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Conformation of poly(L-glutamic acid) at the air/water interface

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The conformation of the monolayer of poly(L-glutamic acid) on subsolutions of different pH values was studied by the film-balance technique, obtaining surface pressure measurements, together with polarized infrared spectroscopy and Raman spectroscopy. The monolayers of poly(L-glutamic acid) gave different surface pressure-area curves on subsolutions of various pH values. It was found that the conformation of poly(L-glutamic acid) monolayer spread at the air/water interface differs from that in solution. It can be presumed that poly(L-glutamic acid) in a monolayer is in the form of an α-helix at pH 2.0, in the β-form at pH 3.5 and in an 'intramolecular' heterogeneous conformation (consisting of a random coil and an α-helix) at pH 4.0.

The interface often plays an important role in biological systems. Conformational evaluation of proteins and polypeptides at the interface is of great interest in understanding the structure and the function of biological membranes. It has been found that poly(D-glutamic acid) or poly(L-serine) adsorbed from the solution changes into the β-form independent of its structure in solution [1,2]. This suggests that proteins or polypeptides are able to adopt a conformation at the interface different from that in solution.

In this study, the spreading monolayer of poly(L-glutamic acid) was formed on subsolutions of differing pH values below pH 4.0, where poly(L-glutamic acid) is in the α-helix form in aqueous solution. The conformation of poly(L-glutamic acid) at the interface is deduced from film-balance surface pressure measurements, from polarized infrared spectroscopy and from Raman spectroscopy.

Sodium poly(L-glutamate) (M_r 26 000) (Sigma) was dialyzed overnight with two changes of 200 ml of 0.1 M HCl, and lyophilized. The poly(L-glutamic acid) obtained was spread from a solution in 0.01 NaOH/5% (v/v) isopropyl alcohol. The spreading solution was deposited from a mycrosyringe onto the surface of subsolution, and left to stand for 15 min. The surface pressure was measured by the Wilhelmy method. All measurements were carried out at 20°C. The details of the instruments and methods for the measurements of the surface pressure of the monolayers have been described previously [3]. Polarized infrared spectra of collapsed films were measured by a JASCO DS-701G spectrometer. The monolayer was spread and compressed until collapse under the same conditions, as in the case of surface pressure measurement. In order to remove the monolayer, it was compressed between two Teflon barriers, until the separation was about 1.5 cm. The polymer was then removed by drawing a stainless steel net (or silver chloride plate) across the trough between the barriers. The transferred collapsed film was dried at room temperature [3,4]. For Raman spectra (5145 Å Argon

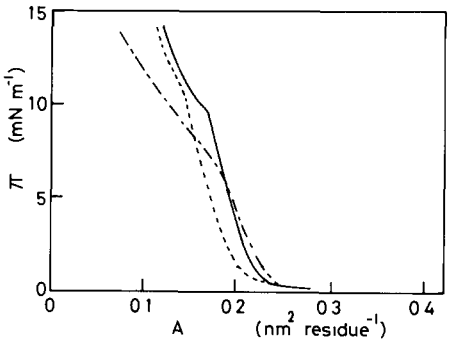

Fig 1. Surface pressure-area curves for poly(L-glutamic acid) monolayers on subsolutions adjusted to pH 2 0 (———), 3 5 (- — - —) and 4 0 (- - - - -)

ion laser, about 450 mW) (JASCO R-800), the film was submerged quickly in 5 ml of the aqueous solution adjusted to pH 2.0, 3.5 or 4.0 after transferring the film onto a 'fine glass tip' from the surface of the subsolution.

The shape of the surface pressure-area (π-A) curves of poly(L-glutamic acid) monolayers depends on the pH of the subsolution. As typical examples, the π-A curves of monolayers on the subsolutions adjusted to pH 2.0, 3.5 and 4.0 are shown in Fig. 1. In general, at a pressure which appears characteristic for polypeptides, a transition is observed which is either an inflection or an almost flat plateau in the π-A curve [4,5]. The inflection which is seen in all the π-A curves is associated with the collapse of the monolayer or the transition from a two-dimensional oriented state to a three-dimensional disoriented state [4,5]. Fig. 2 shows the transition pressure for the poly(L-glutamic acid) monolayer as a function of pH. At first glance it seems possible to assign three

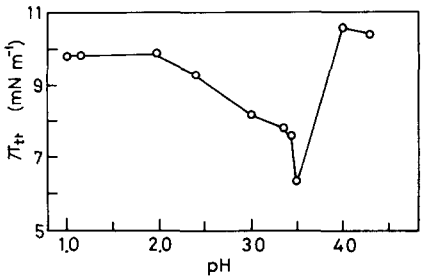

Fig. 2. Transition pressure for poly(L-glutamic acid) monolayer as a function of pH of the subsolution

different values to the transition pressure, namely about 9.8 $mN \cdot m^{-1}$ in the vicinity of pH 1.0 to 2.0, 6.5 $mN \cdot m^{-1}$ at pH 3.5 and 10.8 $mN \cdot m^{-1}$ at pH 4.0. The areas per residue which we obtained by extrapolation of the first steep rise of the π-A curve to zero surface pressure were 0.215 nm^2 at pH 2.0, 0.230 nm^2 at pH 3.5 and 0.195 nm^2 at pH 4.0, respectively (Fig. 1). It is noteworthy that for the spread monolayer there exists a difference in the π-A curves on pH 2.0, 3.5 and 4.0 subsolutions but that poly(L-glutamic acid) adopts a single conformation, the α-helix, in aqueous solution below pH 4.0. This difference could arise from either of the following two possibilities. First, it could represent a difference in the degree of spreading of the poly(L-glutamic acid) monolayers caused by different pH values. Second, it could result from differing poly(L-glutamic acid) conformations such as the α-helical or β-form. If the differences in the π-A curves were due to differing degrees of spreading of the poly-L-glutamic acid) monolayer, the area per residue obtained should follow the change of pH of the subsolution. However, the area decreases in the order pH 3.5 > pH 2.0 > pH 4.0. These areas can, however, be explained by reasonable monolayer conformations [5]. Dioxane is well-known stabilizer of polypeptide helix [6]. Coiled poly(L-glutamic acid) is converted into the helical conformation at high dioxane concentration (above 30% (v/v)) [7]. Fig. 3 shows the π-A curves of poly(L-glutamic acid) monolayers on 10% dioxane aqueous solution adjusted to pH 2.0, 3.5 and 4.0. The π-A curves, which we distinguish from those obtained on the dioxane-free subsolu-

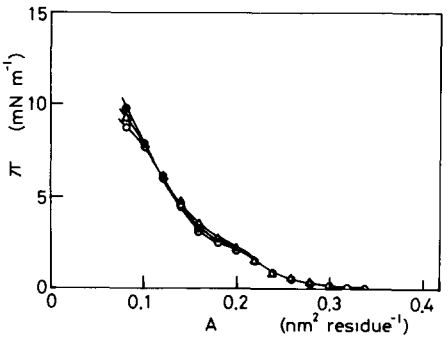

Fig 3 Surface pressure-area curves for poly(L-glutamic acid) monolayers on 10% (v/v) dioxane aqueous solutions adjusted to pH 2 0 (○———○), 3 5 (△———△), and 4 0 (●———●)

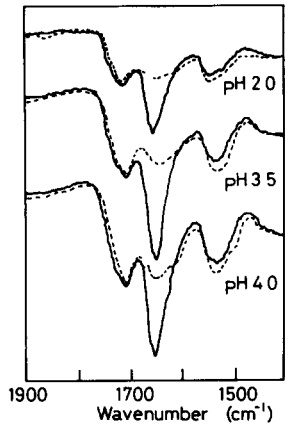

Fig 4 Polarized infrared spectra for collapsed poly(L-glutamic acid) films formed on subsolutions adjusted to pH 2.0, 3.5 and 4.0. ———, Electric vector parallel to the barrier used to collapse the film, - - - - - -, electric vector perpendicular

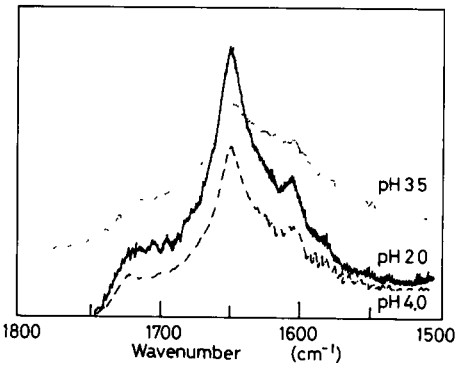

Fig. 5 Raman spectra for collapsed poly(L-glutamic acid) films submerged into aqueous solutions adjusted to pH 2 0, 3 5 and 4.0

tion, are virtually independent of pH. This suggests the existence of a single conformation on 10% dioxane aqueous solution. The effect of dioxane is to solvate poly(L-glutamic acid) monolayer at the air/water interface [8]. This result indirectly indicates that the differences in the π-A curves on the dioxane free subsolutions at different pH values are due to conformational changes of poly(L-glutamic acid) in monolayer (Fig. 1). Fig. 4 shows the polarized infrared spectra for collapsed films of poly(L-glutamic acid) obtained on the subsolutions adjusted to pH 2.0, 3.5 and 4.0. The spectra of these air-dried films were independent of pH. The amide I and II bands (about 1650 cm^{-1} and about 1550 cm^{-1}) indicate that poly(L-glutamic acid) is in α-helical conformation which is the same as that in the solid state. By air-drying, poly(L-glutamic acid) may be converted to an α-helical form because both a β-form and a random coil for poly(L-glutamic acid) are established by the presence of water. Therefore, we tried to obtain conformational information, still in the presence of water, by Raman spectroscopy (Fig. 5). The collapsed films were exposed to water by submerging them in aqueous solution adjusted to pH 2.0, 3.5 and 4.0. In the amide I region, the collapsed films exhibit a Raman band at about 1650 cm^{-1}, in good agreement with the data reported for the α-helix [9]. The view that the α-helix is stable at the air/water interface has been supported [5,10], as has the view that poly(L-glutamic acid) is α-helical in a pH 2.0 aqueous solution [7]. It can therefore be presumed that poly(L-glutamic acid) is in α-helical conformation on a pH 2.0 subsolution.

The shoulder at about 1665 cm^{-1} for the collapsed film at pH 3.5 was associated with the antiparallel-chain β-form (Fig. 5) [9]. Of interest is the fact that at pH 3.5, the β-form is found in the presence of water. Ishii [1] found that poly(D-glutamic acid) adsorbed from solution changes to a β-form in every case independent of the structure in the solution. This suggests that the β-form at the interface is stable. The height of the transition pressure, which can be regarded as a measure of adhesion force of monolayer to water, is lower at pH 3.5 than at pH 2.0 (Figs. 1 and 2). The β-form of the polypeptide precipitates readily in moderately concentrated solution. Nemethy and Scheraga [11] have indicated that the β-form allows extensive hydrophobic interactions. we also have postulated that the β- form of poly(L-glutamic acid) owes a large part of its stability to hydrophobic interactions among intermolecular methylene side-groups in addition to hydrogen bonding. The dichroism shown in Fig. 4 indicates that the horizontally oriented poly(L-glutamic acid) molecules at the interface are aligned in a direction parallel to the barrier used to collapse the film. The improved alignment of poly(L-glutamic acid) molecules in a monolayer might also favor the hydrophobic side-chain interaction in addition to the intermolecular hydrogen bonding, that is, the

formation of the β-form, in a close-packed state. Consequently, we presume the existence of the β-form of poly(L-glutamic acid) on a subsolution of pH 3.5.

It is actually difficult to determine the conformation of poly(L-glutamic acid) on a pH 4.0 subsolution because of the presence of partly charged $-COO^-$ side-groups in poly(L-glutamic acid). 10–15% of the $-COO^-$ groups in poly(L-glutamic acid) become charged at pH 4.0 in aqueous solution. On the other hand, poly(L-glutamic acid) at the air/water interface is charged over 15% because of the higher pH of the surface [12,13]. Such charged $-COO^-$ groups favor the formation of coiled poly(L-glutamic acid) at the interface and also increase the affinity of poly(L-glutamic acid) molecules for water. As shown in Figs. 1 and 2, the film transition pressure has a maximum at pH 4.0. This suggests that a conformation, which is distinct from an α-helix or a β-form, exists in the poly(L-glutamic acid) monolayer on a pH 4.0 subsolution. It thus appears that poly(L-glutamic acid) is in a random coil at pH 4.0. However, the surface pressure for the coiled poly(L-glutamic acid) obtained on a pH 5.5 subsolution was not observed because of the diffusion of the coiled poly(L-glutamic acid) molecules into the subsolution (data not shown). Therefore, the surface pressure observed on a pH 4.0 subsolution may be due to the presence of 'intramolecular' heterogeneous conformation in a monolayer. The area per residue of poly(L-glutamic acid) monolayer is smaller at pH 4.0 than at pH 2.0 and 3.5 (Fig. 1). This can be correlated to the volume per residue of poly(L-glutamic acid) conformation in aqueous solution, namely coil < α-helix [14]. At pH 4.0 poly(L-glutamic acid) can be regarded as being in an 'intramolecular' heterogeneous conformation consisting of a random coil and an α-helix.

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