realistic than the freely jointed chain model. These two features are first, an orientation correlation factor, related to preferred conformations, and second, a different scaling of bond length, due to next-neighbor correlations. Both seem to be supported by recent molecular dynamics calculations.

We looked for a more precise description of the neutron quasi-elastic spectrum when $q\sigma \simeq l$ by an exact solution of the mode equation. This requires heavier calculation and numerical solutions. Thus we restricted ourselves to small-ring polymers in the Rouse regime and chains of 50 atoms or less.

In the classical freely jointed chain model, the mode analysis leads to line shapes $S(q,\omega)$ that show a q-dependent deviation from Lorentzian shape. This deviation can be larger than that predicted by de Gennes in the asymptotic limit.

The conformational constraints taken into account in the NNDC model strongly reduce this deviation and for small rings hinder the appraisal of a polymer-like behavior.

Since our model takes into account the effective interatomic distances, it must fit experiments over the whole q range of interest by varying only two parameters i.e., the elementary frequency W and the correlation parameter γ. Then comparison with experiment would provide not only a severe check of the model but also an opportunity to measure γ and to get an absolute evaluation of the elementary frequency.

The γ value could be compared with conformational energies or Monte Carlo calculations.[23] This would lead to an interesting insight into the relation between static and dynamic rigidity.

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Stoichiometric Complexation of Human Serum Albumin with Strongly Acidic and Basic Polyelectrolytes

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ABSTRACT: Colloid titrations of human serum albumin (HSA) with poly(diallyldimethylammonium chloride) (PDDA) and potassium poly(vinyl alcohol sulfate) (KPVS) were carried out at different pHs. The mole numbers of quaternary ammonium groups in PDDA and sulfate groups in KPVS, which are bound to the acidic (carboxyl, mercapto, and phenolic OH) groups and the basic (amino, imidazolyl, and guanidyl) groups in HSA by salt linkages, respectively, were evaluated by the titration data in the pH range where the ionizable groups in HSA are dissociated completely, i.e., pH >12.5 for the acidic groups and pH <2.5 for the basic groups. The results obtained approximately agree with the contents of the acidic and basic groups in HSA, which are calculated on the basis of the amino acid sequence of HSA. Thus it is indicated that the complexation of HSA with PDDA and also with KPVS follows a stoichiometric relationship in the pH ranges above 12.5 and below 2.5, respectively.

Colloid titration is a useful method for obtaining information about the stoichiometry of the salt linkage formation between the ionizable groups in oppositely charged polyions.[1,2] Previously, we applied this titration technique to study the complexation of human carboxyhemoglobin (Hb) with strongly acidic and basic polyelectrolytes and reported that three kinds of basic groups in Hb stoichiometrically form salt linkages with potassium poly(vinyl alcohol sulfate) (KPVS).[3] Moreover, the acidic groups remaining in the Hb-KPVS complex are salt-linked with poly(diallyldimethylammonium chloride) (PDDA) to form a three-component PDDA-Hb-KPVS complex[4] which is insoluble in aqueous solvents and functions as a cyanide ion exchanger.[5] Thus it is also interesting to study extensively the complexation of protein with polyelectrolyte in connection with the preparation of other polyelectrolyte complexes which have functions as a biomedical material and an immobilized enzyme.

In the present study, human serum albumin (HSA) was chosen as the protein component in view of the available

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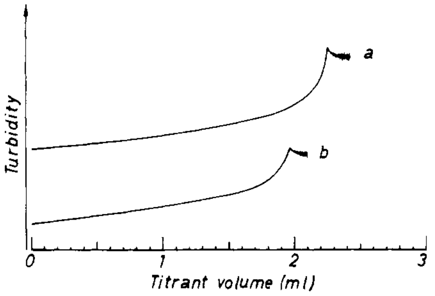

Figure 1. Turbidimetric titration curves of HSA with 0.00501 N PDDA (a) and 0.00497 N KPVS (b) solutions. The titrations with PDDA and KPVS were made for 50 mL of the sample solution containing 6.60 mg of HSA at pH 12.85 and 2.25, respectively. The titration curve was not represented by absolute turbidity because the change in turbidity was measured with an automatic recording titrator.

information on the amino acid composition[6,7] and the amino acid sequence.[8,9] The mole number of ionizable groups in the polyelectrolyte that are bound to the protein by salt linkages was evaluated at different pHs by means of colloid titration. The results obtained were compared with the ionizable group contents of HSA determined on the basis of the amino acid sequence and are discussed in terms of the stoichiometry of the complexation of HSA with PDDA and with KPVS.

Experimental Section

Materials. HSA was purchased from Sigma Chemical Co. The purity was confirmed by amino acid analysis and electrophoresis in polyacrylamide gel. KPVS and PDDA were the same samples used previously.[1-5] The equivalent weights are 166 for KPVS and 158 for PDDA. The number-average degree of polymerization for KPVS is about 1500. The intrinsic viscosity of PDDA is 1.67 dL/g, as determined in 1 N NaCl at 25 °C.

Colloid Titration. The titration was carried out at 25 ± 0.1 °C in a nitrogen atmosphere, using a Hirama automatic recording titrator. HSA (1–8 mg) was dissolved in 50 mL of distilled water, and the pH of the sample solution was adjusted with 0.1–1 N HCl or NaOH. The sample solution was titrated with 0.00497 N KPVS or 0.00501 N PDDA solution adjusted to the pH of the sample solution. The end point of the titration was indicated by measuring turbidity at 500 nm.

IR Spectroscopy. IR spectra for HSA and the complex were measured in a KBr disk with a Hitachi 250-50 IR spectrophotometer in order to obtain information about the salt linkage formations of HSA with PDDA and KPVS. The HSA samples were obtained by lyophilizing the acidic and alkaline solutions of HSA, which were adjusted to pH 2.25 and 12.85, respectively. The complexes were prepared by titrating HSA with the polyelectrolyte solution until the end point (at pH 12.85 for the HSA–PDDA complex and at pH 2.25 for the HSA–KPVS complex), separating by centrifugation, washing with 0.1 N HCl (for the HSA–PDDA complex) or 0.1 N NaOH (for the HSA–KPVS complex), and drying at 50 °C for 1 day under reduced pressure.

Results and Discussion

Typical turbidimetric titration curves of HSA with PDDA and KPVS are presented in Figure 1. A precipitate or turbidity develops when PDDA is added to the alkaline solution of HSA. The same result is obtained by titrating the acidic solution of HSA with KPVS. Thus it is found that PDDA and KPVS ions form a polyion complex with HSA in the basic and acidic regions, respectively.

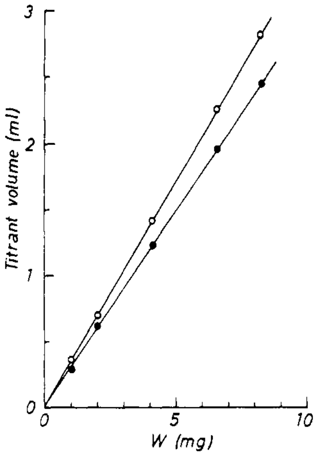

Figure 2. Linear relationships between titrant volume and HSA weight: (○) 0.00501 N PDDA titrant, pH 12.85; (●) 0.00497 N KPVS titrant, pH 2.25. *W* is in grams of HSA in 50 mL of sample solution.

The complexation of HSA with both polyions was examined by comparing the IR spectra of HSA with those of the complexes. The absorption at 1720 cm^{-1} (shoulder), which is assigned to the carboxyl groups in HSA, is observed in the spectrum for the lyophilized sample of the acidic solution of HSA. In the case of the HSA–PDDA complex, however, the absorption at 1720 cm^{-1} was completely extinguished even when the complex was washed satisfactorily with 0.1 N HCl. This indicates salt linkage formation between the quaternary ammonium ions in PDDA and the carboxyl groups in HSA because it has been established that carboxyl groups free of salt linkages with PDDA ions are detectable by means of the IR absorption band at 1720 cm^{-1} (see ref 4). Salt linkage formation of the sulfate groups in KPVS with the amino groups in HSA is supported by the fact that the absorption at 2650 cm^{-1} (shoulder), assigned to the ammonium salt of the primary amino groups in HSA, is observed in the spectrum for the HSA–KPVS complex prepared by leaching with 0.1 N NaOH, whereas this absorption is not detectable for the lyophilized sample of the alkaline solution of HSA. Consequently, it becomes apparent that the complexation is due to salt linkage formations of the acidic and basic groups (at least carboxyl and amino groups) in HSA with PDDA and KPVS ions, respectively.

Figure 2 shows the dependence of the titrant volumes[10] of PDDA and KPVS on the weight of HSA. The titration was made at the pH range where the ionizable groups in HSA are dissociated completely. It is found that the plots of the titrant volume against the sample weight are expressed by straight lines passing through the origin. This finding is similar to the results obtained for Hb[3,4] and could mean that the ionizable groups in HSA are quantitatively salt-linked with PDDA and KPVS ions to form the corresponding complex.

In order to obtain information about the stoichiometry of the salt linkage formation, the mole numbers (M_s) of the quaternary ammonium ions in PDDA and the sulfate groups in KPVS bound by salt linkages to the ionizable groups of 1 g of HSA were estimated from the titrant volumes at different pHs, and the colloid titration curve was constructed by plotting M_s against pH (figures 3 and 4). The result obtained with PDDA titrant shows a gradual increase of M_s with increasing pH, followed by a constant value in the pH range above 12.5. In the case of KPVS titrant, on the other hand, the M_s value rapidly increases with decreasing pH and reaches a constant value in the pH range below 2.5. As was mentioned previously,[1-5] it is not necessary to take into account the pH change in the normality of both titrants because the dissociation of the quaternary ammonium ions in PDDA and of the sulfate groups in KPVS is unaltered with pH. Therefore the change in M_s with pH can be related to the dissociation change of the ionizable groups in HSA,[11] and the M_s value,

Table I

Comparison of the Ionizable Group Contents for HSA Determined on the Basis of the Amino Acid Sequence with the M_s Values Evaluated by Colloid Titration

ionizable group	amino acid residue with ionizable groups	number	content,[a] mmol/g	M_s,[b] mmol/g
		Acidic Groups		
carboxyl	aspartyl	36	1.76	1.71 ± 0.06
	glutamyl	61		
	C terminal	1		
phenolic OH	tyrosyl	18		
mercapto	cysteinyl	1[c]		
		Basic Groups		
amino	lysyl	59	1.51	1.48 ± 0.03
	N terminal	1		
imidazolyl	histidyl	16		
guanidyl	arginyl	24		

[a] Calculated by dividing the numbers of the total acidic and basic groups by the molecular weight (66 436) of HSA, which was determined on the basis of the amino acid sequence presented in ref 8. [b] Evaluated by the colloid titration curves (Figures 3 and 4) at pH ranges above 12.5 for the acidic groups and below 2.5 for the basic groups. [c] One mercapto group was adopted in the calculation of the acidic group content because it has been found that 34 cysteinyl residues are bound to each other by disulfide linkages (see ref 12).

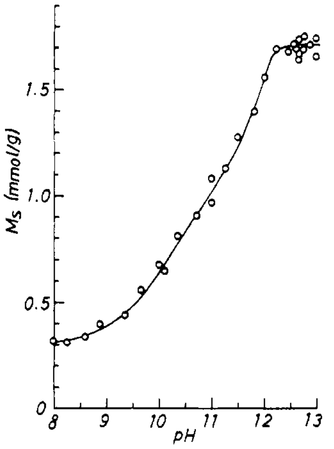

Figure 3. Colloid titration curve of HSA with 0.00501 N PDDA solution.

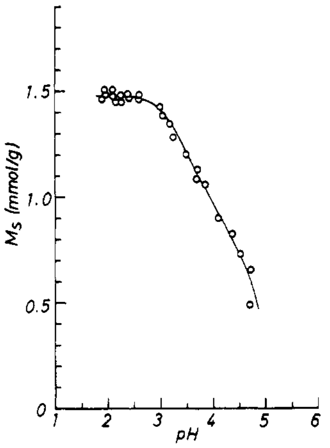

Figure 4. Colloid titration curve of HSA with 0.00497 N KPVS solution.

which is unchanged with pH, is regarded as an intrinsic value under the condition where the ionizable groups in HSA are dissociated completely.

Recently, the amino acid sequence of HSA has been independently published by Meloun et al.[8] and Behrens et al.;[9] thus the contents (in mol/g) of the acidic and basic groups in HSA can be calculated from the number of ionizable groups and the molecular weight. In this study, the calculation was made on the basis of the data presented by Meloun and co-workers (see ref 12). The results are listed in Table I, together with the number of each amino acid residue having an ionizable group. Saber et al.[13] have reported that 17 pairs of a total of 35 cysteinyl residues in HSA are bound to each other by disulfide linkages and only one residue exists in the free form. Taking into account this fact, we adopt one mercapto group in the calculation of the content of the total acidic groups in HSA, since we can assume that the cleavage of the disulfide bonds could not take place during the course of the complexation between HSA and PDDA.

In Table I, the intrinsic values of M_s obtained from Figures 3 and 4 are included to compare with the calculated results of the ionizable group contents. The agreement between the observed values of M_s and the calculated contents of ionizable groups is found to be within the usual limit of the variation (ca. 1–5%) of the colloid titration. Therefore it is indicated that salt linkage formations of the acidic and basic groups in HSA with PDDA and KPVS ions follow a stoichiometric relationship if the complexation is made under the condition where the acidic or basic groups are dissociated completely. This is supported by the fact that the sulfur contents for the HSA–PDDA complex (0.48 mmol/g) and the HSA–KPVS complex (1.74 mmol/g) are in agreement with those (0.51 mmol/g for the HSA–PDDA complex; 1.78 mmol/g for the HSA–KPVS complex) calculated by assuming a stoichiometric complexation between HSA and PDDA or KPVS. Needless to say, the sequence of these events reveals that the disulfide linkages in HSA are not severed during the course of the complexation.

On the basis of the results obtained here and reported previously,[3,4] it may be concluded that the stoichiometric relationship for polyelectrolyte complex formation appears not only in the case of oppositely charged synthetic polyions but also in the case of the polyion and protein if the complexation is made under the condition where the acidic or basic groups in the protein are dissociated completely.

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(10) The titrant volume was confirmed by an indirect titration method (see ref 1): An excess of KPVS (or PDDA) was added to the HSA sample solution to precipitate the HSA-KPVS (or PDDA) complex and then the excess of KPVS (or PDDA) in the supernatant from which the precipitated complex was removed was back-titrated with PDDA (or KPVS) titrant.

(11) When the colloid titration curve for weak polyelectrolyte was obtained by using a strongly acidic or basic polyelectrolyte as a titrant, the degree of dissociation (α) for the weak polyelectrolyte can be estimated from the M_s vs. pH curve by $\alpha = (1/M_s)/(1/M_s^0)$, where M_s^0 represents the value of M_s at $\alpha = 1$ (see ref 1 and 2). For polyampholytes, however, the change in M_s with pH in the range $\alpha < 1$ deviates from this relationship because of intramolecular interaction between the anionic and cationic groups. Thus, it is possible to analyze quantitatively the titration data for HSA only under the condition where the M_s value is independent of pH, i.e., $\alpha = 1$.

(12) This is because the result by Behrens et al. involves indefinite amino acid residues: Glx, residues 266, 268, 382, and 397; Asx, residues 267 and 269. However, the ionizable group contents (1.70 mmol/g for the total acidic groups and 1.51 mmol/g for the total basic groups), calculated by assuming that Glx and Asx are equal to Glu and Asp (acid form) or Gln and Asn (amide form), approximately agree with those shown in Table I.

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Diffusion Model for Volume Recovery in Glasses[†]

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ABSTRACT: Glasses are known to undergo spontaneous densification during isothermal annealing. This volume recovery process can be viewed as collapse of free volume. We have modeled this change in free volume during annealing as a vacancy diffusion process, where the diffusion constant depends on the local free volume (as defined by the Simha-Somcynsky theory) through the Doolittle equation. Good agreement was obtained with estimates of experimental volume recovery results for poly(vinyl acetate). Since volume recovery is sample-size independent, the characteristic length for diffusion cannot be identified with macroscopic dimensions. Several possibilities exist that can reconcile a diffusion picture with the known sample-size independence of the volume recovery process. These possibilities include internal annihilation of vacancies, density fluctuations, and a coupling of diffusive and uniform lattice motions.

Introduction

It is well-known that glasses can undergo spontaneous changes in density during annealing below their glass transition temperature. As shown by Kovacs,[1] this volume recovery process exhibits both nonlinear and thermal history effects. Kovacs and co-workers[2] have developed a phenomenological model that reproduces most of the experimental observations. The essential feature of their model is a distribution of relaxation times, each of which depends upon the instantaneous deviation from the equilibrium volume. In the present investigation, we propose a microscopic interpretation for the volume recovery process. Specifically, we consider the kinetics of this process to be controlled by diffusion of free volume. The essential features of our model are that the free volume is localized in the form of vacancies, and these vacancies diffuse to (or from) a boundary where they are annihilated (or created).

Recently, Robertson developed a molecular model for the relaxation of internal energy[3a] and volume[3b] in polymer glasses. In this model, Robertson considers the detailed conformational changes of the backbone chains which are coupled to discrete local free volume states. These coupled motions were analyzed as a stochastic process and the results agree with many of the features observed in volume recovery experiments. In our theory we also allow the local free volume to change. Whereas Robertson considered the local free volume to vary by virture of *chain conformational changes*, we allow the local free volume to change by a *free volume diffusion* process. In essence, we replace Robertson's stochastic equation, which contains the structural details of the chains, by a diffusion equation. Thus in our approach we suppress the structural details considered in the Robertson model.

The idea of free volume diffusion has been applied to glasses before by Hirai and Eyring,[4] who assumed that vacancies can be annihilated by conversion to phonons and vice versa. The entire diffusion and annihilation process was modeled as an activated rate process with an unspecific activation energy. As pointed out by Kovacs,[1] difficulties are encountered when one compares this theory with experiment.

In our approach we describe the spatial free volume distribution by a nonlinear diffusion equation. The only adjustable parameter in our theory is related to a length scale for diffusion. The introduction of this length scale is somewhat arbitrary since there is no obvious molecular interpretation for it. We will discuss some possible interpretations later in this paper.

Free Volume Diffusion

Although many different quantitative definitions of free volume have been proposed, the qualitative concept of free volume has been useful in explaining many properties of polymer liquids and glasses.[5] In this work we will define the free volume fraction as the fraction of empty lattice sites or holes in the theory of Simha and Somcynsky[6] for equilibrium polymer liquids. We have recently generalized

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