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Research Paper

Self-organization of lysozyme—Ionic surfactant complexes at the aqueous-air interface as studied by tritium bombardment

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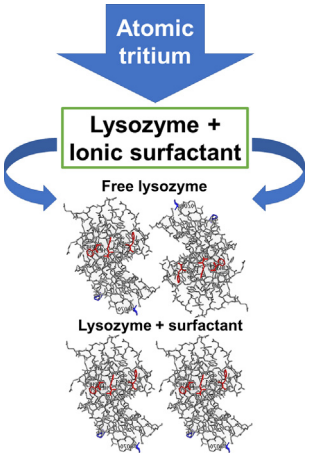
HIGHLIGHTS

- Adsorption of lysozyme at the water – air interface was analyzed by tritium planigraphy.
- We studied the adsorption of lysozyme mixed with SDS or DTAB at the water – air interface.
- An association between the protein and both SDS and DTAB is formed.
- The peculiarities of surfactant interactions with amino acid residues are described.

GRAPHICAL ABSTRACT



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ABSTRACT

The self-organization of lysozyme with cationic (dodecyltrimethylammonium bromide) and anionic (sodium dodecylsufate) surfactants at an aqueous-air interface was studied by means of tritium planigraphy. It was found that even if the surfactant concentration is too low to induce significant structural changes in the protein globule, the surfactant can bind with the protein via both electrostatic and hydrophobic interactions and can displace the protein from the interface. Different mechanisms of complex formation are suggested for lysozyme mixed with SDS or DTAB. SDS molecules screen positively charged groups of amino acid residues via electrostatic interactions and form hydrogen bonds with polar groups of amino acid residues. SDS also penetrates the protein globule via hydrophobic interactions. In contrast, DTAB interacts with hydrophobic amino acid residues, screening them from tritium atoms. For both surfactants, it was found that lysozyme maintains a longways-on orientation in the mixed adsorption layer.

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1. Introduction

The formation of interfacial films by proteins and proteins mixed with surfactants plays a key role in the field of biological mem-

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branes and protein solubilization [1,2]. The interaction between globular proteins and ionic surfactants is complex and involves different types of intermolecular forces [3]. In the model experiments performed in this field, egg-white lysozyme is one of the most commonly used proteins. It is composed of 129 residues and is stabilized by four disulfide bonds.

Lysozyme forms complexes with sodium dodecyl sulfate (SDS) when the protein and surfactant possess an opposite charge. These complexes were studied by means of spectroscopic and tensiometry techniques and were described relatively well for high SDS concentrations (in most cases, the SDS concentration is close to the critical micelle concentration) in terms of phase behavior and structural peculiarities.

Lad and co-authors described the lysozyme-SDS system by means of isothermal titration calorimetry and FTIR spectroscopy [4]. At SDS concentrations lower than 0.3 mM, the electrostatic-specific binding is the most appropriate mechanism of lysozyme – surfactant binding in solutions of low ionic strength. The formation of insoluble SDS-lysozyme complexes was observed when seven or eight SDS molecules neutralized the net positive charge of the protein. High SDS concentrations result in the resolubilization of the complexes. The phase behavior of lysozyme-SDS and free SDS is described in refs [5,6]. The addition of SDS to the neutral complexes results not only in the increase of hydrophobic attractive forces but also in the introduction of charge, which enters the structure together with counterions and water. Both opposing phenomena contribute to the formation of a network structure. Further increase of SDS concentration leads to a strong net repulsive electrostatic interaction between the complexes and, thus, the formation of an isotropic solution.

The other group of studies described the structural peculiarities of lysozyme-SDS complexes. The position of SDS in lysozyme-SDS complexes was defined using three-dimensional X-ray diffraction [7]. In the complexes, SDS is in three locations: (1) SDS is bound to the surfaces of two molecules and causes only small local disorder in this region; (2) SDS penetrated the protein between two aromatic side chains, displaced them, and induced some local disorder; (3) SDS penetrated deep into the hydrophobic core of the protein with the formation of a salt between a lysine residue of the protein and the sulfate group of the surfactant. The last case is characterized by perturbation of the entire lysozyme molecule. If the SDS concentration is below 0.1 M, Valstar and coauthors showed the formation of complexes that can be described as a “necklace model”, where SDS micelle-like clusters are bound to unfolded protein [8]. However, by means of small-angle X-ray scattering, the preservation of the compact structure when it complexes with SDS was observed [9]. In the cited paper, Narayanan and co-authors suggest that lysozyme can partially penetrate the SDS micelle, specifically, the complex looks like a swollen micelle with a protein lying near the shell, in native or unfolded form. From a rheological viewpoint, isotropic solutions behave as Newtonian fluids with very low viscosity values [10].

The complexes of lysozyme with cationic surfactants are not as clearly described. Binding of cationic surfactants is strongly dependent on pH, and binding strength increases at high pH [11]. In the cited paper, it is emphasized that at pH 7 and 9 hydrophobic interactions play a major role, while at pH 5 only electrostatic interactions play a prominent role in the binding of these detergents. A cooperative interaction of proteins with cationic surfactants is described in Ref. [6]. The effects of cationic surfactants are smaller, compared to the effects of anionic detergents. Such effects are explained by the high availability of positively charged amino acid residues such as Arg and Lys because of their longer side chains compared with negatively charged amino acid residues (Asp and Glu) [12]. Thus, the cooperative binding step will start at a higher concentration for cationic relative to anionic surfactant [6]. Structural changes of native lysozyme when the protein is reduced by 1,4-dithio-DL-threitol in the presence of either cationic or anionic surfactant were described by CD, fluorescence, and infrared techniques [13]. The degree of helicity recovered was less in the presence of cationic detergent, although the fluorescence changes indicate an interaction with surface Trp residues. The interaction of reduced lysozyme with micelles of both cationic and anionic surfactants gave the same results, illustrating the role of disulfides in the interaction mechanism.

The cited papers are concerned with the surfactant concentrations close to or higher than the critical micelle concentration (CMC) of the detergent. In previous work, we used the scintillation phase method to characterize the mixed adsorption layer of lysozyme with anionic (SDS), and cationic (DTAB) detergents at the aqueous – organic liquid interface [14,15]. We found that low surfactant concentrations of both SDS and DTAB resulted in the same changes in the colloidal behavior of the protein. The interactive process is associated with the formation of the lysozyme-ionic surfactant complexes by means of protein binding sites followed by co-adsorption with SDS and by step-by-step hydrophilization and displacement from the adsorption layer by DTAB. An increase in the detergent concentration typically results in the substitution of protein from the adsorption layer that was observed [15]. To determine the structural changes of the protein in the mixed adsorption layer at the aqueous-air interface, tritium planigraphy can be used even if the surfactant concentration is too low to reveal changes by other techniques. The basis of the tritium planigraphy method and some examples of its application are summarized below.

1.1. Tritium planigraphy as a method for the investigation of biomolecules at the aqueous-air interface

According to a definition given in ref. [16], the method of tritium planigraphy, which provides comprehensive information on the accessible surface of macromolecules, allows an attempt at reconstructing the three-dimensional structure of a protein from the experimental data on residue accessibility to labeling. A frozen protein solution is subjected to the reactive tritium atoms that form on the surface of a hot W-filament (Langmuir dissociation of hydrogen [17]). The next step is the determination of the intramolecular distribution of the tritium label. To this end, macromolecules are subjected to defragmentation into radiolabeled species and determination of the radioactivity of each fragment. In the case of protein, it is decomposed into peptides and amino acids. On the basis of the assumption that the reaction primarily occurs during a single interaction of the atom with the macromolecule, the radioactivity of the species reflects its location in the molecules at the aqueous-air surface. The last step of the experiment is the modeling of the spatial structure of the macromolecule.

The potential for an amino acid residue to react with the atomic tritium also influences the results of the intramolecular distribution of the label. To characterize the amino acid residues by their propensity for tritium introduction, the special coefficients that were obtained in the experiments with short peptides as a model of a completely unfolded chain are used. To determine these coefficients, Valynskaya and co-authors used 10 g/L of a protein solution that was subjected to tritium bombardment [18]. A linear correlation between the accessible surface of the amino acid residue and the amount of tritium present was shown in ref [19], where peptides were tritiated. Goldanskii and co-authors illustrated the coefficients obtained for lysozyme, and normalized per unit of accessible surface [20]. All of these coefficients are summarized in Table 3 in the “Results and discussion” section.

If the determination of total amino acid composition of the surface is required, it is necessary to define the tritium label introduction into amino acids residues of different types. The first step is purification from the labile tritium, namely tritium in the functional

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