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Dynamic surface properties of lysozyme solutions. Impact of urea and guanidine hydrochloride

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ABSTRACT

Urea and guanidine hydrochloride (GuHCl) have different influence on surface properties of lysozyme solutions. The increase of GuHCl concentration leads to noticeable changes of kinetic dependencies of the dynamic surface elasticity and ellipsometric angles while the main effect of urea reduces to a strong drop of the static surface tension. The difference between the effects of these two denaturants on the surface properties of other investigated globular proteins is significantly weaker and is mainly a consequence of a different extent of the globule unfolding in the surface layer at equal concentrations of the denaturants. The obtained results for lysozyme solutions are connected with the strongly different denaturation mechanisms under the influence of urea and GuHCl. In the former case the protein preserves its globular structure in the adsorption layer at high urea concentrations (up to 9 M) but without tightly packed interior of the globule and with a dynamic tertiary structure (molten globule state). On the contrary, the increase of GuHCl concentration leads to partial destruction of the protein tertiary structure in the surface layer, although this effect is not as strong as in the case of previously studied bovine serum albumin and β-lactoglobulin.

1. Introduction

The X-rays crystallography and NMR spectroscopy give detailed structures of the globules of numerous proteins. The relations between these unique and stable structures on one hand, and protein functions or the properties of protein solutions on the other hand are the main problems of the classic protein physics [1,2]. Meantime, disordered or partly disordered protein structures are widespread in biological and industrial systems but so far have been investigated to a significantly less extent [2–5]. These can be, for example, intrinsically disordered proteins [2,5], some precursors of protein aggregates [6–8] or proteins unfolded due to the changes of environment, in particular, under the influence of chemical denaturants [4]. The intensive studies of the most of these disordered protein structures have started only recently and the obtained information has rather been limited until now [5–8].

At the same time, the disordered or partly disordered protein structures are a well known subject in surface chemistry. Proteins are frequently used in food, pharmaceutical and cosmetic industries to stabilize foams and emulsions. The classic point of view is that the protein molecules are entirely unfolded at the liquid–gas interface. According to Langmuir and Schaefer "the protein layer structure is like that of a net made to float on the surface of water by corks (hydrophobic groups of side chains) distributed over the surface of the net" [9]. The subsequent studies admitted also a possibility of the adsorption of some intact globules at high protein concentrations [10,11]. The advent of the neutron reflectometry led to a reconsideration of the classic ideas and indicated the preservation of the globular structure of the main model proteins at the liquid surface [12–17]. This conclusion, however, is not generally accepted. Different authors using similar experimental techniques give sometimes opposite answers to the question on the destruction or preservation of the protein globular structure at the liquid surface. For example, studies of the X-ray reflectivity allowed the authors to conclude that lysozyme globules are entirely unfolded in the surface layer [18–20] while the neutron reflection studies does not confirm strong changes of the tertiary structure of this protein in the course of adsorption [12,13,16]. Another sensitive method, the external reflection FTIR spectroscopy, gives evidence of noticeable changes of the secondary structure of lysozyme in the surface layer but does not allow direct estimation of the tertiary structure [21]. The lack of sufficiently reliable information on the protein tertiary structure in the adsorption layer is obviously caused by an extremely limited number of suitable experimental techniques.

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It has been shown recently that the dilational surface rheology can give additional information on the protein conformation at the liquid–gas interface [22–25]. This approach is based on a strong difference between kinetic dependencies of the dynamic surface elasticity for globular protein solutions and for solutions of non-globular or unfolded proteins. In the former case the kinetic dependencies are monotonic and resemble the corresponding results for aqueous dispersions of charged solid nanoparticles. In the latter case the kinetic dependencies are similar to those for solutions of amphiphilic polymers where the dynamic surface elasticity goes through a strong local maximum and approaches relatively low values close to equilibrium in agreement with the theory of the surface viscoelasticity of polymer solutions [25]. Measurements of the dynamic surface elasticity of the mixed solutions of bovine serum albumin (BSA) and β-lactoglobulin (BLG) with guanidine hydrochloride and urea as a function of surface age allowed tracing the globule unfolding and the formation of the distal region of the surface layer [22–24].

In this work the developed approach is applied to lysozyme solutions. Although lysozyme is one of the most frequently studied model proteins, the information of the structure of its adsorption layers at the liquid–gas interface is still rather controversial [11–13,16,18–21]. It has a more rigid globule than BSA and BLG and belongs to the group of "hard" proteins [26]. The molecule of lysozyme with the molecular weight of 14,300 Da consists of 129 aminoacid residues. The closed packed globule stabilized by four disulfide bonds has the size of 4.5 × 3.0 × 3.0 nm and consists of two main domains [27]. Lysozyme tertiary structure is relatively stable against the action of high temperatures [28] and chemical denaturants [28–30], in particular of urea and GuHCl. These substances are frequently used in the studies of the protein unfolding, however, the mechanism behind the denaturating power of urea and GuHCl is still not well understood [1,3,28]. Their effect on lysozyme globules is strongly different [28]. Urea has almost no influence on the secondary structure and the protein preserves its globular conformation up to high urea concentrations (>10 M). On the contrary, the Raman spectroscopy indicates the unfolding of lysozyme in concentrated solutions of GuHCl (>5 M). The distinctions in the denaturating mechanism of urea and GuHCl result in different influence on the amyloid fibril formation in lysozyme solutions. GuHCl accelerates this process at high concentrations but urea does not display any noticeable effect [31]. Studies of the dynamic surface properties of the mixed solutions of lysozyme and these two denaturing agents with strongly different impact on lysozyme globules give a possibility to elucidate further the relation between these properties and the protein conformation at the liquid–gas interface. This is the main aim of this work. The data on neutron reflection from the adsorbed layer of unfolded lysozyme molecules facilitate this task [16]. To the best of our knowledge the corresponding information for lysozyme/urea solutions has not been determined yet. Another aim is to estimate the degree of the destruction of lysozyme tertiary structure in the surface layer at different concentrations of the denaturants.

2. Materials and methods

Lysozyme (Sigma–Aldrich, Germany) was used as received. Lysozyme solutions of required concentrations in phosphate buffer at pH = 7 were prepared by dilution of the solution with concentration of 0.05 mM. GuHCl and urea (Sigma–Aldrich, Germany) were used as received. These substances were dissolved in a small quantity of phosphate buffer before the addition to the protein solution. The volume of the solution was then increased up to the required value.pH of all the solutions was adjusted to 7 by adding components of the Na_2HPO_4–NaH_2PO_4 buffer mixture (Sigma–Aldrich, Germany). The ionic strength of all the solutions was 0.04 M. The solutions were prepared using triply distilled water. The glass apparatus was used in the last two steps of the distillation. The surface tension of the pure buffer solution was 72.8 mN/m.

All lysozyme solutions were used without storing and measurements of the surface properties were started in several minutes after preparation of the fresh solution. All the measurements were carried out at 20 ± 1 °C.

The dynamic dilatational surface elasticity was measured by the oscillating ring [32,33] and oscillating barrier [22,23,33] methods. In the first case the surface of the solution under investigation was periodically expanded and compressed as a result of oscillations of a glass ring along its axis. The ring was partly immersed into the liquid with its axis perpendicular to the liquid surface and its internal surface was grounded to improve wetting. The ring oscillations led to regular oscillations of the liquid surface area and surface tension of the solution as a result of periodical changes of the meniscus shape at the internal surface of the ring. The surface tension of the investigated liquid was measured inside the ring by Wilhelmy plate method. The main advantage of the oscillating ring technique consists in almost pure dilational deformations of the liquid surface and thereby in a negligible contribution of shear stresses to experimental results. The relative amplitude and frequency of the solution surface area oscillations were 10% and 0.1 Hz, respectively.

In the case of the oscillating barrier method the liquid surface area oscillated due to the sinusoidal motion of a hydrophobic barrier gliding back and forth along polished brims of a Langmuir trough. The induced oscillations of the surface tension were also determined by Wilhelmy plate method. The application of the oscillating barrier method was possible only at the oscillation frequencies less than approximately 0.2 Hz when the length of surface longitudinal waves far exceeded the length of the Langmuir trough and the surface tension oscillations in the center of the trough were uniform. Therefore the oscillation frequency of the barrier was 0.1 Hz and the relative amplitude was 3%.

The real ε_r and imaginary ε_i components of the dilational dynamic surface elasticity ε were calculated from the amplitudes of oscillations of the surface tension $\delta\gamma$ and surface area δS, and the phase shift φ between the oscillations of these two quantities by the following relation

$$\varepsilon = \frac{d\gamma}{d\ \ln S} = \varepsilon_r + i\varepsilon_i = \frac{S\delta\gamma}{\delta S}\cos\ \varphi + i\frac{S\delta\gamma}{\delta S}\sin\ \varphi \quad (1)$$

The imaginary part of the complex dynamic surface elasticity of the solutions under investigation proved to be much less than the real part. Therefore, only the results for the real part are discussed below. The experimental errors of both the oscillating ring and oscillating barrier methods are mainly determined by the errors of surface tension measurements and are less than ± 5%. However, the distinctions between the kinetic dependencies of surface properties can be higher due to the insufficient reproducibility of the induction period (cf. next section).

A null-ellipsometer Multiskop (Optrel GBR, Germany) at a single wavelength of 632.8 nm was applied to estimate the adsorbed amount using a fixed compensator (±45°) and a 2-zone averaging nulling scheme. The scheme of this apparatus has been described in detail elsewhere [34]. All the ellipsometric measurements were performed near the incidence angle of 50° close to the Brewster angle because this condition ensured the highest sensitivity of the ellipsometric angles to the properties of the adsorption layer.

The elliptically polarized light consists of two components with the electric vectors oscillating parallel and perpendicular to the plane of incidence. The reflection at the interface results in different changes of the phase and amplitude of these two components. These changes depend on the optical properties of the interface and can be characterized by two ellipsometric angles Ψ and Δ connected with the Fresnel reflectivity coefficients of