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ORIGINAL CONTRIBUTION

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Study of complexes between lysozyme and sodium alkyl sulfates in the solid state

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Abstract Complexes between lysozyme and sodium alkyl sulfates (decyl, dodecyl, tetradecyl and hexadecyl) in the solid state were prepared by mixing aqueous solutions of lysozyme and of the surfactant, separating the precipitated complex and purifying it. The stoichiometry of the complexes was investigated by elemental analysis and was found to correspond to about 8 ± 1 alkyl sulfate ions per lysozyme. Low- and wide-angle X-ray scattering were used to investigate the structure of the complexes. The scattering curves showed one fairly large scattering maximum, revealing a low state of organization of the complexes. The characteristic length characterizing the complexes was calculated from the value of the wave vector corresponding to the maximum of the scattered intensity. This length increased by about 0.233 nm per additional methylene group in the surfactant alkyl chain. A model where spherical aggregates of alkyl sulfate ions are arranged in a disordered simple cubic structure, dispersed in a matrix of lysozyme, provides a possible explanation of the results.

Keywords Structure of protein/surfactant complexes · Solid-state protein/surfactant complexes · Sodium alkyl sulfate/lysozyme interaction · X-ray scattering by protein/surfactant complexes

Introduction

The interaction between surfactants and polymers or proteins is attracting considerable attention because many formulations contain these two types of compounds [1, 2]. The interaction can involve a wide variety of systems differing in the nature of both the surfactant and the polymer. Most investigations concerned relatively dilute aqueous systems. In the case of polyelectrolytes and of oppositely charged surfactants precipitation of a solid polyelectrolyte/surfactant complex or gelation occurs at relatively low concentration of each component [3, 4, 5, 6]. Recently it has been discovered that these complexes are often mesomorphic, i.e., the surfactant and polymer are organized in space [7, 8, 9]. The organization is often similar to that found in water/surfactant binary systems. Hexagonal, cubic and lamellar phases have been evidenced. As already stated surfactants can also interact with proteins [10]. The interaction is more complex than with polyelectrolytes owing to the amphoteric nature of the protein. The protein/surfactant complexes are often soluble in water. In some instances the complexes are insoluble. This is the case of the lysozyme/sodium alkyl sulfate (SAS) complexes [11, 12, 13]. Phase diagrams of water/lysozyme/SAS systems have been reported [11, 12]. They clearly show the range of precipitation of lysozyme/SAS complexes. The stoichiometry of the complexes has been determined for short-chain SAS (hexyl, octyl, decyl and dodecyl) and was found to be of eight alkyl sulfate ions per lysozyme [11, 12]. However the structure of the solid lysozyme/alkyl sulfate ion complexes has not been

investigated. In particular, it is not known whether those complexes are mesomorphic and, if so, what their structure is. This contribution attempts to address this problem. The reason for selecting lysozyme/SAS systems in the present investigation is that their phase diagrams are known [11, 12]. Besides the binding isotherms of SAS to lysozyme have been determined and the binding has been shown to be cooperative for surfactant alkyl chains longer than octyl [14, 15]. Also, the lysozyme/SAS complexes can be fairly easily isolated and studied by X-ray scattering [16, 17]. The results reported here show that the degree of organization of the complexes is low.

Experimental

Materials

The lysozyme was obtained from Fluka (from hen egg white, lot 329190/1 1293). The SAS investigated had alkyl chains with 10, 12, 14 and 16 carbon atoms (referred to as SDeS, SDS, STS and SHS, respectively). They were obtained from Fluka (SDeS, STS), Touzart-Matignon (SDS) and Merck (SHS) with a high purity grade and were used as received.

The preparation of the solid complexes made use of the phase diagrams for lysozyme/SAS systems reported by Morén and coworkers [11, 12]. This preparation is detailed in the case of SDS. A solution of SDS (0.2 g in 10 ml water) was added dropwise to a stirred lysozyme solution (2 g in 150 ml water; the pH during the precipitation was around 6.5 as in Ref. [11]). A white precipitate appeared very rapidly and in increasing amount as the addition proceeded. Once the addition was completed, the heterogeneous mixture was stirred for another 2 h and left to settle at 5 °C overnight. The solid was separated by filtration, mixed with cold water, stirred, and left to settle overnight. It was then filtrated and the whole procedure repeated once more in order to eliminate unreacted compounds. With SDeS and SHS the precipitate did not settle well. It was therefore isolated by centrifugation (1 h at 10,000 rpm and 10 °C). The solid was stirred with water, centrifuged again and the procedure repeated once more. The isolated complexes were dried under vacuum (40 °C, 1 mm Hg, in the presence of P_2O_5) and an elemental analysis was performed. The composition of the complex was calculated on the basis of the value of its nitrogen content as compared to that of lysozyme. Indeed SASs do not contain nitrogen. The presence of the surfactant in the complex results in a decrease in the nitrogen content with respect to that in pure lysozyme. This difference permits the calculation of the number, n, of bound surfactants per lysozyme. The calculations assumed that the lysozyme binds alkyl sulfate ions but no sodium counterions and yielded the values of n listed in Table 1. The values of n for SDeS and SDS agree with reported ones [11], within the experimental accuracy of the experiments (± 1 unit). Our results suggest a small increase in n with the alkyl chain length but this increase is nearly within the experimental error. A previous study of lysozyme/SAS systems for surfactants with an alkyl chain containing 6–12 carbon atoms reported a value of $n = 8$, irrespective of the surfactant chain length [11]. The weight fractions of the alkyl sulfate ion in the complexes were also obtained from the elemental analysis data. Their values increased from about 0.10 for the decyl sulfate to around 0.17 for the hexadecyl sulfate (Table 1). The volume fractions of the alkyl sulfate ion in the complexes are not expected to differ by more than 10% from the values of their weight fractions.

Methods

The X-ray scattering measurements were performed using an experimental device equipped with a Guinier-type camera for wide angles or a Luzzatti–Baro camera [18] for small angles operating in a vacuum. The X-ray beam was generated with a copper anticathode sealed tube supplied with a Philips PW1130 generator operating at 35 kV and 35 mA. The X-ray beam was of a linear type and was collimated with two slits. It was made monochromatic and focused with a bent quartz crystal, providing radiation of wavelength 0.1541 nm ($K_{\alpha 1}$ emission ray of copper). The X-ray diffraction pattern was recorded with a photographic film or an imaging plate (Molecular Dynamics) set perpendicular to the beam. The film or plate was scanned and the data stored for analysis. The width and height of the X-ray beam were such that the correction for infinite slit applied to the experimental data. The range of the scattering vector, q, investigated was between 0.3 and 30 nm^{-1} ($q = 4\pi / \lambda \sin\theta$; diffraction angle 2θ). The samples were contained in a sealed capillary or between two mica windows in a sealed cell.

Results and discussion

The variations of the scattered intensity with the scattering vector for the four complexes are shown in Fig. 1. Each plot shows a broad maximum, particularly for the decyl sulfate complex, in the q range between 1 and 3 nm^{-1}. The intensity plots showed no other feature in the q range between 3 and 30 nm^{-1} (not shown in Fig. 1). No change in the intensity and the position of the intensity maximum occurred when the temperature was varied between 25 and 95 °C. Also the same scattering curve was obtained at 25 °C and after heating the sample to 95 °C and cooling it back to 25 °C.

The broadness of the scattering maximum in Fig. 1 reveals a low degree of organization of the lysozyme/alkyl sulfate complexes. Nevertheless, Fig. 1 shows that the width of the scattering maximum decreases and the

Table 1. Characteristics of the lysozyme/alkyl sulfate ion complexes in the solid state

Surfactant	Sodium decyl sulfate	Sodium dodecyl sulfate	Sodium tetradecyl sulfate	Sodium hexadecyl sulfate
Surfactant ion weight fraction	0.10	0.13	0.13	0.17
n	7.0 ± 1.0 (8.0)[a]	8.2 ± 1.0 (8.0)[a]	7.4 ± 1.0	9.3 ± 1.0
q_{max} (nm^{-1})	0.242	0.168	0.156	0.133
l_c (nm)	2.60 ± 0.2	3.75 ± 0.13	4.00 ± 0.16	4.71 ± 0.1

Values in parentheses from Ref. [11]

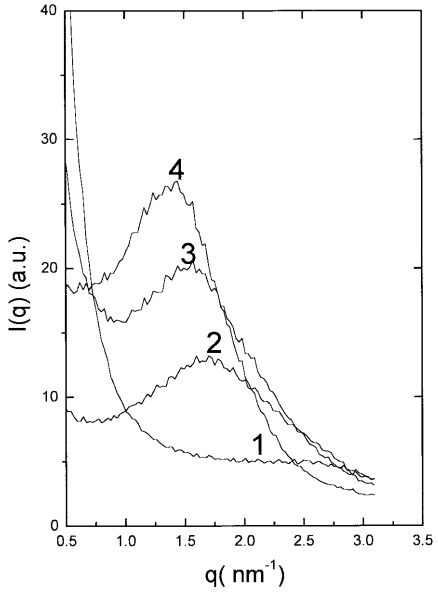

Fig. 1. Variations of the scattered intensity with the wave vector for lysozyme/decyl sulfate (*1*), lysozyme/dodecyl sulfate (*2*), lysozyme/tetradecyl sulfate (*3*) and lysozyme/hexadecyl sulfate (*4*) at 25 °C

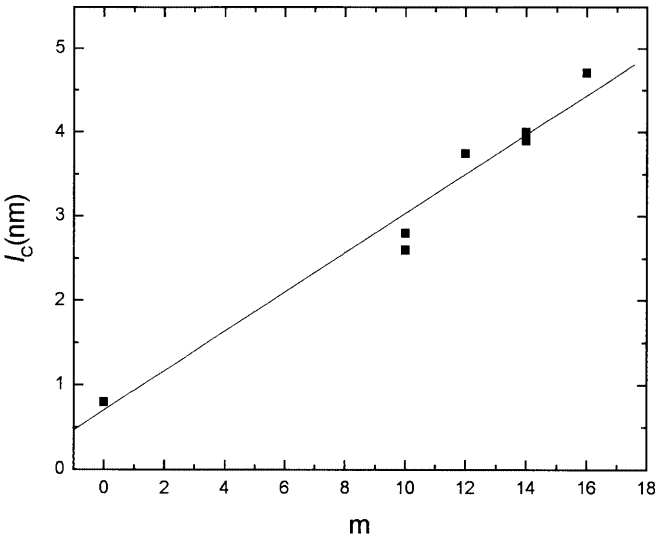

Fig. 2. Variation of the characteristic length, l_c, with the number, m, of carbon atoms in the surfactant alkyl chain

intensity at the maximum increases upon increasing surfactant chain length, indicating a slight increase of the organization. This may reflect the stronger hydrophobic character of the complexes arising from the stronger interactions between the longer alkyl chains of the surfactant. Figure 1 also shows that the value of the wave vector corresponding to the maximum, q_{max}, decreases as the chain length of the surfactant increases.

The values of q_{max} and the characteristic length, $l_c = 2\pi / q_{max}$, of the system are listed in Table 1. The error in the values of q_{max} and l_c is large for the lysozyme/decyl sulfate ion complex. Figure 2 shows that the variation of the characteristic length with the carbon number, m, of the surfactant alkyl chain is linear, within experimental error. For a more accurate determination of the slope of this plot we introduced at $m = 0$ the estimated value of twice the size of the sulfate head group (i.e., about 0.8 nm). The least-squares fitting of the data yielded a slope of 0.233 ± 0.015 nm per additional methylene group in the surfactant alkyl chain. Since the binding of alkyl sulfates to lysozyme is cooperative for alkyl sulfates longer than octyl sulfate [14, 15] the lysozyme-bound alkyl sulfate ions must be in an aggregated form in the lysozyme/alkyl sulfate complexes.

These results raise the question of the type of structure responsible for the broad maximum of the scattering intensity and its variation with the surfactant chain length seen in Fig. 2. The small volume fraction of alkyl sulfate ions in the complexes does not support a structure of the lamellar type, however disordered such a structure may be. Indeed, calculations based on this model yield for the surfactant layer a thickness of about 0.8 nm in the case of the hexadecyl sulfate. Such a low value implies that the surfactant alkyl chains are extremely tilted with respect to the lamellar surface or lie almost flat. Our results are more in favor of a disordered simple cubic structure of small surfactant aggregates, of spheroidal shape, with a center-to-center distance between aggregates equal to l_c and with the lysozyme filling the space between surfactant aggregates. On this assumption l_c would increase by twice the length of the projection of a C–C bond on the alkyl chain axis, i.e., by 0.253 nm, for each additional methylene group in the surfactant alkyl chain. This value is close enough to the experimentally determined increment of 0.233 nm. The model can be simply tested for the hexadecyl sulfate containing complex as follows. Assuming equal values of the weight and volume fractions of the surfactant ion [19, 20], the ratio R/l_c (R is the aggregate radius, l_c is the length of the cubic structure) is found to be 0.345 and the aggregate radius to be 1.63 nm.[1] Using a value of 0.534 nm^3 for the volume of the hexadecyl sulfate ion [19, 20] yields a surfactant aggregation number of about 34. Since one lysozyme molecule binds about eight alkyl

[1]The partial molal volume of the dodecyl sulfate ion in the micellar state is 251.5 cm^{3}mol^{-1} on the basis of the values –5.1 and 246.4 cm^{3}mol^{-1} reported for the partial molal volume of the sodium ion [19] and of the micellar SDS [20], respectively. Using the value 17.2 cm^{3}mol^{-1} for the partial molal volume per micellar methylene group [20] yields for the hexadecyl sulfate ion a partial molal volume of 320.3 cm^{3}mol^{-1}; thus, a volume of 0.534 nm^3 per ion. Note that the density of the micellar hexadecyl sulfate ion is 1. Thus, the volume fraction and the weight fraction of aggregated hexadecyl sulfate are equal. This result supports the assumption made in the calculation.

sulfate ions, this results indicates that the structural unit cell is made up of about four lysozyme molecules and one surfactant aggregate. The numbers would be different if the structure is assumed to be the more ordered centered cubic; however, our results do not warrant the use of a structure more ordered than the simple cubic one. Note that the aggregates are probably polydisperse in size. This by itself introduces fluctuations in the structural unit cells and thus disorder, thereby contributing to the broadness of the scattering maximum and the absence of other scattering features in the spectra between 3 and 30 nm^{-1}. The increase in intensity seen at $q < 1\ nm^{-1}$ (Fig. 1) is probably due to the presence of some large dust particles collected during the isolation of the solid complexes.

Our results for the lysozyme/alkyl sulfate complexes are very similar to those reported in an X-ray study of the solid-state complexes formed by an anionic polysaccharide and alkyltrimethylammonium ions with an alkyl chain containing 12, 14 and 16 carbon atoms [21]. Thus, a single broad scattering peak characterized each complex and the characteristic length increased with the carbon number of the surfactant alkyl chain in a manner very similar to that for lysozyme/alkyl sulfate complexes, by 0.15–0.4 nm per additional methylene group. The volume fraction of surfactant ion in the polysaccharide/alkyltrimethylammonium complexes was around 0.5, a value that is larger than the alkyl sulfate volume fraction in the lysozyme/alkyl sulfate complexes, which ranged from about 0.10 to 0.17 (Table 1).

Conclusions

We have shown that the structure of the solid-state complexes formed by lysozyme and sodium alkyl sulfates is rather disordered, in contrast to the ordered structures often found for complexes formed by polyelectrolytes and oppositely charged surfactants. This may reflect the amphoteric nature of lysozyme. It may be worth extending this study to other water-insoluble protein/surfactant and polyampholyte/surfactant complexes.

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Note added in proof Since this work was submitted for publication Stenstam et al. [22] reported for the lysozyme/dodecyl sulfate complex exactly the same composition as that listed in Table 1.