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Lysozyme-surfactant adsorption at the aqueous-air and aqueous-organic liquid interfaces as studied by tritium probe

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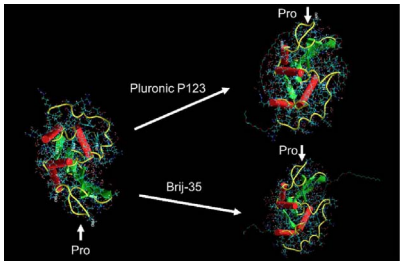
GRAPHICAL ABSTRACT



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ABSTRACT

Self-organization of proteins and non-ionic surfactants at the aqueous-air and aqueous-organic liquid interfaces is controlled by hydrophobic interactions. Unfortunately, little is quantitatively known about the effects of high- and low-molecular weight non-ionic surfactants on protein adsorption and aggregation at aqueous-air and aqueous-organic liquid interfaces. A tritium probe technique was applied to the systems lysozyme – Pluronic P123 and lysozyme – Brij-35. For complex analysis, UV and fluorescent spectrometry were also used for solutions with the chosen protein and surfactants concentrations. Non-ionic surfactants form a flexible complex with lysozyme at the aqueous-air interface. We observed that both Pluronic P123 and Brij-35 result in displacement of 40–75 % of protein, even in a surfactant concentration range less than the CMC and rotation of residual protein (or protein in complex with surfactant) in the mixed adsorption layer compared to the adsorption layer formed by free lysozyme. In the mixed adsorption layer, lysozyme globules are separated, and protein becomes more available for tritium atoms compared to free lysozyme.

1. Introduction

Protein-surfactant systems have wide applications, including pharmacy, the food industry, cosmetics and bioscience [1–3]. In particular, non-ionic surfactants are often added to protein solutions to prevent aggregation and unwanted adsorption during purification, filtration, transportation, freeze-drying, spray-drying, and storage [4]. Three main factors affect the dynamic interfacial characteristics and foam properties of protein aqueous solutions: the particular protein, the level of association/dissociation of these proteins, and, the competitive adsorption between protein and surfactant in the aqueous phase [5]. Mechanisms of binding proteins with ionic surfactants are well studied and include four binding steps that depend on surfactant concentration. Increasing surfactant concentrations result in electrostatic interactions

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that dominate until the available charges in the protein molecule are compensated by the surfactant ions, thus forming electroneutral complexes of higher surface activity compared to native protein. Upon further increases in surfactant concentration, hydrophobic interactions become more important, making the complex more hydrophilic and less surface active in a step-by-step fashion. Due to competition in the adsorption layer, the protein-surfactant complexes with less surface activity become incapable of displacing the free surfactant molecules adsorbed earlier (because their concentration in the solution is high) so that finally, and usually at the critical micelle concentration (CMC) of the surfactant, the adsorption layer is formed mainly by surfactant molecules [6].

For non-ionic surfactants, the mechanism of interaction is different. Unlike ionic surfactants, the effective charge of the proteins is not affected by the presence of surfactant, even at high concentrations [7]. In the case of non-ionic surfactant, the aggregation numbers of surfactant clusters in the presence of protein are lower than the aggregation numbers of free micelles, and the effect is stronger at lower surfactant concentrations, as is the case for ionic surfactants [8]. The presence of non-ionic surfactants, in particular Tween20 and Tween80, prevents protein unfolding and increase solubility, protecting the protein from heat-induced aggregation [9–12]. These interactions between proteins and non-ionic surfactants result in changes in the process of the adsorption layer formation.

During the initial stage of competitive adsorption, the rate of adsorption of surface-active constituents is proportional to their concentration in solution. Therefore, if the surfactant concentration is essentially higher than the protein concentration, the surfactant is first adsorbed and can then be displaced by the surfactant and the protein. Above a certain concentration of surfactant, the interface may be covered by free surfactant molecules [6,13]. However, Day and co-authors have shown that in the cases of a protein-non-ionic surfactant mixture, non-aggregated protein diffuses and adsorbs to the surface more rapidly than aggregated protein, while the protein–surfactant complex then adsorbs to the interface over longer timescales and the presence of the surfactant in the film reduces the coefficient of ellipticity accordingly [14]. In the cited paper, free non-ionic surfactant forms structurally similar adsorption layers at different interfaces, namely, hexadecane–-water, triolein–water and air–water.

Special attention in this area was paid to protein intermolecular depletion interactions induced by polyethylene glycol (PEG). By means of measuring fluorescence anisotropy and lysozyme with a fluorescent label as a tracer, the role of PEG molecular weight, concentration and the ionic strength of the aquatic media was studied [15]. It was shown that increasing NaCl concentration from 0 to 0.2 M results in an increase in attractive interactions between PEG10000 and lysozyme due to screening of electrostatic repulsion. The experimental results reveal that at polymer concentrations close to the crossover concentration and 0.2 M NaCl, long-range attractive protein interactions induced by PEG are characterized by increasing the degree at which protein rotational diffusion is slowed when protein concentration is increased. Protein interactions consist of noncovalent forces, including van der Waals, electrostatic, hydrophobic, and hydrodynamic interactions that are short-range in nature; long-range depletion forces are induced by polymer such as PEG.

Three block copolymers of ethylene oxide (EO) and propylene oxide (PO) with the general formula EO_n-PO_m-EO_n, known under trade names pluronics, poloxamers or synperonics, are used as a shear-protective excipient to enhance cell yield in agitated cultures and reduce cell adhesion in stationary cultures [16]. These *in vivo* studies have demonstrated that the polymers are tissue-compatible, nonimmunogenic, and less toxic than low molecular-mass surfactants [17]. Due to their amphiphilic character, these copolymers efficiently form nanoparticles [18]. Using optical waveguide lightmode spectroscopy, Kim and co-authors compared the adsorption kinetics exhibited by poloxamer 188, also known as Pluronic F68, and polysorbates 80 and 20, in the presence and absence of hen egg white lysozyme and human granulocyte colony-stimulating factor at hydrophilic silica–titania surfaces [19]. The kinetic results showed that polysorbates 80 and 20 can only inhibit protein adsorption by their preferential location at an interface to which they show sufficient affinity and not by formation of less surface-active, protein–surfactant complexes. However, poloxamer 188 is able to inhibit protein adsorption via formation of protein–surfactant complexes of low adsorption affinity (i.e., high colloidal stability) and not by preferential location at the interface. The authors suggest that protein accelerates the adsorption of these surfactants by disrupting their self-associations, releasing surfactant monomers. The increased concentration of surfactant monomers may promote surfactant adsorption or the formation of stable, surfactant–protein complexes having little or no effect on the surfactant adsorption rate.

Unfortunately, little is quantitatively known about the effect of Pluronics (and other non-ionic surfactants containing EO-gropes as a hydrophilic part) on protein adsorption and aggregation at aqueous-air and aqueous-organic liquid interfaces. The goal of this study was to reveal peculiarities of the interaction pf protein with non-ionic surfactant in the mixed adsorption layers formed at the aqueous-air and aqueous-organic liquid interfaces. To achieve this, we applied a tritium probe technique to the systems of hen egg white lysozyme (a model protein) – Pluronic P123 and lysozyme – Brij-35 to reveal the effect of non-ionic surfactants on globular protein adsorption at the aqueous-air and aqueous-organic liquid interfaces. The behavior of macromolecules at the liquid-liquid interface plays a key role in biological events such as interactions of biomolecules with membranes [20–22]. Non-ionic surfactants were chosen for their different molecular mass and hydrophobic fragments (Table 1). Moreover, both non-ionic compounds possess high binding affinity in relationship with cellular membrane [23].

The tritium probe technique includes the combination of two methods that use tritium as a radioactive tracer: (i) tritium planigraphy of the aqueous-air interface [27], and (ii) liquid scintillation spectrometry as a scintillation phase tool [28]. The combination of the results obtained by these methods characterize the system by orientation of the protein molecule in the adsorption layer and by its colloidal properties, such as hydrophobicity and surface activity. To clarify the results obtained here, let us first briefly describe the techniques we used.

The method of tritium planigraphy provides comprehensive information on the accessible surface of macromolecules, and allows an attempt at reconstructing the three-dimensional structure of a protein from experimental data for residue accessibility to labeling. A frozen solution containing biomacromolecules is subjected to reactive tritium atoms that form on the surface of a hot tungsten filament (Langmuir dissociation of hydrogen [29]). 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 the determination of the radioactivity of each fragment. Protein is decomposed into peptides and amino acids. Based on 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 molecules at the aqueous-air surface. The last step of the experiment is modeling of the spatial structure of the macromolecule. This technique was successfully used in the investigation of the structural peculiarities of membrane proteins [30], viruses [31,32], and cell

Table 1
Properties of non-ionic surfactants used in this study.

Non-ionic surfactant	Nominal formula	Molecular weight	HLB [24]	CMC at 25 °C, M
Pluronic P123	$EO_{20}PO_{70}EO_{20}$	5800	11	5.2×10^{-5} [25]
Brij-35	$C_{12}H_{25}(OCH_2CH_2)_{23}OH$	1221	16	7.4×10^{-5} [26]