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Influence of guanidine hydrochloride and urea on the dynamic surface properties of lysozyme solutions

Mikhail M. Tikhonov, Alexander V. Akentiev and Boris A. Noskov*

Department of Colloid Chemistry, St. Petersburg State University, 198504 St. Petersburg, Russian Federation. E-mail: borisanno@rambler.ru

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Guanidine hydrochloride and urea have different effects on the surface properties of lysozyme solutions due to different interaction mechanisms between the protein and denaturants: an increase in the guanidine hydrochloride concentration leads to noticeable changes in the dynamic surface elasticity, while the main effect of urea consists in a significant drop of the static surface tension.

Protein conformational transitions and partial unfolding at the water/air interface can strongly influence technological processes in food and pharmaceutical industries. A way to induce the unfolding in a bulk phase is the addition of chemical denaturants to protein solution. The aim of this work was to study the destruction of the tertiary structure of a globular protein, lysozyme, in the surface layer under the influence of guanidine hydrochloride (GuHCl) and urea. The complex dilatational dynamic surface elasticity (DSE) ε and surface tension were measured by the oscillating ring method as a function of the surface age and denaturant concentration at pH 7 and a fixed protein concentration of 0.005 mmol dm^{-3}. The periodical oscillations of the surface tension due to the oscillations of the liquid surface inside an oscillating glass ring were determined by the Wilhelmy plate method.[1] The ratio of the oscillation amplitudes of the surface area and surface tension and the phase shift between the oscillations of these two parameters allowed us to calculate ε values. The ellipsometric angle Δ was measured by a null-ellipsometer (Optrel GBR, Germany), and it was approximately proportional to the adsorbed amount Γ.[2]

The kinetics of surface properties of pure lysozyme solutions are characterized by an induction period of ~1 h, as was also observed previously.[3] All these kinetic dependencies of DSE or the dependencies on surface pressure are monotonic and the modulus of DSE reaches a plateau close to an equilibrium (~80 mN m^{-1}) [Figure 1(*a*)]. These data are typical of the solutions of globular proteins,[4–7] and they indicate that lysozyme preserved its globular structure in the surface layer. The increase in GuHCl concentration up to 2 mol dm^{-3} results in a gradual decrease in the induction period and a slight acceleration of the changes in the surface properties due to the growth of the solution ionic strength.[4–6] At the same time, one can observe a slight decrease in the maximum values of $|\varepsilon|$ from ~80 to ~65 mN m^{-1} indicating a slight loosening of the lysozyme globular structure [Figure 1(*a*)]. According to Perriman *et al.*,[8] this GuHCl concentration range corresponds to a change of the globule orientation in the surface layer.

The transition of GuHCl concentration from 2 to 4 mol dm^{-3} results in the entire elimination of the induction period and a dramatic acceleration of changes in the surface properties. One can also observe a local maximum of the kinetic dependencies of DSE and its dependencies on surface pressure [Figure 1(*a*)], which indicates the globule unfolding, at least partial, and can be explained within the framework of the theory of surface dilatational viscoelasticity of polymer solutions.[9] If the affinity of segments to the surface is sufficiently high, the adsorbed macromolecules at the beginning of adsorption have almost flat

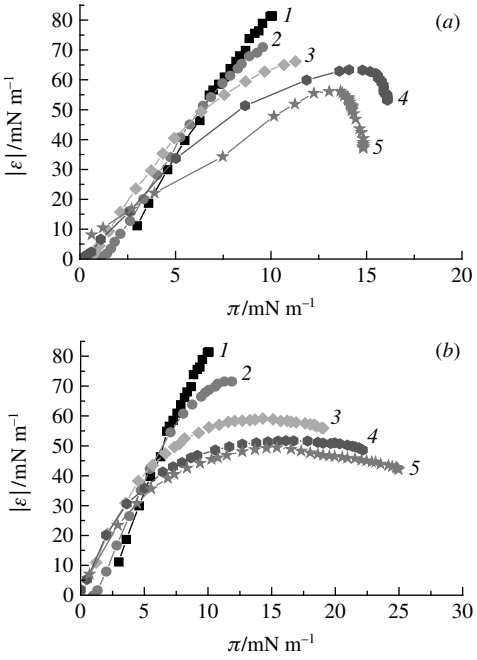

Figure 1 Modulus of the dynamic surface elasticity *vs.* surface pressure: (*a*) for lysozyme/GuHCl solutions at GuHCl concentrations of (*1*) 0, (*2*) 0.5, (*3*) 2, (*4*) 4 and (*5*) 6 mol dm^{-3}; (*b*) for lysozyme/urea solutions at GuHCl concentration of (*1*) 0, (*2*) 2, (*3*) 4, (*4*) 6 and (*5*) 8 mol dm^{-3}.

two-dimensional conformations without long loops and tails. The growth of the adsorbed amount in the course of adsorption leads to stronger repulsion between the segments and, consequently, to the increase in $|\varepsilon|$. Gradually some loops and tails appear and the surface stresses can be relaxed at the expense of the segment exchange between the proximal and distal regions of the surface layer. The dynamic surface elasticity starts to decrease due to the increase in the number of loops and tails and, thereby, goes through a local maximum. Similar dependencies of $|\varepsilon|$ with local maxima are also observed for solutions of non-globular proteins or unfolded globular proteins at high concentrations of different denaturants.[4–7,10]

Figure 1(*a*) shows that the unfolding of lysozyme globules in the surface layer starts at GuHCl concentrations of 2–4 mol dm^{-3}, *i.e.*, at higher concentrations than in the bulk phase where this process occurs at concentrations of ~1 mol dm^{-3}.[8,11] This distinction means that the water/air interface has a stabilizing influence on lysozyme globules unlike the case of bovine serum albumin (BSA) and β-lactoglobulin (BLG) solutions, where the

interface has an opposite effect and the globule destruction occurs at lower concentrations than in the bulk phase.[4–6] Another distinction between lysozyme solutions and the solutions of other globular proteins consists in a slighter decrease of DSE beyond the maximum in the former case when $|\varepsilon|$ diminishes to ~40 mN m^{-1}. Even at GuHCl concentrations > 2 mol dm^{-3}, the conventional model of a concentrated proximal region of the surface layer and a dilute distal region of loops and tails protruding into the bulk phase does not adequately describe the lysozyme adsorption layer. Nevertheless, the neutron reflectivity shows the increase in the layer thickness at GuHCl concentrations higher than 2 mol dm^{-3}.[8] The partial protein unfolding probably results in the formation of short loops, and a segment exchange between the two regions of the surface layer can lead to the relaxation of surface stresses but to a lesser extent than in the case of BSA and BLG. The high stability of lysozyme globules and the formation of a more rigid adsorption layer can be connected with a special stabilizing role of disulfide bonds. In the molecules of other proteins, they connect neighboring amino acid residues and do not limit strongly the flexibility of unfolded macromolecules. In the case of lysozyme, the disulfide bonds between the 6th and 127th and between the 30th and 115th amino acid residues make the globule more rigid[12] and hence do not allow the formation of long loops and tails in the surface layer.

The influence of urea at low concentrations (< 4 mol dm^{-3}) on the dynamic surface properties of lysozyme solutions is similar to the effect of GuHCl [Figure 1(*b*)]. The induction period disappears and the rate of change in the surface properties is noticeably raised with denaturant concentration. The maximal value of $|\varepsilon|$ also diminishes indicating slight changes in the globular structure. The main difference between the surface properties of lysozyme/GuHCl and lysozyme/urea solutions in this concentration range consists in a decrease in the surface tension to lower values in the latter case (Figure 2). The results at higher denaturant concentrations display more differences that are significant. The surface tension of lysozyme/urea solutions changes during the whole time of experiment (250 min), while for lysozyme/GuHCl solutions it reaches a constant value within the error limits in about 30 min after the surface formation. Furthermore, the dependencies of $|\varepsilon|$ *vs.* surface pressure do not display any noticeable local maxima at urea concentrations lower than 8 mol dm^{-3} [Figure 1(*b*)] thereby differing strongly from the data for lysozyme/GuHCl solutions. The decrease in the surface elasticity with urea concentration is probably connected with the softening of the globular structure without its destruction. At the same time, the strong drop of the surface tension at high urea concentrations (Figure 2) implies the growth of the concentration of hydrophobic amino acid residues in the proximal region of the surface layer close to the gas phase. This is possible if the globules are soft,

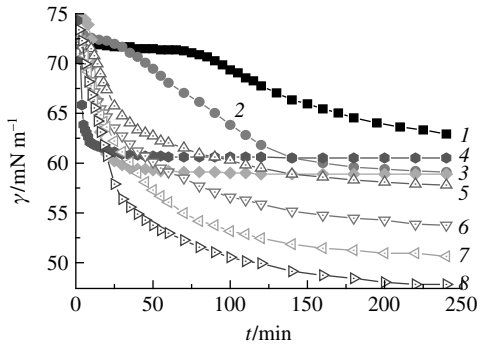

Figure 2 Kinetic curves of the dynamic surface tension of lysozyme/GuHCl solutions at GuHCl concentrations of (*1*) 0.5, (*2*) 2, (*3*) 4 and (*4*) 6 mol dm^{-3} and lysozyme/urea solutions at urea concentrations of (*5*) 2, (*6*) 4, (*7*) 6 and (*8*) 8 mol dm^{-3}.

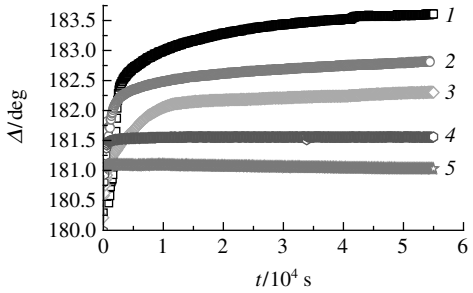

Figure 3 Ellipsometric angle Δ *vs.* time for lysozyme/GuHCl solutions at GuHCl concentrations of (*1*) 0, (*2*) 0.5, (*3*) 2, (*4*) 4 and (*5*) 6 mol dm^{-3}.

the hydrophobic groups are mobile and some of them can move from the interior of the globule to its surface. These features are consistent with the molten globule state of lysozyme molecules, as was assumed by Hedoux *et al.*,[11] who studied lysozyme denaturation in a bulk phase. Raman spectroscopy showed that urea does almost not influence the secondary structure of lysozyme, but it makes the tertiary structure more mobile. GuHCl and urea interact with different groups of protein molecules – hydrophobic in the former case and polar groups in the latter.[11,13] These distinctions result in different denaturation mechanisms and in significantly different effects of these two denaturants on the surface properties of the protein solution. Unlike urea, GuHCl leads to the hardening of lysozyme dynamics.[11] Although the unfolding releases some hydrophobic groups from the globule interior, most of them can interact between themselves and do not move to the boundary with a gas phase. As a result, the surface tension decreases to a lesser extent than in the case of lysozyme/urea solutions.

The kinetic dependencies of the ellipsometric angle Δ corroborate this conclusion. All the dependencies for lysozyme/urea solutions are similar and close to the results for pure protein solutions. These data do not reveal strong changes in the adsorption layer structure due to urea. On the contrary, the addition of GuHCl provides a significant drop of Δ close to equilibrium with the increase in GuHCl concentration (Figure 3).

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