efflux time of the solution, t_{solu}, to that of solvent, t_{solv}, was regarded as the experimental relative viscosity, $\eta_r = t_{solu}/t_{solv}$. The obtained weight concentration of the solution in g/g was converted to weight–volume concentration in g/mL by applying density correction. The kinetic energy correction and drainage correction were neglected.[40]

RESULTS AND DISCUSSION

The dependence of the relative viscosities of BSA aqueous solution on the concentration is shown in Figure 1. The results of linear regression analysis

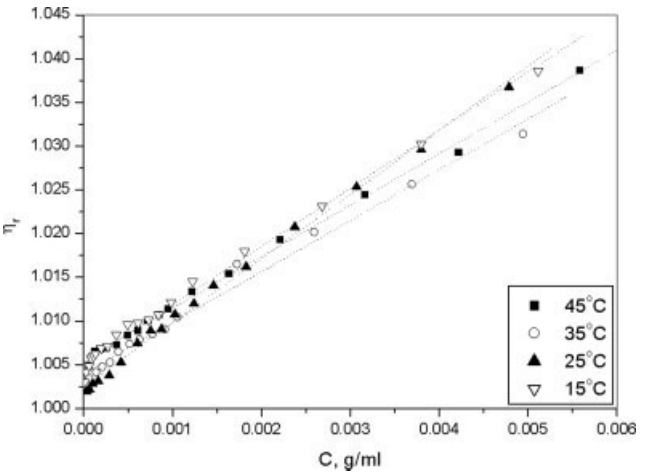

Figure 1 Plots of relative viscosity versus concentration of BSA aqueous solution in the extremely dilute concentration region.

listed in Table I showed that the linearity of the variation in relative viscosity with concentration was not bad, but the straight lines never pass through the origin point ($\eta_r = 1$ at $C = 0$). Therefore, Eq. (4) is not suitable to describe the change of experimental η_r with c herein. The plots of reduced viscosity against concentration are shown in Figure 2. The curves show upward turns at extremely dilute concentration region at all temperatures. The influence of the experimentally measured error derived from the accuracy of measured flow time on the viscosity measurement in the extremely dilute concentration has been examined. It was found that the experimental error does not distinctly affect the observed viscosity abnormalities appearing at extremely dilute concentration. This means that the viscosity abnormalities are not ascribed to the experimental error. Since

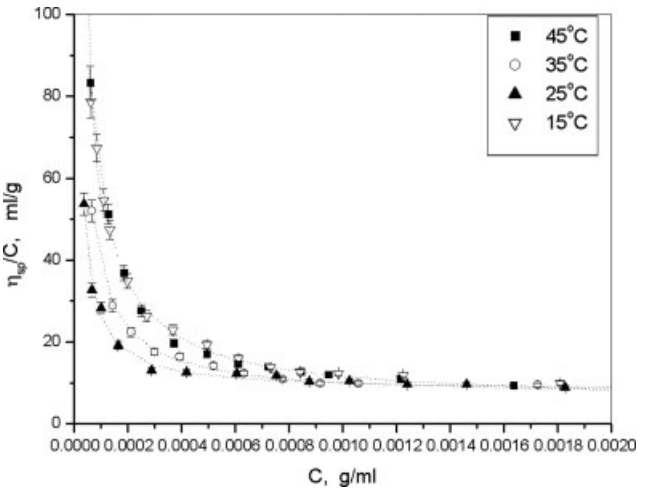

Figure 2 Plots of reduced viscosity versus concentration of aqueous BSA solution in the extremely dilute concentration region.

TABLE II
Viscosity Parameters of Aqueous Solution of BSA Obtained by Fitting the Reduced Viscosity Measured to Eq. (16)

Temperature (°C)	$k \times 10^3$	b_{eff} (nm)	[η] mL/g	$C_a \times 10^5$ g/mL	$A \times 10^{17}$ g/mL	K_m	Corr. coef
15	4.60	229.3	8.60	1.0E-14	5.7	0	0.98
25	1.91	95.4	8.06	0.55	2	0	0.99
35	3.64	181.6	6.45	1	1	0	0.99
45	6.55	326.2	5.17	2	0.1	0	0.99

reduced viscosity did not change linearly with c in the extremely dilute concentration, as is seen in Figure 2, Eq. (5) is not suitable to describe the change of experimental η_r with c herein too. Therefore, the adsorption effect is responsible for viscosity abnormalities observed in the extremely dilute concentration. According to Eq. (16), the reduced viscosity data has been treated by an iterative fitting procedure, the obtained parameters K_m, C_a, k, A, and [η] are listed in Table II. The coincidence of the calculated curve with the experimental points is excellent.

BSA is postulated to be an oblate ellipsoid with dimensions of 140 Å × 40 Å, i.e., the axial ratio is 3.5 : 1.[41] It shows less particle aggregation than any other protein, so it is often used as an ideal hard-sphere model to exam the folding and unfolding behavior in chemical denaturants.[16] The parameter K_m represents the ability of aggregation for polymer. Since the value of K_m at all test temperatures is zero in Table II, which indicated that the BSA molecules dispersed in the dilute solution in separated state and the change of dimension of molecules with temperature could only be related to the self-change of conformation of molecules.[42,43] The change of intrinsic viscosity with temperature is shown in Table II According to Flory,[44] the ratio of root-mean-square end-to-end length $(\bar{r^2})^{1/2}$ scales with [η] as:

$$[\eta] = \Phi \cdot \frac{(\bar{r^2})^{3/2}}{M_w} \tag{17}$$

The parameter Φ is a universal constant with a value of 2.1×10^{21}. The reported M_w of BSA is about 66,500.[45] Then the calculated $(\bar{r^2})^{1/2}$ are 139.7 Å, 136.6 Å, 126.9 Å, 117.8 Å at 15, 25, 35, and 45°C, respectively, which are over the range of literature radii values from Uversky and Fink for unfolded, 81.8 Å, and folded, 33.9 Å, BSA.[42,43] The fundamental type of folding of the polypeptide chains for native BSA is 55% α-helix and 45% random conformation from X-ray scattering studies, and the increasing of temperature will cause a partial loss of α-helical structure and the formation of β-sheet.[46] The change of dimension for BSA in this article indicated that molecules were unfolded in all test temperatures and the degree of unfolding increased with the descending of temperature.

As Figure 3 shows, the values of A reduced with the increasing of temperature, which indicated that the lower the temperature is, the larger the amount of adsorption is. However, since all these values are very low, far less than the test concentration of solution, the adsorption of solute did not lessen the test concentration actually. As we know, the BSA molecule contains net negative charge in neutral condition, and the static repulsive force between molecules makes them difficult to accumulate, which might be the main reason why the BSA molecules often take single layer adsorption method The values of C_a listed in Table II increased with temperature. When the temperature decreased to 15°C, the value of C_a was so low that it was reasonable to believe that the adsorption of BSA to the inner surface of capillary could be regarded as saturated.

The values of k and calculated b_{eff} according to Eq. (4) listed in Table II varied with temperature, but did not agree with the change of adsorption amount, as Figure 3 shows. Since the effective adsorbed layer thickness far exceeded the actual dimension of BSA molecule, indicating that the effective adsorbed layer included two parts, the actual adsorbed layer and the boundary layer.[47] The protein was initially transferred from the bulk liquid to the liquid–solid interface, then the protein bound to an unoccupied site on the solid surface. On account of the static repulsion between BSA molecules, an exclusive layer may

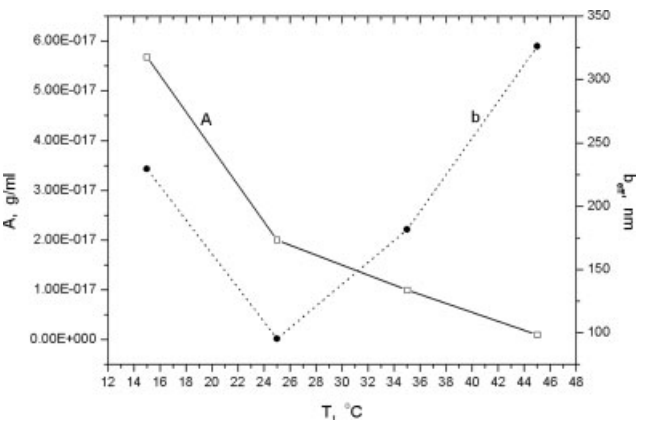

Figure 3 Comparison of adsorption amount and adsorption layer thickness for BSA on the inner surface of glass capillary.

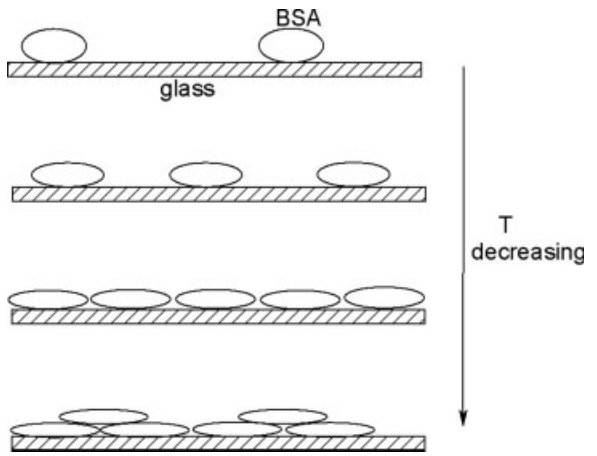

Figure 4 Model of the BSA adsorption onto inner surface of glass capillary during viscosity measuring.

exist between adsorbed and flowing solutes.[48,49] If the values of b_{eff} and A from 15 to 45°C were divided by those at 25°C, the ratios are 2.4 : 1 : 1.9 : 3.4 for b_{eff} and 2.8 : 1 : 0.5 : 0.05 for A at 15, 25, 35, and 45°C, respectively. It is found that when the temperature is lower than 25°C, the two ratios are close, indicating that the more BSA was adsorbed, the thicker the adsorbed layer was. It is reasonable to imagine that multilayer adsorption had happened below 25°C. However, when the temperature is higher than 25°C, the two ratios has opposite changes. To explain this irregularity, a model was proposed on the following assumptions:

1. The BSA molecule is regarded as an oblate ellipsoid[41] and it binds to the glass surface in a sideways-on conformation[50];
2. The BSA molecule prefers to adsorb as a monolayer, with some flattening because of unfolding or denaturizing.[51,52]

As Figure 4 shows, at high temperature, the adsorption amount was very low, the BSA molecules occupied fewer sites on the glass surface and the spaces between the absorbed molecules were large due to the static repulsive force. With the decreasing of temperature, more BSA molecules bound to the surface. Along with the further unfolding of molecules in bulk solution, the adsorbed molecules occupied more sites on the glass surface till the maximum monolayer adsorption coverage was reached. However, the planar extending resulted in the height decreasing in structure for adsorbed molecules, so the thickness of adsorption monolayer decreased on the contrary. After the first-layer adsorption amount reached the maximum, the second-layer adsorption began. From this time, the increasing of adsorption layer thickness began to keep consistent with the adsorption amount.

CONCLUSIONS

Adsorption of BSA has been investigated on inner wall of glass capillary by measuring the viscosity of BSA aqueous solution at different temperatures. The obtained viscosity data has been treated by an iterative fitting procedure and five parameters of K_m, C_a, k, A, and [η] were obtained simultaneously. The parameter K_m kept zero at all temperatures, showing that there's no aggregation happened in the solution. The change of [η] disclosed that the dimension of BSA molecule increased with the descending of temperature. The low value of A indicated that the adsorption of solute nearly did not lessen the actual concentration of solution. However, the actual radius of glass capillary was narrowed due to the solute adsorption. The effective adsorbed layer thickness was far larger than the actual dimensions of molecules even considering multiple-layer adsorption, indicating that there existed an exclusive layer because of the static repulsion between BSA molecules. A model has been proposed to explain the contrary change of adsorption amount and adsorbed layer thickness with temperature. It seemed that the conformational change may be the major reason for such abnormality.

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