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Effect of Urea on Hydrophobic Side Chain Interactions in Polypeptides at the Air-Water Interface

Akira SHIBATA,* Shinsuke YAMASHITA, and Takuya YAMASHITA
Faculty of Pharmaceutical Sciencs, Tokushima University, Shomachi, Tokushima 770

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Synopsis The surface activity of urea at the polypeptide monolayer–water interface was investigated by the surface pressure analysis. The expansion of the monolayer is attributed to the penetration of polypeptide monolayer by urea in the subsolution. The hydrophobic side chain interactions in polypeptides are weakened by the action of urea. The surface activity of urea depends on the difference in the chemical composition and the geometrical arrangement of side chains between benzyloxycarbonyl derivatives of basic poly(α-amino acid) and benzyl esters of acidic poly(α-amino acid).

In view of the intense current interest concerning the mode of action of denaturants on biological macromolecules, it seems desirable to advance our understanding of this phenomenon by appropriate experimental studies. Considerable evidence exists from model compound studies to the effect that urea (1) interacts strongly with peptide backbone groups, thus weakening interpeptide hydrogen bonds,[1,2] and (2) enhances the solubility of nonpolar groups, thus reducing hydrophobic interactions.[3] It is difficult to assess the relative significance of such effects in studies with natural biopolymers. Thus far synthetic polypeptides as protein analogs have been provided useful informations concerning protein structure and function.

The purpose of this work is to clarify the effect of urea on the hydrophobic side chain interactions in synthetic polypeptides at the air–water interface.

Experimental

Synthetic polypeptides used here were poly(N^{δ}-benzyloxycarbonyl-L-ornithine) (PLO(Z)), poly(N^{ε}-benzyloxycarbonyl-L-lysine) (PLL(Z)), poly(β-benzyl L-aspartate) (PBLA), and poly(γ-benzyl L-glutamate) (PBLG). The samples and spreading solvents were the same as those used in the preceding work.[4] Urea solutions were treated with activated charcoal in order to remove surface active contaminants. A 10 to 1 compression of the surface of the urea subsolutions, after being left to stand for 20 min, produced less than 0.1 mN m^{-1} film pressure. The surface pressure was measured by the Wilhelmy method at 25±0.5 °C. The films were compressed at a rate of 0.01 nm^2 residue^{-1} min^{-1}.

Results and Discussion

Figure 1 shows the π-A curves for PLO(Z) spread on distilled water, and on 1.0, 2.0, 3.0, and 4.0 M (1 M=1 mol dm^{-3}) urea subsolutions. Plateaux are seen in the curves. The plateau which is characteristic of a number of synthetic polypeptide monolayers is associated with the collapse or transition of the monolayer in the α-helical conformation from a two-dimensional oriented state to a three-dimensional disoriented state.[4,5] The surface tension of water increases with an increase in urea concentration.[6] This is due to the desorption of urea from the subsolution surface. Increasing the concentration of urea in the subsolution progressively expanded the condensed regions of PLO(Z) monolayer (Fig. 1). By measuring the change in surface pressure ($\Delta\pi$) due to the change in mole fraction of urea(Δx) in subsolution, the surface concentration of urea was calculated from:[6,7]

$$(1/RT)(\Delta\pi)/(\Delta x)|_A = \Gamma_2/x - \Gamma_1/(x-1) \tag{1}$$
$$= I/x(1-x),$$

where x is the mole fraction of urea, R is 8.3×10^7 ergs per mole, T is the absolute temperature, Γ_1 is the surface concentration of water, Γ_2 is the surface concentration of urea, A is the area per residue of PLO(Z). I is defined by the expression

$$I = (1-x)\Gamma_2 - x\Gamma_1. \tag{2}$$

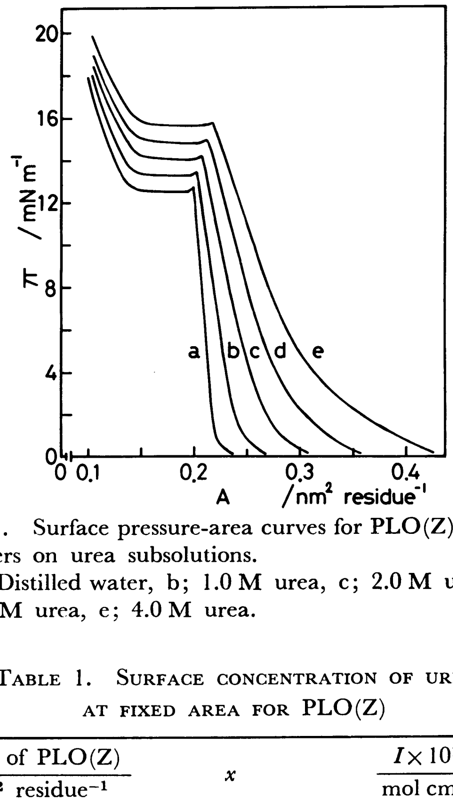

Fig. 1. Surface pressure-area curves for PLO(Z) monolayers on urea subsolutions.
a; Distilled water, b; 1.0 M urea, c; 2.0 M urea, d; 3.0 M urea, e; 4.0 M urea.

TABLE 1. SURFACE CONCENTRATION OF UREA AT FIXED AREA FOR PLO(Z)

Area of PLO(Z) / nm^2 residue^{-1}	x	$I\times 10^{10}$ / mol cm^{-2}
0.23	0.018	1.37
0.23	0.035	2.85
0.23	0.051	3.85
0.23	0.067	4.81
0.25	0.035	2.85
0.25	0.051	3.73
0.25	0.067	4.97
0.27	0.051	3.60
0.27	0.067	4.89

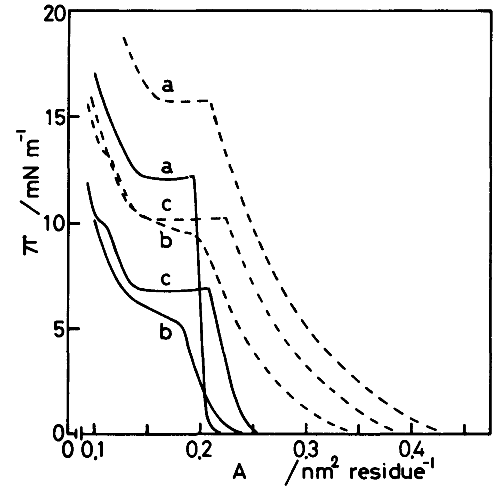

Fig. 2. Surface pressure-area curves for PBLA(a), PBLG(b), and PLL(Z)(c) monolayers on distilled water(——) and 4.0 M urea subsolution(----).

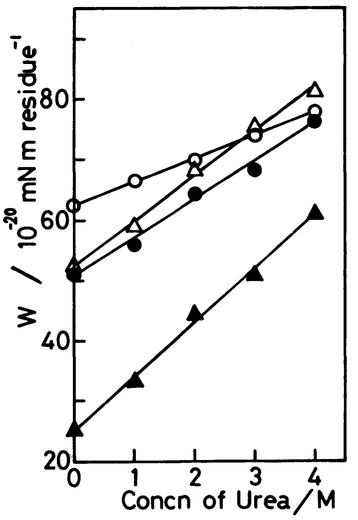

Fig. 3. Work of collapse for PLO(Z)(○), PLL(Z)(●), PBLA(△), and PBLG(▲) monolayers as a function of urea mole fraction.

At the mole fractions of urea used here ($x \leq 0.067$) I is approximately the surface concentration of urea. The results are given in Table 1. When we take the order of magnitude of the effective thickness of the interfacial region to be 10^{-7} cm, for 1, 2, 3, and 4 M bulk concentrations, the concentration of urea in a bulk volume 1 cm×1 cm×10^{-7} cm would be 1×10^{-10}, 2×10^{-10}, 3×10^{-10}, and 4×10^{-10} ml/l, respectively. Therefore, the results indicate that the surface concentration of urea required to provide energy for the observed expansions of the PLO(Z) monolayers is of the same order of magnitude as the bulk concentration of urea (Table 1). There was no surface excess concentration of urea. The film expansion with increase in the concentration of urea can be attributed to the penetration of PLO(Z) monolayer by urea in the subsolution.

Figure 2 shows the π-A curves for PBLA, PBLG, and PLL(Z) spread on distilled water and 4.0 M urea subsolution. The presence of urea brings about the film expansion and the increase in transition pressure for all polymers. The transition pressures were 12.5 mN m^{-1} for PLO(Z), 6.5 mN m^{-1} for PLL(Z), 12.1 mN m^{-1} for PBLA, and 5.3 mN m^{-1} for PBLG, respectively. The addition of one -CH$_2$- group to the β-C is the direct cause of the lowering of the plateau by 6—7 mN m^{-1}. The lowering of the plateau may arise from the reduction of the adhesion of the polymer to the subsolution by direct hydrophobic interaction with the water, because the methylene side group is close to the backbone.[8]

The work required for collapse of monolayers provides an information concerning the effect of urea on the hydrophobic side chain interactions in polypeptides. The work required for collapse of monolayer, W, is given by

$$W = \pi_{\mathrm{tr}}\cdot\Delta A, \qquad (3)$$

where π_{tr} is the transition pressure, and ΔA is the difference between initial and final areas of collapse. Figure 3 shows the work of collapse for the monolayers of PLO(Z), PLL(Z), PBLA, and PBLG as a function of urea concentration. The work of collapse for these polymers increases linearly with an increase in concentration of urea, C. The values of dW/dC($\times10^{-20}$ mN m residue^{-1} M^{-1}) were 3.9 for PLO(Z) 6.0 for PLL(Z), 7.6 for PBLA, and 9.0 for PBLG respectively. The addition of one -CH$_2$- group to the β-C increases the value of dW/dC. This suggests that the hydrophobic side chain interactions in polypeptides are weakened by the action of urea. The obtained values are also larger for benzyl esters of acidic poly(α-amino acid) than for benzyloxycarbonyl derivatives of basic poly(α-amino acid). The surface activity of urea depends on the difference in the chemical composition and geometrical arrangement of side chains between the benzyl esters of acidic poly(α-amino acid) and the benzyloxycarbonyl derivatives of basic poly(α-amino acid).

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