Monolayer Studies on Polyanhydro-L-glutamic and Polyanhydro-L-aspartic Acids

By Shoichi IKEDA and Toshizo ISEMURA

(Received August 15, 1960)

When poly-γ-benzyl-L-glutamate is reduced to remove the benzyl group in an anhydrous state, poly-α-L-glutamic acid is obtained1). However, even if poly-β-benzyl-L-aspartate is treated in the same way, no poly-α-L-aspartic acid but only polyanhydro-L-aspartic acid can be obtained2). Berger et al.3) has reported that poly-α-L-aspartic acid is prepared by this method, but Noguchi et al.2) has suggested that the polymer obtained by Berger et al. would be a copolymer of L-aspartic acid with its andydride. Furthermore, Asai et al.4) has shown that polyanhydro-L-glutamic acid can be produced by dehydrating poly-α-L-glutamic acid with acetic anhydride. Polyanhydro-L-glutamic acid may be identified with either poly-L-glutarimide (I) or poly-L-pyrrolidone-2-carboxylic acid (II), and polyanhydro-L-aspartic acid with either poly-L-succinimide (III) or poly-L-propiolactam-2-carboxylic acid (IV). Noguchi et al.2,4) has demonstrated from the

---

1) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, **1950**, 3239; E. R. Blout and M. Idelson, *J. Am. Chem. Soc.*, **78**, 497 (1956); M. Idelson and E. R. Blout, ibid., **80**, 4631 (1958).

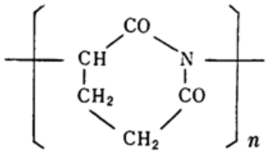
2) J. Noguchi, T. Saito and M. Asai, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 622 (1960).

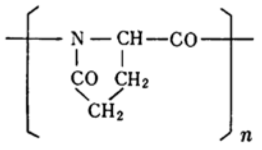
3) M. Frankel and A. Berger, *J. Org. Chem.*, **16**, 1513 (1951); A. Berger and E. Katchalski, *J. Am. Chem. Soc.*, 73, 4084 (1951).

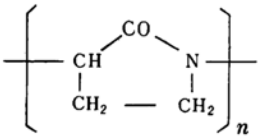
4) M. Asai, K. Imai and J. Noguchi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 624 (1960).

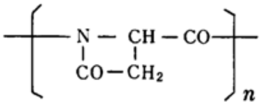
syntheses of appropriate polymers and the comparison of their infrared spectra that polyanhydro-L-glutamic acid is poly-L-glutarimide itself and polyanhydro-L-aspartic acid is poly-L-succinimide.

We have examined the monolayers of polyanhydro-L-glutamic acid, poly-DL-pyrrolidone-2-carboxylic acid, polyanhydro-L-aspartic acid and poly-L-succinimide at air-water interfaces and attempted to obtain further information concerning the structure of the polyanhydro acids. Some of these polymers are interesting in themselves for monolayer studies, since they are composed of chains of rings directly connected with each other and expected to have highly rigid structures.









Experimental

Polyanhydro-L-glutamic acid and poly-DL-pyrrolidone-2-carboxylic acid were prepared by Professor Noguchi and his collaborators[4]. Polyanhyro-L-aspartic acid was prepared by reducing poly-β-benzyl-L-aspartate[5] in glacial acetic acid with anhydrous hydrobromic acid. Poly-L-succinimide was also prepared by Professor Noguchi et al.[2]

Surface pressure, potential and viscosity were measured by the methods as described previously[5]. Surface moment was calculated by the usual Helmholtz equation. Area was expressed in the unit of Å^2 per residue, a residue being assumed to consist of an anhydro acid residue plus a water molecule. The spreading solvent was a mixture of dichloroacetic acid-benzene (1 : 1 v/v) throughout the present investigation.

Results and Discussion

Polyanhydro-L-glutamic Acid.—Two kinds of polymers, i. e., poly-L-glutarimide and poly-L-pyrrolidone-2-carboxylic acid, are possibly assumed as polyanhydro-L-glutamic acid. The former has a structure composed of rings exclusively, each connected directly by a C–N linkage, and is expected to be rigidly folded. The latter has a structure similar to poly-L-proline but is different from poly-L-proline by the presence of a carbonyl group in the pyrrolidone ring and expected to be highly hydrophilic. Poly-DL-pyrrolidone-2-carboxylic acid did not spread on distilled water but gave stable films on aqueous salt solutions, as illustrated in Fig. 1. The films of poly-DL-pyrrolidone-2-carboxylic acid are of condensed type, in contrast to the expanded nature of poly-L-proline film. The reason why the film is condensed is not clear, since an expanded nature of polypeptide monolayer is ascribed to the structure devoid of hydrogen bonding[6] and polypyrrolidone carboxylic acid can include no hydrogen bond as polyproline.

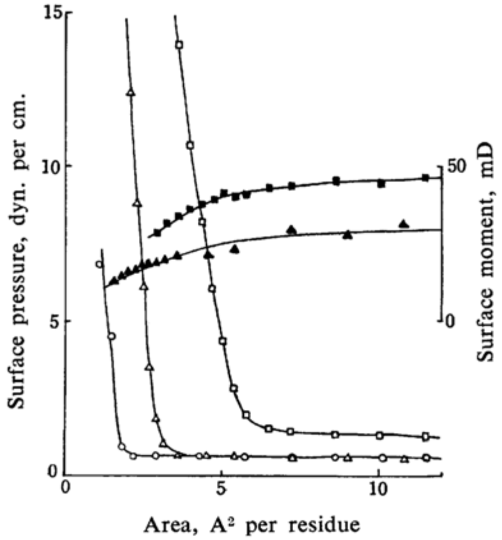


Fig. 1. Plots of surface pressure and surface moment of poly-DL-pyrrolidone-2-carboxylic acid against area (10°C). Aqueous subphases: potassium chloride solutions; ○, 0.01 M; △, ▲, 0.1 M; □, ■, 1 M.

On the other hand, polyanhydro-L-glutamic acid gave a stable monolayer of condensed type on distilled water, as shown in Fig. 2. This already indicates that polyanhydro-L-glutamic acid is not identical with poly-L-pyrrolidone-2-carboxylic acid but is poly-L-glutarimide itself. The limiting areas from surface pressure and surface viscosity were equally 13 A^2, which was smaller than those assigned to a

---

[5] S. Ikeda and T. Isemura, This Bulletin, 34, 416 (1961).

[6] T. Isemura and S. Ikeda, ibid., 32, 178 (1959).

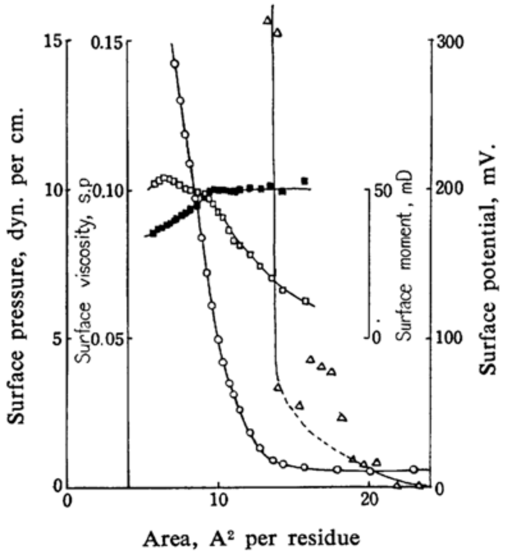


Fig. 2. Monolayer of polyanhydro-L-glutamic acid on distilled water (10°C).
○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

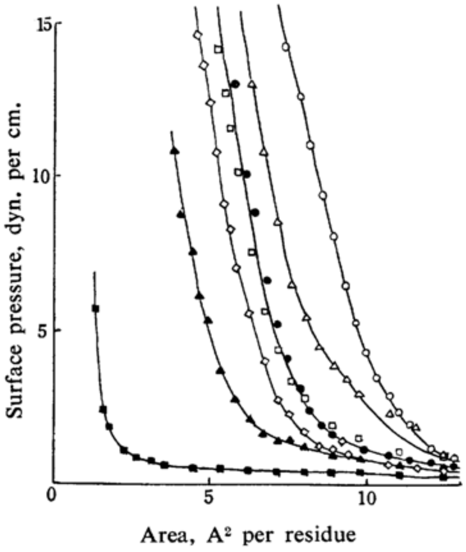


Fig. 3 Variation of surface pressure-area curves of polyanhydro-L-glutamic acid with alkalinity of aqueous subphase (10°C).
○, H_2O; △, 0.01 M, □, 0.1 M; ◇, 0.2 M K_2CO_3; ●, 0.01 M; ▲, 0.02 M; ■, 0.05 M KOH.

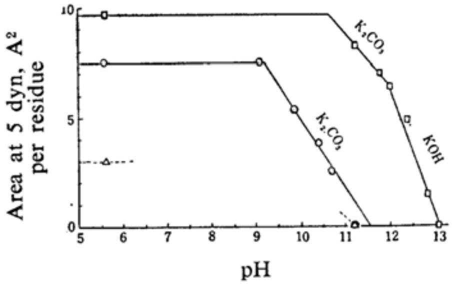


Fig. 4. Plots of area at 5 dyn. per cm. against pH.
□, polyanhydro-L-glutamic acid; ○, poly-L-succinimide; △, polyanhydro-L-aspartic acid.

β-configuration[6,7]. The surface potential was also low and the surface moment was about 50 mD. The low values of limiting area and surface moment also suggest that polyanhydro-L-glutamic acid might be poly-L-glutarimide, which can not be spread on aqueous surface as a monomolecular layer but has a folded configuration in film, as expected from its structure consideration.

The condensed nature of the monolayer of poly-L-glutarimide may be attributed to the rigid structure of this polymer, i. e., to its low configurational entropy. On the contrary, poly-L-proline has a relatively loose configuration in film, since its film is of an expanded type. Thus the pyrrolidine rings would not influence the flexibility of polymer chain in film very profoundly.

As referred to later, the monolayer of polyanhydro-L-glutamic acid behaves very similarly to that of poly-L-succinimide; this also supports the identity of polyanhydro-L-glutamic acid with poly-L-glutarimide. All these are consistent with the results of Asai et al.[4] and Bruckner et al.[8]

Since the ring of poly-L-glutarimide can be cleaved by dilute alkali[8,9], we have examined the monolayers of polyanhydro-L-glutamic acid on alkaline solutions of different pH's. The results on surface pressure-area relations are shown in Fig. 3 and the areas at 5 dyn. per cm. are plotted against the pH in Fig. 4. It can be seen that the film occupies smaller area as the rise of pH at the region of pH higher than 10.5 and no film can be formed at pH 13. This indicates that poly-L-glutamic acid produced by the cleavage of ring of polyanhydro-L-glutamic acid does not cause any expansion of film. When polyanhydro-L-glutamic acid was spread from its alkaline solution, no film was obtained on distilled water and even on 0.01 N hydrochloric acid or on potassium chloride solution. This suggests that the poly-L-glutamic acid thus formed should be composed of both α- and γ-linkages of peptide bonds, because poly-α-L-glutamic acid

---

[7] C. W. N. Cumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**, 235 (1950).

[8] V. Bruckner, J. Kovács and K. Medzihradszky, *Naturwiss.*, **42**, 96 (1955).

[9] V. Bruckner, M. Szekerke and J. Kovács, ibid., **42**, 179 (1955).

can make films on the acid side[10], but poly-γ-L-glutamic acid forms no film on aqueous subphases of various compositions[11]. Although the cupric salt of the poly-L-glutamic acid is insoluble[8], the poly-L-glutamic acid spread from an alkaline solution gave no film on cupric sulfate solution.

Polyanhydro-L-aspartic Acid.—We will proceed to examine the relation of polyanhydro-L-aspartic acid with poly-L-succinimide. The behavior of the monolayer of poly-L-succinimide on distilled water is shown in Fig. 5. The monolayer was of a condensed type and the limiting area determined from both surface pressure and viscosity was about 11 Å^2, and the surface potential was low and the surface moment was 20 to 25 mD. These low values may be attributed to the rigidly folded configuration of this polymer, which is maintained even in the spread state. Since poly-L-succinimide can be converted into poly-L-aspartic acid by dilute alkaline solutions[12-14], as poly-L-glutarimide to poly-L-glutamic acid[8,9], we have examined the monolayer properties on alkaline solutions of different pH's. The results are shown in Figs. 6 and 4. The cleavage of ring occurred at less alkaline pH for poly-L-succinimide than for poly-L-glutarimide; the film spreads less completely as the rise of pH at the region of pH higher than 9.0 and no film can be formed at pH 11. By the same procedures as for poly-L-glutarimide, it was found that the poly-L-aspartic acid thus formed might include both α- and β-linkages. This is in accord with the result of Kovács et al.[14]

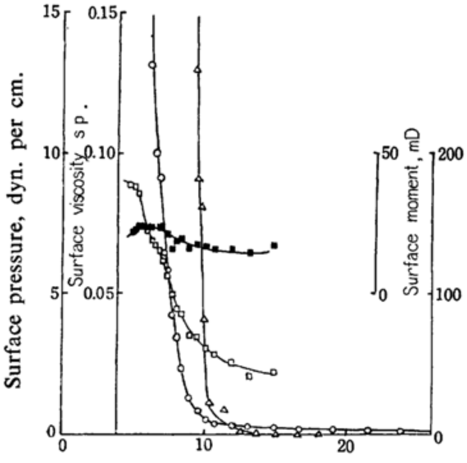


Fig. 5. Monolayer of poly-L-succinimide on distilled water (17°C).
○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

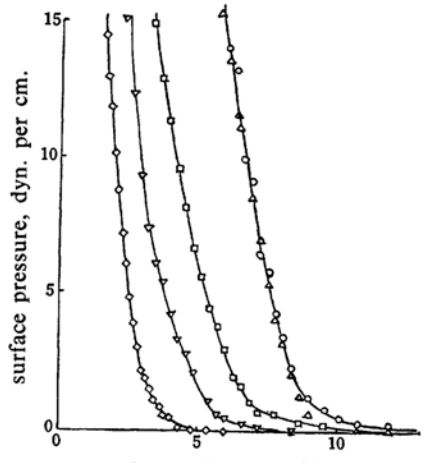


Fig. 6. Variation of surface pressure-area curves of poly-L-succinimide with alkalinity of aqueous subphase (17°C).
○, H_2O; △, 0.0002 M; □, 0.001 M; ▽, 0.003 M; ◇, 0.005 K_2CO_3.

Polyanhydro-L-aspartic acid gave a stable monolayer on distilled water, as shown in Fig. 7. The spreading of this polymer was far less than that of poly-L-succinimide, and it could not be increased by the addition of salt into the aqueous subphase. Except for the

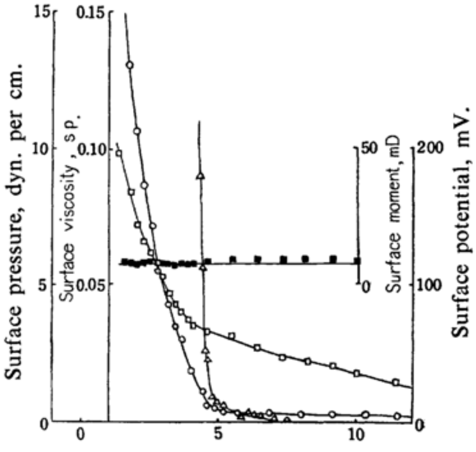


Fig. 7. Monolayer of polyanhydro-L-aspartic acid on distilled water (12°C). ○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

---

[10] T. Isemura and K. Hamaguchi, This Bulletin, 27, 339 (1954).

[11] T. Yamashita, personal communication.

[12] J. Kovács, J. Könyves and J. Császár, *Naturwiss.*, 41, 575 (1954).

[13] A. Vegotsky, K. Harada and S. W. Fox, *J. Am. Chem. Soc.*, 80, 3361 (1958).

[14] J. Kovács, I. Könyves and A. Pustzai, *Experientia*, 9, 459 (1953); J. Kovács and I. Könyves, *Naturwiss.*, 41, 333 (1954).

poorer spreading, however, polyanhydro-L-aspartic acid behaved very similarly to poly-L-succinimide in the monolayer state; e. g., no film was formed on an alkaline solution of pH 11, as shown in Fig. 4. Noguchi et al.[2] has confirmed that polyanhydro-L-aspartic acid is just poly-L-succinimide itself but never poly-L-propiolactam-2-carboxylic acid. Further they have referred to the Berger's polyaspartic acid[3] and concluded that it might be a copolymer of L-succinimide and L-aspartic acid. Our polyanhydro-L-aspartic acid might also be the same kind of copolymer since it occupies smaller area than poly-L-succinimide.

Summary

To examine the structures of polyanhydro-L-glutamic acid and polyanhydro-L-aspartic acid, we have studied their monolayer properties and compared them with those of poly-DL-pyrrolidone-2-carboxylic acid and poly-L-succinimide. It was found that polyanhydro-L-glutamic acid is not identical with polypyrrolidone carboxylic acid but with poly-L-glutarimide, which has a rigidly folded configuration even in monolayer. This conclusion is in agreement with the results obtained by other methods. It was shown, however, that polyanhydro-L-aspartic acid can not be identified with poly-L-succinimide but it might be a copolymer of L-succinimide and L-aspartic acid.

We are grateful to Professor J. Noguchi of Kanazawa University for the gift of the polymers as well as for his guidance in preparing polyanhydro-L-aspartic acid.

Faculty of Science and
Institute for Protein Research
Osaka University
Kita-ku, Osaka