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Dilational surface viscoelasticity of protein solutions. Impact of urea

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ABSTRACT

The dilational surface rheology is applied to solutions of globular proteins (bovine serum albumin and β-lactoglobulin) in presence of urea. The kinetic dependencies of the dynamic dilational surface elasticity become non-monotonic if the denaturant concentration exceeds a certain critical value indicating the adsorption of unfolded protein molecules. The unfolding in the surface layer occurs at lower urea concentrations than in the bulk phase similar to the case of mixed solutions of the proteins and guanidine hydrochloride. At the same time, the influence of urea on the dilational surface rheological properties of protein solutions has some peculiarities. In particular, the high values of the dynamic surface elasticity close to equilibrium indicate the limited flexibility of unfolded BLG molecules in the surface layer.

1. Introduction

Proteins alone or in mixtures with surfactants are frequently used as stabilizing agents for foams and emulsions (Damodaran, 2005; Dickinson, 2011; Murray, 2011; Wierenga & Gruppen, 2010). Adsorption layers of these substances are characterized by strong cohesion and stability to external mechanical perturbations. It is generally recognized that these properties are ensured by the network of durable intermolecular bonds arising in the course of protein adsorption (Martin, Cohen Stuart, Bos, & van Vliet, 2005). At the same time, the protein conformations at the liquid–gas interface are known to a less extent. The degree of globular protein unfolding in the surface layer is still a subject of intensive discussion (Maldonado-Valderrama & Patino, 2010; Murray, 2011; Noskov et al., 2009; Noskov, Mikhailovskaya, Lin, Loglio, & Miller, 2010; Perriman, Henderson, Holt, & White, 2007; Wierenga & Gruppen, 2010; Yano et al., 2009). For example, circular dichroism spectroscopy of bovine serum albumin (BSA) adsorption layers indicated the increase of protein disordered structure and a high degree of unfolding of the protein (Damodaran, 2003) while according to the data of Fourier transform infrared reflection absorption (FTIR) spectroscopy the secondary structure of various proteins including BSA changed only a little upon adsorption at the air–solution interface (Lad, Birembaut, Matthew, Frazier, & Green, 2006; Martin, Meinders, Bos, Cohen Stuart, & van Vliet, 2003). If X-ray reflectivity evidenced complete unfolding of the lysozyme globules in the surface layer (Postel, Abillon, & Desbat, 2003; Yano, Uruga, Tanida, Toyokawa, Terada, Takagaki, &Yamada, 2009), the neutron reflectivity led to the opposite conclusions that the globules are intact (Lu, Su, Thomas, Penfold, & Webster, 1998; Perriman, Henderson, Evenhuis, McGillivray, & White, 2008). The disagreement between different authors on the destruction or preservation of the protein globular structure in the course of adsorption is probably caused by a limited number of suitable experimental techniques. The classic methods of surface chemistry are usually not sensitive enough to the conformation of macromolecules in surface layers.

It has been shown recently that the dilational surface rheological properties change strongly when the macromolecules start to form the region of loops and tails in the adsorption layer (distal region of the surface layer) (Noskov, 2010; Noskov, Akentiev, Bilibin, Zorin, & Miller, 2003; Noskov, Loglio, Lin, & Miller, 2006; Noskov, Loglio, & Miller, 2011). The transition from a thin almost two-dimensional adsorption layer to a thicker layer consisting of the proximal and distal regions is accompanied by a strong peak in the kinetic dependency of the dilational dynamic surface elasticity. One can observe this peak also in the case of adsorption of non-globular proteins at the liquid–gas interface (Noskov, Latnikova, Lin, Loglio, & Miller, 2007). On the other hand, the adsorption of protein globules results in monotonic kinetic dependencies of the dynamic surface elasticity (Noskov et al., 2010). This strong distinction between the kinetic dependencies for globular and non-globular

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proteins gives a possibility to study protein unfolding in surface layers under the influence of denaturing agents. Using this approach it was shown that the destruction of the tertiary structure of β-lactoglobulin (BLG) and BSA at a liquid surface occurs at lower concentrations of guanidine hydrochloride (G.HCl) than in the bulk phase (Noskov et al., 2009; Noskov et al., 2010). At the same time, the influence of an anionic surfactant on the dilational surface properties of BLG and BSA solutions shows that the protein can preserve some elements of the secondary structure and probably of the tertiary structure even close to the critical micellar concentration of the surfactant (Mikhailovskaya, Noskov, Lin, Loglio, & Miller, 2011).

In this work the dilational surface rheology is used to study the BSA and BLG unfolding at the solution–air interface under the influence of another frequently used denaturant – urea. This substance unlike G.HCl does not dissociate in aqueous solutions and therefore its interaction with protein globules has some peculiarities (England & Haran, 2011). The protein interactions with urea resulting in the globule unfolding have been studied in numerous papers (Busti, Scarpeci, Gatti, & Delorenzi, 2002; Czarnik-Matusewicz, Kim, & Jung, 2009; D'Alfonso, Collini, & Baldini, 2002; Das, Chitra, Choudhury, & Ramanadham, 2004; Eberini et al., 2011; England & Haran, 2011; Ikeguchi, Nakamura, & Shimizu, 2001; Leggio, Galantini, Konarev, & Pavel, 2009; Tayyab, Sharma, & Khan, 2000; Tobi, Elber, & Thirumalai, 2003) but the details of the interaction still remain to be elucidated. Moreover, it is still unclear whether the denaturant molecules modulate significantly the solvent properties (indirect mechanism of the protein denaturation) or interact with the protein directly (direct mechanism) and what type of interaction (polar, hydrophobic, van der Waals) is the main driving force for the protein denaturation (England & Haran, 2011).

Although BLG and BSA are both globular proteins, their tertiary and secondary structures differ strongly. BLG consists of 162 amino acid residues forming a single domain. Its secondary structure comprises 9 antiparallel β-sheets (50%) and one α-helix (15%). Eight β-sheets form a structure resembling a flattened cone with a hydrophobic cavity – a calix (Brownlow et al., 1997; Wong, Camirand, & Pavlath, 1996). At pH close to 7 BLG forms dimers in aqueous solutions. BSA comprises 573 amino acid residues and the globule of this protein consists of three domains. Each of them can be divided into two subdomains. 67% of the BSA secondary structure are α-helices (Carter & Ho, 1994; Peters, 1985).

2. Materials and method

2.1. Materials

BSA (Sigma–Aldrich) and urea (Sigma–Aldrich) were used as received. BLG (Sigma-Aldrih) was purified by addition of activated charcoal (mass ratio charcoal:BLG was 6:1) according to the method of Clark et al., 1995. The solution was stirred intensively for half an hour and then the charcoal was removed by centrifugation.

Urea (Sigma–Aldrich) was used as received. It does not display any surface activity in aqueous solutions but increases slightly the surface tension at high concentrations, up to about 75 mN/m in 10 M at 20 °C (Siskova, Hejtmankova, & Bartovska, 1985) probably due to the influence on hydrogen bonds in water.

2.2. Preparation of solutions

The BSA and BLG solutions in phosphate buffer at pH 6.7 and 7.0 respectively were prepared by dilution of a stock solution, which had been stored in a refrigerator at 2 °C not longer than one week.

It is known that the change of BSA concentration does not influence the denaturation mechanism under the action of urea (Aswal, Chodankar, Kohlbrecher, Vavrin, & Wagh, 2009; Chodankar, Aswal, Kohlbrecher, Vavrin, & Wagh, 2008). On the other hand, preceding data of our group show that the protein concentration does not influence the shape of the kinetic curves of surface properties at different concentrations of the denaturant (Mikhailovskaya et al., 2011). Therefore all measurements in this work were carried out at a single BSA concentration of 0.03 μM and a single BLG concentration of 0.5 μM. The solution pH was regulated by the addition of NaH_2PO_4 and Na_2HPO_4. The ionic strengths of BSA and BLG solutions were 0.02 and 0.01 M correspondingly. Triple-distilled water was used for the preparation of all the solutions.

2.3. Surface tension and dilation surface elasticity measurements

The surface tension was measured by the Wilhelmy plate method using a roughened glass plate attached to an electronic balance. Measurements of the complex dynamic surface elasticity were executed by the oscillating barrier method at a fixed frequency of 0.1 Hz. The excitation of longitudinal surface waves results in the decrease of the accuracy at higher frequencies. On the other hand, the decrease of the frequency up to 0.01 Hz results only to insignificant changes of the dynamic surface elasticity. The experimental procedure was described in detail elsewhere (Noskov et al., 2003). The oscillations of the solution surface area in a polytetrafluoroethylene (PTFE) Langmuir trough were produced by a movable PTFE barrier sliding along the polished brims of the trough. A mechanical generator transformed the rotation of an electric motor into the translational motion with reversion and gave the possibility to control the oscillation amplitude and frequency. The moving part of the generator was connected to the barrier by a steel rod. The barrier glided back and forth along the Langmuir trough and produced oscillations of the liquid surface area δS with a relative amplitude of 3%. The corresponding oscillations of the surface tension γ were measured by the Wilhelmy plate method. The dynamic surface elasticity ε was determined from the oscillations of the surface tension γ and surface area according to the following relationship

$$\varepsilon(\omega) = \varepsilon_r + i\varepsilon_i = \frac{\delta\gamma}{\delta \ln S} \quad (1)$$

where ε, $\delta\gamma$ and δS are complex quantities.

The elasticity modulus was determined from the ratio of the oscillation amplitudes, while the phase shift between the oscillations of the two measured parameters determined the phase angle of the dynamic surface elasticity. At frequencies less than about 0.2 Hz the length of surface longitudinal waves far exceeds the length of the Langmuir trough, and they did not influence the surface tension oscillations in the trough. However, these oscillations are not completely uniform mainly due to the influence of the trough walls on the liquid flow. To exclude this effect all measurements in this work corresponded to a fixed position of the Wilhelmy plate in the centre of the Langmuir trough allowing a reduction of the relative experimental error.

The imaginary part of the complex dynamic surface elasticity of protein solutions under investigation proved to be much less than the real part. Therefore only the results for the real part are discussed below.

3. Results and discussion

Figs. 1 and 2 show the kinetic dependencies of the surface pressure for BSA/urea and BLG/urea solutions, respectively. The surface pressure of the protein solutions without the denaturant is