Structure and Fractal Dimension of Protein-Detergent Complexes

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Small-angle neutron-scattering experiments were made on bovine serum albumin (BSA)–lithium dodecyl sulfate (LDS) complexes in buffer solutions. As increasing amounts of LDS are added, the scattering data indicate that BSA molecules are successively transformed into random coil conformations with LDS forming globular micelles randomly decorating the polypeptide backbones. A cross-section formula is developed which successfully fits small-angle neutron-scattering spectra over the entire Q range. The fractal dimension, the micellar size, and the extent of the denatured protein are simultaneously extracted.

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It has been suggested from electron-spin relaxation measurements on low-spin iron in several proteins and from an independent analysis of their x-ray crystallographic data,[1,2] that the spatial distribution of the amino-acid residues in these globular proteins has a chain fractal dimension in the vicinity of 1.67. This dimension relates the end-to-end length of the chain to the number of the amino-acid residues. Following this idea, it is interesting to speculate what would be the topological character of a denatured protein when it is transformed into a random coil. Since a precise structural study of the random coil is difficult on an atomic scale, a possible way of visualizing the global structure of a denatured protein is to introduce, in a random way, a local probe, such as small micelles, into the polypeptide backbone. It is well known that detergent sodium dodecyl sulphate (SDS) induces denaturation in many globular proteins. The molecular weight of the denatured protein can be determined by measurement of its electrophoretic mobility in a suitable polymer gel. In this connection, it is important to elucidate the structure of the protein-detergent complex which makes this technique workable.[3,4] It was observed by Shirahama, Tsujii, and Takagi[5] that the free electrophoretic mobilities of the saturated polypeptide-SDS complexes are virtually constant regardless of the molecular weight of the polypeptides, and that the mobility was compatible with that of the SDS micelle. This can be reasonably explained by our postulating that micellelike clusters of bound SDS molecules are arranged statistically along the polypeptide chain and that the complex behaves like a flexible polyelectrolyte.[6] This picture of the SDS-protein complex was also independently suggested from a quasielastic light-scattering experiment of Tanner *et al.*[7]

In this Letter we undertake a series of SANS experiments on lithium dodecyl sulfate–bovine serum albumin (LDS-BSA) complexes. The complexes are made by the addition of increasing amounts of LDS until the saturation binding is reached. We verify that the LDS-BSA complex indeed consists of a string of micelles randomly distributed along the polypeptide chain, and can therefore be visualized pictorially as a "pearl necklace." In a D_2O solvent, the major contrast is between D_2O and the hydrophobic hydrocarbon core of the micelles, and the SANS sees effectively the pearls and not the backbones. We found that we can describe the randomly distributed pearl necklaces in solution as an open structure of fractal dimension D. A schematic picture for this situation is shown in Fig. 1. Figure 1(a) depicts a native protein

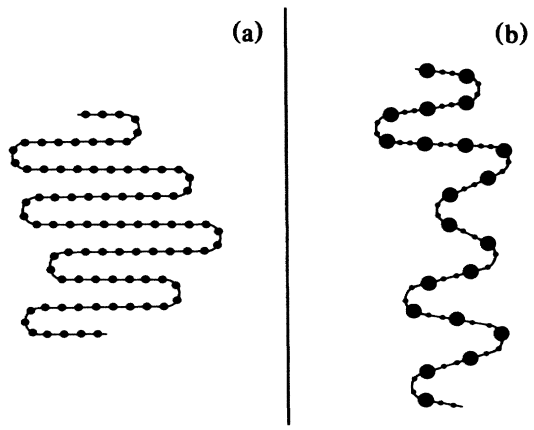

FIG. 1. Schematic representation of the protein configuration. (a) Native protein; (b) denatured protein. Small dots: amino-acid residues. Black dots: micelles.

configuration with small dots representing each amino-acid residue. Figure 1(b) depicts a denatured protein decorated randomly with micelles (large black dots). In a solution, we imagine a large number of such chains suspended in random orientations.

SANS experiments were carried out at the PACE spectrometer of Laboratoire Léon Brillouin. Neutron wavelengths $\lambda = 4.3$ and 8.1 Å were used in the measurements. The Q ranges [$Q = 4\pi\sin(\theta/2)/\lambda$, θ the scattering angle] covered were from 0.0053 to 0.056 $Å^{-1}$ and from 0.023 to 0.24 $Å^{-1}$ in two separate measurements. The sample cells were flat quartz cells of path length 2 mm and 1-mm-thick windows. The protein stock solution was prepared by solution of 1 wt.% of BSA in D_2O buffer solution. The buffer solution had a pH = 5.2 (nearly the isoelectric pH of BSA) and contained 500 mM of NaCl. The BSA sample was of high-purity form supplied by the European Molecular Biology Laboratory at Grenoble.[8] The LDS sample was the highest purity form supplied by Sigma Chemical Company. The samples were prepared by addition of 1, 2, and 3 wt.% of LDS into the protein stock solution. We shall denote by 1/1, 1/2, and 1/3 the samples containing respectively 1, 2, and 3 g of LDS in 100 g of protein stock solution. The temperature was maintained at 25 ± 1 °C during the experiment. The normalized SANS data are expressed as scattering cross sections per unit volume of sample $I(Q)$.[9]

$I(Q)$, for a collection of uniform spherical objects of radius R, can be written as

$$I(Q) = N_p(\Delta\rho)^2 V_p^2 P(Q) S(Q). \tag{1}$$

N_p is the number density of the spheres, $\Delta\rho$ the difference of the scattering-length densities between the spheres and the solvent, and V_p the volume of each sphere. $P(Q)$ denotes the particle structure factor, which, in the case of spheres, can be written as

$$P(Q) = \left[3\frac{\sin(QR) - QR\cos(QR)}{(QR)^3}\right]^2. \tag{2}$$

$S(Q)$ is the interparticle structure factor which takes into account the interparticle correlations. We can derive simply the asymptotic behavior of $S(Q)$ at small Q by the following argument: In a D-dimensional space, the number $N(r)$ of individual scatterers within a sphere of radius r is given by $N(r) = (r/R)^D$. This definition of the fractal dimension D concerns the spatial distribution of the individual scatterers distributed along the chains and consequently is not directly related to the spatial distribution of amino-acid residues along the chain. The derivative of this equation can be related to the asymptotic pair-correlation function $g(r)$ through the relation

$$g(r) = \frac{1}{4\pi r^2}\frac{dN}{dr} = \frac{D}{4\pi N_P}\frac{1}{R^D} r^{D-3}. \tag{3}$$

This will be the correct asymptotic behavior of $g(r)$ for $r \gg R$ in an infinite system. However, in our model of the detergent-protein complex, we expect that the correlation among micelles has a limited range which is of the order of the dimension of the denatured protein. Therefore, the micelle-micelle correlation will have a finite range ξ and we introduce a "simple" cutoff factor $\exp(-r/\xi)$ into Eq. (3). The expression for $S(Q)$ can be obtained straightforwardly by

$$S(Q) = 1 + N_p\int_0^\infty dr\, 4\pi r^2 g(r)\frac{\sin Qr}{Qr} = 1 + \frac{1}{(QR)^d}\frac{D\Gamma(D-1)}{\frac{1}{2}(D-1)}\sin[(D-1)\tan^{-1}(Q\xi)]\left[1 + \frac{1}{Q^2\xi^2}\right]. \tag{4}$$

A similar formula has been derived by Sinha, Freltoft, and Kjems,[10] except that in their formula the important term 1 is missing and the sphere radius R is not included. The general behavior of $I(Q)$ in Eq. (1) with use of Eq. (2) and Eq. (4) can be classified into the following regions: $Q \le 1/\xi$ (Guinier region), $1/\xi < Q \le 2/R$ (fractal region), and $Q > 2/R$ (asymptotic region). We can see these three regions clearly in our scattering data. It should be emphasized that a common procedure of simply extracting a slope from a log-log plot of $I(Q)$ vs Q, in the fractal region, will lead to a misleading evaluation of D.

The SANS spectrum for 1% BSA solution in the buffer has already been studied before and is well known.[11] In Fig. 2 we compare the SANS spectra of the 1/1 sample to the reference 1% protein solution. It is clear from the figure that both size and number of the scattering units in the 1/1 sample are markedly different from the 1% protein solution. Moreover, this figure shows that dimers do not contribute significantly in the Q region studied, a result that justifies *a posteriori* the picture of a "pearl necklace" for the protein observed at the micellar size scale. We can now demonstrate that the scattering unit is actually a micellar aggregate of LDS. At sufficiently large Q (such that $Q\xi > 10$), where the structure factor, $S(Q)$, is nearly unity, one can write approximately

$$I(Q) = N_p(\Delta\rho)^2(4\pi R^3/3)^2\exp(-Q^2R^2/5).$$

From the knowledge of $\Delta\rho = 6.75\times10^{-6}$ $Å^{-2}$ for D_2O-hydrocarbon contrast and assuming $R = 18$ Å,[12] we can deduce from values of $I(Q)$ for Q around 0.1 $Å^{-1}$ that the number density of the scattering centers is $N_p = 2.4\times10^{-17}$ cm^{-3}. From the known concentration

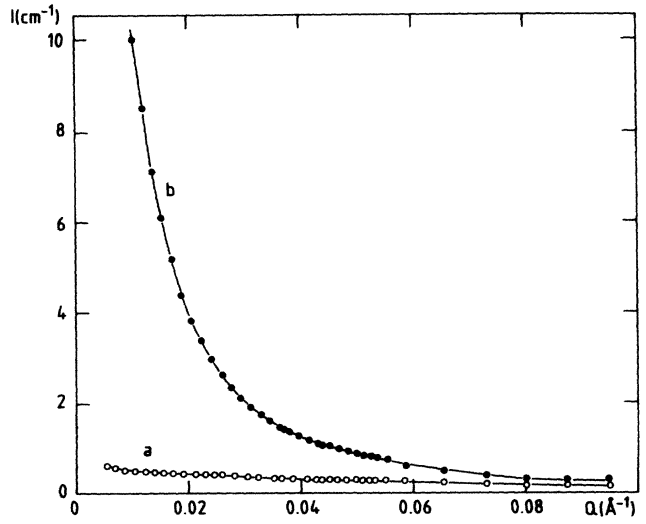

FIG. 2. Scattering cross section $I(Q)$ (a) of the native protein in the buffer solution and (b) of the 1/1 sample, plotted vs Q for $\Gamma < 0.1$ Å^{-1}.

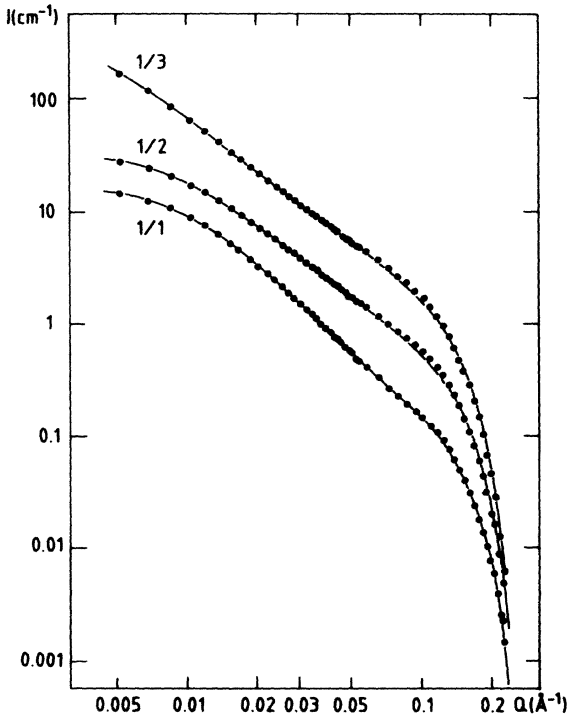

FIG. 3. Scattering cross section $I(Q)$ for the three studied samples plotted vs Q in a log-log diagram. Dots: experimental points. Solid lines: best theoretical fits.

of the detergent, assuming that all detergent is micellized, we can easily deduce that the aggregation number of the micelles is 70 ± 20. Knowing that the steric volume of the hydrocarbon tail of LDS is 350 Å^3 and its fully stretched chain length is $b = 16.7$ Å,[12,13] we can easily calculate that the micelle is a prolate ellipsoid with $a/b = 1.25$. This volume is consistent with an equivalent sphere of radius 18 Å, postulated in the beginning of the calculation.

Once we have established the fact that the scattering centers are globular micelles of dimension 18 Å, it is appropriate to discuss the scattering due to the ensemble of the micelles. The total volume of the micellar aggregates, including the hydrophobic tail and the polar head group, can be estimated to be 1.5% for the 1/1 sample. From previous experiments,[12] for a collection of ionic micelles, if the micelles are freely suspended in solution, they must show a substantial first diffraction peak in the structure factor, so that the scattered intensity distribution will show an interaction peak at around 0.05 Å^{-1}. In Fig. 3 we plot three spectra corresponding to samples 1/1, 1/2, and 1/3 as a log$I$ versus log$Q$. It is clear from the figure that there is no evidence of the intermicellar correlation of the short-range-type characteristic of a liquid. Instead, the scattering data suggest that the intermicellar arrangement has a fractal character. This is because the micellization occurs at the hydrophobic patches distributed along the polypeptide backbones. The distribution of micelles in this case is dictated by the topology of the backbones which is of a lower dimensionality than three. If we assume that the positional correlations of micelles are like strings of pearl necklaces randomly distributed in solution, then the formula that we developed previously can be used to calculate the scattered intensity.

The result of the analysis is shown as solid lines, which are in excellent agreement with the data. The analysis assumes three independent parameters—R, ξ, and D—and the values obtained from the analyses are given in Table I. The facts that R remains constant for the three samples studied and that the large-Q Porod region is well reproduced by the analyses reinforce beyond a doubt that the scattering units are the micellar aggregates. The parameter ξ increases from approximately the dimension of the globular protein for the 1/1 sample to the size of the denatured protein at each stage of the detergent binding. This result agrees very well with a previ-

TABLE I. Fractal dimension D, correlation length ξ, and micellar radius R for protein-detergent complexes with different detergent concentrations. D' is the fractal dimension obtained directly from the slope of the linear region of $S(Q)$.

Sample	D	R (Å)	ξ (Å)	D'
1/1	2.30	18	84	1.92
1/2	1.91	18	108	1.53
1/3	1.76	19	338	1.54

ous inference obtained from a quasielastic light-scattering study.[7] However, the most surprising finding from the analysis is that the fractal dimension D changes successively from 2.30 to 1.91 and to 1.76. Note that a determination of the fractal dimension directly from the slope of the linear region of the scattering function gives values D' which are different from D (see Table I).[14] We feel that this way of determining the fractal dimension is not justified because, for systems where the ratio ξ/R is not sufficiently large, the linear region merges continuously into the Guinier region.

The valves obtained for D are consistent with the intuitive picture that, as the denaturation proceeds, the globular protein transforms gradually from a compact object to a more open random coil. Thus D may be taken as an index of the topology of the denatured protein. We have made another independent series of measurements involving samples 1/0.5, 1/1, 1/1.4, 1/2.5, 1/3.5, and 1/4. From our results, it appears that the SANS spectra change continuously when the LDS concentration increases from 0.5% to 4%. The saturated level of binding, which is 1.4% for SDS[4] apparently has no effect on the micellar distribution we measured.

In conclusion, the measured fractal dimension of the micellar spatial distribution on the "backbones of" denatured proteins appears to be larger than the commonly accepted value for D for the diffusion-limited aggregation model,[15] implying a less open structure than that of the diffusion-limited aggregates. The structure of the detergent-protein complex is well described by a string of constant-size micelles distributed randomly along the hydrophobic patches of the denatured random coil. This picture agrees with the existing literature on studies of SDS-BSA complexes and is the basis for the functioning of the SDS polyacrylamide gel electrophoresis.[6] The fractal dimension D together with ξ that we obtained can perhaps be used to characterize the degree of the denaturation of proteins.

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