Holloway, C. T., Bond, R. P. M., Knight, I. G., and Beechey, R. B. (1967), *Biochemistry 6*, 19.

Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry (1956), Supplement II, Part I, Briscoe, H. V. A., Eldridge, A. A., and Dyson, G. M., Ed., London, Longmans Green, p 934.

Perlgut, L. E., and Wainio, W. W. (1967), *Biochemistry 6*, 15.

The Conformational Transitions of Uncharged Poly-L-lysine. α Helix–Random Coil–β Structure*

Betty Davidson and Gerald D. Fasman†

ABSTRACT: The heat-induced transition of poly-L-lysine, α helix–random coil–β form, has been studied, mainly by optical rotatory dispersion (ORD). Below T_β, the transition has the properties of a reversible α-helix–random coil equilibrium. Above T_β, the transition is not reversible during the period of the measurements; it proceeds at a rate dependent upon polymer concentration, temperature, pH, and solvent. Evidence is presented that above T_β the thermal transition represents the conversion of α-helical poly-L-lysine to an intermediate random coil and finally to an intermolecular antiparallel β structure. At 50° the β form is the most stable of the three conformations of poly-L-lysine, and its conversion from the helix is essentially complete. At 4° the α helix is the most stable form; however, β–α conversion is very slow. In comparison to H_2O, the β form is destabilized by 15% ethylene glycol and is probably stabilized by 0.2 M NaCl. The α helix is destabilized by 2.0 M LiBr and stabilized by 50% methanol. The different behavior of the two conformations with respect to temperature and solvent is consistent with the proposal that α-helical poly-L-lysine is stabilized largely by intraamide hydrogen bonds while β poly-L-lysine owes a large part of its stability to hydrophobic interactions between lysyl residues.

Of the three conformations known to exist in proteins and polypeptides (the α helix, β-pleated sheet, and random coil), the β structure (the pleated-sheet structure) in solution has been the least well characterized. The pleated-sheet structures were first studied and characterized by means of X-ray diffraction (Meyer and Mark, 1928; Astbury and Woods, 1930; Astbury and Marwick, 1932; Pauling *et al.*, 1951; Pauling and Corey, 1951), and the results were correlated with infrared absorption data (Astbury *et al.*, 1948; Ambrose and Elliot, 1951). These early studies classified the β structure as an intermolecularly hydrogen-bonded pleated sheet, further aggregated by three-dimensional stacking (Pauling and Corey, 1951; Marsh *et al.*, 1955). The polypeptide chains have either the same (parallel) or alternating (antiparallel) direction (Pauling and Corey, 1951). A third type of β structure, the intramolecularly hydrogen-bonded "cross β," has also been described (Dickerson and Bailey, 1935; Parker and Rudall, 1957). The parallel and antiparallel forms have been considered in a theoretical treatment of the amide I and II infrared absorption bands (Miyazawa and Blout, 1961).

Both the α-helical and the β forms have been found to be energetically favorable conformations for the polypeptide chain (Pauling *et al.*, 1951; Pauling and Corey, 1951; Ramachandran *et al.*, 1963a,b; Némethy and Scheraga, 1965; De Santis *et al.*, 1965). The preferential formation of the β structure in some high molecular weight synthetic polypeptides was found to depend on the presence of bulky substituents or hetero atoms on the β carbon of the amino acid side chain (Bloom *et al.*, 1962; Blout, 1962).

Early studies of the β structure were carried out on samples in the solid state owing in part to the insolubility of this aggregated conformation in aqueous solution. Recently, this difficulty has been overcome and the study of the β conformation in aqueous solution has been reported (Davidson *et al.*, 1966; Sarkar and Doty, 1966). Optical rotatory dispersion (ORD)

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* Contribution No. 492 of the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received January 16, 1967.* The work was supported in part by grants from the National Institute of Arthritis and Metabolic Disease of the National Institutes of Health, U. S. Public Health Service (AM0582), the National Science Foundation (GB-5576), the American Cancer Society (Mass. Division), Inc., and the U. S. Army Medical Research and Development Command, Department of the Army, under Research Contract DA49193-MD-2933. For the previous paper in this series see Davidson *et al.* (1966).

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in the near-ultraviolet–visible spectral region had been useful in studying the α-helical and random conformations of polypeptides in solution (Blout, 1960; Urnes and Doty, 1961; Fasman, 1963), and an extension of these considerations to include the β form was attempted by several groups (Wada *et al.*, 1961; Schellman and Schellman, 1961; Troitski, 1965; Timasheff *et al.*, 1966). The Moffitt parameters, a_0 and b_0 (Moffitt, 1956; Moffitt and Yang, 1956), were determined for several β polypeptides and found to vary widely over the range $a_0 = -62$ to $+840$ and $b_0 = -240$ to $+420$ (Fasman and Blout, 1960; Wada *et al.*, 1961; Bradbury *et al.*, 1962; Harrap and Stapleton, 1963; Ikeda *et al.*, 1964; Imahori and Yahara, 1964; Davidson *et al.*, 1966; Sarkar and Doty, 1966; S. Ikeda and G. D. Fasman, manuscript in preparation).

Recently, improved instrumentation has permitted ORD measurements to be made in the spectral region of the optically active peptide chromophore. Far-ultraviolet ORD spectra of films of various β-forming polyamino acids have been described for poly-L-isoleucine (Blout and Shechter, 1963), poly-L-serine (Davidson *et al.*, 1966), and many other β formers (Fasman and Potter, 1967). These β structures fall into two categories. Either the ORD spectra show a peak at 205 mμ and a trough at 230 mμ, or the entire Cotton effect is displaced about 7 mμ toward higher wavelengths (Fasman and Potter, 1967). In aqueous solution, the α-helical and random conformations have been characterized by far-ultraviolet ORD spectra (Blout *et al.*, 1962; Yang and McCabe, 1965; Iizuka and Yang, 1965). Recently, the far-ultraviolet ORD spectrum of an aqueous solution of the β form of poly-L-lysine has been described by this laboratory (Davidson *et al.*, 1966) and also by Sarkar and Doty (1966). This spectrum is similar to one class of β films. The circular dichroism (CD) spectrum of β poly-L-lysine has also been studied (Sarkar and Doty, 1966; Townend *et al.*, 1966). The latter group used the same sample of poly-L-lysine and the same conditions as were used in the present work. The intrinsic Cotton effects of aqueous solutions of other β-forming polypeptides have now been studied (Tooney, 1966; Ikeda and Fasman, manuscript in preparation). In addition, some β structure has been postulated in several proteins, including lysozyme (Blake *et al.*, 1965; Tomimatsu and Gaffield, 1965; Greenfield *et al.*, 1967), β-lactoglobulin (Timasheff and Townend, 1965; Timasheff and Susi, 1966), and silk fibroin (Iizuka and Yang, 1966). Their far-ultraviolet ORD and CD spectra have been studied and correlated with X-ray or infrared data. Infrared spectra in the amide I absorption region have also been useful in characterizing the β form of polypeptides in D_2O solution (Blout and Lenormant, 1953; Blout, 1957).

The α-helix–random coil transition of poly-L-lysine has been shown to be dependent upon the extent of ionization of the ϵ amino groups of the polymer (Applequist and Doty, 1958, 1962). The work to be described herein is a study of the thermal transition of uncharged poly-L-lysine from the α-helical to the β conformation. Some of these results have been reported in preliminary communications (Davidson and Fasman, 1966; Davidson *et al.*, 1966).

Previous studies utilizing the techniques of ultraviolet and infrared spectroscopy and X-ray diffraction have shown that under conditions of dehydration (Blout and Lenormant, 1957; Schmueli and Traub, 1965) or heating (Rosenheck and Doty, 1961; Applequist and Doty, 1958, 1962), poly-L-lysine undergoes a transition from the α-helical to the β form. These findings were corroborated in this laboratory in the course of a temperature study of the α-helix to random coil transition of poly-L-lysine. The behavior of the polymer, as observed by ORD, was found to be more complicated than expected for a simple α-helix–random coil equilibrium. The temperature transition appeared to have a dual character. From 11° to a temperature designated as T_β, the transition had the properties of a simple helix–coil equilibrium. Above T_β, which varied from 23 to 56°, depending upon solvent and polymer concentration, the transition was no longer rapidly reversible upon cooling. The rate was dependent upon pH, concentration, temperature, and solvent. Evidence will be presented that the transition from 11° to T_β represents an α-helix–randomcoil equilibrium, while above T_β the transition represents the formation of an intermolecular β structure from the random form of poly-L-lysine.

Materials and Methods

Poly-L-lysine·HCl was synthesized as previously described (Fasman *et al.*, 1961). The lyophilized material was dissolved in 0.2 M NaCl at a concentration of about 0.23%, pH 11.75, and heated at 30° for 5 min in order to remove the least soluble fraction. Slight opalescence was observed. The sample was then cooled in ice, filtered through a sintered-glass funnel of medium porosity, adjusted to pH 4.3 with HCl, and dialyzed against five successive changes of 0.01 M HCl. The pH of the final dialysate was 2.4. This material (BD-1-10-28) was lyophilized and used for all of the work reported here. It had an intrinsic viscosity of 0.67 in 1 M NaCl (pH 4.0, estimated mol wt 55,000). There was one chlorine atom for every two nitrogen atoms as determined by potentiometric chloride determination and by Nessler micro-Kjeldahl nitrogen analysis (Lang, 1958).

Concentration of Poly-L-lysine. Concentration was determined by a modification of a microbiuret procedure (Zamenhof and Chargaff, 1963). The same filtered samples were used for both ORD and microbiuret determinations. Standard solutions were prepared from samples of poly-L-lysine·HCl (BD-1-10-28) previously dried over P_2O_5 at 50° *in vacuo* to constant weight. Aliquots of standard were stored at −20° and removed as needed. Concentrations determined by this method agree within 2% with Nessler determinations of the nitrogen content of this polymer.

In some early experiments (data of Figures 1 and 2

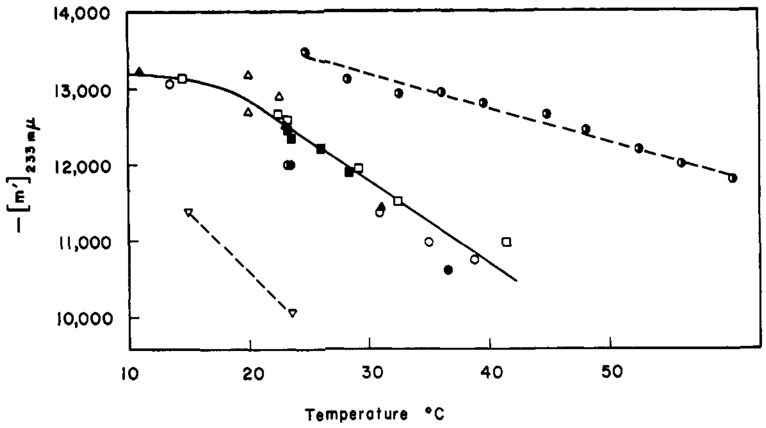

FIGURE 1: Poly-L-lysine: α-helix–random coil transition as measured by $[m']_{233}$ as a function of temperature in a variety of solvents. The pH was 11.6–11.7. Data obtained in 2 M LiBr (▽– –▽) and in 50% methanol–0.2 M NaCl (◑– –◑). The solid line represents data obtained in H_2O (△), 0.2 M NaCl (■), 0.2 M NaCl–0.05 M LiBr (▲), 15% ethylene glycol–0.2 M NaCl (○), 15% ethylene glycol–H_2O (●), and 15% ethylene glycol–0.2 M NaCl–0.05 M LiBr (□). A water-jacketed cell of 1-mm path length was used.

and most of Table I), the concentrations were based on weighed amounts of undried poly-L-lysine·HCl. Since these samples contained from 12 to 15% water, $[m']$ values based on these concentrations are low.

pH measurements were made with a Radiometer Model 25 SE pH meter (Copenhagen, Denmark) and a Sargent Model DR pH meter. The accuracy of these measurements is ±0.01 pH unit.

ORD measurements were made with a Bendix-Ericcson Polarmatic 62 spectropolarimeter (slit width, 1.8 mm) and with a Cary Model 60 recording spectropolarimeter (slit width programmed to maintain a 15-A half-band width). All measurements below 220 mμ and all reported ORD spectra were obtained using the Cary instrument. ORD spectra of poly-L-lysine were compared with ORD curves of the appropriate solvents, adjusted within 0.03 unit to the pH of the sample.

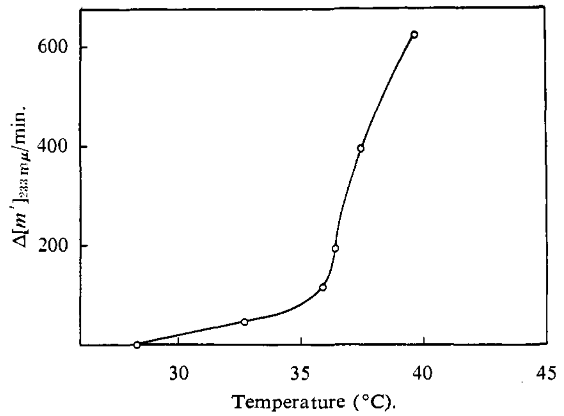

FIGURE 2: The rate of β formation of poly-L-lysine, $\Delta[m']_{233m\mu}$/min, as a function of temperature. The optical path length was 1 mm, the pH 11.7, and the concentration 0.114% in 0.2 M NaCl.

All solutions were filtered through Teflon Millipore filters (pore size 5.0 μ) into a 1-mm path-length, fused-quartz optical cell obtained from Optical Cell Co., Brentwood, Md. The cell was water jacketed to permit temperature regulation by means of a Haake type F circulating bath. Temperature was monitored by a TRI-R electronic thermometer and a bimetallic probe placed at the exit of the cell jacket.

Data are reported as $[m']_\lambda$, the reduced rotation per mole of lysine·HCl residue (Fasman, 1963). Measurements in the near-ultraviolet–visible range were made with cells of 1.0- and 5.0-cm optical path length.

*The Conversion of α-Helical to β Poly-*L*-Lysine.* Poly-L-lysine·HCl was dissolved in H_2O and the pH was adjusted to 11.0–11.25 with NaOH. The polymer concentration was kept between 0.012 and 0.027% to avoid precipitation upon heating. The solution was filtered into the optical cell and the ORD of this helical sample was recorded from 250 to 187 mμ, temperature 22.5°. The monochromator was then set at 198.6 mμ and the rotation at the Cotton effect peak recorded for about 3 min. The jacketed cell was connected to a circulating water bath set at around 50°. Cell and contents were heated *in situ*. The decrease in rotation at 198.6 mμ was recorded until the rotation again became constant. (This took 15–25 min at the pH and concentrations used.) The cell and contents were cooled to 22.5° for 10–20 min and the ORD of the β form was recorded over the same wavelength range. There was no discernible precipitation at the conclusion of the experiments. The final ORD of the β form was independent of the heating temperature over a range of 50 to 75°. Maximal total absorbances in the 190–195-mμ range (polymer and solvent) were 1.5 as compared with glass-distilled water. A Cary 14 spectrophotometer purged with nitrogen was used for the absorbance measurements.

TABLE I: The Effect of Solvent on the Formation of β Poly-L-lysine.

Solvent (M)	Concn (%)	T_β[a]	Rate Measurement Temp (°C)	Rate of β Formation[a,e]
50% CH_3-OH-(0.2) NaCl	0.1142	No β formation observed at any temperature		
15% ethylene glycol-LiBr (0.05)-NaCl (0.2)	0.0942		41.3	0
	0.0942	44–46	46.3	115
	0.0942		51.7	537
15% ethylene glycol-NaCl (0.2)	0.0942	38–39	38.8	38
	0.0942		50	323
	0.0942		55.3	821
15% ethylene glycol-H_2O	0.0594	40–42	42	80
	0.0594		48	105
15% ethylene glycol-H_2O	0.02	53–55	55	565
	0.02		56.5	2280[d]
H_2O	0.0075[b]	49–50		
	0.044	38–39	39	43
	0.055[c]	31.5	38	246
	0.059	31–33	33.8	65
	0.059		38	107
	0.059		43	593
NaF (0.2)	0.100	33–34	34	44
NaCl (0.2)	0.1142	32	32.7	45
			35	115
			39.7	620
NaF (0.2)	0.055[c]	33–34		
NaF (0.2)	0.0082[b]	47		
LiBr (0.05)-NaCl (0.2)	0.0942	39–41	41.8	70
LiBr (0.5)-NaCl (0.2)	0.0942	23	23	23
	0.0942		32.3	93
	0.0942		37	705
LiCl (2)	0.046	<25	25	120
LiBr (2)	0.059	24–25	30.8	354
LiBr (1.25)	0.059	—	30.9	150
			39.0	824

[a] Measured at pH 11.6–11.7. [b] Measured at pH 11.0. [c] Measured at pH 12.25. [d] $\Delta[m']$/min measured at 198 mμ instead of 233 mμ. [e] Given as $\Delta[m']_{233\ \mathrm{m}\mu}$/min.

The Rate of Formation of the β Structure. At specified conditions of pH, temperature, and solvent, the monochromator was set to either 233 or 198.6 mμ, and the decrease in rotation was recorded as a function of time ($\Delta[m']_{233\ \mathrm{m}\mu}$/min or $\Delta[m']_{198.6\ \mathrm{m}\mu}$/min). The measured rates were as close as possible to initial rates within the limits of the time required for attainment of thermal equilibrium (approximately 2 min).

The Measurement of T_β. The monochromator was set at either 233 or 198.6 mμ, and the temperature was raised by increments of 3–4°. The temperature range in which $\Delta[m']$/min changed from zero to a small number (around 50) was taken as T_β.

Index of Refraction. The indices of refraction necessary to calculate the $[m']$ values were obtained as follows.

(1) H_2O AND 0.2 M NaCl were taken from tables of Fasman (1963). Values of n for 0.2 M NaCl, 0.2 M NaF, and all concentrations of LiBr and LiCl were assumed to be the same as for H_2O.

(2) ETHYLENE GLYCOL (15%). The refractive index of 100% ethylene glycol and 15 and 50% solutions in H_2O was measured at 589, 546, and 436 mμ, using a Bausch and Lomb refractometer. At the wavelengths measured, the index of refraction of pure ethylene glycol was found to be greater than that for pure water by a constant amount.

$$\left(\frac{3}{n^2+2}\right)_{H_2O} - \left(\frac{3}{n^2+2}\right)_{\text{ethylene glycol}} = 0.0545$$

Refractive indices of ethylene glycol-H_2O mixtures were proportional to ethylene glycol concentration. Thus, low-wavelength values for $(3/(n^2 + 2))_{15\%\ \text{ethylene glycol}}$ were estimated by the use of the equation, $(3/(n^2 + 2))_{H_2O} - 0.15(0.0545)$.

(3) METHANOL (50%)-NaCl (0.2 M). The index of refraction of this solvent was measured at 589, 546, and 436 mμ, using a Bausch and Lomb refractometer. In addition, the change in $n_{589\ \mathrm{m}\mu}$ over a temperature range 25–64° was measured.[1] The refractive index at 233 mμ was calculated by means of the Sellmeier approximation (Fasman, 1963), $(n^2 - 1) = a\lambda^2/(\lambda^2 - \lambda_0^2)$, where $a = 0.7857$ and $\lambda_0 = 95.6$ mμ, and was corrected for temperature effects assuming the same temperature dependence for $n_{233\ \mathrm{m}\mu}$ and for $n_{589\ \mathrm{m}\mu}$.

Infrared Absorption Spectra. FILMS. A solution of poly-L-lysine·HCl of about 4 mg/0.05 ml was prepared and the pH was altered as desired. The solution was unidirectionally stroked on a AgCl plate until dry. The film was further dried *in vacuo* over P_2O_5 for approximately 18 hr at 35°.

Spectra in the 1200–2500-cm^{-1} region were recorded using a Perkin-Elmer Model 621 infrared spectrophotometer purged with dry CO_2-free air. A clean AgCl plate was mounted in the reference beam.

SOLUTION. Poly-L-lysine·HCl was dissolved in D_2O. After 30–40 min, the sample was frozen and lyophilized. For the spectral determination, the deuterated polymer was dissolved in D_2O immediately before use (concentration ≈0.24%). The pD was adjusted to 12.3

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[1] The decrease in refractive index (n_D) with temperature was found to be 25–40° (2.5×10^{-4})/deg; 40–52.5°, (2.8×10^{-4})/deg; and 52.5–64°, (3.1×10^{-4})/deg.

by addition of NaOD. NaOD was prepared by dropping clean sodium metal into D_2O until a pD of 13–14 was reached. The polymer solution was put into a water-jacketed sealed cell of 0.05-mm path length, equipped with CaF_2 windows (Beckman-Limit Co.). (It has since been found that a demountable cell equipped with Teflon spacers is more suitable since it can easily be cleaned. The contents do not evaporate at 50°.) Spectra were recorded from 1300 to 1950 cm^{-1} at temperatures of 15, 34, and 47° as controlled by a circulating water bath. The 0.05-mm reference cell contained NaOD, pD = 12.3, and remained at the ambient temperature of the sample compartment. A tenfold ordinate scale expansion was used for these measurements.

Fogging could be prevented at 15° by purging the instrument with dry CO_2-free air. Suitable precautions were taken to exclude H_2O from all deuterated samples.

Results

The α-Helix–Random Coil Transition. TEMPERATURE DEPENDENCE. At temperatures below T_β, the thermal stability of helical poly-L-lysine can be measured. Figure 1 shows the temperature-dependent conversion of the α-helical to the random form of poly-L-lysine measured in several solvents. The magnitude of $[m']_{233}$ is taken as a measure of the helical content (Simmons *et al.*, 1961; Yang and McCabe, 1965; Sage and Fasman, 1966). The fully helical polypeptide was found to have $[m']_{233} = -14{,}700$[2] (Greenfield *et al.*, 1967). The value reported here of −13,100 has not been corrected for the water of hydration of the polypeptide. The relative thermal stability of helical poly-L-lysine is similar in water, 0.2 M NaCl, 0.05 M LiBr, 15% ethylene glycol, and combinations of these four solvents with each other. The transition is not sharp over the temperature range indicated. In the solvent mixture 50% CH_3OH–0.2 M NaCl, the helical stability is enhanced. The helical conformation of poly-L-lysine appears to be destabilized in 2 M LiBr and 2 M LiCl, but the data on these solvents are scanty because T_β is very close to room temperature. Of interest, however, is the observation that at 22° the helical conformation of poly-L-lysine is more than 80% intact in 2 M lithium halide. Under these conditions, poly-L-glutamic acid has lost virtually all of its helical structure (Fasman *et al.*, 1964).

The standard enthalpy for the helix–coil transition in H_2O and 0.2 M NaCl was obtained from the slope of the straight line resulting from a plot of ln K *vs.* $1/T$, where the slope $= -\Delta H°/R\sigma^{1/2}$ (Zimm and Bragg, 1959; Applequist, 1963). The ratio, [random coil]: [α helix] = K, was obtained from the data of Figure 1, using a value of $[m']_{233\ m\mu} = -13{,}100$ for the fully helical form and $[m']_{233\ m\mu} = -2{,}200$ for the fully random form. σ was assumed to be 3×10^{-3} (Rifkind and Applequist, 1964; Snipp *et al.*, 1965). The thermodynamic parameters, calculated at $T = 298°K$, for the formation of the random form of poly-L-lysine from the α-helical form are $\Delta H° = +890$ cal/mole of residue, $\Delta F° = +1500$ cal/mole of residue, and $\Delta S° = -2$ eu. The decrease in entropy on going to the random form might reflect either a σ value which is too small for this polypeptide and degree of polymerization or an increased ordering of water molecules around the lysyl side chains of the solvated random conformation. The $\Delta H°$ value is in good agreement with that obtained from pH titrations of poly-L-lysine as reported by Hermans (1966). However, the $\Delta F°$ value reported here is 1 kcal larger than $\Delta F°$ values derived from titration data of Hermans (1966), Applequist and Doty (1962), and Miller and Nylund (1965). These investigators report an increase in entropy upon formation of the random coil while the present study indicates that the entropy of the system has decreased in the α-helix–coil transition. The exact values of the calculated thermodynamic parameters are open to serious question, because, as mentioned above, the cooperative nature of the helix–coil transition, as expressed through the σ value, may be incorrect. The accessible portion of the transition shown in Figure 1 is indeed too broad to be termed cooperative. It should be noted, however, that if a totally noncooperative transition is assumed, an improbable value of ~16 kcal/mole of residue is obtained for $\Delta H°$. The σ value used here was derived from both thermal measurements and pH titration data of poly-L-glutamic acid (Rifkind and Applequist, 1964; Snipp *et al.*, 1965). This polymer is similar to poly-L-lysine. The pH-mediated helix–coil transition appears cooperative for both polymers (Applequist and Doty, 1958; Rifkind and Applequist, 1964; Miller and Nylund, 1965) while the thermal transition of poly-L-glutamic acid is very broad (Fasman *et al.*, 1964) as is that reported herein for poly-L-lysine. The observed broadening of the thermal transition is probably due to hydrophobic side-chain interactions which increase in strength, stabilizing the helical structure, as the temperature is raised.

The α-Helix–Random Coil–β-Structure Transition. TEMPERATURE AND CONCENTRATION DEPENDENCE. The rate of the α–random coil–β transition of poly-L-lysine above pH 11 is dependent upon both concentration and temperature. The temperature dependence for 0.2 M NaCl is seen in Figure 2. The rate of β formation was recorded as the time-dependent loss of the trough at 233 mμ ($\Delta[m']_{233\ m\mu}$/min). A similar temperature dependence was found in H_2O.

The temperature at which β formation begins, T_β, decreases (Table I) and the rate of β formation increases (Figure 3) as the polymer concentration increases. The concentration-dependent rate (38–39°) of β formation for three solvents, 0.2 M NaCl, H_2O, and 15% ethylene glycol–0.2 M NaCl, is seen in Figure 3. The initial rates show a greater than proportional increase with increasing polymer concentration. Moreover, the over-all α–β transition is observed to be 1.6

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[2] This represents a revision of the previously reported average $[m']_{233}$ value of −15,700 (Davidson *et al.*, 1966) which included two spuriously high $[m']_{233}$ values.

order, as measured by $\Delta[m']_{198.6\ \mathrm{m}\mu}$/min over the course of the transition. The above findings indicate that the β structure is being formed *via* intermolecular chain aggregation.

Effect of Solvents on the α-Helix–Random Coil–β Transition. The effect of solvent on both T_β and the rate of the transition to the β structure is summarized in Figure 3 and Table I. In the following discussion, comparisons of rates are made at similar concentrations and temperatures; T_β values are compared at similar concentrations.

The rate of β-structure formation increases in the following order as the solvent is changed: ethylene glycol (15%) < H_2O < 0.2 M NaCl or NaF < 1.25 and 2 M LiBr.

T_β decreases as the solvent is changed: ethylene glycol (15%)–0.05 M LiBr–0.2 M NaCl > 15% ethylene glycol–0.2 M NaCl or H_2O = 0.05 M LiBr–0.2 M NaCl > H_2O = 0.2 M NaCl > 0.5 M LiBr = 2.0 M LiBr. Unlike $\Delta[m']_{233\ \mathrm{m}\mu}$/min, T_β does not appear to reflect a change in solvent from H_2O to NaCl.

In summary, the results show that the solvent 50% CH_3OH–0.2 M NaCl does not support β formation over the temperature range studied. The β structure can be formed in ethylene glycol, but T_β is higher and $\Delta[m']$/min is less than at comparable conditions in either H_2O or 0.2 M sodium halide. At concentrations of LiBr greater than 0.5 M, T_β is markedly decreased and the rate of β formation increased with respect to all of the solvents investigated.

pH Dependence on the Rate of β Formation. The rate of the α–random–β transition of poly-L-lysine is increased as the pH is increased under similar conditions of temperature and polymer concentration (Table II).

ORD Studies of the α and β Forms of Poly-L-Lysine. The Moffitt parameters (Moffitt, 1956; Moffitt and Yang, 1956) found for the α-helical and β form of poly-L-lysine are as follows: α helix, $a_0 = +162$, $b_0 = -689$; β structure, $a_0 = -63$, $b_0 = -241$. The wavelength ranges were 460–278 mμ for measurements of the β form and 400–278 mμ for measurements for the α form. A λ_0 of 212 mμ was used. Sarkar and Doty (1966) have obtained $a_0 = -65$ and $b_0 = -580$ for the α helix and $a_0 = -343$ and $b_0 = -147$ for the β form of this polymer. Applequist and Doty (1962) and Cassim and Taylor (1965) report $a_0 = -30$ and $b_0 = -650$ for the helical form dissolved in 0.2 M NaCl.

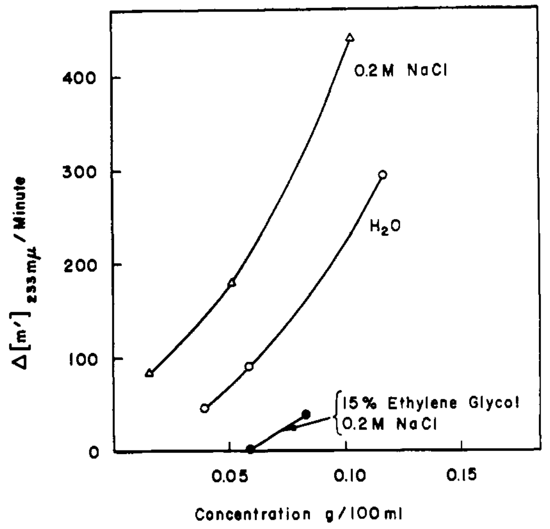

FIGURE 3: The dependence of the rate of the α–β transition of poly-L-lysine, $\Delta[m']_{233\ \mathrm{m}\mu}$/min, on polymer concentration. Rates measured in 0.2 M NaCl (Δ), H_2O (O), and 15% ethylene glycol–0.2 M NaCl (●). The temperature was 38–39°; pH was 11.6–11.7.

TABLE II: pH Dependence of the Rate of α to β.[a]

Solvent (M)	pH	Rate, α–β ($\Delta[m']_{233\ \mathrm{m}\mu}$/min)	Concn (%)
H_2O	12.30	276	0.049
H_2O	11.62	120	0.052
H_2O	11.57	90	0.059
NaF (0.2)	12.27	560	0.049
NaCl (0.2)	11.70	181	0.052

[a] Temperature = 38–39°. Data were chosen from a number of experiments in which the temperature and concentration were approximately the same, but the pH differed as indicated.

The intrinsic Cotton effects of the α-helical and β conformations of poly-L-lysine are shown in Figure 4. Curve α represents the ORD spectrum of the helical form, obtained at 22.5°, pH 11.06, H_2O. After spectrum α was obtained, the cell and its contents were heated at 51° as described in the Methods section. The decrease in the ORD peak at $\lambda_{198.6\ \mathrm{m}\mu}$ was followed as a function of time, as shown in Figure 4 (left side). When $\Delta[m']_{198.6\ \mathrm{m}\mu}$/min approached zero, the cell and sample were cooled to 22.5° and curve β was obtained. This spectrum is assigned to that of the β form of poly-L-lysine. The assignment will be supported by infrared and X-ray data.

The shift in both position and magnitude of the ORD spectrum of poly-L-lysine as it undergoes a thermal conversion from the α-helical to β form is illustrated in Figure 5. The spectra have been photographed from the recorder chart paper. The transition was interrupted at the indicated times by cooling to 23°, thus permitting the ORD spectra to be obtained without change during the measurement.

In Table III are summarized the far-ultraviolet ORD parameters for α, β, and random poly-L-lysine. These are average values obtained from several experiments. Included in Table III are CD data obtained by Townend

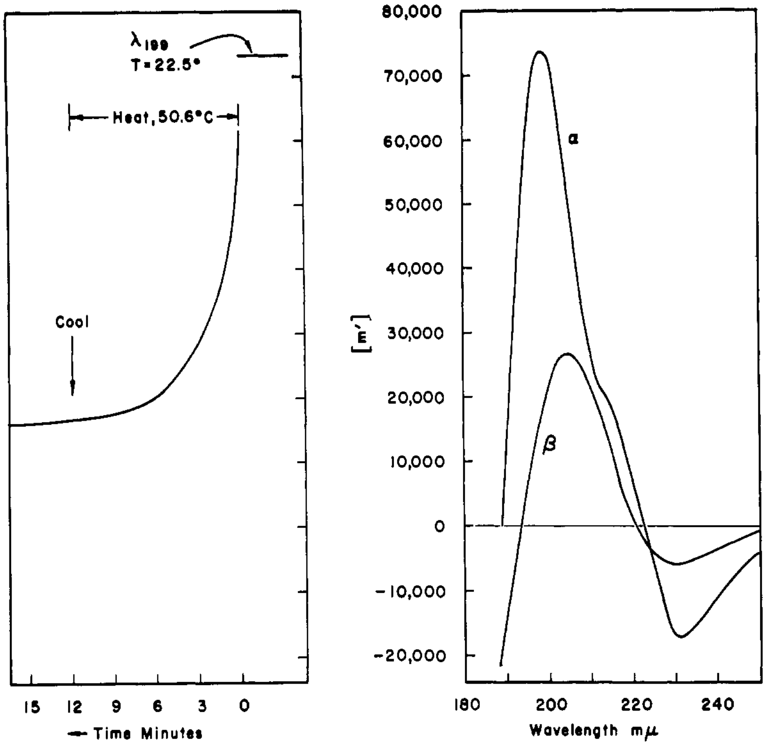

FIGURE 4: The ORD of α and β poly-L-lysine. α: Poly-L-lysine·HCl dissolved in H_2O, pH 11.06, $c = 0.0123\%$. The ORD was determined at 22.5°. β: After obtaining curve α, the sample was heated, 51° *in situ* for about 15 min. When no further decrease in $[m']_{198.6\ m\mu}$ was noted, the cell and contents were cooled to 22.5° and curve β was obtained. The heating process, as followed by $\Delta[m']_{198.6\ m\mu}$/min, is shown in the left-hand portion of the figure. Measurements were made in a water-jacketed cell of 1-mm path length.

TABLE III: The Intrinsic Rotatory Parameters of α, β, and Random Poly-L-lysine.

	ORD Minimum		ORD Maximum		Cross-Over Wavelengths (mμ)	Ultraviolet Spectrum, λ_{max} (mμ)
	Position (mμ)	$[m']$[a]	Position (mμ)	$[m']$[a]		
Poly-L-lysine, α	233	−14,720 ± 990[b]	198.6	+70,860 ± 3620[c]	189, 223	188[f,i] 190[g,h,i]
Poly-L-lysine, β	230	−6,250 ± 546[d]	205	+29,100 ± 1874[e]	194, 220.5	194[f] 194[g]
Poly-L-lysine·HCl, random	205	−21,930 ± 1136[b]			196	193[f] 192[g]
Poly-L-lysine, α calculated from CD data of Townend *et al.* (1966)	233	−16,000	198.0	+57,000	187.5, 222	
Poly-L-lysine, β calculated from CD data of Townend *et al.* (1966)	230	−5,200	206	+21,800	194, 222	

[a] Reduced mean residue rotation; Fasman (1963). [b] Average of eight determinations. [c] Average of nine determinations. [d] Average of five determinations. [e] Average of six determinations. [f] F. Cordelle, B. Davidson, and G. Fasman, unpublished data. [g] Data of Rosenheck and Doty (1961). [h] Data of Tinoco *et al.* (1962). [i] λ_{max} 188 mμ (ϵ_{max} 6085) (F. Cordelle, B. Davidson, and G. Fasman, unpublished data); λ_{max} 190 mμ (ϵ_{max} 4400) (Rosenheck and Doty, 1961; Tinoco *et al.*, 1962).

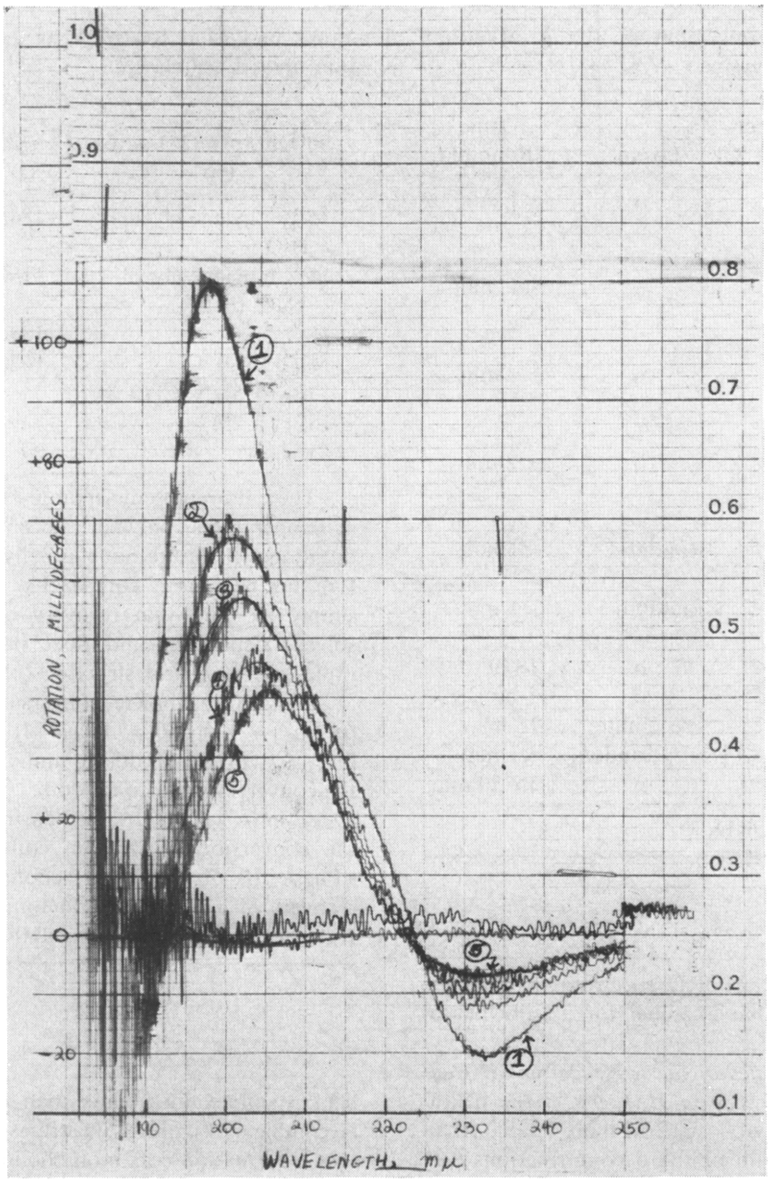

FIGURE 5: The conversion of poly-L-lysine, α helix to β form. The ORD was measured as a function of time. The data have been photographed directly from the Cary 60 recorder chart paper. Curve 1: α-helical poly-L-lysine. Curves 2–5: The α–β transition was initiated by heating to 51° and was interrupted at various times by rapid cooling to 23°. The total heating times were: curve 2, 2.5 min; curve 3, 5.5 min; curve 4, 13.5 min; and curve 5, 26.5 min. Spectrum 5 represents the completed conversion to β poly-L-lysine. The concentration was 0.0195%, pH 11.07, optical path length 1 mm.

et al. (1966) on the same preparation of poly-L-lysine as was used in this work. When transformed into ORD parameters, these data are in reasonable agreement with the results of this study. The ORD spectra of α and β poly-L-lysine obtained by Sarkar and Doty (1966) also are in reasonable agreement with the present study.

The crossover wavelengths of the ORD spectra of α and β poly-L-lysine agree well with the positions of the ultraviolet absorption maxima, as shown in the last column of Table III. The correspondence is not as close in the case of the random conformation. It should be noted that ultraviolet spectra obtained in this laboratory (F. Cordelle, B. Davidson, and G. Fasman, unpublished data) for two of the three forms of poly-L-lysine agree with those reported by Rosenheck and Doty (1961). There is agreement with data for the random and β forms, but not for the α-helical form (see footnotes *f*–*i*, Table III). The results of Rosenheck and Doty for the helical form could be more closely approximated using their conditions of pH and concentration. However, under these conditions,

TABLE IV: The Interconversion of the α, Random, and β Forms of Poly-L-lysine.[a]

Procedure	Temperature (°C)	Product	Time Required
Poly-L-lysine·HCl + OH^- (random)	4	α	<1 min
Poly-L-lysine·HCl + OH^- (random)	25	α	<1 min
Poly-L-lysine·HCl + OH^- (random)	39	α	<1 min
Poly-L-lysine·HCl + OH^- (random)	50	β	<2 min
α poly-L-lysine + H^+	25	Random	<1 min
β poly-L-lysine + H^+	22.5	Random	<1 min
β poly-L-lysine	50–4	α	18 hr
β poly-L-lysine	50–25	β	1 hr
β poly-L-lysine	50–25	Precipitate	18 hr
α poly-L-lysine	20–$<T_\beta$	α + random	<1 min
α poly-L-lysine	20–50	β	15–20 min

[a] As determined by ORD.

TABLE V: Partial Recovery of the Helical Conformation upon Cooling.[a]

Solvent, pH 11.3–11.6 (M)	Phase		
	I[b]	II[c]	III[d]
15% ethylene glycol–H_2O	1845	1105	1765
15% ethylene glycol–NaCl (0.2)	1160	2320	1160
H_2O	1850	1760	1850
NaCl (0.2)	1680	1770	0
LiBr (0.5)–NaCl (0.2)	*e*	4180	0
LiBr (1.25)	*e*	6940	0
LiBr (2)	*e*	3555	0

[a] The data represent separate experiments performed at temperatures, polymer concentrations, and heating durations which are not mutually identical and, therefore, not necessarily comparable. Table V is intended simply to show that recovery of helical structure is solvent dependent and does not exceed the initial rapid loss of helical structure (phase I). Initial $[m']_{233} = -14{,}720$; complete conversion to β would yield $[m']_{233} = -6250$. [b] Phase I: initial decrease in $[m']_{233\,\mathrm{m}\mu}$ observed during initial heating; represents the conversion of α to random. [c] Phase II: further decrease in $[m']_{233\,\mathrm{m}\mu}$ observed during heating; represents the conversion of random coil to the β structure. [d] Phase III: observed after cooling to 20°; represents recovery of α helix from random coil. [e] Phases I and II cannot be separated owing to the fast rate of phase II.

poly-L-lysine exhibits an ORD spectrum that is little more than one-half that expected for a fully helical polypeptide.

*The Relative Stabilities of the α-Helical and β Conformations of Poly-*L*-lysine in H_2O.* At 4° the helical conformation of poly-L-lysine is more stable than either the random chain or the β conformations (pH 11–11.3, 0.01–0.13%), as shown in Table IV. Thus, over a period of 18 hr at 4°, a solution of β poly-L-lysine slowly resumes the α-helical form when cooled from 50°. If base is added to random poly-L-lysine·HCl at 4°, the helical form is immediately obtained.

At 50°, the β form is the most stable conformation of poly-L-lysine. At this temperature the α helix is completely and irreversibly converted to the β form. If base is added to random poly-L-lysine at 50°, the polymer immediately assumes the β conformation with no detectable formation of an α-helical intermediate.

At 25°, the situation is complicated by slow precipitation of the β form. Thus, when β poly-L-lysine is cooled from 50 to 25°, the polymer eventually precipitates out instead of regaining the helical conformation. If base is added to random poly-L-lysine·HCl, at 25°, the α helix is formed (Applequist and Doty, 1962). The above results are summarized in Table IV.

In order to change the conformation of poly-L-lysine from helical to β, the temperature must be held at some value greater than T_β until the transition is complete. Figure 5 illustrates that point, showing that β formation ceases on cooling and the conformation does not immediately revert to α. When β poly-L-lysine is held at 25° for prolonged periods, precipitation of the β aggregates occurs, preventing the system from reattaining equilibrium. In contrast, the data at 4 and 50° are unambiguous. It is seen that somewhere between these temperatures the most stable form of poly-L-lysine changes from the α-helical to the β form. Since hydrophobic interactions are more stable at elevated than at low temperatures (Kauzmann, 1954), it is suggested that these forces are the major ones stabilizing the β conformation of poly-L-lysine.

The Reversibility of the Initial Stages of the α-Random Coil-β Transition. If the heat-induced α-β transition is interrupted in its early stages by cooling to 20°, partial recovery of the helix as measured by the ORD parameter, $[m']_{233\,\mathrm{m}\mu}$, is seen, depending on the solvent system (Table V). If the transition is permitted to go to completion, recovery of helical structure is not observed in any solvent during cooling periods of up to 1 hr.

These changes may be considered to occur in three phases. In all instances where recovery is seen, elevation of the temperature reveals two changes in $[m']_{233\ m\mu}$: a very rapid initial decrease (phase I), followed by the onset (phase II) of the $\Delta[m']$/min characteristic of the particular concentration, temperature, and solvent system. Phase I most probably represents an α-helix–random coil transition, while phase II reflects formation of the β from the random structure. The recovery (phase III) of $[m']_{233\ m\mu}$ upon cooling to 20° is approximately equal to or less than the initial rapid decrease (phase I) observed upon heating.

There is no recovery of helical structure in 0.2 M NaCl, even under conditions where $\Delta[m']_{233\ m\mu}$/min is slow enough to allow observation of the separated transitions. These data suggest that the helical structure which is regained upon cooling is formed from the product of the first rapid decrease in $[m']_{233\ m\mu}$, probably the random form of poly-L-lysine. The β structure probably remains intact during the period of cooling. Thus, the equilibrium position of α-helix–random chain is quickly attained upon changing temperature, while β formation proceeds more slowly. These conclusions hold for all of the solvents investigated.

*The Stability of β Poly-*L*-lysine in Water and 15% Ethylene Glycol.* An attempt was made to measure the temperature-induced β–random chain equilibrium of β poly-L-lysine in water and in 15% ethylene glycol. This experiment would compare the relative stability of the β conformation in the two solvents at elevated temperatures where the α-helix–random coil equilibrium is not a complicating factor. The β form proved to be relatively stable in both solvents, however, and the observed ORD changes were small over the temperature range investigated (60–93°). In 15% ethylene glycol, a 25% diminution of the ORD spectrum of the β form was observed as the temperature was increased from 60 to 93°. One-half of the total decrease was recoverable upon cooling back to 60°, the remainder being lost probably owing to precipitation of some of the β form. When the same experiment was attempted with water as solvent, a portion of the β poly-L-lysine precipitated and there was no recovery of the ORD spectrum upon cooling to 60°. Thus, it appeared that ethylene glycol assisted in melting out the β form.

*Infrared Absorption Spectra of Poly-*L*-lysine.* FILMS. Films of poly-L-lysine were cast from H_2O on AgCl plates at pH 5 and 10. At pH 5, the material appeared to be a mixture of the β form and the random coil and/or α-helical form. The amide I region showed two distinct bands at 1625 and 1651 cm^{-1}, and a small shoulder at about 1691 cm^{-1}. The amide II region showed a broad skewed band at 1532–1545 cm^{-1}.

If the polymer was cast from H_2O at pH 10, the material seemed to be almost completely in the β conformation. The amide I region showed a band at 1625 and another at 1696 cm^{-1}, with possibly a shoulder remaining at around 1650 cm^{-1}. The amide II region showed a component at 1530 cm^{-1} with a shoulder of almost equal intensity at 1542 cm^{-1}.

It has not yet been possible to obtain an oriented film of this material. Thus, the assignment of either a cross β or intermolecular β by infrared spectroscopy remains unresolved.

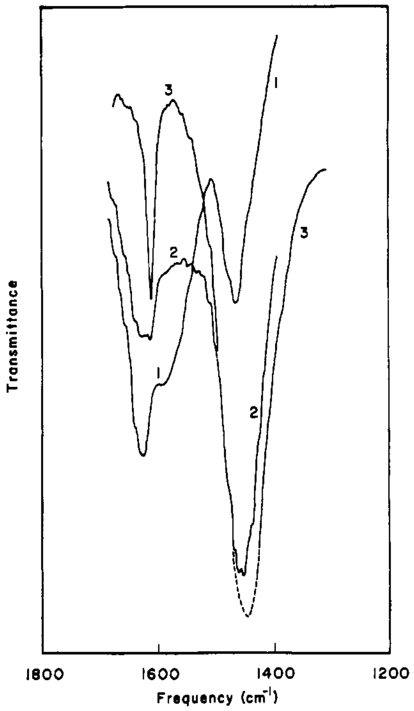

FIGURE 6: The transition of α to β poly-L-lysine, infrared spectra. Poly-L-lysine in D_2O (0.24%) at pD 12.3. Spectra were recorded at 15 (spectrum 1), 34 (spectrum 2), and 47° (spectrum 3). (The dashed portion of curve 3 extended beyond the ordinate scale and has been estimated.) A jacketed CaF_2 cell of 0.05-mm optical path length was used.

D_2O SOLUTION. The conversion by heating of the α form to the β form in D_2O is seen in Figure 6. A D_2O solution of deuterated poly-L-lysine (pD = 12.3, concentration $\approx$0.24%) was prepared as described in the Methods section. Infrared spectra were obtained over the region of the amide I and II absorption bands (1300–1950 cm^{-1}) at 15, 34, and 47°. At 15°, the amide I and II region showed a band at 1628 cm^{-1} with a shoulder at 1585 cm^{-1} and a second band at 1465 cm^{-1}. At 34°, there was a broad band (1612–1632 cm^{-1}) in the amide I region and a second band at 1455 cm^{-1} in the amide II region. At 47°, the solution exhibited a very sharp absorption band at 1612 cm^{-1}, a much smaller band at 1680 cm^{-1}, and an amide II band which extended beyond the ordinate scale, but seemed to be centered at about 1445 cm^{-1} (dashed curve in Figure 6). These results confirm the ORD finding that poly-L-lysine undergoes a thermal conversion from the α to the β form. It should be noted that the amide I band position of the helical form reported here is about 10 cm^{-1} lower than that reported by Townend *et al.* (1966) and by Rosenheck and Doty (1961), but is in agreement with that reported by Sarkar and Doty (1966) and Apple-

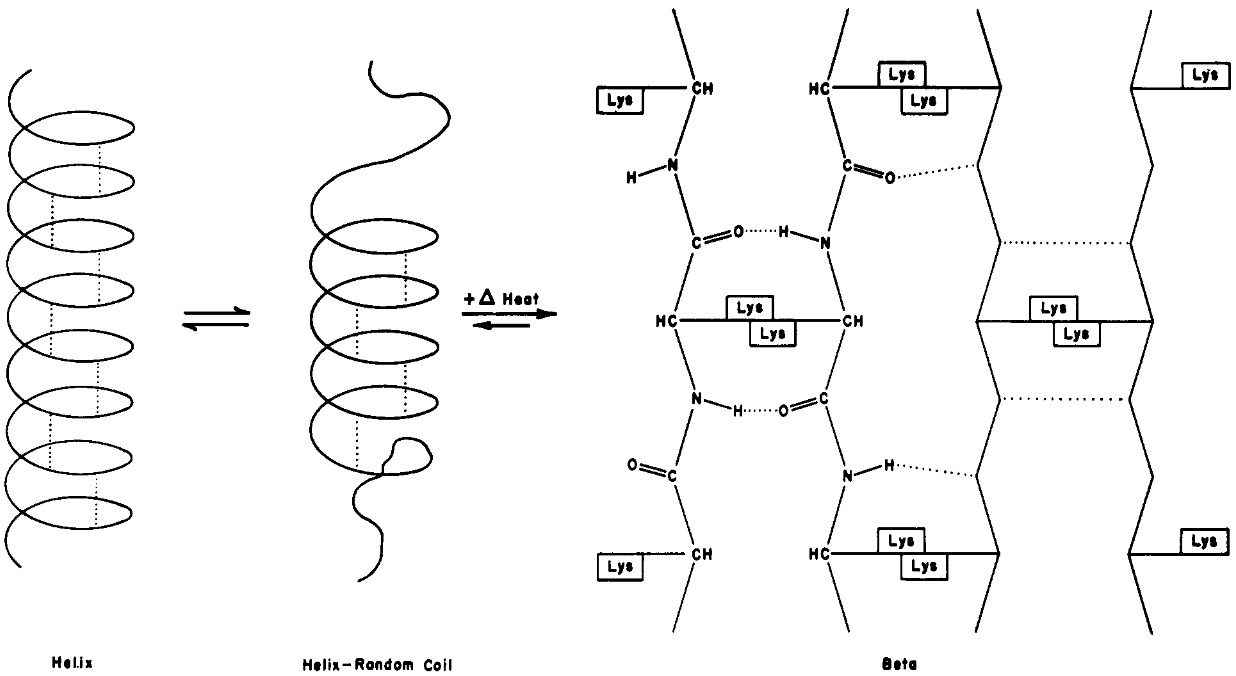

FIGURE 7: A schematic representation of the proposed mechanism of the α–β transition of poly-L-lysine.

quist and Doty (1962). The sample used for this study had been previously deuterated; this may account for the observed differences.

Discussion

Evidence for β Structure. Poly-β-lysine is thermally converted from the α-helical to the β conformation. Briefly, the evidence that the heated product is in the β conformation is as follows. The ORD is similar to that observed for films of polymers known by other criteria to be in the β conformation (Blout and Shechter, 1963; Davidson *et al.*, 1966; Fasman and Potter, 1967). The ORD spectrum (peak at 205 mμ) of heated poly-L-lysine is not explicable as a sum of contributions from the random and helical forms. Such mixtures would always have the ORD peak displaced toward lower wavelengths than 198.6 mμ. (See the following paper (Greenfield *et al.*, 1967) and also Jirgensons, 1966.) Infrared spectroscopy shows that upon heating, the amide I band of deuterated poly-L-lysine shifts from 1628 (helical) to 1612 and 1680 cm^{-1} (β), confirming the original observations of Doty and co-workers (Applequist and Doty, 1958, 1962; Rosenheck and Doty, 1961; Sarkar and Doty, 1966) and Townend *et al.*, (1966). The 1680-cm^{-1} absorption band has been assigned to that of the antiparallel β structure (Miyazawa and Blout, 1961). Infrared spectra of films of poly-L-lysine cast from H_2O (pH 10.0) show that the polymer is in the antiparallel β form (the amide I bands are at 1628 and 1696 cm^{-1}). In D_2O solution, a second small band at 1690 cm^{-1} also indicates that the polypeptide chain orientation is antiparallel. X-Ray powder patterns of the heated product show reflections characteristic of the β form (M. L. Tiffany and S. Krimm, unpublished data). Finally, the rate of formation of the β structure produced by heating is concentration dependent. This would indicate that the β structure is formed by an inter- rather than intramolecular aggregation, in contrast to the suggestion of Sarkar and Doty (1966) that β poly-L-lysine is an intramolecular structure. Their conclusion was based on the comparative sedimentation characteristics of the three forms of poly-L-lysine as determined at 20° by a 50-hr centrifugation in a sucrose gradient. However, the β form has been observed to precipitate slowly over an 18-hr period at the concentration (0.01%) used in their experiments (see Table IV). It is probable that precipitation of aggregated β poly-L-lysine occurred during the 50-hr centrifugation. This would account for the fact that the radioactivity recovered in the β sample appears to be only about 25% of that recovered in the random form of the polymer (Sarkar and Doty, 1966). In this paper, the evaluation of inter- and intramolecular aggregation was based mainly on studies of the initial rate of the α–β transition rather than on consideration of the final β state, thus avoiding the problem of precipitation.

Mechanism of the Transition. It is postulated that the transformation from the α to the β form involves the participation of an intermediate random conformation. The proposed sequence is α helix–random coil–β pleated sheet (Figure 7). The following observations are offered in support of this thesis. Procedures which increase the concentration of random coil also increase the rate of β formation. Thus, partial disruption of the helical conformation, either by heat or by 1–2 M lithium halide, results in an increased rate of β formation. A decreased T_β is observed in the case of lithium salts (Table I). Methanol (50%) stabilizes the helical conformation at elevated temperatures (Figure 1). In this solvent, temperatures approaching the boiling point do not produce enough random poly-L-lysine to allow β structure initiation.

The partial reversibility observed at the early stages of the α–β transformation disappears as the transition proceeds (Figure 5 and Table V). This is consistent with the idea that the α helix can only be re-formed from a species which is present in limited amounts during the course of the transition, but which is depleted as the transition is completed. This intermediate is probably the random form of poly-L-lysine. The data of Table V show that the amount of helical structure recovered corresponds roughly to the amount lost in the initial rapid helix–random coil transition. The postulated random coil intermediate is not formed in sufficient amounts to allow its observation as a blue shift in the 199-mμ ORD maximum. In order to observe the shift postulated by Greenfield *et al.* (1967), the random coil content would have to be above 40%.

Finally, the β conformation is formed much more rapidly from the random form at 50° than from the α form at 50° (Table IV). This indicates that the rate of β formation is limited by the concentration of random coil present.

The over-all transition of α–β is much slower than either α–random or random–β. This is probably due to the fact that the first transition is intramolecular and the second intermolecular. When chains are composed of both helical and random regions, their collisions are not always fruitful in producing β structures, whereas pure random chains have a higher probability of yielding the β form.

Relative Stability of α and β Structures. The above discussion has shown that the concentration of the random coil intermediate is one factor affecting both T_β and the rate of β formation. However, it is not the only factor. Inspection of Figure 1 shows that between 11 and 42°, the thermal stability of helical poly-L-lysine is the same in H_2O, 0.2 M NaCl, 0.05 M LiBr, and 15% ethylene glycol, *i.e.*, the concentration of the random form at a given temperature is the same in all of the above solvents. Nonetheless, T_β is markedly higher in 15% ethylene glycol and 0.2 M NaCl–0.05 M LiBr than it is in water or 0.2 M NaCl (Table I). Thus, it appears that at the same concentration of the random coil intermediate, there is a temperature range in which 15% ethylene glycol and 0.05 M LiBr do not support formation of the β structure while H_2O and 0.2 M NaCl do. Furthermore, the rate of β-structure formation is greatly decreased in 15% ethylene glycol, indicating that the ratio of "successful" to "unsuccessful" β-forming collisions is lower per unit time in this solvent that it is in H_2O or 0.2 M NaCl. Both the increased T_β and the decreased $\Delta[m']$/min are consistent with the suggestion that 15% ethylene glycol and 0.05 M LiBr destabilize the β structure with respect to H_2O. Despite the technical difficulties cited above, the temperature melt out of the β structure in ethylene glycol at elevated temperature also supports this view.

The rate of β formation is increased in 0.2 M NaCl, compared to H_2O (Figure 3). This might indicate that the β form is more stable in 0.2 M NaCl than it is in H_2O. Above T_β, 0.2 M NaCl has a greater stabilizing effect on the β form of poly-L-lysine than on the helical structure. This is not true for water. The experiments summarized in Table V show that if a system containing all three conformations of poly-L-lysine is cooled below T_β, the random regions that are present will assume the α conformation if the solvent is H_2O. No recovery is seen if the solvent is 0.2 M NaCl, implying that the random regions have been completely incorporated into the β structure.

These results may be explained on the following basis. The electrostatic shielding by an electrolyte might be expected to have a greater stabilizing effect on the β form than on the α helix. In the β structure the amino acid side chains are closer together than in the helical conformation (Marsh *et al.*, 1955). Therefore, any residual charge on the lysyl residues would be more disruptive to the former structure.

Lastly, in the presence of salts, the lysyl side chains would almost certainly have a greater tendency to interact with one another. Model studies with leucine, tyrosine (Cohn and Edsall, 1943), and 1-butanol (Reber *et al.*, 1942) have shown that these compounds are strongly "salted out" of aqueous solution by NaCl and KCl.

*Conformational Stabilization Forces of the α Helix and the β Structure of Poly-*L*-lysine. α Helix.* Over the temperature range and solvents investigated, only 50% methanol stabilizes the helical conformation of poly-L-lysine. This is in accord with the concept of the α helix as a hydrogen-bonded structure, the integrity of which mainly depends upon maintenance of $C{=}O\cdots HN$ bonds. In a medium of decreased dielectric constant (50% methanol), more thermal energy would be required to disrupt the interamide hydrogen bonds. Moreover, the helical form behaves like a hydrogen-bonded structure in that it appears to be more stable at low temperatures (4° to approximately 20°) than at high temperatures.

β Structure. The β structure is more stable at high (50–60°) than at low temperatures. This is a property of a structure stabilized by hydrophobic interactions (Kauzmann, 1954). β formation appears to be facilitated by the addition of 0.2 M NaCl or NaF. As previously noted, this "salting-out" phenomenon could be thought of as decreasing the energy required for hydrophobic interactions between lysyl side chains.

Ethylene glycol (15%) appears to destabilize the β structure, but has no effect on the α structure. This compound is a better solvent than is water for apolar molecules (Curme and Johnston, 1952; Nozaki and Tanford, 1965), and might thus be considered capable of disrupting hydrophobic interactions. The partial specific volume, $\bar{v}$, of ethylene glycol reaches a minimum value at a concentration of 15% (Nakanishi, 1960). At this point of minimum $\bar{v}$, the reagent is believed by some investigators (Franks and Ives, 1966) to have a maximal ability to structure solvent water molecules. β-Lactoglobulin undergoes dissociation from a dimeric to a monomeric form in about 15% ethylene glycol, reverting to the dimeric structure in 20% ethylene glycol (Kientz and Bigelow, 1966).

Infrared (Blout and Lenormant, 1957) and X-ray (Schmueli and Traub, 1965) studies of poly-L-lysine have shown that the β structure is increasingly more stable than the α helix as water is removed from the environment. In aqueous solution, the β structure may be thought of as analogous to a micelle, the interior of which has a diminished water content. The solubility of this hydrophobic micelle would be dependent upon its size.

Summary

Poly-L-lysine, at pH 11.2, at 25°, is in the α-helical conformation. Heating to temperatures below T_β causes the transition of the α-helical to the random form. This process is reversible on cooling. Upon heating to temperatures above T_β, however, the concentration of random chains is sufficient to allow their association into a β structure. At $T > T_\beta$, this newly formed structure is more stable than that of the α helix and grows in size, unravelling the helix until the poly-L-lysine has been completely converted to the β form. The greater thermodynamic stability of the β form at elevated temperatures may result from strengthened hydrophobic interactions (Kauzmann, 1954) of the lysyl side chains in the closely packed pleated-sheet conformation (Marsh *et al.*, 1955). The view that hydrophobic interactions play a role in the stabilization of β poly-L-lysine is further supported by the finding that ethylene glycol destabilizes and 0.2 M NaCl probably stabilizes the β form of poly-L-lysine.

The thermodynamics of the transition may be viewed as follows. Upon heating, the energy necessary to break the hydrogen bonds stabilizing the helical structure is supplied. On raising the temperature further, lysyl residues of the random chain are removed from the aqueous environment and are associated, forming the β structure. The positive enthalpy for this association is derived from the heating process. The over-all free energy for the β formation is negative, however, and above T_β, the β form of poly-L-lysine is the most stable conformation.

Although our understanding of the β structure has been aided by this study, one should be cautious in generalizing the conclusion to apply to all β proteins and polypeptides. Recent ORD studies (Fasman and Potter, 1967; Tooney, 1966; S. Ikeda and G. D. Fasman, manuscript in preparation) have revealed at least two categories of β structures which probably represent different β forms. Whether all β structures have similar properties to those described for β poly-L-lysine remains to be investigated.

This work has illustrated that the same polypeptide sequence is capable of assuming more than one definitive conformation when environmental conditions are altered. In analogy to the work presented here, the precipitation of heat-denatured proteins may in part be due to insoluble β-structure formation.

Acknowledgments

We wish to thank Drs. M. L. Tiffany and S. Krimm for their X-ray analysis of heated poly-L-lysine. We wish to thank F. Cordelle for the data concerning the extinction coefficients of the various conformations of poly-L-lysine. The assistance of Mrs. M. Wells is greatly appreciated.

References

Ambrose, E. J., and Elliot, A. (1951), *Proc. Roy. Soc. (London) A206*, 206.

Applequist, J. (1963), *J. Chem. Phys. 38*, 934.

Applequist, J., and Doty, P. (1958), Abstracts, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April, p 32a.

Applequist, J., and Doty, P. (1962), *in* Polyamino Acids, Polypeptides and Proteins, Stahmann, M., Ed., Madison, Wis., University of Wisconsin, p 161.

Astbury, W. T., Dalgleish, C. E., Darman, S. E., and Sutherland, G. B. B. M. (1948), *Nature 162*, 596.

Astbury, W. T., and Marwick, J. C. (1932), *Nature 130*, 309.

Astbury, W. T., and Woods, H. J. (1930), *Nature 126*, 913.

Blake, C. C. F., Koenig, D. F., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R. (1965), *Nature 206*, 757.

Bloom, S. M., Fasman, G. D., De Lozé, C., and Blout, E. R. (1962), *J. Am. Chem. Soc. 84*, 458.

Blout, E. R. (1957), *Ann. N. Y. Acad. Sci. 69*, 84.

Blout, E. R. (1960), *in* Optical Rotatory Dispersion, Djerassi, C., Ed., New York, N. Y., McGraw-Hill.

Blout, E. R. (1962), *in* Polyamino Acids, Polypeptides and Proteins, Stahmann, M., Ed., Madison, Wis., University of Wisconsin, p 275.

Blout, E. R., and Lenormant, H. (1953), *J. Opt. Soc. Am. 43*, 1093.

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

Blout, E. R., Schmier, I., and Simmons, N. S. (1962), *J. Am. Chem. Soc. 84*, 3193.

Blout, E. R., and Shechter, E. (1963), *Biopolymers 1*, 568.

Bradbury, E. M., Elliot, A., and Hanby, W. E. (1962), *J. Mol. Biol. 5*, 487.

Cassim, J. Y., and Taylor, E. W. (1965), *Biophys. J. 5*, 573.

Cohn, E. J., and Edsall, J. T. (1943), Proteins, Amino Acids and Peptides, New York, N. Y., Reinhold, p 239.

Curme, G. D., and Johnston, F. (1952), Glycols, New York, N. Y., Reinhold, p 48.

Davidson, B., and Fasman, G. D. (1966), *Federation Proc. 25*, 412.

Davidson, B., Tooney, N., and Fasman, G. D. (1966), *Biochem. Biophys. Res. Commun. 23*, 156.

De Santis, P., Giglio, E., Liquori, A. M., and Piromonti, A. (1965), *Nature 206*, 456.

Dickerson, S., and Bailey, K. (1935), *Biochem. J. 29*, 2351.

Fasman, G. D. (1963), *Methods Enzymol. 6*, 928.

Fasman, G. D., and Blout, E. R. (1960), *J. Am. Chem. Soc. 82*, 2262.

Fasman, G. D., Idelson, M., and Blout, E. R. (1961), *J. Am. Chem. Soc. 83*, 709.

Fasman, G. D., Lindblow, C., and Bodenheimer, E. (1964), *Biochemistry 3*, 155.

Fasman, G. D., and Potter, J. (1967), *Biochem. Biophys. Res. Commun. 27*, 209.

Franks, F., and Ives, D. J. G. (1966), *Quart. Rev. (London) 1*, 1.

Greenfield, N., Davidson, B., and Fasman, G. D. (1967), *Biochemistry 6*, 1630 (this issue; following paper).

Harrap, B. S., and Stapleton, I. W. (1963), *Biochim. Biophys. Acta 75*, 31.

Hermans, J., Jr. (1966), *J. Phys. Chem. 70*, 510.

Iizuka, E., and Yang, J. T. (1965), *Biochemistry 4*, 1249.

Iizuka, E., and Yang, J. T. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 1175.

Ikeda, S., Maeda, H., and Isemura, T. (1964), *J. Mol. Biol. 10*, 223.

Imahori, K., and Yahara, I. (1964), *Biopolymers Symp. 1*, 421.

Jirgensons, B. (1966), *J. Biol. Chem. 241*, 147.

Kauzmann, W. (1954), *in* The Mechanism of Enzyme Action, McElroy, W. D., and Glass, B., Ed., Baltimore, Md., John Hopkins, p 70.

Kientz, M. L., and Bigelow, C. C. (1966), *Biochemistry 5*, 3494.

Lang, C. A. (1958), *Anal. Chem. 30*, 1692.

Marsh, K. E., Corey, R. B., and Pauling, L. (1955), *Biochim. Biophys. Acta 16*, 1.

Meyer, K. H., and Mark, H. (1928), *Ber. Deut. Chem. Ges. 61*, 1932.

Miller, W. G., and Nylund, R. E. (1965), *J. Am. Chem. Soc. 87*, 3542.

Miyazawa, T., and Blout, E. R. (1961), *J. Am. Chem. Soc. 83*, 712.

Moffitt, W. (1956), *Proc. Natl. Acad. Sci. U. S. 42*, 736.

Moffitt, W., and Yang, J. T. (1956), *Proc. Natl. Acad. Sci. U. S. 42*, 596.

Nakanishi, K. (1960), *Bull. Chem. Soc. Japan 33*, 793.

Némethy, G., and Scheraga, H. A. (1965), *Biopolymers 3*, 155.

Nozaki, Y., and Tanford, C. (1965), *J. Biol. Chem. 240*, 3568.

Parker, K. D., and Rudall, K. M. (1957), *Nature 179*, 905.

Pauling, L., and Corey, R. B. (1951), *Proc. Natl. Acad. Sci. U. S. 37*, 729.

Pauling, L., Corey, R. B., and Branson, H. R. (1951), *Proc. Natl. Acad. Sci. U. S. 37*, 205.

Ramachandran, G. N., Ramakrishnan, C., and Sasisekharan, V. (1963a), *in* Aspects of Protein Structure, Ramachandran, G. N., Ed., New York, N. Y., Academic, p 1.

Ramachandran, G. N., Saisekharan, V., and Ramakrishnan, C. (1963b), *J. Mol. Biol. 7*, 95.

Reber, L. A., McNabb, W. M., and Lucasse, W. W. (1942), *J. Phys. Chem. 46*, 500.

Rifkind, J., and Applequist, J. (1964), *J. Am. Chem. Soc. 86*, 4207.

Rosenheck, K., and Doty, P. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1775.

Sage, H. J., and Fasman, G. D. (1966), *Biochemistry 5*, 286.

Sarkar, P. K., and Doty, P. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 981.

Schellman, J. A., and Schellman, C. G. (1961), *J. Polymer Sci, 49*, 129.

Schmueli, U., and Traub, W. (1965), *J. Mol. Biol. 12*, 205.

Simmons, N. S., Cohen, C., Szent-Gyorgi, A. G., Wetlaufer, D. B., and Blout, E. R. (1961), *J. Am. Chem. Soc. 83*, 4766.

Snipp, R. L., Miller, W. G., and Nylund, R. E. (1965), *J. Am. Chem. Soc. 87*, 3547.

Timasheff, S. N., and Susi, H. (1966), *J. Biol. Chem. 241*, 249.

Timasheff, S. N., and Townend, R. (1965), *Biochem. Biophys. Res. Commun. 20*, 360.

Timasheff, S. N., Townend, R., and Mesanti, L. (1966), *J. Biol. Chem. 241*, 1863.

Tinoco, I., Jr., Halpern, A., and Simpson, W. T. (1962), *in* Polyamino Acids, Polypeptides and Proteins, Stahmann, M., Ed., Madison, Wis., University of Wisconsin, p 147.

Tomimatsu, Y., and Gaffield, W. (1965), *Biopolymers 3*, 509.

Tooney, N. M. (1966), Ph.D. Thesis, Brandeis University, Waltham, Mass.

Townend, R., Kumosinski, T. F., Timasheff, S. N., Fasman, G. D. and Davidson, B. (1966), *Biochem. Biophys. Res. Commun. 23*, 163.

Troitski, G. V. (1965), *Biofizika 10*, 895.

Urnes, P., and Doty, P. (1961), *Advan. Protein Chem. 16*, 401.

Wada, A., Tsuboi, M., and Konishi, E. (1961), *J. Phys. Chem. 65*, 1119.

Yang, J. T., and McCabe, W. J. (1965), *Biopolymers 3*, 209.

Zamenhof, S., and Chargaff, E. (1963), *Methods Enzymol. 3*, 702.

Zimm, B. H., and Bragg, J. K. (1959), *J. Chem. Phys. 31*, 526.