clusion agrees with Jirgensons', *viz.*, serum albumin undergoes a subtle configurational change when the *p*H is above 7.5. It is predicted that there will be an interaction with a common mechanism in the *p*H region between 4.5 and this boundary *p*H (*ca.* 7.5) and that there will be an interaction with another common mechanism between *p*H *ca.* 7.5 and *ca.* 10.

At *p*H 4.4 where the isomerization occurs, DPB converted N into F form. Electrophoretic studies were made on the interaction of BPA with SDS between *p*H 3.5 and 4.5 in the region of mixing ratio between 100/0 and 95/5 where no precipitate was formed. It was found that SDS converted the F into N form,[27] and thus the following equation is given for the *p*H region between 3.5 and 4.5

$$\mathrm{N}\ \underset{\mathrm{SDS}}{\overset{\mathrm{DPB}}{\rightleftarrows}}\ \mathrm{F.}$$

It was found that anionic and cationic detergents had an opposite effect toward the isomerization reaction—an interesting point. However, much more cationic detergents than anionic detergents were needed to this transformation. This is because the affinity of DPB to HSA is lower than the affinity of SDS to HSA.

At *p*H 3.3, the electrophoretic pattern of the system BPA and a cationic detergent had a single boundary, and that of the system egg albumin and this detergent had two boundaries.[11] (This was confirmed also in the present systems HSA–DPB and egg albumin–DPB.) Foster and Yang interpreted the difference in electrophoretic pattern to be caused by the fact that BPA expands at this *p*H, but egg albumin does not. The same result was obtained at *p*H 10.6. When a mixture of HSA and SDS and a mixture of egg albumin and SDS, with the weight mixing ratio (protein/SDS) of 90/10, were analyzed electrophoretically at this *p*H, the former had a single boundary and the latter two boundaries, *i.e.*, egg albumin and a complex. It was found that egg albumin does not expand at this *p*H.[28] Hence the fact that some abnormality (expansion or degradation) occurs in serum albumin above *p*H 10 is considered to be the reason that electrophoretic patterns of the system HSA–SDS at *p*H 10.6 are quite different from those at *p*H 6.8.

Acknowledgments.—The author is sincerely grateful to Prof. Rempei Goto of the Institute for Chemical Research, Kyoto University for his encouragement during this study, and to Mr. Joji Hori for his help in conducting the experiment.

[28] The sedimentation coefficient of egg albumin at *p*H 10.6 was found to be almost the same as that at *p*H 6.8. This indicates that egg albumin exists in the identical state at these two *p*H values.

MIZUHO-KU, NAGOYA, JAPAN

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION]

Compositional Effects on the Configuration of Water-soluble Polypeptide Copolymers of L-Glutamic Acid and L-Lysine[1]

BY E. R. BLOUT[2] AND M. IDELSON

RECEIVED APRIL 11, 1958

The synthesis of four high molecular weight random polypeptide copolymers of L-lysine and L-glutamic acid in various mole ratios is described. Studies of the physical-chemical properties of these water-soluble ionic polypeptides indicate that they exist in molecular configurations which depend on their composition and their environment. Infrared spectra of solid films show only a single α-amide frequency when cast from *p*H 3 solutions; the films cast from *p*H 11 solutions have spectra which show the presence of both α- and β-configurations. Infrared spectroscopic studies on D_2O solutions of these polypeptides indicate that the proportion of random configuration compared to α configuration changes as the *p*D is changed from 3 to 10 but no β-configuration is observed. At low *p*H's in solution the proportion in the helical configuration, as measured by optical rotatory dispersion, decreases with increasing L-lysine content. The solubilities, changes in infrared spectra and changes in optical rotation resemble those observed with certain proteins.

In previous communications we have described the synthesis of high molecular weight poly-L-glutamic acid[3,4] and its configurational changes with *p*H,[3,5] heat[5] and water[6] in both the solid state and in solution. We also have investigated configurational changes in a cationic synthetic polypeptide, poly-L-lysine hydrochloride.[7] Because proteins contain both anionic and cationic groups it was interesting to extend these configurational studies of synthetic poly-α-amino acids to species carrying both negative and positive charges in the side chains of the same molecule. Therefore a series of copolymers of L-glutamic acid (L-glu) and L-lysine (L-lys) were synthesized and in this communication we report both the synthesis and the initial configurational studies on these ionic water-soluble polypeptides. An electrophoretic study of L-lys:L-aspartic acid copolymers has been described by Shavit[8] and some properties of one L-lys:L-glu copolymer are being reported by Doty, Imahori and Klemperer.[9]

Infrared spectroscopic studies indicate that these high molecular weight L-glu:L-lys copolymers exist

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[1] This paper is Polypeptides, XXII. For the previous paper in this series see M. Idelson, and E. R. Blout, THIS JOURNAL, **80**, 2387 (1958).

[2] Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

[3] E. R. Blout and M. Idelson, THIS JOURNAL, **78**, 497 (1956).

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

[6] H. Lenormant, A. Baudras and E. R. Blout, THIS JOURNAL, **80**, in press (1958).

[7] E. R. Blout and H. Lenormant, *Nature*, **179**, 960 (1957).

[8] N. Shavit, Abstracts of the September, 1955, Meeting of the American Chemical Society.

[9] P. Doty, K. Imahori and E. Klemperer, *Proc. Natl. Acad. Sci.*, **44**, 424 (1958).

in α- and β-configurations in the solid state; the proportion depends on the pH of the solution from which the solid film was made. The infrared spectra reveal the presence of a solvated random configuration in D_2O solutions, in addition to the α-configuration. Optical rotatory studies indicate that there is a progressive increase in helix content (which may be correlated with the α-configuration) with decreasing L-lysine content at pH 3; at pH 8 the helix content is low (below 30%) with all L-glu: L-lys polypeptides investigated.

Experimental

Copolymers of L-Glutamic Acid and L-Lysine.—The NCA's of γ-benzyl L-glutamate[10] and ϵ-carbobenzyloxy-L-lysine[11] in mole ratios of 7:3, 6:4, 5:5 and 4:6 were dissolved in benzene freshly distilled from calcium hydride to make 1% solutions. They were initiated with sodium methoxide solution to A/I 200 and allowed to polymerize overnight. Part of each solution was removed and precipitated in 95% ethanol. The reduced specific viscosities at 0.2% in dichloroacetic acid of the organic-soluble polypeptides are given in Table I.

TABLE I

COPOLYMERS OF γ-BENZYL L-GLUTAMATE AND ϵ-CARBOBENZYLOXY-L-LYSINE

Sample	Mole ratio of L-Glu: L-Lys NCA's	$(\eta_{sp}/c)^a$	MW_w[b]	DP_w	Nitrogen, % Calcd.	Found
407A	7:3	1.85	350,000	1500	7.6	7.5
432A	6:4	1.43	260,000	1100	8.1	7.8
430A	5:5	1.49	270,000	1100	8.5	8.4
128A	4:6	1.17	210,000	900	8.9	8.9

[a] c = 0.2 in dichloroacetic acid. [b] Estimated from the viscosity using the molecular weight calibration for poly-γ-benzyl L-glutamate from Doty, Holtzer and Bradbury, THIS JOURNAL, **78**, 947 (1956).

The remainder of each benzene solution was treated with anhydrous hydrogen bromide for 20 minutes, and the mixtures were allowed to stand overnight. The benzene was sucked from the insoluble product through a glass tube with a glass wool plug by means of an aspirator. The products were washed twice with anhydrous benzene, extracted with acetone for 2 hr., and dried at 60°. Yields were quantitative. The nitrogen analyses and the amino acid contents of the acid-hydrolyzed water-soluble polypeptides are given in Table II. All the copolymers were free of benzyl groups as determined from their ultraviolet spectra in aqueous solution.

TABLE II

Mole ratio L-Glu: L-Lys	$[\eta]^{0.2 M \text{NaCl}}_{\text{pH } 3.0}$	$[\eta]^{0.2 M \text{NaCl}}_{\text{pH } 7.3}$	MW_w[a]	DP_w	Analyses, %	Glutamic acid[b]	N
7:3	1.5	1.04	110,000	750	Calcd.	70	11.6
					Found	72	12.0
6:4	0.53	0.30	36,000	250	Calcd.	60	11.9
					Found	63	12.4
5:5	.74	.34	42,000	300	Calcd.	50	12.1
					Found	50	11.9
4:6	.72	.32	39,000	290	Calcd.	40	12.7
					Found	40	11.5

[a] Estimated from the molecular weight calibration of Doty, *et al.* (ref. 9), for a similar copolymer. [b] Determined by hydrolysis of the polymer and then by paper chromatography according to the method of R. E. Kay, D. C. Harris and C. Entennan, *Arch. Biochem. and Biophys.*, **63**, 14 (1956). We are indebted to Dr. G. D. Fasman for these determinations. The error in the determinations is estimated to be ±5%.

Infrared Measurements.—Infrared spectra were measured on a Perkin-Elmer model 21 spectrometer. Samples were prepared as films on silver chloride sheet by evaporation from aqueous solutions. The spectra were measured both on the freshly prepared sample and after drying over P_2O_5 *in vacuo* for a day. Oriented films were prepared by unidirectional stroking of a viscous solution on silver chloride until it was dry. The D_2O solutions were measured in 0.05-mm. calcium fluoride cells at about 5% concentration.

Optical Rotation Measurements.—Optical rotatory dispersion measurements were made with a Rudolph high precision photoelectric polarimeter model 200, using a General Electric H100-A4 mercury lamp as light source. Baird interference and Corning absorption filters were used as follows to isolate the mercury lines: 365 mμ, Corning #5860 and 0-52; 405 mμ, Baird #436 and Corning #5113; 546 mμ, Baird #546, Corning #9780 and 3484; 578 mμ, Baird #578 and Corning #3484. All measurements were made at 25°. The concentration was about 1.0%.

Results and Discussion

The ionic polypeptide copolymers of L-glu and L-lys reported here are readily soluble at both high and low pH's, but show minimum solubility at an intermediate pH depending on their composition and their charge. Thus they show solubility behavior similar to that observed in polyampholytes and certain proteins.

Infrared spectra of the ionic copolymers have been determined in both the solid state and in aqueous (D_2O) solution.

Infrared Spectra. A. Solid State.—Films cast from aqueous solutions at both low pH (~3) and high pH (11 to 12) show characteristic polypeptide absorption spectra. The films of each copolymer cast from low pH solutions show practically identical spectra in the region 1350 to 1800 cm.$^{-1}$ (Fig. 1, curve A). They are characterized by a non-ionized COOH band at 1720 cm.$^{-1}$, a strong (α) amide I (C=O stretching frequency) at 1655 cm.$^{-1}$ a fairly strong amide II absorption at 1550 cm.$^{-1}$, and weaker C–H deformational modes at 1460 and 1410 cm.$^{-1}$.

Films from various poly-L-glu:L-lys high pH solutions also show characteristic infrared bands (Fig. 1, curve B). In these cases, two amide I bands are observed, one lying at 1650–1655 cm.$^{-1}$ correlated with α-configurations and the other at 1625–1630 cm.$^{-1}$ correlated with β-configurations. In addition there is a strong band at 1575 cm.$^{-1}$ as well as a weaker one at 1400 cm.$^{-1}$ due to COO_a^- and COO_s^- vibrations, respectively. These films show no COOH frequency, but a weak band at 1690 cm.$^{-1}$ characteristic of β-configurations is always seen. In these films the 1625 cm.$^{-1}$ (β) amide I band increases in intensity relative to the 1655 cm.$^{-1}$ (α) amide I band with the amount of L-lys in the polymer. It is interesting to note that a film of the 5 glu:5 lys copolymer cast from a solution at pH 8 shows no COOH band, and no 1625 cm.$^{-1}$ (β) band.

Preliminary studies with oriented films of the ionic copolymers prepared from the low pH solutions indicate that orientation produces a strong 1625 cm.$^{-1}$ (β) amide I band in addition to the 1655 cm.$^{-1}$ band. The 1655 cm.$^{-1}$ band shows parallel dichroism, while the 1625 cm.$^{-1}$ band shows perpendicular dichroism. Also two amide II bands are observed, 1545 cm.$^{-1}$ (with perpendicular dichroism) and 1525 cm.$^{-1}$ (with parallel dichroism). A few experiments, performed to study the effects of various relative humidities on these oriented

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[10] E. R. Blout and R. H. Karlson, THIS JOURNAL, **78**, 941 (1956).

[11] M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935).

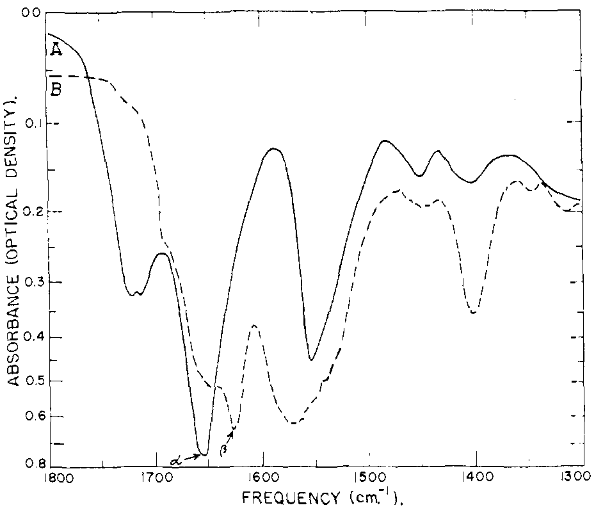

Fig. 1.—The infrared spectra of films of 6:4 copoly-L-glutamic acid:L-lysine: A, ———, cast from pH 3 aqueous solution; B, - - - -, cast from pH 11 aqueous solution. Note the β-carbonyl amide band which appears in film B.

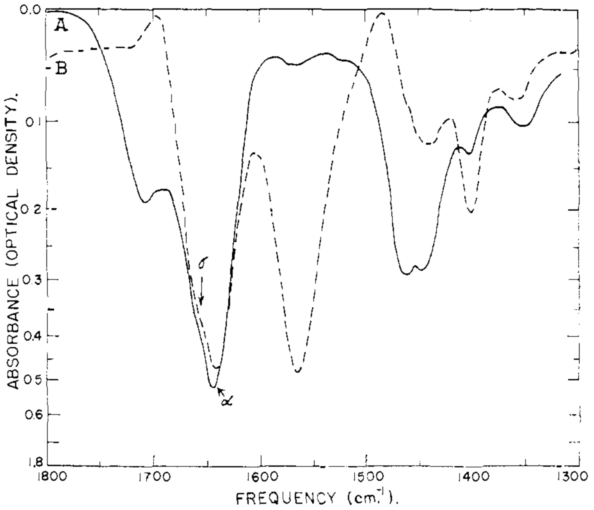

Fig. 2.—The infrared spectra of D_2O solutions (concn. ~5%) of 1:1 copoly-L-glutamic acid:L-lysine. The spectra were measured after the exchange of the amide hydrogens by deuterium was essentially complete: A, ———, pD 3 solution; B, - - - -, pD 11 solution. Note the σ (solvated) carbonyl amide inflection and the absence of the β-carbonyl amide band.

films, indicate that their configuration is sensitive to the amount of water surrounding them as has been observed with oriented films of poly-L-lysine hydrochloride[7] and sodium poly-L-glutamate.[6]

B. Solutions.—The infrared spectra of the several copolymers of L-glu:L-lys have been determined at various pD's between 3 and 13 both in D_2O solution and in D_2O containing 0.2 M NaCl. All measurements were made on solutions in which all the amide hydrogens had been exchanged for deuterium. Measurements also have been made on the rate of exchange of the amide hydrogens with deuterium and these will be reported separately.[12]

The D_2O solutions of all the copolymers show similar spectra at pD ~3 (see Fig. 2, curve A). In the region between 1300 and 1800 cm.$^{-1}$ they show a non-ionized carboxyl band at 1710 cm.$^{-1}$, an amide I band between 1640 and 1645 cm.$^{-1}$ (characteristic of the deuterated α-configuration), a deuterated amide II band at 1450 cm.$^{-1}$, as well as bands at 1465, 1405 and 1350 cm.$^{-1}$.

As the pD of these solutions is raised, the 1710 cm.$^{-1}$ band disappears and concomitantly there appear bands of COO^- at 1565 and 1400 cm.$^{-1}$ (Fig. 2, curve B) similar to those observed with the solid films described above. Also as the solution is raised in pD a shoulder or band at 1660 cm.$^{-1}$, due to solvated deuterated amide I groups,[5,16] shows in all spectra. This band, called a σ-band, also appears as a shoulder in the 4 glu:6 lys polymer at pD ~3.5. The intensity of the 1660 cm.$^{-1}$ band varies with pH. In the spectra of the D_2O solutions of each polymer it increases in intensity in the region between pD 6 and 10 and decreases at the extremes of pD. The fact that the σ-band is only observed in solution indicates that the band is characteristic of a solvated deuterated amide group such as expected in random configurations. Although no measurements of intensities of the solvated amide (σ) band have been made, because of the overlap with the α-amide band, it does appear to change in intensity with pD in the range 6 to 10. This change is compatible with the optical rotation results (see below) which indicate a decreasing helix content as the pH is raised. The spectra with 0.2 M NaCl in D_2O were qualitatively similar to those measured without salt.

Experiments were performed in which D_2O solutions of sodium poly-L-glutamate and D_2O solutions of poly-L-lysine hydrochloride were mixed. In these cases the resulting solutions (pD 6 to 7) showed a large increase in viscosity and some precipitation. Figure 3 shows the infrared spectra of the 1:1-L-lys:L-glu *mixture* of polymers compared with the D_2O solutions of poly-L-lys and poly-L-glu from which the mixture was made. The spectrum of the mixture shows amide I bands at 1640 and 1615 cm.$^{-1}$, the latter being characteristic of the deuterated β-form of polypeptides.[6,9] Raising the pD to 13 did not dissolve the precipitate nor decrease the intensity of the 1615 cm.$^{-1}$ band.

The frequencies observed in the infrared spectra and tentative assigments are given for the 6:4 L-glu:L-lys copolymer in Table III. The data for the other copolymers are qualitatively similar but vary quantitatively as indicated in the text.

Optical Rotation. Solutions.—Optical rotatory dispersion measurements of solutions of the several ionic copolymers of L-glu and L-lys were made at pH 3 and at pH 8. These data, along with similar data for poly-L-glutamic acid and poly-L-lysine, are summarized in Table IV.

At pH 3 the specific optical rotations become more negative with increasing lysine content. If the b_0 values of these solutions are calculated as described by Moffitt and Yang[13] it is seen that the b_0's become less negative as the lysine content increases (in the pH 3 solutions). At pH 8 the op-

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[13] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.*, **42**, 596 (1956).

TABLE III

FREQUENCIES (IN CM.$^{-1}$) OBSERVED IN THE SPECTRA OF THE L-GLU:L-LYS COPOLYMERS

			Amide I					
	-COOH	"β"	σ (solvated or random)	α (helical)	β (extended)	Amide II	COO_a^-	COO_s^-
Unoriented film (from pH 3 soln.)	1720			1655		1550		
Unoriented film (from pH 12 soln.)		$\sim$1690		$\sim$1650	1628	$\sim$1535	1570	1400
Oriented film (from pH 3 soln.)	1715			1650	1625	1545, 1525		
D_2O solution (pD $\sim$3)	1710			1645		1460, 1450		
D_2O solution (pD $\sim$8)	1710		$\sim$1665	1643		1440	1565	1400
D_2O solution (pD $\sim$12)			$\sim$1660	1645		1440	1565	1400

TABLE IV

Polymer composition L-glu: L-lys	Solutions in water					
	pH 3			pH 8		
	$[\alpha]^{25}_{546}$	b_0[a]	Estimated[b] % helix	$[\alpha]^{25}_{546}$	b_0[a]	Estimated[b] % helix
10:0	− 8°[c]	−625[d]	100	−116°	0	0
7:3	−12	−586	>90	− 88	0	0
6:4	−27	−439	70	− 60	−140	25
5:5	−51	−310	50	− 66	−196	30
4:6	−69	−131	<25	− 62	[e]	
0:10	−92	0	0	− 63	[f]	0

[a] Calculated assuming $\lambda_0 = 212$ mμ. [b] Based on the assumption that $b_0 = 0$ in a completely random configuration and $b_0 = -625$ in the completely helical form of poly-L-glutamic acid and poly-L-lysine. [c] At pH 4.5. [d] From Moffitt and Yang.[13] [e] These data did not fit either a Drude or Moffitt plot. [f] These data fit a Drude (single term, normal dispersion) plot.

posite situation exists in that with increasing lysine content the specific optical rotations of the copolymers become less negative and the b_0 values become more negative.

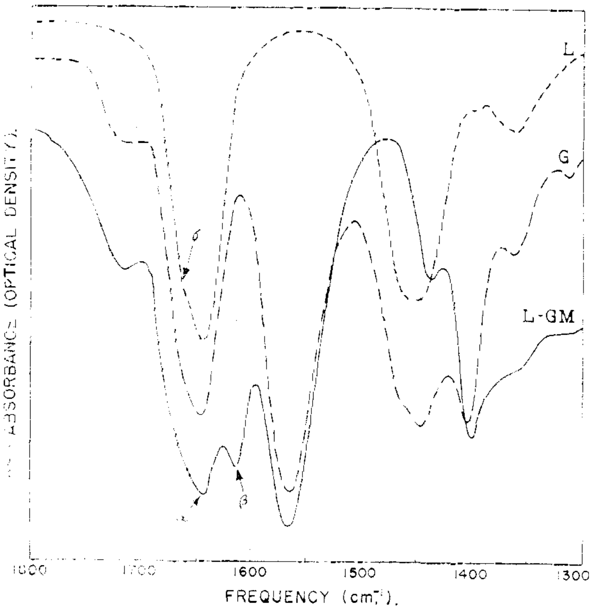

Fig. 3.—L, --------, the infrared spectrum of a D_2O solution of poly-L-lysine hydrochloride, pD 3, concn. 5%; G, – – – –, the infrared spectrum of a D_2O solution of sodium poly-L-glutamate, pD 7, concn. 5%; L-GM, ———, the infrared spectrum obtained when the D_2O solutions of poly-L-lysine hydrochloride and sodium poly-L-glutamate that gave the L and G spectra were mixed. The viscosity increased and some precipitation occurred.

Cohen and Szent-Gyorgi have suggested[14] that the magnitude of b_0 can be used as a measure of the helix content of proteins and polypeptides. This appears to be a reasonable assumption for a series of two-component polypeptides in which the configurations of the extreme members of the series, in this case poly-L-lysine and poly-α,L-glutamic acid, have been characterized by several physical-chemical methods. Therefore we have calculated the helix content of the L-glu:L-lys copolymers from the b_0 values. The results, given in Table IV, show that at pH 3 the helix content decreases with increasing L-lys content. At pH 8 in aqueous solution the values of b_0 indicate the absence of helix, or low helix contents.

We also have determined the optical rotatory dispersion for the 6:4 glu:lys copolymer at pH's 3 and 8 in the presence of 0.2 M NaCl. At pH 3 the b_0 is −440, which is practically identical with the value observed in the absence of salt. At pH 8 with salt, $b_0 = -73$, whereas without salt $b_0 = -140$. Using these b_0 values as a measure of helix content, one must conclude that compared with aqueous solutions the addition of 0.2 M salt does not increase the helix content of this polypeptide.

The "optical titration" curves for three of the copolymers are shown in Fig. 4 along with that of poly-α,L-glutamic acid. The sharp change in optical rotation in a very small pH interval is qualitatively similar to that observed with poly-L-glutamic acid[3-5] and with poly-L-lysine.[15] The more highly negative $[\alpha]$ values observed in the copolymers at high pH's probably reflect the lower helix contents at high pH compared with the helix contents at low pH.

Conclusions

The following conclusions may be drawn about the configuration of the L-glu:L-lys copolymers from the data presented here: (1) The infrared spectra indicate that in the solid state, at low pH, unoriented films of the copolymers show only α-configuration; oriented films show α- and β-configurations. In the solid state, at high pH's, all the copolymers show strong infrared amide bands characteristic of both α- and β-configurations[7,16] even in the unoriented films. In these films the amount of β-configuration increases as the molar L-lys content increases.

(2) The infrared spectra of D_2O solutions at pD's > 6, show absorption at 1660 cm.$^{-1}$ either as a discrete band or a shoulder on the 1640 cm.$^{-1}$ α-

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(15) E. R. Blout and M. Idelson, to be published.

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amide band. The 1660 cm.$^{-1}$ band (called a σ-band) is characteristic of a solvated deuterated amide group and appears in all the copolymers. Thus in solution infrared absorption frequencies of the amide groups are observed which can be correlated with random and α-configurations of polypeptides. No quantitative measurements of the relative amounts of these forms have been attempted using these frequencies.

(3) Infrared spectra of D_2O solutions of the copolymers show no evidence of amide bands characteristic of the β-configuration. However equimolar *mixtures* of sodium poly-L-glutamate and poly-L-lysine hydrochloride gel and precipitate, concomitantly show the appearance of a β-amide I band. It may be suggested that this β-band results from the coprecipitation of the two polymers due to meeting of fairly long sequences of carboxyl and amine groups producing thereby a type of "ionic" crosslinking.

(4) Using the optical rotation data at *p*H 3, and assuming that the magnitude of b_0 represents a measure of the helix content, it may be concluded that the helix content decreases when the molar lysine content increases. From these data it appears that 30 mole per cent. L-lysine in a glutamic acid copolymer does not decrease the helix content significantly at *p*H 3, but 60 mole per cent. L-lysine decreases it to less than 25%. Estimates of helix content using optical rotatory dispersion should be regarded as preliminary[17] but are qualitatively confirmed by infrared results. Using the optical rotatory dispersion data in the manner described it appears that at approximate neutrality (*p*H 8) the helix content of these polypeptides ranges from zero in the copolymer with 30% lysine to a maximum of 30% in that with 50% lysine.

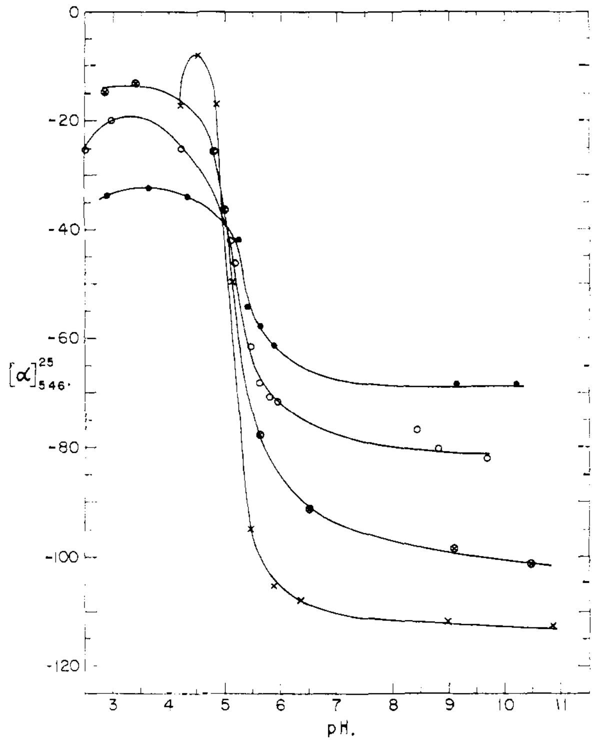

Fig. 4.—The optical rotation, $[\alpha]^{22}_{546}$, as a function of *p*H in aqueous solution: ●—●—●, 5:5 copoly-L-glutamic acid:L-lysine; ○—○—○, 6:4 copoly-L-glutamic acid:L-lysine; ⊗—⊗—⊗, 7:3 copoly-L-glutamic acid:L-lysine. The poly-L-glutamic acid data, ×—×—× (ref. 4), is shown for comparison.

Acknowledgment.—We are pleased to acknowledge the support of this work by the Office of the Surgeon General, Department of the Army, as well as the valuable assistance of Miss Evelyn DesRoches in the preparative work, that of Mrs. A. A. Ferguson in the infrared studies, and that of Mr. Kenneth Norland in the viscometric measurements.

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[Contribution from the Lankenau Hospital Research Institute and the Institute for Cancer Research]

Studies in Valine Biosynthesis. II. α-Acetolactate Formation in Microörganisms

By Katherine F. Lewis and Sidney Weinhouse

Received February 7, 1958

Extracts of *Saccharomyces cerevisiae* and *Escherichia coli* have been shown to be capable of converting pyruvate to α-acetolactate.

In an isotope tracer study of the biosynthesis of valine in *Torulopsis utilis*, Strassman, Thomas and Weinhouse[1] proposed a mechanism of valine biosynthesis which involves a condensation of pyruvate and acetaldehyde to yield α-acetolactate, followed by an intramolecular migration of one of the methyl groups of acetolactate from the α- to the β-carbon to yield the valine carbon skeleton. α-Acetolactate was first proposed as a biological intermediate by Krampitz,[2] who synthesized this compound and showed it to be decarboxylated to acetylmethylcarbinol (acetoin) in extracts of *Staphylococcus aureus*. Juni[3] subsequently demonstrated that α-acetolactate was an intermediate in acetoin formation in *Aerobacter aerogenes*, and found later[4] that a "pyruvic oxidase" preparation of *Escherichia coli* synthesized α-acetolactate from

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[3] E. Juni, *J. Biol. Chem.*, **195**, 715 (1952).

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