Surface Chemistry of Synthetic Protein Analogues. VI. The Interaction of Urea and Salt with Non-electrolytic Polypeptides

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When protein is spread on the surface of water as a monolayer, it suffers in general surface denaturation. On the other hand, it is also denatured by the interaction with some reagent such as urea. Moreover, it is well known that urea forms adduct compounds[1] with most compounds having a relatively long, straight hydrocarbon chain. The behaviour of the synthetic polypeptides such as poly-DL-α-aminolauric acid etc. which had been investigated as a protein model in the previous experiments,[2] has been studied on the substrate of urea solution by measuring surface pressure-area and surface viscosity-area relations. Meanwhile, the interaction of salt solution on these films of non-electrolytic polypeptides was also investigated. Urea affects the pressure-area relation profoundly, in spite of the absence of -S-S- or -SH groups in these polypeptides, as compared with the protein which contains such groups.

Experimental

The Method and Materials. — The surface pressure was measured by the surface balance of Langmuir-Adam type. The torsion thread used was phosphorbronze wire, 0.12 mm. in diameter. The pressure was measured up to 5 dynes/cm. The surface viscosity was measured by the dumping of the oscillatory rotation of disc. The details of the measurements are essentially the same as those in the previous investigations.[3] The materials were poly-DL-α-aminolauric acid, poly-DL-α-aminocapric acid, poly-DL-α-aminocaprylic acid and poly-γ-benzyl-L-glutamate. The urea used was a commercial product of Toyo Koatsu Kogyo Co., which was used after repeated recrystallization and adsorption by active carbon. Potassium chloride was used as an example of salt in this case. It was a chemical reagent grade and doubly recrystallized.

1) A.E. Smith, *J. Chem. Phys.*, 18, 150 (1950).
2) T. Isemura and K. Hamaguchi, This Bulletin, 25, 40 (1952).
3) T. Isemura and K. Hamaguchi, ibid., 27, 125 (1954).

Experimental Results

The measured surface pressure-area relations are shown in Fig. 1–4. The film expanded considerably on the substrate containing urea. The more urea existed in the substrate, the film the more expanded. The viscosity-area relations on the substrates with or without urea are shown in Fig. 5. The viscosity of the film on the urea containing substrate was rather irregular and the reproducibility of the results was relatively unsatisfactory. The products of pressure and area FA were plotted against pressure F in the low pressure region. Satisfactorily linear relations were obtained as shown in Fig. 6. In each figure, all straight lines intersect FA-axis at a single point.

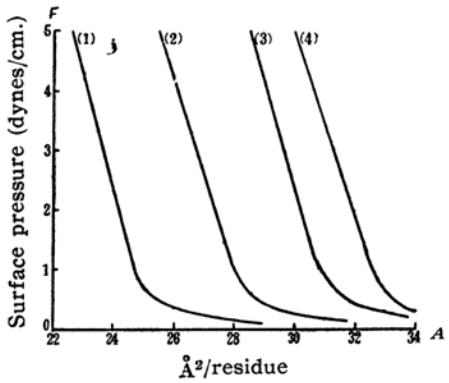


Fig. 1. Surface pressure-area curves of poly DL-α-aminolauric acid. (1) on distilled water; (2) on 10 % urea solution; (3) on 20 % urea solution; (4) on 30 % urea solution.

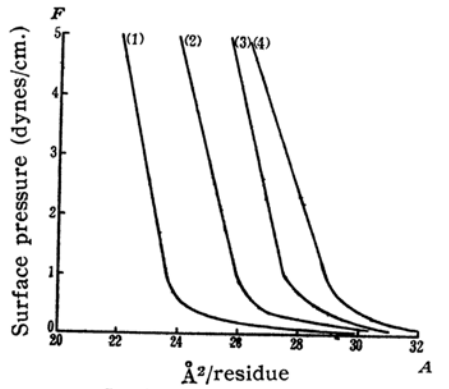


Fig. 2. Surface pressure-area curves of poly DL-α-aminocapric acid. (1) on distilled water; (2) on 10 % urea solution; (3) on 20 % urea solution; (4) on 30 % urea solution.

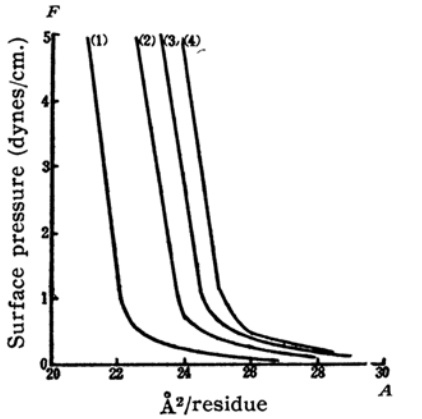


Fig. 3. Surface pressure-area curves of poly DL-α-aminocaprylic acid. (1) on distilled water; (2) on 10 % urea solution; (3) on 20 % urea solution; (4) on 30 % urea solution.

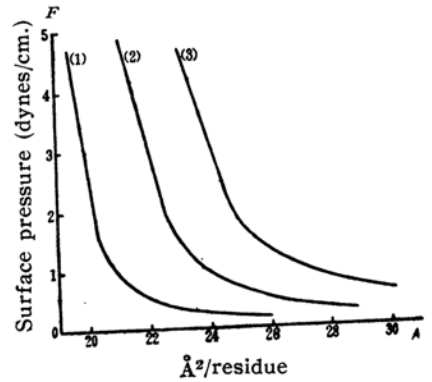


Fig. 4. Surface pressure-area curves of poly γ-benzyl-L-glutamate. (1) on distilled water; (2) on 10 % urea solution; (3) on 30 % urea solution.

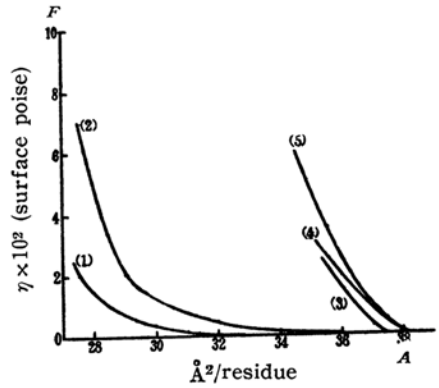


Fig. 5. Surface viscosity-area curves of poly-γ-benzyl-L-glutamate. (1) on distilled water or 0.1 % urea solution; (2) on 0.3 % urea solution; (3) on 0.5 % urea solution; (4) on 5 % urea solution; (5) on 10 % urea solution.

The effect of potassium chloride is shown in Fig. 7. While potassium chloride affects considerably the F–A curve in the case of electrolytic polypeptide as reported in the preceding paper[4] in the concentration as low

4) K. Hamaguchi and T. Isemura, ibid., 28, 9 (1955).

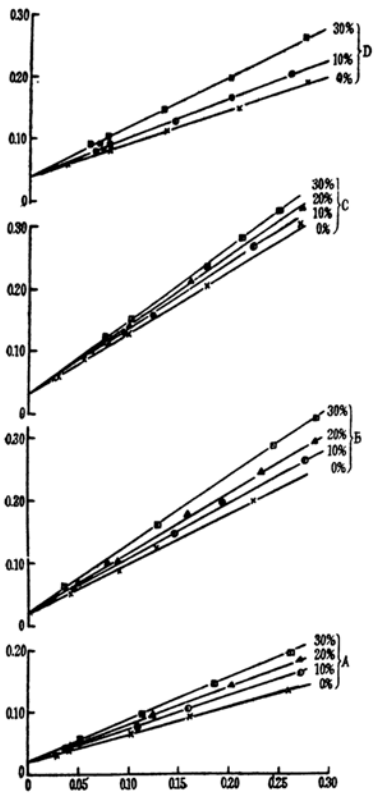


Fig. 6. Plot of FA versus F for poly DL-α-aminolauric acid (A), poly DL-α-aminocapric acid (B), poly DL-α-aminocaprylic acid (C), and poly γ-benzyl-L-glutamate (D).

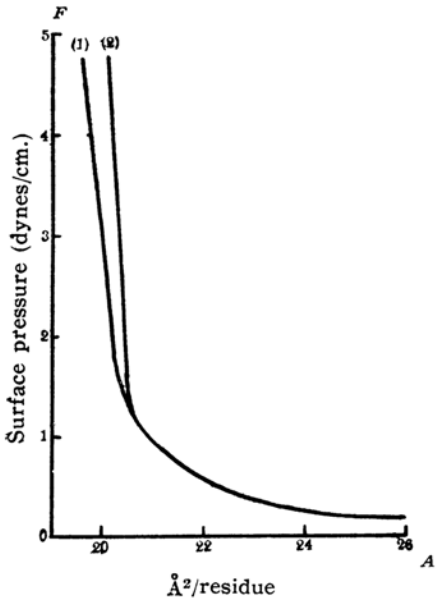


Fig. 7. Surface pressure-area curves of poly γ-benzyl-L-glutamate on distilled water (1) and on 10 % KCL solution (2).

as 0.5 %, it did not affect that of the non-electrolytic polypeptide in the concentration of 1 %. However, the film expanded slightly on 10 % potassium chloride solution.

Discussion

From these experimental results, it might be concluded that the Guastalla's relation[5],

$$FA=\alpha F+\beta$$

holds for the polypeptide films on distilled water as well as on urea solutions. The estimated molecular weights of these polypeptides are shown in Table I.

TABIE I

MOLECULAR WEIGHT DETERMINED ON VARIOUS SUBSTRATES

Substrate sample	Distilled water	10% urea solution	20% urea solution	30% urea solution
Poly-DL-α-amino caprylic acid	74,500	73,500	74,000	74,000
Poly-DL-α-amino capric acid	122,000	111,700	111,700	111,700
Poly-DL-α-amino lauric acid	121,000	122,000	111,500	111,800

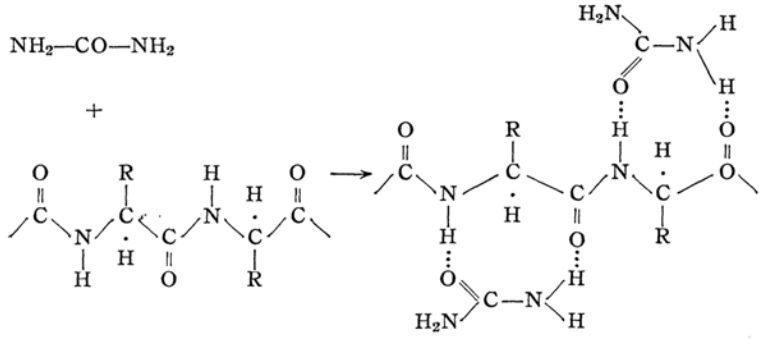
The coincidence of points of intersection made by the FA axis and FA–F lines indicates that the molecule of the polypeptide suffers neither association nor dissociation on the substrate of urea solution. The slopes of these FA–F lines become steeper with the increase of the concentration of urea. As generally accepted, the slopes of these lines correspond to the co-area of the molecule.[5] Therefore, it is very probable that the polypeptide molecule interacts in some way with urea and forms a labile addition compound which occupies a larger area. The interaction was non-stoichiometric because the slope of FA–F lines became steeper when the solution was more concentrated. It does not seem to be caused by the aduct formation, because the expansion of the film was observed on the dilute aqueous urea solution as low as 5 %. This concept is also valid in view of the fact that the film of poly-γ-benzyl-L-glutamate which should not form urea aduct, also expanded on the urea solution, and that the formation of aduct is generally agreed to be difficult with compounds of branched chain or those containing a large group such as benzene in the chain.

If the effect of urea found in the present experiments stands with the urea denaturation of protein, the view that the sulfhydryl groups play a special part in denaturation might be incorrect. Bonding of urea to the

5) J. Guastalla, *Compt. rend.*, 208, 1078 (1938); H.B. Bull, *J. Biol. Chem.*, 185, 27 (1950).

polypeptide linkage replaces the intramolecular hydrogen bondings in protein and leads to the cleavage of salt bridges causing the unfolding of the polypeptide. In our present experiment the polypeptide was unfolded by spreading it as a surface film. Hence, the urea solution as dilute as 5% in concentration will readily interact with polypeptide linkage. The appearance of -SH groups in the protein denatured by the interaction with urea rather means the removal of steric hindrance by neighbouring peptide chains against the reaction with the reagent such as nitroprusside.

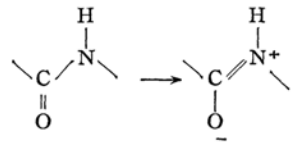
With a given polypeptide sample, all *FA–F* lines obtained with the substrate of different urea content intersect *FA*-axis at a single point as mentioned above. This is a very striking fact, which may be comprehended to be such that the number of polypeptide molecules does not change by the urea interaction. Nevertheless, the co-area of the molecule of film substance increased with the increase of urea concentration in the substrate. It is said that the denaturing action of solution of urea on protein is probably due to the formation of hydrogen bonds with the peptide linkages of the peptide chains[5]. Hence, the expansion of the film might be caused by the bonding of urea to the polypeptide linkage as follows:



Once this bonding was formed, it would be fairly strong. When the substrate water was partially replaced by dropping distilled water of equal volume to that of the through into it at a corner and overflowing the substrate water through syphone from it at its farthest corner without causing any disturbance on the film surface, the film area did not decrease.

A view may be expressed that the expanding effect of urea on the film of polypeptide would be caused by the breaking of the inter- and intrachain hydrogen bonds. However, if that were the case, the number of molecules on substrates of different urea concentrations would have changed and the *FA–A* lines should not intersect *FA* axis at a single point.

It was reported in the previous paper[4] that electrolyte such as potassium chloride exerts a profound influence on the nature of film of amphoteric copolypeptide. However, on the films of non-electrolytic polypeptide as in the present experiment was scarcely detected. On the highly concentrated solution the film non-electrolytic in nature was slightly affected as shown in Fig. 7. The reasons for this expansion of the film might be explained by the formation of rather looser network, by the interaction of polarized backbone as follows.



From the results of the viscosity measurements, the influence of urea was found to be predominant at the concentration more than 0.5 % in the substrate. Although the *F–A* curve tends to shift to the larger area on the more concentrated urea solution, the *η–A* curves on the substrates of concentrated urea solution were independent of urea concentration.

Summary

The interaction of urea and potassium chloride with non-electrolytic polypeptide monolayers was investigated. The film of polypeptide was expanded considerably in the presence of urea in the substrate. The number of molecules was, however, not changed by the interaction with urea. The molecular weights of polypeptides determined by surface pressure measurement on the substrates with

6) F. Haurowitz. "Chemistry and Biology of Proteins," Acad. Press, New York, (1950) p. 130.

or without urea were the same. Whereas the co-area of the molecule was increased by increase of urea concentration in the substrate. Urea might be bound to the peptide linkages in backbone by hydrogen bonding and the formation of aduct seems improbable.

Although potassium chloride affected the nature of electrolytic polypeptide film profoundly, it scarcely affected that of the non-electrolytic polypeptide film. The interaction with urea was discussed in relation to the protein denaturation.

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