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A light scattering investigation of the sodium dodecyl sulfate–lysozyme system

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Dynamic and static light scattering measurements have been made on lysozyme solutions at 25 °C in 0.1 M phosphate buffer, pH 6.88, in the presence of sodium dodecyl sulfate (SDS). The concentration of SDS has been varied over a wide range (0–0.2 g/ml). It is found that under these conditions both SDS micelles and lysozyme(Lys)–SDS complexes coexist. Addition of lysozyme at constant SDS concentration leads to formation of more lysozyme–SDS complex. The translational diffusion coefficient of the complex increases with SDS concentration. It is also found that two lysozyme molecules are associated in the protein–surfactant complex. © 1996 American Institute of Physics. [S0021-9606(96)51219-2]

I. INTRODUCTION

The binding of SDS to lysozyme has been the subject of a number of studies during the last 20 years; see for example, Refs. 1 and 2. It was shown that, for very low concentrations of SDS, the initial highly cooperative binding is mainly due to electrostatic interaction leading to the precipitation of the complex when the complex formed is neutral, i.e. with seven SDS molecules per lysozyme molecule (see Experiment). Then, if the concentration of SDS is increased, the complex redissolves and further binding occurs, mostly driven by hydrophobic interactions. A binding ratio of at least 70 mol/mol is found for SDS concentrations above 10^{-3} g cm $^{-3}$ (pH 5.8, 0.1 M NaCl).³ At high SDS concentrations SDS micelles and the SDS–protein complexes coexist.⁴ The structure of this complex is at present not well understood. Jones and Manley⁵ assume on the basis of thermodynamic data that the protein unfolds during the second binding process. But Murata *et al.*¹ argue that lysozyme is too small and too rigid to unfold and use this interpretation to explain the observed differences in behavior between the SDS–lysozyme and SDS–BSA systems. Circular dichroism measurements⁶ have shown, however, that the conformation of lysozyme in the complex is close to that of a random coil. Furthermore, SANS studies of the analogous SDS–BSA complex⁷ are in good agreement with the necklace model in which micelle-like SDS clusters are randomly distributed along the unfolded polypeptide chain. The degree of unfolding and the number of SDS micellelike structures bound are determined by the amount of SDS in the solution at saturation of the chain. An earlier static light scattering study⁸ reported that, in the case of SDS–lysozyme, several molecules of protein may be involved in the structure of the complex. In this work, we have studied the SDS–lysozyme complex using static and dynamic light scattering. The combination of these two techniques provides information on the size and the number of protein molecules in the complex with SDS.

II. EXPERIMENT

A. Sample preparation

Egg-white lysozyme (Lys) (Sigma, ref. L6876, batch 111H7010) is a small globular protein. It contains 129 resi-

dues and 4 disulfide bridges between residues: 6–127, 30–115, 64–80, and 76–94. Its molar mass is close to 14350 g mol $^{-1}$, and its isoelectric pH is high: 11.35 in 0.1 M salt.⁹ All the solutions were prepared in phosphate buffer, pH 6.88, 0.1 M (from Merck), and NaN $_3$ (200 ppm) was added to avoid bacterial growth. In these conditions, the protein has an excess charge of seven positive charges¹⁰ and there is an equilibrium between the monomeric and dimeric forms.⁹ Sodium dodecyl sulphate (SDS), especially pure grade, was obtained from BDH. SDS and Lys mixtures were prepared by dialysing, at 25 °C, 5 ml of Lys solution against a large volume of SDS solution, using Spectra Por cellulose ester membranes with a cut-off mass of 5000 g mol $^{-1}$. Equilibrium was reached in four days. Concentrations, of pure lysozyme in phosphate buffer were determined by measurements at 281.5 nm using an extinction coefficient of 25.9 g $^{-1}$ 1 cm $^{-1}$.⁹ Calibration curves in the presence of SDS were made to allow determination of Lys concentrations after dialysis. The samples were filtered through 0.1 μ m pore size Anotop filters into glass ampoules that had been soaked in chromic acid and rinsed with distilled water and filtered ethanol.

B. Static and dynamic light scattering measurements

Measurements were made in the same apparatus using a frequency-stabilized Coherent Innova Ar-ion laser operating at 488 nm with changeable output power. The light was vertically polarized with a Glan–Thompson polarizer, extinction better than 10^{-6} . A typical laser power was 500 mW. The scattered light passed through a Glan–Thompson polarizer with an extinction coefficient better than 10^{-7} whose orientation was adjusted to give the maximum intensity for a dilute solution of a high molecular weight polystyrene in ethyl acetate. The scattering cells (10 ml sealed cylindrical ampoules) were immersed in a large-diameter thermostated bath of index-matching liquid (*trans*-decalin). The detector optics employed a 4 μ m diam monomodal fiber coupled to an ITT FW130 photomultiplier the output of which was digitized by an ALV amplifier-discriminator. The polarized (I_{vv}) DLS measurements, in the self-beating (homodyne) mode, were performed using as the signal analyzer an ALV-5000 digital

multiple- τ correlator, Langen GmbH, with 288 exponentially spaced channels. It has a minimum real time sampling time of 0.2 μ s and a maximum of ≈ 100 s. The intensity-intensity autocorrelation function, $g_2(t)$, was measured. The measurements were made at different angles. The temperature was controlled to within ± 0.02 °C. In the present study the temperature was 25 °C. Toluene has been used as a reference in static measurements with a value of 3.1×10^{-5} cm⁻¹ for the Rayleigh ratio, R (Ref. 11). $\partial n/\partial c$ measurements were also performed on the same solutions and at the same wavelength using a specially constructed instrument having Rayleigh interference optics. The value 0.139 ml/g lysozyme at 25 °C was determined over the concentration range 7 to 20% w/w SDS.

C. Data analysis

The dynamic light scattering data were analysed by non-linear regression procedures. $g_2(t)$ is related to the normalized field autocorrelation function, $g_1(t)$, through the Siegert relation:

$$g_2(t) - 1 = \beta \cdot |g_1(t)|^2,$$

where β is a nonideality factor ($0 < \beta \leq 1$). β was determined using a standard solution (a dilute solution of polystyrene in ethyl acetate). The value 0.957 ± 0.03 was obtained $g_1(t)$ can be written as the Laplace transform of the distribution of relaxation rates, $G(\Gamma)$:

$$g_1(t) = \int_0^\infty G(\Gamma) \exp(-\Gamma \cdot t) d\Gamma,$$

where Γ is the relaxation rate. For relaxation times, τ , $g_1(t)$ will be expressed as

$$g_1(t) = \int_0^\infty A(\tau) \exp(-t/\tau) d\tau,$$

where $\tau A(\tau) \equiv \Gamma G(\Gamma)$. $\tau A(\tau)$ was obtained by regularized inverse Laplace transform (RILT) of the experimental data using a constrained regularization calculation algorithm called REPES.¹²⁻¹⁴ The translational mutual diffusion coefficient, D_m , was calculated from the average relaxation rate, Γ^- , using

$$D_m = \bar{\Gamma}/q^2 \quad (q \rightarrow 0),$$

where $q = 4\pi n_s/\lambda_0 \sin(\theta/2)$, with n_s the refractive index of the solution, λ_0 the wavelength of the radiation in vacuum and θ the scattering angle. At $\theta = 90^\circ$, the condition $q \rightarrow 0$, is fulfilled due to the small size of the particles in the solution. In the limit of small concentrations, we have¹⁵

$$D_m(c) = D_0(1 + k_D c) \quad c \rightarrow 0,$$

where D_0 is the translational diffusion coefficient at zero concentration and k_D (cm³ g⁻¹) a constant and c (g cm⁻³) the particle concentration. D_0 is related to the hydrodynamic radius, R_h , through the Stokes-Einstein relation

$$D_0 = \frac{k_B T}{6\pi\eta R_h},$$

where k_B is the Boltzmann constant, T the absolute temperature and η the viscosity of the solvent. k_D can be expressed as¹⁶

$$k_D = 2MA_2 - k_f - 2v_2,$$

where A_2 (mol cm³ g⁻²) is the second virial coefficient, M (g mol⁻¹) the molar mass, k_f (cm³ g⁻¹) the coefficient of the linear term in the development of the friction factor and v_2 (cm³ g⁻¹) the partial specific volume of the particle.

The static light scattering data were analyzed using the classical Zimm equation:¹⁷

$$\frac{K_Z c}{R(\theta)} = \frac{1}{M} + 2A_2 c \quad (c \rightarrow 0, \theta \rightarrow 0),$$

where K_Z (mol cm³ g⁻²) is a constant depending on the apparatus and on the particles analyzed, $R(\theta \rightarrow 0)$ is the Rayleigh ratio at θ tending to zero (once again, due to the small size of the particles, the condition is already achieved at $\theta = 90^\circ$). The general expression for K_Z is

$$K_Z = \frac{[2\pi n_s (\partial n/\partial c)]^2}{N_a \lambda_0^4},$$

where $\partial n/\partial c$ (cm³ g⁻¹) is the refractive index increment due to the introduction of particles at concentration c in the solvent and N_a the Avogadro number.

The Rayleigh ratio is defined as

$$R_\theta = R_{\text{ref}} \frac{(I_s(\theta) - I_0(\theta)) \sin(\theta) / V_s(90^\circ)}{I_{\text{ref}}(90^\circ) / V_{\text{ref}}(90^\circ)},$$

where $I_s(\theta)$, $I_0(\theta)$, are the intensities scattered at θ by the solution and the solvent respectively, $I_{\text{ref}}(90^\circ)$, the intensity scattered by the reference at 90° , R_{ref} is the Rayleigh ratio of the reference, $V_s(90^\circ)$ and $V_{\text{ref}}(90^\circ)$ are the scattering volumes of the solution and the reference at 90° respectively. If the scattered light is collected through a pin hole (at the center of the beam), then¹⁸

$$V_s(\theta) = (n/n_{\text{ref}})^2 V_{\text{ref}}(\theta),$$

where n_{ref} is the refractive index of the reference and n that of the solution. From the value of A_2 , we can calculate the radius, R_{A_2} , of the sphere having the same excluded volume:

$$A_2 = \frac{N_a 16\pi/3 R_{A_2}^3}{M^2}.$$

III. RESULTS AND DISCUSSION

In Fig. 1(a), we note that the classical linear behavior of $D_m(c)$ is not followed. This clearly shows the dissociation of the dimer of Lys upon dilution. In order to roughly estimate a dissociation constant, K_d , we can fit the experimental data to a simple equilibrium model, using the fact that we measure a z -averaged translational diffusion coefficient of a mixture of monomers and dimers and that the hydrodynamic radius of a dimer is 4/3 larger than that of a monomer:¹⁹

$$\begin{aligned} &K_d \\ &D \leftrightarrow M + M, \end{aligned}$$

FIG. 1. (a) The mutual translational diffusion coefficient as a function of the lysozyme concentration in phosphate buffer (pH 6.88, 0.1 M). The solid line corresponds to the fit with the model described in the text, using the dissociation constant K_d . (b) $K_z c / R(\theta \rightarrow 0)$ as a function of c for lysozyme solutions in phosphate buffer (pH 6.88, 0.1 M). The solid line corresponds to the fit with the model described in the text, using the dissociation constant K_d .

D and M represent the dimer and the monomer, respectively, and $[D]$, $[M]$ are the molar concentrations. The total molar concentration expressed as the monomer is c_0 . We have the following relations:

$$c_0 = [M] + 2[D],$$

$$K_d = \frac{[M]^2}{[D]},$$

leading to

$$[M] = \frac{1}{4}(-K_d + \sqrt{K_d^2 + 8K_d c_0}),$$

$$[D] = \frac{1}{8}(K_d + 4c_0 - \sqrt{K_d^2 + 8K_d c_0}).$$

The z -averaged mutual diffusion coefficient can then be expressed as

$$D_m = D_0 \frac{[M] + 3[D]}{[M] + 4[D]} (1 + k_D c),$$

where D_0 is the translational diffusion coefficient of the monomer at zero concentration. We obtain $pK_d \approx 4$, with $R_H = 1.6$ nm. This value of R_H is close to that given by Colvin.²⁰ Strictly speaking, K_d is ill defined in the range of concentration used but gives valid information within an order of magnitude. Figure 1(b) shows data from static light scattering experiments on the same solutions. The dissociation of the dimer is also noticeable. Once again, using the same dissociation model and considering that the weight average molar mass and a z -averaged second virial coefficient are measured in the static light scattering experiment, the second virial coefficient of the monomer, A_{20} and K_d can be extracted from the data. Assuming that both the monomer and the dimer have the same excluded volume expressed in cm^3/g , we then have

$$\frac{K_z c}{R(\theta)} = \frac{c_0}{[M] + 4[D]} \left(\frac{1}{M_0} + 2A_{20}c \right),$$

where M_0 is the molar mass of the monomer. We obtain $pK_d \approx 3$ and $R_{A2} = 1.6$ nm. The static and dynamic light scattering experiments are thus consistent with the Lys monomer having a radius close to 1.6 nm and pK_d lying between 3 and 4.

The critical micelle concentration (cmc) has been determined by surface tension measurements as described in Ref. 21 (typical data shown in Fig. 2) and $\text{cmc} = 1.58$ mM. This value is in very good agreement with that given in the literature²² in 0.1 M salt at 25 °C and is negligible comparing to the concentrations of SDS used in this study. Thus the cmc can be neglected in the static light scattering concentra-

FIG. 2. The surface tension of an SDS solution as a function of SDS concentration at 25 °C. The cmc is 1.58 mM in the phosphate buffer solution (pH 6.88, 0.1 M).

(a)

(b)

FIG. 3. (a) The mutual translational diffusion coefficient as a function of the SDS concentration in phosphate buffer (pH 6.88, 0.1 M). The solid line corresponds to the fit with the model described in the text. (b) $K_z c/R(\theta \rightarrow 0)$ as a function of c for SDS solutions in phosphate buffer (pH 6.88, 0.1 M). The solid line corresponds to a fit with the model described in the text.

tions for SDS solutions [Fig. 3(b)]. Figure 3(a) shows the evolution of D_m as a function of c for SDS solutions. We find $R_h = 2.5$ nm and $k_D = 15.6$ cm³ g⁻¹, again in good agreement with literature values.²² From Fig. 3(b), we find $M_w = 25\,430$ g mol⁻¹, leading to an aggregation number of 88 molecules of SDS per micelle and $A_2 = 1.05 \times 10^{-3}$ mol cm³ g⁻², in good agreement with the literature.²² Using the values of A_2 and M_w , we find $R_{A_2} = 4.1$ nm. Comparing this value with R_h , we conclude that the electrostatic interactions are not totally screened in the solution. Using R_h to estimate v_2 , we find an overestimated value of 1.5 cm³ g⁻¹, which gives a figure for k_f close to 35 cm³ g⁻¹. We have experimentally determined the value $v_2 = 0.888$ ml g⁻¹ at 25°C.

For Lys and SDS concentrations higher than 0.003 and

FIG. 4. Relaxation time distribution of a lysozyme 0.01 g cm⁻³ and SDS 0.13 g cm⁻³. A_s is the slow mode contribution to the distribution.

0.03 g cm⁻³ respectively, we can readily resolve two relaxation modes (Fig. 4). Both are q^2 dependent, showing two distinct diffusional species in the solution. We call A_s the slow mode contribution to the time relaxation distribution. In Fig. 5, we see that the fast mode corresponds to free SDS micelles and the slow mode to the complex. Moreover, for SDS concentrations higher than 0.03 g cm⁻³ and when the two peaks can be resolved, we note that

$$A_s \approx \frac{I_{\text{Lys+SDS}} - I_{\text{SDS}} - 2I_0}{I_{\text{Lys+SDS}} - I_0},$$

where $I_{\text{Lys+SDS}}$, I_{SDS} , and I_0 are the scattered light intensities from the Lys+SDS solution, from the pure SDS solution with which it is in equilibrium, and from the phosphate

FIG. 5. The mutual translational diffusion coefficient as a function of SDS concentration for a lysozyme concentration of 0.01 g cm⁻³. (●) for the slow mode. (O) for the fast mode. The solid line corresponds to pure solutions of SDS.

(a)

(b)

FIG. 6. (a) The mutual translational diffusion coefficient of (●) free SDS micelles and (○) the complex as a function of the lysozyme concentration for SDS concentration of 0.13 g cm⁻³. (b) The contribution of the complex to the relaxation time distribution as a function of the lysozyme concentration for SDS concentration of 0.13 g cm⁻³.

buffer solvent. Under our conditions, I_0 is negligible. The pure SDS solution may then be regarded as the solvent for the Lys+SDS solution with which it is in equilibrium.

From Fig. 6(a), we can see that, over the range used, the Lys concentration has no influence on the value of the mutual translational diffusion coefficient, but that the value of A_s is an increasing function of the Lys concentration [Fig. 6(b)]. We thus conclude that, for the complex, D_m is very close to D_0 . Addition of Lys at constant SDS chemical potential thus leads to the formation of more complex molecules without a significant change in their size.

Figure 7 shows the evolution of the hydrodynamic radius of the Lys-SDS complex, using the viscosity of the pure SDS solution in the Stokes-Einstein equation as an approximation. We note a small increase its size with increase of

FIG. 7. The hydrodynamic radius of the complex as a function of SDS concentration.

SDS concentration. For comparison, R_h of the SDS micelle, the monomer and the dimer have been added in the figure. Even if the increase is considered significant, it is not large which is probably due to the four disulfide bridges retaining the protein structure in a compact form. This seems to support the interpretation of Murata *et al.*¹ Let us call α the mass fraction of Lys in the complex. Then the mass concentration of complex, c_C , is related to the mass concentration of Lys, c_{Lys} by: $\alpha c_C = c_{Lys}$. We have also $\alpha M_C = M_{Lys}$, where M_C and M_{Lys} are the molar mass of the complex and the molar mass of Lys in the complex (gram of Lys per mole of complex), respectively. We will now consider the pure SDS solution with which the mixture Lys+SDS is in equilibrium, as the solvent. Both $\partial n/\partial c$ and the excess scattered intensity are then expressed in relation to the latter. We have also the

FIG. 8. Static light scattering data at different SDS concentrations. See text for details.

following relation: $\partial n / \partial c_C = \alpha \partial n / \partial c_{\text{Lys}}$. The Zimm expression for the complex is

$$\frac{K'_Z (\partial n / \partial c_C)^2 c_C}{R(\theta)} = \frac{1}{M_C} + 2A_{2c} c_C \quad (c_C \rightarrow 0, \theta \rightarrow 0),$$

where $K'_Z = K_Z / (\partial n / \partial c_C)^2$ and A_{2c} is the second virial coefficient of the complex. If we use the relations introduced before, we have

$$\frac{K'_Z (\partial n / \partial c_{\text{Lys}})^2 c_{\text{Lys}}}{R(\theta)} = \frac{1}{M_{\text{Lys}}} + \frac{2A_{2c}}{\alpha^2} c_{\text{Lys}} \quad (c_{\text{Lys}} \rightarrow 0, \theta \rightarrow 0).$$

So using the Zimm approach and comparing only to the Lys concentration, we can extract the molar mass of Lys in the complex. Figure 8 shows that whatever the concentration of SDS (in the range where pure SDS solution can be regarded as the solvent), the complex contains two molecules of Lys. Jones and Manley,⁸ using the same method, found that the Lys-SDS complex at 25 °C, pH 6, in water and in 0.15 M NaCl, contains three monomers of Lys. The state of aggregation of Lys inside the complex thus seems to be very sensitive to the charge of the protein and to the electrostatic interactions in the solution.

IV. CONCLUSIONS

We show that the size of the Lys-SDS complex is only a function of the SDS concentration with which the solution is in equilibrium. Note that the ratio $[\text{SDS}]/[\text{Lys}]$ is not a relevant parameter to describe the system over the concentration ranges used. Even if the increase in R_h is significant, it is not large due to the role of the four disulfide bridges in maintaining the essential features of the structure.

We find that the complex contains two lysozyme molecules. Jones and Manley⁸ on the other hand found three molecules but at a different pH and ionic strength condition (pH 6, 0.15 M NaCl). A more complete study is being undertaken in our laboratory to determine the relationship between the state of aggregation of the protein in the complex, the pH and the ionic strength. Binding curves are also being determined to estimate the amount of SDS bound to the protein. But the precise structure of the complex appears very difficult to elucidate. Small angle neutron scattering (SANS) could be used, where the protein is index-matched using D₂O solution,⁷ but the presence of a large excess of free SDS

micelles makes the calculation of the bound SDS structure factor uncertain. This problem seems to have been ignored by Chen and Teixeira⁷ when studying LDS-BSA complex, although Chen noted the presence of free SDS micelles when using dynamic light scattering. In theory, the SDS could be index-matched using a solution containing 13% w/w D₂O, but here the large proportion of H₂O (87%) leads to a large incoherent scattering and a poor signal-noise ratio and prevents an accurate calculation of the Lys structure factor in the complex. An expensive solution would be to use deuterated SDS in D₂O instead.

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