

## ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE LABORATORIES OF PHYSIOLOGIE DES FONCTIONS, FACULTE DES SCIENCES, UNIVERSITE DE PARIS, AND THE CHILDREN'S CANCER RESEARCH FOUNDATION, BOSTON]

Reversible Configurational Changes in Sodium Poly- $\alpha$ ,L-glutamate Induced by Water<sup>1</sup>BY H. LENORMANT, A. BAUDRAS AND E. R. BLOUT<sup>2</sup>

RECEIVED JUNE 11, 1958

Oriented films and aqueous ( $D_2O$ ) solutions of high molecular weight sodium poly- $\alpha$ ,L-glutamate and poly- $\alpha$ ,L-glutamic acid have been examined by means of infrared spectroscopy. It has been shown that oriented films of sodium poly- $\alpha$ ,L-glutamate exist in a  $\beta$ -configuration at relative humidities less than 70% and in an  $\alpha$ -configuration at higher relative humidities. A reversible structural change occurs in this synthetic polypeptide with variations in relative humidity. In aqueous solutions this polypeptide shows three forms: two corresponding to the  $\alpha$ - and  $\beta$ -forms observed in the solid state, the third being assigned to a solvated ( $\sigma$ ) random configuration. Poly- $\alpha$ ,L-glutamic acid exists in both an  $\alpha$ - and a  $\beta$ -form in the solid state, but no reversible interconversion of these forms has been obtained which does not involve complete solution and ionization changes.

The structure of high molecular weight poly- $\alpha$ ,L-glutamic acid<sup>3,4</sup> (hereafter designated PGA) and its sodium salt (hereafter designated SPG) has been studied by means of infrared spectroscopy both in the solid state<sup>4,5</sup> and in solution.<sup>6</sup> Using oriented films it has been shown that the non-ionized acid shows infrared dichroism which is in agreement with that obtained from an  $\alpha$ -helical structure,<sup>7</sup> whereas the sodium salt shows dichroism corresponding to that of a  $\beta$ -structure. These changes in configuration have been shown to be reversible, depending on the state of ionization of the PGA.<sup>4</sup>

Recently we have observed that high molecular weight polymers of poly-L-lysine hydrochloride in the solid state show a reversible structural change which is induced by a simple variation of relative humidity.<sup>8</sup> This has led us to examine whether an analogous phenomenon is produced with PGA and its sodium salt (SPG). Because PGA is soluble in dioxane-water mixtures, we also have examined the effects of vapors of this mixed solution upon solid samples of PGA. Finally, we have studied the deuteration of films of SPG and PGA, and the effect of temperature and pH on their aqueous ( $D_2O$ ) solutions.

## Experimental

The work reported in this paper was performed using a sample of sodium poly- $\alpha$ ,L-glutamate prepared by the method previously described.<sup>3,4</sup> This polymer (#S-2035-186-C) had  $[\eta]$  in 0.2 molar salt at pH 7.3 = 1.88, and had a weight average molecular weight of 80,000. The oriented films were obtained by unidirectional smearing with the aid of a spatula. A concentrated aqueous solution of the SPG was rubbed during the entire time necessary to complete evaporation of the solvent, and the PGA was treated similarly after solution in a 4:1 dioxane-water mixture (by volume). The preparations were mounted in a cylindrical cell (2 cm. in length) closed at its ends by two infrared

transparent end-plates, one of which carries the film under examination. The lateral face of the cell carried a short open tube which may be sealed with a slightly hollowed out stopper. The relative humidity in that enclosure may be controlled by introducing in a cavity of the stopper one or two drops of a suitable salt solution. We have used  $P_2O_5$  in order to obtain humidities lower than 10%, for a relative humidity around 30% we used a saturated solution of  $CaCl_2$ , for a relative humidity around 60% we used a saturated solution of  $NaNO_2$ , and for a relative humidity around 90% a saturated solution of  $Na_2SO_4$  was used. With the same apparatus one is able to expose the preparation to dioxane vapors or to those of heavy water.

A Perkin-Elmer double beam spectrometer (model 21) was used with a salt prism. The polarized infrared radiation was obtained with the aid of a polarizer using six sheets of silver chloride, which was based on the principle described by Makas and Shurcliff.<sup>9</sup>

## Results

**I. Sodium Polyglutamate—Oriented Solid Films. The  $\beta$ -Forms ( $\beta''$ ).**—Sodium poly- $\alpha$ ,L-glutamate (SPG) is oriented with some difficulty and the qualities of the film depend on the conditions under which it has been obtained. When the film is prepared in an atmosphere whose relative humidity is around 70% and when orientation is continued for some time after the crystallization of the material, *i.e.*, until the complete disappearance of water, one obtains a specimen which is strongly birefringent in the visible. If this film is examined in an atmosphere having a relative humidity in the order of 60–70%, the film shows a typical spectrum of a  $\beta$ -polypeptide chain (Fig. 1(A)).

The amide I band appears at 1615  $cm^{-1}$  and shows strong perpendicular dichroism. The amide II band is more difficult to observe because it is seen as a shoulder on the strong band of the ionized carboxyl. However, one is able to estimate the position of its maximum at about 1525  $cm^{-1}$  with parallel dichroism. There is also a small band at 1690  $cm^{-1}$  which shows strong parallel dichroism. This band is always observed with polypeptides in the  $\beta$ -form.<sup>10,11</sup> The ionized carboxyl groups give rise to two bands, one,  $COO^-_a$ , is observed around 1560–1565  $cm^{-1}$  and is very intense; the other,  $COO^-_b$ , is less intense and lies around 1410  $cm^{-1}$ . The dichroism of these bands is feeble, but we have observed without exception in some score of spectra that it actually appears to exist as parallel dichroism in the  $COO^-_a$  band and perpendicular dichroism in the  $COO^-_b$  band. Hereafter we shall term the form which corresponds to the spectrum just described as the  $\beta''$ -form.

**The  $\beta'$ -Form.**—When an oriented film of SPG is placed in an atmosphere having a relative humidity less than 10%, the spectrum remains typically  $\beta$ . However, some minor variations are produced (Fig. 1(B)). The amide I band is displaced to 1625  $cm^{-1}$  (a displacement of approximately 10  $cm^{-1}$ ); the  $COO^-_a$  band is displaced to 1570–1575

(1) This paper is Polypeptides. XXIII. For the previous paper in this series see E. R. Blout and M. Idelson, *THIS JOURNAL*, **80**, 4909 (1958).

(2) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

(3) M. Idelson and E. R. Blout, *THIS JOURNAL*, **80**, 4631 (1958).

(4) E. R. Blout and M. Idelson, *ibid.*, **78**, 497 (1956).

(5) E. M. Ambrose, Appendix to W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(6) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(7) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 205 (1951).

(8) E. R. Blout and H. Lenormant, *Nature*, **179**, 960 (1957).

(9) A. S. Makas and W. A. Shurcliff, *J. Opt. Soc. Am.*, **45**, 998 (1955).

(10) E. R. Blout and A. Asadourian, *THIS JOURNAL*, **78**, 955 (1956).

(11) A. Elliott and B. R. Malcom, *Trans. Faraday Soc.*, **52**, 528 (1956).

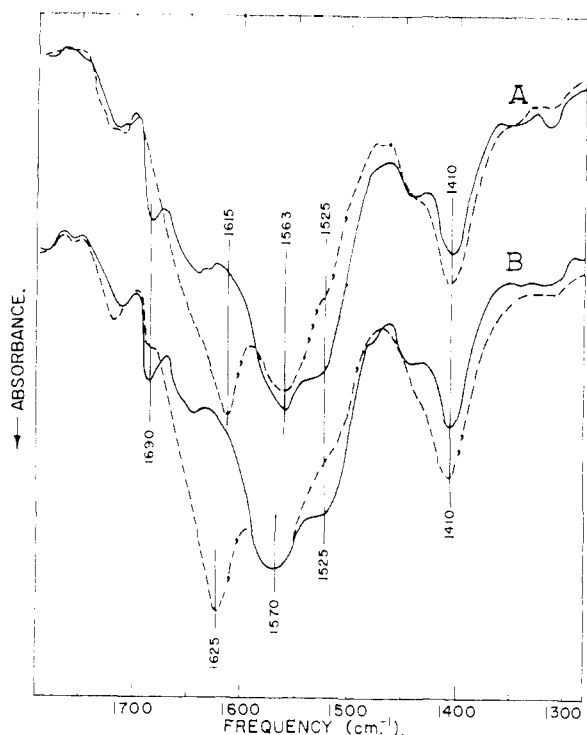


Fig. 1.<sup>a</sup>—The infrared spectra of oriented films of sodium poly- $\alpha$ ,L-glutamate (SPG): A, the  $\beta''$ -form, relative humidity 60%; B, the  $\beta'$ -form, relative humidity <10%.

<sup>a</sup> In all figures: —, electric vibration direction of incident polarized radiation parallel to orientation direction; ----, electric vibration of incident polarized radiation perpendicular to orientation direction.

$\text{cm.}^{-1}$  (a displacement of  $10 \text{ cm.}^{-1}$ ). These displacements, although feeble, are real, having been observed in many spectra. The other bands remain unchanged, and we designated the new spectra as corresponding to a form  $\beta'$ .

**The  $\alpha'$ -Forms ( $\alpha''$ ).**—Upon raising the relative humidity surrounding the oriented preparation of SPG to 90% or more the spectrum is radically transformed (Fig. 2(A)). The amide I band is shifted to  $1635\text{--}1640 \text{ cm.}^{-1}$  and its dichroism becomes parallel. The amide II band, formerly seen at  $1525 \text{ cm.}^{-1}$ , disappears and seems to blend into the  $\text{COO}^-$  band. The dichroism is feebly perpendicular. In the very best spectra, however, one is able to distinguish the amide II band at  $1550 \text{ cm.}^{-1}$  which is seen in the spectral curve obtained with the plane of polarization perpendicular to the direction of the orientation. The curve obtained with the plane of polarization parallel to the direction of orientation shows only the maximum  $1560 \text{ cm.}^{-1}$  corresponding to  $\text{COO}^-$ . The  $\text{COO}^-$  band remains unchanged around  $1408 \text{ cm.}^{-1}$ . The structures corresponding to this spectrum (Fig. 2(A)) will be designated as form  $\alpha''$ .

If this film remains in a very humid atmosphere for a long time, more or less disorientation results. If, however, before the disorientation is produced, the film is replaced in a dry atmosphere, the spectrum shows the characteristic  $\beta$ -bands, occasionally with noticeable disorientation, but often very good orientation and strong dichroism are conserved.

**The  $\alpha'$ -Form.**—When a film of SPG is prepared in an atmosphere having a relative humidity between 30–50%, one obtains specimens with little visible birefringence. The spectra of these films are obviously hybrid, part  $\beta$  and part  $\alpha$ . The characteristic bands of the  $\beta$ -part are fairly highly dichroic; those of the  $\alpha$ -part do not appear as dichroic. Also one notes that the position of the amide I band in this form is at  $1645\text{--}1650 \text{ cm.}^{-1}$ , that is, at a frequency slightly higher than the amide I band of the  $\alpha''$ -form described above. We have been able to obtain in a pure state this new form, which we call  $\alpha'$ , under the following conditions: The solution is rubbed in an atmosphere of relative humidity less than 50%. Just before crystallization, the SPG is

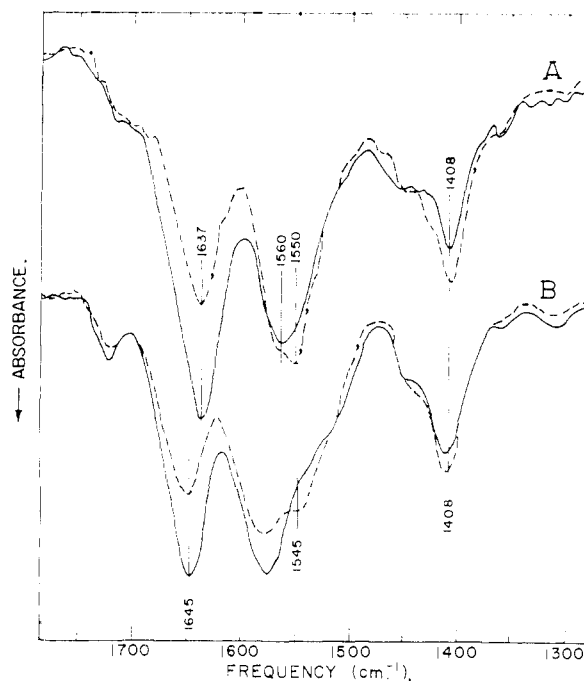


Fig. 2.—The infrared spectra of oriented films of sodium poly- $\alpha$ ,L-glutamate (SPG): A, the  $\alpha''$ -form, relative humidity 90%; B, the  $\alpha'$ -form, film obtained by precipitation with alcohol and measured at relative humidity <10%.

quickly precipitated by the addition of a large amount of absolute alcohol. The alcohol is drained off rapidly and evaporated in a current of warm, dry air. The films so obtained are highly birefringent in the visible and remain stable in a very dry atmosphere (rel. hum. <10%). When studied in that state, the spectrum in Fig. 2(B) is observed: an amide I band at  $1645\text{--}1650 \text{ cm.}^{-1}$  with parallel dichroism, an amide II band easily visible at  $1545\text{--}1550 \text{ cm.}^{-1}$  in the form of a shoulder (with perpendicular dichroism) on the strong  $\text{COO}^-$  band which lies at  $1570 \text{ cm.}^{-1}$ . The  $\text{COO}^-$  band is situated around  $1408 \text{ cm.}^{-1}$ .

When the film is placed in a very humid atmosphere (rel. hum. >90%), the amide I band is displaced slightly and appears at  $1635\text{--}1640 \text{ cm.}^{-1}$ , and the  $\text{COO}^-$  band is seen at  $1560 \text{ cm.}^{-1}$  (cf. Fig. 2(A)). If then the relative humidity is lowered to 30–70%, the film changes toward the  $\beta$ -form. In this case, then, it is impossible to obtain again the amide I band at  $1645\text{--}1650 \text{ cm.}^{-1}$ , but one easily obtains the reversible transformation mentioned above (Fig. 2(A)  $\rightleftharpoons$  Fig. 1(B)) as a function of the relative humidity.

**II. Polyglutamic Acid—Oriented Solid Films. The  $\alpha$ -Form.**—Poly- $\alpha$ ,L-glutamic acid (PGA) when oriented in the solid state from a solution of the polymer in dioxane–water mixture (4:1) shows, as has been previously described,<sup>4</sup> a typical  $\alpha$ -polypeptide spectrum (Fig. 3(A)). The amide I band is at  $1645\text{--}1650 \text{ cm.}^{-1}$  with quite strong parallel dichroism. The amide II band is observed at  $1550 \text{ cm.}^{-1}$  and shows strong perpendicular dichroism. It should be noted, however, that the  $\alpha$ -form is not absolutely pure; an indication of the bands of the  $\beta$ -form is seen. A non-dichroic  $\text{C=O}$  band, due to the non-ionized carboxyl, is observed around  $1705 \text{ cm.}^{-1}$ . This spectrum does not change after equilibration of the sample with various relative humidities ranging from less than 10% to greater than 90%.

If the film is exposed to vapors of pure dioxane or dioxane–water mixture (4:1), the bands associated with the peptide bonds remain at the same frequencies and show the same dichroism. On the other hand, the bands of the non-ionized carboxyl change from  $1705$  to  $1725 \text{ cm.}^{-1}$ . This change is not immediate, taking about three to five hours to occur, and is totally and rapidly reversible upon the evaporation of the dioxane.

**The  $\beta$ -Form.**—One is able to obtain an oriented film of non-ionized PGA in the  $\beta$ -form by the following experimental artifice: An oriented film of SPG, showing the typical  $\beta'$ -

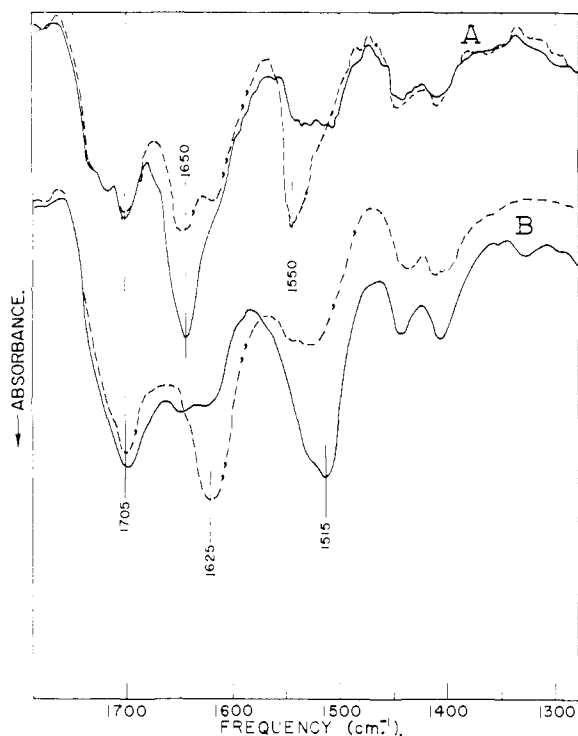


Fig. 3.—The infrared spectra of oriented films of poly- $\alpha$ ,L-glutamic acid (PGA): A, the  $\alpha$ -form; B, the  $\beta$ -form.

spectrum when completely dry, is exposed to gaseous dry HCl. During this period the ionization is lost without change of structure. At the same time the film becomes completely insoluble in water and may be washed with water without producing any disorientation. The two bands,  $\text{COO}^-$ , and  $\text{COO}^-$ , disappear and at the same time a strong band around  $1700\text{ cm}^{-1}$  appears which corresponds to a non-ionized carboxyl. The spectrum (Fig. 3(B)) also shows the following bands: the amide I band is at  $1625\text{ cm}^{-1}$  with very strong perpendicular dichroism. The amide II band is much more easily seen than in the case of the sodium salt; its maximum is at  $1515\text{ cm}^{-1}$  and shows strong parallel dichroism. When this film is exposed to various humidities between 10 and 100%, no other spectral modifications are produced. However, when such a film is exposed for a sufficiently long time (18 hours) to the vapors of a mixture of dioxane-water (4:1), a  $\beta \rightarrow \alpha$  transformation is produced, although it is incomplete and does not appear to go to more than 60–70% of completion. At the same time the  $1705\text{ cm}^{-1}$  carboxyl band shifts to  $1720\text{ cm}^{-1}$ , but, as noted previously, returns to  $1705\text{ cm}^{-1}$  when the dioxane is removed.

**III. Deuteration Studies on Oriented Solid Films of SPG and PGA.**—We have observed<sup>12</sup> that in  $\text{D}_2\text{O}$  solution SPG is instantly deuterated, while the PGA requires a long time (greater than 90 hours) to effect deuteration of the amide NH group. The same difference in behavior is found with these materials in the solid state exposed to the  $\text{D}_2\text{O}$  vapors. Orientated films of PGA exposed to relative humidities of 92% in  $\text{D}_2\text{O}$  exchange their amide hydrogens very slowly and very incompletely—the deuteration being less than 50% after 100 hours of exposure. This is true whether the free acid is in the  $\alpha$ - or  $\beta$ -form in the oriented film.

Conversely, SPG exchanges with deuterium very rapidly. An oriented film in the  $\beta$ -form exposed to a relative humidity of 30% in  $\text{D}_2\text{O}$  is deuterated to about 70% in two hours. The same film exposed to a relative humidity of 92% in  $\text{D}_2\text{O}$  is deuterated totally in one-half to one hour, in which time it changes in structure to  $\alpha$ . The exchange in the inverse sense, that is, from the deuterated state to the non-deuterated state, is also very rapid for SPG and very slow for PGA. In the latter case it has been possible to examine the deu-

terated material spectroscopically by working in the following way: PGA is conveniently deuterated<sup>13</sup> and dissolved in a mixture of dioxane: $\text{D}_2\text{O}$  (4:1). The oriented film is prepared by evaporation of the solution in air and the spectroscopic data may be obtained without any protection against back exchange with the water in the air. For many hours the deuteration of the material remains complete. Oriented films of deuterated PGA prepared as described above show spectra like Fig. 4(A). There is a non-dichroic, non-ionized COOD band at  $1700\text{ cm}^{-1}$ , and an amide I band at  $1640\text{ cm}^{-1}$  with strong parallel dichroism, and an amide II band at  $1440\text{ cm}^{-1}$  with perpendicular dichroism. These latter two bands are characteristic of deuterated polypeptides in the  $\alpha$ -form.

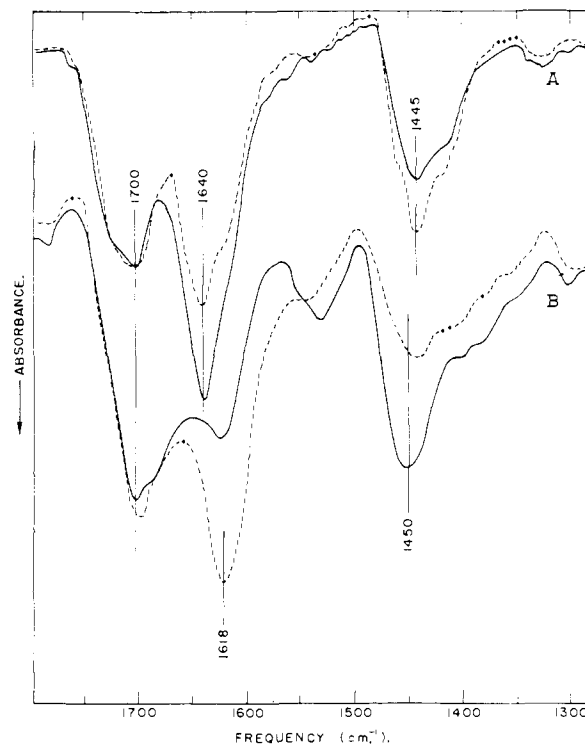


Fig. 4.—The infrared spectra of oriented films of deuterated poly- $\alpha$ ,L-glutamic acid: A, the  $\alpha$ -form; B, the  $\beta$ -form.

An oriented film of deuterated PGA in the  $\beta$ -form may be obtained easily by treatment of a film of SPG (in the  $\beta$ -form) with the vapors of dry DCl. The absorption frequencies observed then are: non-ionized COOD at  $1700\text{ cm}^{-1}$  (non-dichroic), amide I about  $1620\text{ cm}^{-1}$  with strong perpendicular dichroism, and amide II at  $1445\text{--}1450\text{ cm}^{-1}$  with strong parallel dichroism (Fig. 4(B)). Compared with hydrogenic material, the deuterated PGA is differentiated by a slight shift of the amide I band (approximately 5 to 10  $\text{cm}^{-1}$  for both  $\alpha$ - and  $\beta$ -form) and by the position of the amide II band near  $1445\text{--}1450\text{ cm}^{-1}$ .

Deuterated SPG exposed to a relative humidity lower than 70% in  $\text{D}_2\text{O}$  shows a typical  $\beta$ -spectrum with slight differences depending on whether the humidity is less than 10% or higher than 30%. The spectra are shown in Fig. 5 (A and B) and a tabulation of the bands is seen in Table II.

When this film is exposed to a relative humidity of 90% in  $\text{D}_2\text{O}$ , the absorption curve undergoes the following important and curious changes. In a first step of transformation (Fig. 6(A)), the amide I band shows a completely reversed dichroism which becomes strongly parallel, but the maximum measured on the parallel curve remains at a relatively low frequency (about  $1625\text{ cm}^{-1}$ ). The corresponding maximum on the perpendicular curve is at a different

(13) In order to obtain deuterated PGA quickly, one dissolves SPG in  $\text{D}_2\text{O}$  then precipitates the free acid by lowering the pH with DCl. After washing with  $\text{D}_2\text{O}$ , in order to eliminate the sodium chloride formed, the deuterated PGA is dissolved in dioxane- $\text{D}_2\text{O}$ .

(12) E. R. Blout and A. A. Ferguson, to be published.

frequency (1640–1645  $\text{cm}^{-1}$ ). In fact there seem to exist two contiguous bands at 1625 and 1640  $\text{cm}^{-1}$  of which the parallel dichroism is maximum for the 1625 band. The amide II band at 1445  $\text{cm}^{-1}$  decreases strongly and its dichroism becomes slightly perpendicular.

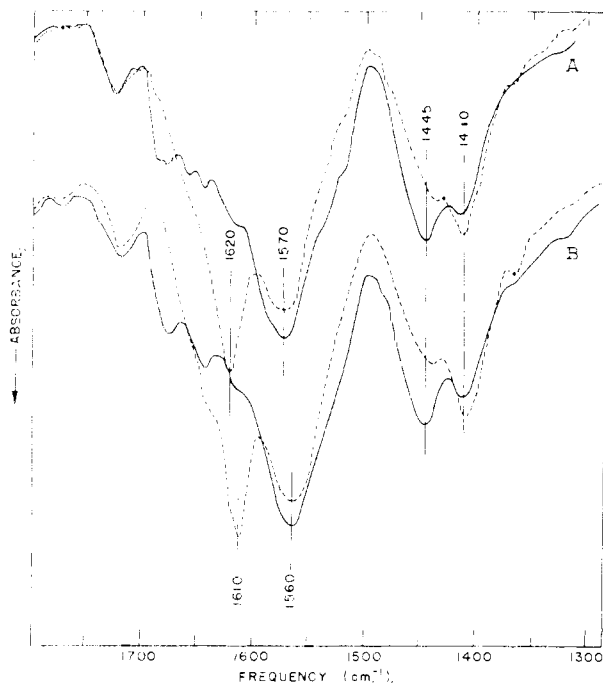


Fig. 5.—The infrared spectra of oriented films of deuterated sodium poly- $\alpha$ ,L-glutamate: A, the  $\beta'$ -form, relative humidity  $<10\%$ ; B, the  $\beta''$ -form, relative humidity  $\sim 66\%$ .

If the exposure at high humidity (90%) in  $\text{D}_2\text{O}$  is maintained during a long time (10 hours), a new transformation takes place. The amide I maximum of the parallel curve shifts at 1640–1645  $\text{cm}^{-1}$  and now coincides with the one of perpendicular curve (Fig. 6(B)). At the same time the dichroism progressively disappears. The same frequency—but dichroic—can be obtained when a very strong gel of SPG in  $\text{D}_2\text{O}$  is rubbed between two slides until crystallization (Fig. 6(C)). The dichroism of the amide I is parallel.

IV. Studies of SPG and PGA in  $\text{D}_2\text{O}$  Solutions.—At  $\text{pH}$ 's higher than 5.2 solutions of the partial or complete sodium salt of PGA in  $\text{D}_2\text{O}$  show a characteristic amide I band between 1640 and 1645  $\text{cm}^{-1}$ . The position of this band shifts to higher frequencies (as high as 1660  $\text{cm}^{-1}$ ) when the temperature of the solution is raised from 25 to 95°. Upon cooling the heated solution to 25°, the band reverts to its normal position between 1640 and 1645  $\text{cm}^{-1}$ . It appears that the new band, around 1660  $\text{cm}^{-1}$ , is characteristic of a deuterated solvated carbonyl amide group, similar to the solvated band ( $\sigma$ ) previously described.<sup>8,10</sup> If the  $\text{pD}$  of the  $\text{D}_2\text{O}$  solution is lowered below 5, the 1660  $\text{cm}^{-1}$  band does not appear upon heating, but a new band appears in the region 1610 to 1615  $\text{cm}^{-1}$ . For example, solutions at  $\text{pD}$  4.6 (with 2 molar salt) show only a single amide I band at temperatures up to 50°. At 67° a second band appears at 1615  $\text{cm}^{-1}$  with a shoulder around 1640  $\text{cm}^{-1}$ . Heating this solution to 95° results in the disappearance of the amide I band around 1640 and an increase in intensity of the amide I band at 1615  $\text{cm}^{-1}$ . Concurrent with this appearance of the 1615  $\text{cm}^{-1}$  band the solution gels. Cooling the solution to 25° results in the partial or complete reappearance of the original band around 1640  $\text{cm}^{-1}$ . If the  $\text{pD}$  of the solution is lowered still further, a gel results and the 1615  $\text{cm}^{-1}$  band appears even at 25°, along with the shoulder at 1640  $\text{cm}^{-1}$ . Heating these solutions results in the disappearance of the 1640  $\text{cm}^{-1}$  band, and upon cooling to 25° the 1640  $\text{cm}^{-1}$  band does not reappear.

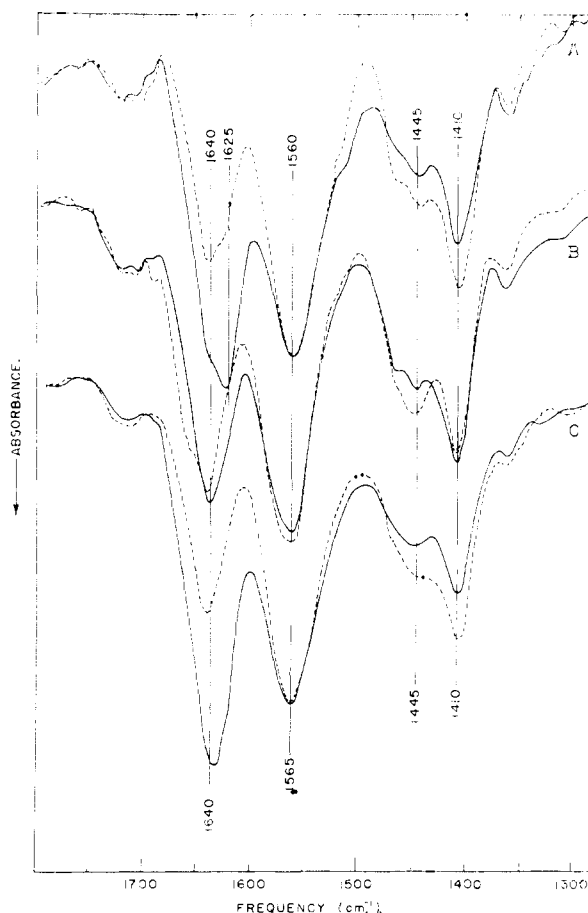


Fig. 6.—The infrared spectra of oriented films of deuterated sodium poly- $\alpha$ ,L-glutamate: A, after 5 hours at 90% relative humidity; B, after 12 hours at 90% relative humidity; C, a gel oriented between two slides.

### Discussion

The experiments which we have reported above show clearly that upon variation of the ionization or the hydration of oriented films of poly- $\alpha$ ,L-glutamic acid, this polypeptide is obtained in several structures. From a purely spectral point of view, we may distinguish the following four different forms of sodium poly- $\alpha$ ,L-glutamate in the solid state (Table I).

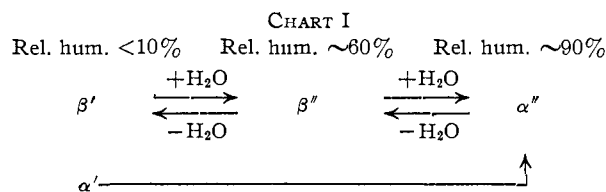
TABLE I  
INFRARED SPECTRAL BANDS OBSERVED WITH SODIUM POLY- $\alpha$ ,L-GLUTAMATE (SPG) IN THE SOLID STATE

Form	Amide I <sup>a</sup>	Amide II <sup>a</sup>	COO <sup>-</sup> <sub>a</sub>	COO <sup>-</sup> <sub>b</sub>	Relative humidity %
$\beta'$	1625 (L)	1525 (  )	1570–1575	1408	$<10$
$\beta''$	1615 (L)	1525 (  )	1560–1565	1408	30–70
$\alpha'$	1645–1650 (  )	1550 (L)	1570–1575	1410	$<10$
$\alpha''$	1635–1640 ( )	1550 (L)	1560	1410	$\geq 90$

<sup>a</sup> The dichroism is noted in parentheses.

It is apparent that the  $\alpha''$ - and  $\beta''$ -forms are only observed at higher humidities (rel. hum.  $>30\%$ ), while the  $\alpha'$ - and  $\beta'$ -forms exist only at very low humidities (rel. hum.  $<10\%$ ). It is thus seen that the  $\alpha'$ - and  $\alpha''$ -forms correspond to the dehydrated and hydrated forms of the same  $\alpha$ -structure;

likewise the  $\beta'$ -form corresponds to a hydrated  $\beta$ -structure and the  $\beta''$ -form to a dehydrated  $\beta$ -structure. Chart I indicates the interconversions of these forms under the influence of various relative humidities.



The important point is that at relative humidities between 70 and 90% the variation in the humidity produces a reversible structural change between the hydrated  $\alpha$ -form ( $\alpha''$ ) and the hydrated  $\beta$ -form ( $\beta''$ ). When the humidity is decreased the  $\beta$ -form appears; upon increasing the humidity the  $\alpha$ -form appears. If the humidity is lowered to about 30%, one obtains the dehydrated form ( $\beta'$ ), from which one is easily able to return to the two preceding forms. However, it is not possible to obtain the dehydrated  $\alpha$ -form ( $\alpha'$ ) in that way. To observe this form, it is necessary to pass the critical zone of dehydration under such conditions that the  $\alpha'' \rightarrow \beta''$  transformation cannot take place, for example, by precipitating the SPG by absolute alcohol. An equally remarkable fact is that the reversible water-induced transformation  $\alpha'' \rightleftharpoons \beta''$  is made without noticeable loss of orientation, although the orientation of the  $\alpha''$ -form is unstable at high humidities.

Turning now to non-ionized polyglutamic acid, PGA, it is observed that this polypeptide exists spontaneously in the  $\alpha$ -form at all relative humidities. The amide I band positions correspond exactly to those observed in the  $\alpha'$ -form of the sodium salt, that is, the dehydrated form. However, PGA has been obtained in a  $\beta$ -form in the solid state with the observed amide I and II frequencies being the same as those of the dehydrated sodium salt—the  $\beta'$ -form. Thus, PGA, in contrast to SPG, tends to remain either in the  $\alpha$ -form or in the  $\beta$ -form, whatever the relative humidity to which it is exposed. This is probably a consequence of the insolubility of PGA in water.

Also note should be made of the variations of the frequency of the non-ionized carboxyls in the PGA films, which change from 1705 to 1725  $\text{cm}^{-1}$  when the film is exposed to dioxane vapors. These changes indicate that normally the carboxyls are strongly hydrogen bonded one to another—perhaps intramolecularly—and that the effect of the dioxane is to solvate them and to break that association.

As indicated in the results section, corresponding changes appear in PGA and SPG upon exposure to  $\text{D}_2\text{O}$ . However, the frequencies are slightly lower in the deuterated materials, due to the greater mass of the deuterated polypeptide groups. The behavior of deuterated PGA (Fig. 4) is exactly the same as that of non-deuterated PGA (Fig. 3), and the observed spectral differences between the two compounds may be correlated with the deuteration effect alone.

With deuterated SPG, when the relative humidity is less than 70%, both  $\beta'$ - and  $\beta''$ -forms can be identified (Fig. 5). However, when the relative humidity is raised to 90%, a more complicated situation appears to exist than with the non-deuterated material. However, as with the non-deuterated material, the increased humidity serves to effect a  $\beta \rightarrow \alpha$  transformation as is evident from the dichroism. We have observed two different spectra with parallel dichroism of the amide I band (Fig. 6). The one with a relatively low frequency (1625  $\text{cm}^{-1}$ ) seems to correspond to the  $\alpha''$  (hydrated) form. Like the  $\alpha''$ -form of non-deuterated SPG, this deuterated  $\alpha$ -form can be reversibly changed into  $\beta$ -form. The other spectrum with a relatively high amide I frequency (1640  $\text{cm}^{-1}$ ) should correspond to an  $\alpha'$ -deuterated form but it must be noted that this deuterated  $\alpha$ -form appears at high relative humidity and with hydrated gels. Therefore, the exact identification of these deuterated  $\alpha$ -forms is uncertain.

The bands observed in deuterated SPG are indicated in Table II.

TABLE II  
INFRARED SPECTRAL BANDS OBSERVED IN DEUTERATED  
SODIUM POLY- $\alpha$ -L-GLUTAMATE

Form	Amide I <sup>a</sup>	Amide II <sup>a</sup>	COO <sup>-</sup> <sub>a</sub>	COO <sup>-</sup> <sub>s</sub>	Rel. humidity (in $\text{D}_2\text{O}$ ), %	State
$\beta'$	1620 ( $\perp$ )	1445 ( $\parallel$ )	1570-1575	1410	<10	Solid
$\beta''$	1610 ( $\perp$ )	1445 ( $\parallel$ )	1565	1410	60	Solid
$\alpha$ (?)	1625-1640 ( $\parallel$ )	1445 <sup>b</sup>	1565	1410	90	Solid
$\sigma$	1660	1445	1565	1405	100	Soln. (pH 7.3)
$\beta$	1615	1445	1565	..	100	Gel-soln. (pH 4.7)

<sup>a</sup> The dichroism is noted in parentheses. <sup>b</sup> Very weak dichroism.

In  $\text{D}_2\text{O}$  solutions of SPG the main amide I band appears around 1640-1645  $\text{cm}^{-1}$  with a higher frequency band (1660  $\text{cm}^{-1}$ ) appearing as an inflection at 25° but as a discrete band at high temperatures. This 1660  $\text{cm}^{-1}$  absorption is the solvated amide I ( $\sigma$ ) band. The band appearing at 1615  $\text{cm}^{-1}$  in  $\text{D}_2\text{O}$  solutions at low pH is that seen when the polypeptide is in the  $\beta$ -form in the solid state,<sup>10</sup> and indeed the 1615  $\text{cm}^{-1}$  ( $\beta$ ) amide I band appears only in gelled solutions and may be considered the onset of the solid state.

Finally, it should be emphasized that the water-induced reversible configurational changes of sodium poly- $\alpha$ -L-glutamate described here correspond almost exactly to those observed previously<sup>3</sup> with high molecular weight poly-L-lysine hydrochloride. Since the same type of configurational changes now have been detected in both anionic and cationic synthetic polypeptides, it is reasonable to inquire (a) whether such changes occur in other water-soluble synthetic polypeptides and (b) whether such changes may occur in naturally occurring polypeptides, *i.e.*, proteins.<sup>14</sup>

PARIS, FRANCE, AND  
BOSTON 15, MASS.

(14) This work was supported, in part, by the Office of the Surgeon General, Department of the Army.