Sorption of Water Vapor on Poly-L-lysine Hydrobromide

SHOICHI IKEDA, AKIO KITO, TOYOKO IMAE, AND HIROSHI MAEDA

Department of Chemistry, Faculty of Science, Nagoya University, Nagoya, Japan

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Sorption of water vapor on poly-L-lysine hydrobromide in the forms of powder and film is investigated by means of Bull's method. The sorption rate measured with the powder reveals some characteristics indicative of conformational changes occurring during the course of the sorption process. The powder and film of poly-L-lysine hydrobromide obtained by the usual drying procedure are found to contain some amounts of water still left, which can be removed after swelling caused by the sorption of water. The sorption isotherm belongs to type II of Brunauer's classification, and from the effect of temperature on the isotherm the isosteric heat of sorption is determined. By means of infrared and X-ray studies, it is shown that the β-form is the most stable when the polypeptide sorbs a mole of water per residue. Further dehydration destroys the β-form into the random coil, while higher hydration makes it the α-helix.

INTRODUCTION

It was reported more than a decade ago (1) that poly-L-lysine hydrochloride was subject to conformational transitions when its film cast from aqueous solution was exposed to different relative humidities. When an oriented film was regenerated from a concentrated solution, its infrared spectra showed two amide I bands at 1655 and 1625 cm^{-1} with different dichroism. Exposure of the film to relative humidities from 65 to 90% caused the 1655 cm^{-1} band to disappear. While the 1655 cm^{-1} band was attributed to the α′-form of the polypeptide, the 1625 cm^{-1} band was assigned to the β-form. Raising the surrounding humidity beyond 90%, the amide I band appeared at 1646 cm^{-1}, and the conformation was designated as the α″-form. Similar phenomena were observed for hydrobromide and hydroiodide of poly-L-lysine (2). Later work on oriented films of poly-L-lysine hydrochloride (3) showed that the β-form can be converted into the α′-form, when dried over phosphorus pentoxide, and it suggested that the α″-form could be identified with the α-helix, while the α′-form was the random coil.

Oriented fiber of poly-L-lysine hydrochloride was also investigated by X-ray diffraction (4) which similarly indicated that the fiber underwent a conformational change from the β-form to the α-helix upon raising the relative humidity beyond 84%. The X-ray study would detect the conformational change within the crystalline region of the fiber.

We have drawn the sorption isotherm of water vapor on poly-L-lysine hydrobromide, because we have expected that the conformational transitions of the solid described above could be more directly related to the amount of water sorbed, rather than to the relative humidity of the surrounding atmosphere. It was found, however, that the sorption isotherm was dependent on the aggregation state of the polypeptide sample, whether it was in powder or in film, and the conformational stability was further complicated by the dependence on the history of the film. For comparison, we have also done similar work on poly-L-lysine hydrochloride.

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Sorption of water vapor on synthetic polypeptides has not been investigated in detail, but only few data have been accumulated (5–9). Recently, sorption isotherms of water vapor on many polypeptides, including poly-L-lysine hydrobromide, were published by Breuer and Kennerley (10). Their results were, however, restricted to the film state of polypeptides, owing to their application of a newly developed technique, and they were not interested in the polypeptide conformation.

EXPERIMENTAL

Materials. Poly-L-lysine hydrobromide was prepared by the polymerization of ϵ-carbobenzoxy-*N*-carboxy-L-lysine anhydride with sodium methoxide at A/I 200 in dioxane, followed by treatment with hydrogen bromide gas in chloroform-dioxane (11).

Precipitated poly-L-lysine hydrobromide was isolated by passing dry air and evaporating the solvent, and was then washed thoroughly with dry acetone and ethyl ether. The obtained powder was stored over concentrated sulfuric acid in a vacuum desiccator. H1031, $[\eta] = 0.75$ dl/g in 0.2 *M* NaCl at 25°C.

Poly-L-lysine hydrochloride was obtained by dialyzing poly-L-lysine hydrobromide against 0.01 *N* HCl and finally against distilled water (11). It was isolated by addition of acetone and washed with acetone and ethyl ether. H801, $[\eta] = 1.30$ dl/g in 0.2 *M* NaCl at 25°C.

The film of those poly-L-lysine hydrohalides was prepared by drying an aliquot of aqueous solution of the polypeptides on a clean mercury surface. Average thickness of the film was made about 10 μm, adequate for infrared absorption measurements, by adjusting the concentration of spreading solution.

Procedure of sorption experiments. Sorption measurements were performed by a method similar to that of Bull (12). About 100 mg of sample was weighed in a weighing bottle (18 mmϕ × 30 mm), and it was put in a glass container (75 mmϕ × 170 mm) having a screw cap with a rubber gasket, together with about 200 cm^3 of aqueous sulfuric acid solution. An empty weighing bottle was also put in the same container, which could serve as a standard for checking the weighing balance. The container was immersed in a water thermostat at 25, 35, 40, or 45°C, or put in a cold room at 5°C. During the course of sorption, the weighing bottle was intermittently weighed, until equilibrium was demonstrated.

Before and after each run, the concentration of aqueous sulfuric acid solution was determined by alkaline titration. Its concentration did not change more than 3% during the sorption process, which assured the substantially constant relative humidity, P/P_0.

Apparatus. Infrared spectra were recorded on a Jasco DS-402 G spectrophotometer at 25°C from 4000 to 700 cm^{-1}. The polypeptide film was mounted in a brass box with silver chloride windows, specially designed to keep the humidity constant. The X-ray diffraction was recorded on a Rigaku Denki X-ray diffractometer using a Ni filter for Cu$K\alpha$ radiation.

RESULTS

Determination of dry weight. The samples of poly-L-lysine hydrohalide presented some complications in the determination of their dry weight. Before the first run at 25°C, the sample was stored over concentrated sulfuric acid until a constant weight was attained, when a loss of about 2% in weight was observed. The weight of the sample did not decrease further even after it was dried over phosphorus pentoxide for a week, or after it was heated to 80°C *in vacuo* for several hours. The rate curves shown in Fig. 1 were drawn with reference to this sample weight.

When the powder sample was once equilibrated with a humid circumstance and then desorbed over concentrated sulfuric acid, however, a definite loss of weight was observed, amounting to 2–5% as compared with the original dry weight. The amount of loss seemed to depend on the relative humidity once equilibrated with. Even after being kept at a relative humidity as low as 6%, the loss was about 1%. If the sample was once more ex-

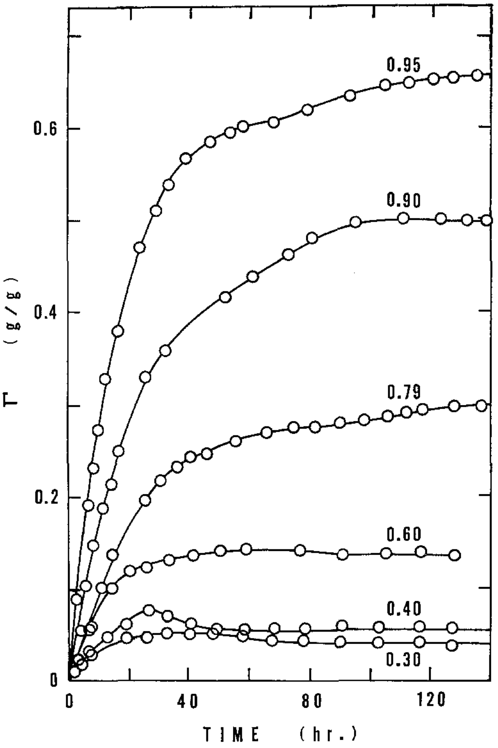

FIG. 1. Sorption rate of water vapor on poly-L-lysine hydrobromide powder at 25°C. Values of relative humidity are indicated.

posed to the same relative humidity at the same temperature, 25°C, the total weight of the sample was found to be always equal to that obtained in the first sorption equilibrium. Thus the loss of the dry weight encountered after the first sorption–desorption cycle can be attributed to the result of liberation of occluded water from the interior of powder such as the ink bottle pores when the powder was swollen by sorption.

Because of this complication, we had to determine the dry weight of the powder, after exposing the original sample to a humid atmosphere, equilibrating with it, and then desorbing. It is not yet certain how high the relative humidity must be raised to drive off all the occluded water from the original dry sample. Then, all the experiments on sorption equilibrium were performed on the samples once equilibrated with humidities higher than about 10% relative humidity. With this pretreatment of the samples, the original dry weight of the powder decreased by about 5% for poly-L-lysine hydrobromide and by about 2% for poly-L-lysine hydrochloride. After the second sorption run, the dry weight remained equal to that obtained after the first run. Similar behavior was observed for films of the two poly-L-lysine hydrohalides.

Sorption rate. Figure 1 shows some of the rate curves of sorption of water vapor on the powder of poly-L-lysine hydrobromide, where Γ denotes the amount of water sorbed. At low humidities from 1 to 40%, the sorption equilibrium was attained after the amount of water sorbed passed a maximum. This is illustrated in the case of 30 and 40% relative humidities in Fig. 1. To confirm that the maximum has not come from the deviation from constant relative humidity during the course of sorption, an experiment was performed by using a saturated solution of magnesium chloride to keep the relative humidity to 33%, and a similar rate

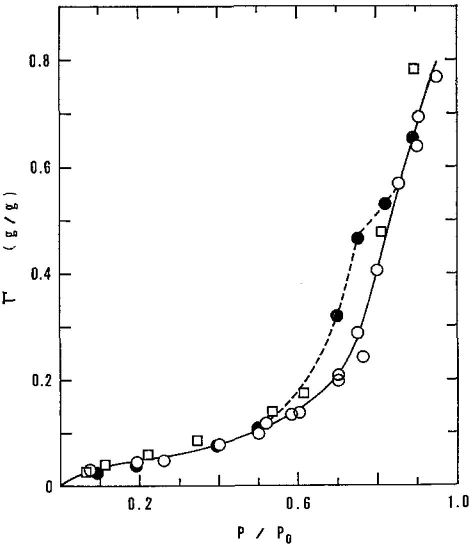

FIG. 2. Sorption isotherm of water vapor on poly-L-lysine hydrobromide powder at 25°C. ○—sample sorbed successively by exposing to increasing relative humidities (including values for two runs); □—sample sorbed at a definite relative humidity (each from the dry state); ●—sample desorbed successively (from 95% relative humidity).

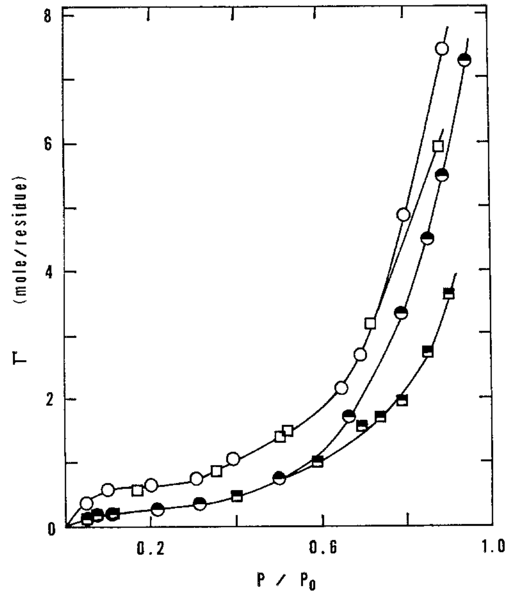

FIG. 3. Sorption isotherm of water vapor on poly-L-lysine hydrohalides at 25°C. Open symbols are for hydrochloride and half-filled symbols for hydrobromide; circles, powder; and squares, film.

curve passing a maximum sorption was observed.

When the relative humidity was 50–60%, the sorption rate approximately followed first-order kinetics. However, the rate process again deviated from first-order kinetics at relative humidities higher than 70%, and the curve indicates that at least two sorption stages are involved.

It was confirmed that a similar sorption rate process occurred with the samples which had been exposed to a humid circumstance and then desorbed.

Sorption isotherm. Figure 2 shows the sorption isotherm of water vapor on poly-L-lysine hydrobromide at 25°C. It can be seen that different courses of sorption to equilibrium do not influence on the equilibrium amount, Γ. Preliminary results on lyophilized samples also indicated that their sorption isotherm was scarcely distinguishable from that presented in Fig. 2. However, the desorption isotherm of a sample exposed to 95% relative humidity was not coincident with the sorption isotherm at relative humidities higher than 50%.

Figure 3 compares the sorption isotherm of poly-L-lysine hydrobromide and hydrochloride, both in powder and film states. The hydrobromide sorbs less water than the hydrochloride over all regions. At high relative humidities, the film state can sorb water with greater difficulty than the powder state.

The isotherms of poly-L-lysine hydrobromide powder at five different temperatures are shown in Fig. 4. The isosteric heat of sorption, Q_{st}, was obtained by means of the Clapeyron–Clausius equation,

$$[\partial \ln P/\partial(1/T)]_{\Gamma} = -Q_{st}/R,$$

where T is the temperature and R is the gas constant. The isosteric heat of sorption is shown (13) to be nearly equal to the differential heat of sorption for the process (14, 15).

$$\text{Polymer (dry at } P = 0) + \Gamma\, H_2O \text{ (at } P_0)$$
$$= \text{Polymer (with } \Gamma\, H_2O \text{ at } P).$$

Figure 5 shows the isosteric heat of water vapor sorption on poly-L-lysine hydrobromide powder plotted against the amount of water

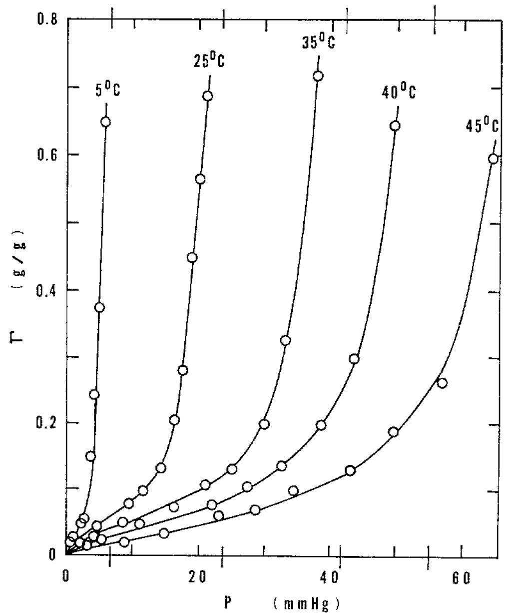

FIG. 4. Sorption isotherm of water vapor on poly-L-lysine hydrobromide powder at different temperatures. Temperatures are indicated. Bars on abscissas represent the values of P_0 at those temperatures.

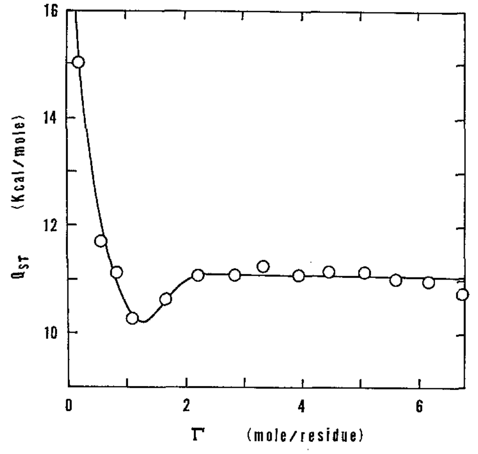

FIG. 5. Heat of sorption as a function of the amount of water sorbed on poly-L-lysine hydrobromide powder

sorbed. At the region where more than 2 moles of water are sorbed per mole of residue, the value of heat of sorption is nearly equal to the heat of liquefaction of water, which suggests that simple condensation of water vapor occurs. At the region of less sorption the heat is generally high and the interaction of water with polypeptide is definitely involved. It is noted that there is an endothermic contribution over the heat of condensation of water when about 1 mole of water is sorbed per mole of residue.

Conformational changes. In order to observe the possible conformational changes, we have made several attempts to detect the conformation by infrared spectra and X-ray diffraction. It was found very difficult to disperse the powder uniformly into nujol or potassium bromide disk, but the infrared spectra of poly-L-lysine hydrobromide powder poorly dispersed in potassium bromide or nujol indicated that the amide I band located at 1655 cm^{-1} as well as at 1626 cm^{-1} at the dry state, while it was at 1648 cm^{-1} at relative humidities higher than 80%. Then we may identify the conformation stable at the dry state as a mixture of the β-form and the random coil (1,3) and the conformation stable at the high humidities as the α-helix (1, 3). We failed to measure the spectra of the powder equilibrated with medium humidities, owing to the difficulty in dispersing it.

The X-ray diffraction was measured on poly-L-lysine hydrobromide powder after equilibration with various humidities. The diffraction pattern was broad, indicating low crystallinity of the samples. At low relative humidities, 0–60%, the spacings at 3.79–3.79, 4.62–4.57, and 8.05–8.10 Å were observed, while at 90% relative humidity the reflection corresponding to 4.6 Å disappeared and the spacings at 3.79, 5.54, 7.13 and 8.35 Å were observed. With reference to the data published by Shmueli and Traub (4) it is clear that the spacings observed at low humidities can be assigned to the β-structure, while those observed at 90% relative humidity would be characteristic of the α-helix. As the random coil form would be amorphous in the polypeptide, it could not necessarily be identified by the X-ray, and only the β-structure would have been detected at the dry state.

Furthermore, we have found that the conformation of poly-L-lysine hydrobromide in the film state is not solely determined by the humidity, but is largely influenced by the way the film was cast. In some film preparations we observed good dependence of the conformation on humidity. That is, at the dry state the amide I band appeared at 1654 and 1626 cm^{-1}, the former being stronger; with increasing humidity the latter band became stronger and was the strongest at 1624 cm^{-1} when the relative humidity was at 60%, both bands having almost equal intensity; at higher humidities the band at 1625 cm^{-1} became weaker again and at 90% relative humidity the amide I band appeared at 1645 cm^{-1}. These observations are in good agreement with those reported (1–3) and it seems clear that the β-form is the most stable at 60% relative humidity or at 1 mole of water sorbed per residue.

We may thus assume that the stable conformation of poly-L-lysine hydrobromide is the β-form at 60% relative humidity, while it is the α-helix at relative humidities of more than

90%. Dehydration of the polypeptide to less than 1 mole of water per residue would sometimes destroy the β-form, making it the disordered or the randomly coiled form. The random coil form must be metastable at the dry state but should be stable at the highly hydrated state or in dilute solutions.

The degree of destruction of the β-form would depend on the method of film regeneration, because in some other film preparations we failed to reproduce results similar to those given above and found that either the 1654 or 1625 cm^{-1} band was very strong and its intensity did not change with humidity. In those films a single conformation, either the random coil or the β-form, had been frozen in when they were prepared. In order to prepare a film with definite properties reproducibly, it would be necessary to control the drying conditions during the period of evaporating water from a spreading solution.

DISCUSSION

As was illustrated in Fig. 2, all the sorption isotherms of water vapor on poly-L-lysine hydrobromide powder of different origins approximately coincided with one another. This suggests that the sorption process occurring in the powder form would not be involved simply on the surface of polypeptide powder but should be regarded as the molecular process. The conformational stability dependent on the humidity also supports this view.

The profiles of the rate process, as shown in Fig. 1, would then be most adequately interpreted as a result of conformational changes occurring more slowly than the sorption. At low relative humidities of less than 40%, a conformation at the dry state, i.e., the metastable random coil, sorbs water rapidly at the first stage, but a conformational change gradually occurs from the random coil to the β-form. Then the β-form must be less hydrated than the random coil form, if both conformations could be equilibrated with the same humidity. The random coil form is first rapidly hydrated and then partly transformed into the β-form of less hydration. Thus a maximum hydration should occur at some stage of the course of sorption. This kind of rate process of sorption has been reported in the case of sorption of trifluoroacetic acid on poly-L-valine (16) and it was attributed to the formation of a more ordered state of order, or possibly, to the conformational change from random coil to the β-form.

The β-form is the most stable at relative humidities from 50 to 60% or at 1 mole of water sorbed per residue. At higher relative humidities, the α-helix is more stable and thus another conformational change occurs from the β-form to the α-helix. The α-helix can be hydrated more than the β-form, if both conformations were exposed to the same humidity. The conformational change would proceed as fast as the sorption rate of the random coil, thus without revealing a maximum sorption on the rate curve.

The isosteric heat of sorption obtained above contains the contributions of those conformational changes. The transition from the random coil to the β-form is endothermic, while the change from the β-form to the α-helix is exothermic. Thus the heat of sorption curve in Fig. 5 exhibits a minimum at the region where the β-form is the most stable. However, no abrupt change such as that reflecting the conformational change is observed on the sorption isotherm itself.

The observed sorption isotherm belongs to type II of the Brunauer's classification but is somewhat close to type III, especially in the case of hydrobromide. The amount of water sorbed is rather low at low humidity regions, while it increases drastically at high humidity regions. For example, on the basis of moles per residue, our data on poly-L-lysine hydrobromide were always lower at low relative humidities than those for globular proteins (12), while they exceeded all of them at high relative humidities. On the other hand, the amount of water sorbed on those poly-L-lysine hydrohalides was more than twice as high as those on nylon (12) and copoly-(glycine, DL-alanine)

(6) even at low humidity regions. Those observations, together with the difference in sorption between the two hydrohalides, suggest that the first site of hydration on poly-L-lysine hydrohalides would be the side chain ammonium or halide ions. It can be seen from Fig. 5 that the first mole or two of water sorbed play important roles in determining the polypeptide conformation. A mole of water sorbed stabilizes the β-form, and its removal would sometimes break the β-form partially. At high humidities, hydration occurs simply as the condensation of water, and thus the capillary condensation in this case would be that at the molecular interstices of the α-helix, finally leading to solution. It is noted that at the region where the capillary condensation takes place a hysteresis loop is observed, as illustrated in Fig. 2.

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