ordered conformation in TFE and TMS, while some conformational transition is induced by the addition of MSA.

Acknowledgments. We wish to thank Mr. R. A. Veneski for providing certain samples. We wish to thank Dr. G. Montaudo for his suggestions. One of us (Y. S.) is grateful to Toray Industries, Inc., Japan, for giving him the opportunity to perform this work at The University of Michigan. We also thank The University of Michigan, Rackham Fund, for Chemistry fellowship support through the Macromolecular Research Center and the Department of Chemistry. The authors are grateful for financial support by the U. S. Army Research Office-Durham, under Grant No. DAHCO4-69-C-0050.

Solution Properties of Synthetic Polypeptides. Circular Dichroism Studies on Poly-L-histidine and on Random Copolymers of L-Histidine and L-Lysine in Aqueous Solution

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ABSTRACT: The conformational properties of poly-L-histidine (PLH) and of random copolymers of lysine and histidine in aqueous solution have been investigated by CD techniques in the spectral range 185–250 nm. It was found that PLH undergoes a cooperative conformational transition on varying the extent of protonation of the polymer side chains. CD studies on the random copolymers show that increasing amounts of histidine residues cause a linear change of the CD spectrum on going from pure protonated poly-L-lysine (PLL) to pure protonated PLH. On the basis of these data, it was concluded that completely protonated PLH exists as a random coil in aqueous solution. Similar copolymer studies have been carried out in a methanol–water solution containing 92% methanol. In this solvent mixture protonated PLL exists as a right-handed α helix, and *deprotonated* PLH is in the same conformation as in water. Increasing amounts of *deprotonated* histidine residues in the *protonated* PLL chain cause a sharp conformational transition which occurs when the histidine content is higher than 80%. From these results it was concluded that the ordered form of charge-free PLH in water *is not* that of a right-handed α helix.

At the present time the conformation of poly-L-histidine (PLH) in aqueous solution has not been safely established. Previous investigations carried out by Norland, *et al.*,[1] and by Beychok and coworkers[2] have shown that PLH undergoes a conformational transition induced by pH changes. At a pH lower than 3, the random coil was assumed to be the most probable conformation, on the basis of viscosity and CD data. At pH 5.8, Beychok and coworkers[2] assumed that PLH is in the right-handed α-helical form on the basis of the presence of a negative CD band at 223 nm. On the contrary, Norland, *et al.*,[1] on the basis of optical rotation measurements and other evidence tentatively concluded that PLH in aqueous solution at pH 5.8 exists as a left-handed α helix. None of these assignments should be considered definitive, since the interpretation of ORD and CD data in terms of conformation is complicated by the presence of imidazole side-chain groups which may contribute to the total optical activity.

In previous papers[3-6] we have studied the conformation in solution of aromatic poly(α-amino acids) by measuring the CD properties of copolymers of the aromatic amino acids with a simple amino acid whose homopolymer has a known conformation in solution. These copolymer studies allowed us to draw conclusions concerning the conformation in solution of poly-L-tryptophan,[3-4] poly-L-phenylalanine,[5] and poly(1-benzyl-L-histidine).[6]

The present paper reports CD and potentiometric studies in aqueous solution on a highly purified sample of PLH and on random copolymers of L-lysine and L-histidine of various composition in order to investigate the perturbations of the CD pattern induced by increasing amounts of histidine residues in the peptide chain. The spectroscopic studies have been extended down to 185 nm.

Experimental Section

Solvents and Materials. Reagent grade dioxane (Carlo Erba RP) was dried over potassium–anthracene complex as previously described[3] and distilled immediately before use. The water content in the distillate was less than 0.002% by weight. Petroleum ether (40–70°, Carlo Erba RP) was refluxed over sodium wire and then fractionally distilled. Spectrograde methanol (Fisher Scientific Co.) was used as received. Dichloroacetic acid (DCA) and trifluoroacetic acid (TFA), both Carlo Erba RP products, were used without any further purification. Triethylamine (Carlo Erba RP) was refluxed over KOH and then fractionally distilled from potassium metal, rejecting the first 10% of the distillate. Nitromethane (Carlo Erba RP) was dried over P_2O_5 and fractionally distilled.

1-Benzyl-L-histidine *N*-carboxyanhydride (Bz-His-NCA) was obtained from *N*-carbobenzoxy-1-benzyl-L-histidine[7] and PCl_5 follow-

(1) K. Norland, G. D. Fasman, E. Katchalski, and E. R. Blout, *Biopolymers*, **1**, 277 (1963).
(2) S. Beychok, M. N. Pflumm, and J. E. Lehmann, *J. Amer. Chem. Soc.*, **87**, 3990 (1965).
(3) A. Cosani, E. Peggion, A. S. Verdini, and M. Terbojevich, *Biopolymers*, **6**, 963 (1968).
(4) E. Peggion, A. Cosani, A. S. Verdini, A. Del Pra, and M. Mammi, *ibid.*, **6**, 1477 (1968).
(5) E. Peggion, A. Cosani, A. S. Verdini, and E. Scoffone, *Macromolecules*, **2**, 170 (1969).
(6) M. Terbojevich, M. Acampora, A. Cosani, E. Peggion, and E. Scoffone, *ibid.*, **3**, 618 (1970).
(7) B. G. Overell and V. Petrow, *J. Chem. Soc.*, 232 (1955).

TABLE I
CHARACTERIZATION DATA FOR RANDOM COPOLYMERS OF LYSINE AND HISTIDINE

Copolymer	His/Lys mol ratio[a]	His/Lys mol ratio (R)[b]	His content, mol %	H_2O content,[c] wt %
No. 1	0.200	0.198	16.5	11.4
No. 2	0.500	0.488	32.8	9.5
No. 3	1.00	0.869	46.5	13.0
No. 4	3.00	2.775	73.5	8.8
No. 5	5.00	4.264	81.0	7.3
No. 6	8.00	7.548	88.3	10.0

[a] Molar ratio in the initial monomer mixtures. Ratios of N^{ϵ}-carbobenzoxy-L-lysine NCA to 1-benzylhistidine NCA used in the preparation of the blocked copolymers. [b] Molar ratio found after removing the blocking group. Ratios of lysine/histidine in the final deblocked copolymers, as determined by amino acid analysis. [c] Average values of elemental analysis, uv absorption measurements, amino acid analysis, and direct determination of the water content.

ing the procedure of Patchornik, *et al.*[8] The product so obtained was purified by several recrystallizations from nitromethane.

N^{ϵ}-Carbobenzoxy-L-lysine N-carboxyanhydride (Z-Lys-NCA) was prepared from N^{ϵ}-carbobenzoxy-L-lysine[9] and phosgene in dioxane according to the literature.[10]

Polymers and Copolymers. Poly-L-histidine (PLH) was prepared from poly(1-benzyl-L-histidine) by reaction with sodium in liquid ammonia, according to the procedure of Norland, *et al.*[1] In a three-necked 250-ml flask, 150 ml of ammonia was condensed, with rigorous exclusion of moisture. Small pieces of sodium metal (previously cleaned with ethanol) were added to the liquid until a persistent blue color appeared. Then, 3 g of poly(1-benzyl-L-histidine) was added with stirring. Sodium metal was added during the reaction in order to maintain the blue color of the reaction mixture. The reaction was complete in 2 hr. Solid NH_4Cl was added in order to eliminate the excess of sodium, and the liquid ammonia was slowly evaporated at room temperature. The residual white powder was dissolved in 1 N HCl, and the by-products formed in the debenzylation reaction were extracted with ethyl ether. The hydrochloride solution was then exhaustively dialyzed against water, using a 4465-A2 dialyzing tubing (A. Thomas Co., Philadelphia, Pa.), which retains materials with molecular weight 12,000 or higher. The dialysis procedure was necessary in order to remove the low molecular weight fractions. Undialyzed samples give CD spectra much less intense than do the samples purified by dialysis. The aqueous solution of PLH hydrochloride was treated with an excess of 1 N NaOH and methanol. PLH separates as a white voluminous precipitate which was isolated by decantation and exhaustively washed with 1:1 methanol–water solution until disappearance of Cl^- ions. The polymer was subsequently washed with anhydrous acetone and ethyl ether, dried under vacuum at 100° for several hours to constant weight, and stored in a vacuum dessicator over P_2O_5. The water content of this sample, as determined by a modified Fisher apparatus,[11] was 10.0% by weight. The absence of benzyl groups was checked by uv absorption spectroscopy in the 260-nm region. The content of benzyl groups was less than 0.1% (by weight).

Anal. Calcd for $C_6H_7N_3O$: C, 52.55; H, 5.11; N, 30.68. Found: C, 47.38; H, 5.15; N, 27.26. These data are consistent with the water content of the polymer (calcd for PLH + 10% water: C, 47.3; N, 27.6; H, 5.70). The intrinsic viscosity in 1 N HCl was 0.20 dl/g.

Random Copolymers of Lysine and Histidine. Random copolymers of lysine and histidine of various composition were obtained from the parent copolymers of N^{ϵ}-carbobenzoxy-L-lysine and 1-benzyl-L-histidine[6] by treatment with sodium metal in liquid ammonia. The procedure was the same as for the preparation of PLH from poly(1-benzyl-L-histidine), but required a longer reaction time (5 hr), since removal of the carbobenzoxy group from the ϵ-amino groups of the lysine residues is quite slow. All copolymers were obtained as hydrochlorides by exhaustive dialysis against 0.02 N HCl (4465-A2 dialyzing tubing) and subsequent liophylization. Storage as the hydrochloride was necessary since the copolymers as free bases showed a strong tendency to become insoluble, probably because of β-structure formation. The molar ratio R of histidine to lysine in each copolymer was determined by amino acid analysis after hydrolysis of copolymer samples in 6 N HCl for 22 hr.

The water content of the final samples was checked by direct analysis using the Fisher procedure,[11] and by determination of the copolymer title both by amino acid analysis and by uv absorption measurements at 220 nm in 0.01 N HCl. In this last case, we determined the molar extinction coefficients of pure poly-L-lysine and of pure PLH in 0.01 N HCl. From the determined values [$(\epsilon_{220})_{His}$ = 4625, and $(\epsilon_{220})_{Lys}$ = 518], the molar amount of histidine in a copolymer solution was determined by the following equation

$$[\text{His}] = \frac{A_{220}R}{[(\epsilon_{220})_{His}R + (\epsilon_{220})_{Lys}]l}$$

R being the molar ratio of histidine to lysine in the copolymer, l being the optical cell path (in centimeters), and A_{220} being the total absorbance at 220 nm. The amino acid analysis also gives directly the title of the copolymers. The uv method requires additivity of the absorption bands of lysine and histidine residues; this condition holds in 0.01 N HCl, where both the homopolymers (PLH and poly-L-lysine) and the copolymers are in the random-coil conformation (see Results and Discussion). The absence of benzyl groups in the copolymers was checked by uv absorption measurements in the 260-nm region.

All the characterization data on the copolymers are collected in Table I.

Poly-L-lysine hydrochloride (PLL·HCl) was prepared from poly(N^{ϵ}-carbobenzoxy-L-lysine) (PCBL) by reaction with dry gaseous HBr in dry dioxane–chloroform solution, according to the procedure of Fasman, *et al.*[12] The water content was 8.2% by weight, and the intrinsic viscosity in 1 N HCl was $[\eta]$ = 1.0 dl/g at 25°.

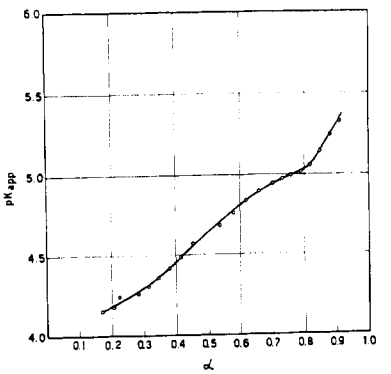

Figure 1. Potentiometric titration curve of PLH in aqueous solution.

(8) A. Patchornik, A. Berger, and E. Katchalski, *J. Amer. Chem. Soc.*, **79**, 5227 (1957).
(9) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N. Y., 1961, p 893.
(10) M. Sela and A. Berger, *J. Amer. Chem. Soc.*, **77**, 1893 (1955).
(11) We thank Dr. M. Acampora of our institute for the measurements.
(12) G. D. Fasman, M. Idelson, and E. R. Blout, *J. Amer. Chem. Soc.*, **83**, 709 (1961).

Apparatus and Measurements. Potentiometric titrations were carried out with a Metrohm Model E 388 precision potentiometer equipped with Metrohm UX combined glass electrodes or Beckman glass and calomel electrodes. Standard solutions of carbonate-free KOH were prepared according to the literature.[13] Titrations were carried out in the absence of added salt. The polymer concentration was always on the order of 0.2 g/l.

Circular dichroism (CD) measurements were carried out by a Cary Model 60 spectropolarimeter equipped with the 6002 CD accessory unit. The molar ellipicity values [θ] and the molar dichroic absorption values $\Delta\epsilon$ were calculated directly from the recorded spectra according to well-known equations.[14]

Uv absorption measurements have been carried out using a Cary 15 spectrophotometer equipped with a thermostatable cell assembly. In all spectroscopic measurements, fused quartz cylindrical cells of 0.5- or 1-mm path, with Suprasil windows, were used.

Results and Discussion

The results of potentiometric titrations of PLH in the absence of added salt are shown in Figure 1. The usual polyelectrolyte plot, pK_{app} *vs.* α, was calculated from the equation

$$pK_{app} = pH - \log \alpha/(1 - \alpha)$$

where α is the degree of ionization of the imidazolinium side chains (the extent of protonation is of course $(1 - \alpha)$).

Quite evident from the graph is an inflection point occurring at α values between 0.50 and 0.80 (*i.e.*, between 50 and 20% protonation of the basic side chains). This behavior is consistent with the occurrence of a conformational transition of the polymer induced by pH changes.

Figure 2 shows typical CD spectra of PLH recorded in the far-uv region (250–185 nm) at various extents of protonation. At $\alpha = 0$ (complete protonation), the spectrum is characterized by a positive band centered at 222 nm ($\Delta\epsilon = 3.05$) followed by a negative shoulder at 198 nm and by a strong negative band located near 187 nm ($\Delta\epsilon = 6.7$). Deprotonation of the imidazole side chains causes a marked change in the CD pattern. The positive band at 222 nm disappears and a negative band centered at *217 nm* is formed, followed by a positive band at 203 nm and by a negative one centered at 188 nm. At $\alpha = 0.879$ (*i.e.*, at ~12% protonation), the $\Delta\epsilon$ values of the three bands at 217, 203, and 188 nm are −2.5, +9.0, and −14.0, respectively. The CD spectrum in the 215–250-nm region is not consistent with that reported by Beychok, *et al.*[2] These authors in fact report for PLH at pH 5.78 a negative band at *223 nm* (Figure 3 of ref 2), while, according to our results, the negative band is located at *217 nm*. Furthermore, the intensity of the positive band at 222 nm of completely protonated PLH is ~30% higher in our spectra than that reported by the above-mentioned authors. In our opinion these discrepancies are due to the limited instrumentation used by these early workers.

Neither the spectrum at $\alpha = 0$ nor that at $\alpha = 0.879$ is easily interpretable in terms of conformation. In fact, these spectra do not resemble any of the spectra of polypeptides in the various known conformations[15] and certainly contain contributions from the side-chain aromatic chromophores.

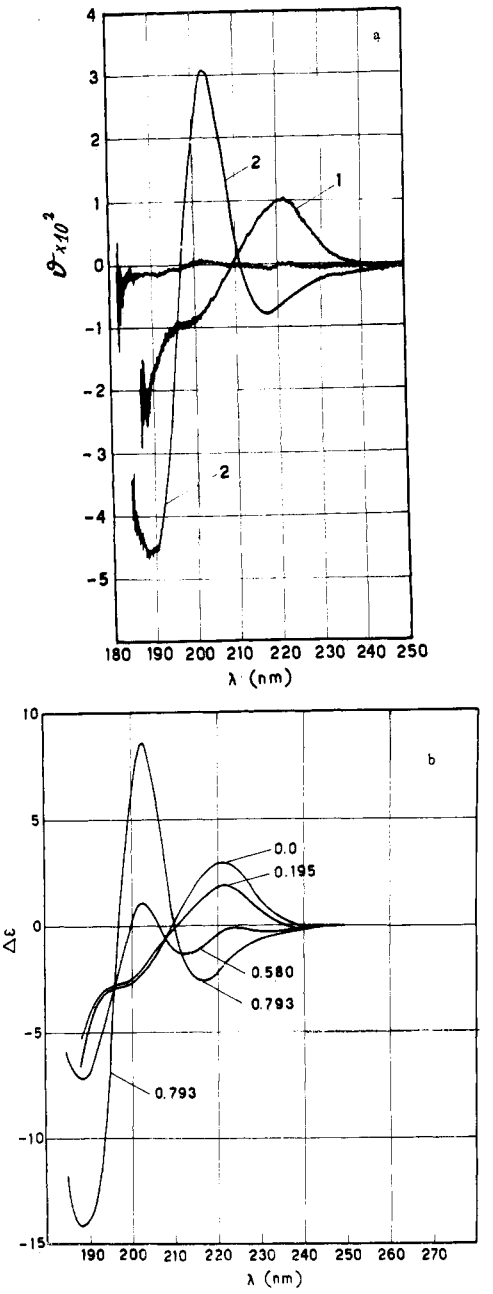

Figure 2. (a) Original CD spectra of PLH at $\alpha = 0$ (curve 1, 100% protonation) and at $\alpha = 0.879$ (curve 2, 12.1% protonation). The polymer concentration was 2.02×10^{-3} *M* in a 0.5-mm cell. In this and in subsequent figures, where original CD spectra recorded with the Cary spectropolarimeter are reported, the ordinate scale gives the measured ellipticity (degrees), from which the molar ellipiticity [θ] and the molar circular dichroism $\Delta\epsilon$ can be calculated.[14] In this case the ordinate scale corresponds to the 0.1 sensitivity of the instrument. (b) Some CD spectra of PLH at various degrees of ionization, calculated in terms of molar dichroic absorption values $\Delta\epsilon$.

In order to decide whether the observed changes of the CD pattern merely reflect the change of the nature of the side-chain chromophores because of protonation, the $\Delta\epsilon$ values at 203 and 222 nm have been reported as a function of the degree of ionization α. The results are shown in Table II and in Figures 3 and 4. The plot of the $\Delta\epsilon$ values at 203 nm at various protonation extents (Figure 3) unambiguously shows that PLH undergoes a pH-induced conformational transition. The α value at which the transition starts is

(13) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962, p 24.
(14) Instruction manual for the Cary Model 6002 CD accessory unit, p 368.
(15) S. Timasheff and M. G. Gorbunoff, *Annu. Rev. Biochem.*, **36**, 13 (1967).

TABLE II
Δε VALUES OF PLH MEASURED AT 222 AND 203 NM FOR VARYING EXTENTS OF PROTONATION OF THE POLYMER SIDE CHAINS

α^a	$\Delta\epsilon_{203}$	$\Delta\epsilon_{222}{}^b$
0	−2.25	3.00
0.152	−2.55	2.04
0.195	−2.30	1.92
0.220	−2.70	1.80
0.247	−2.70	1.74
0.273		1.60*
0.308	−2.40	1.50
0.315		1.51*
0.345		1.54*
0.416	−1.92	1.30
0.418		1.27*
0.426		1.00*
0.495	−1.08	0.97
0.516		0.60*
0.562		0.40*
0.580	1.08	0.15
0.609		0.15*
0.619		0.11*
0.701		−0.55*
0.705	5.70	−0.30
0.746		−0.61*
0.793	8.60	−1.30
0.833	8.80	−1.50
0.879	9.00	−1.75

[a] α is the degree of ionization of the imidazole side chains; the degree of protonation is therefore $1 - \alpha$. [b] Values marked with an asterisk were obtained using a 5-mm path-length cell. The remaining values were obtained using either 0.5- or 1-mm cell, which allow measurements to be carried out down to 185 nm.

nearly coincident with that at which the inflection point in the potentiometric titration curve is observed (Figure 1).

The conformational change appears to be complete at $\alpha \simeq 0.9$. CD measurements at $\alpha > 0.9$ are prevented by polymer precipitation.

On the other hand, the plot of the Δε values of the 222-nm band as a function of α does not clearly show a transition (Figure 4), indicating that the variation of this band, both in position and intensity, is mainly a consequence of the change of the nature of the side-chain chromophore because of

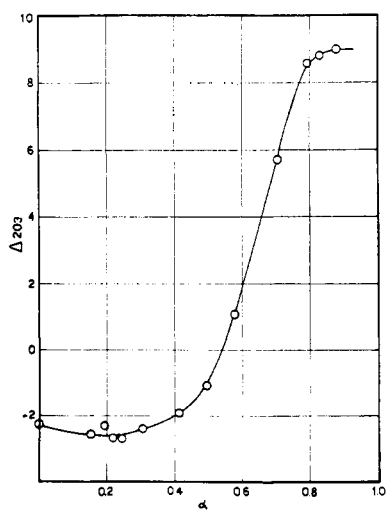

Figure 3. Δε values of PLH at 203 nm reported as a function of the degree of ionization α.

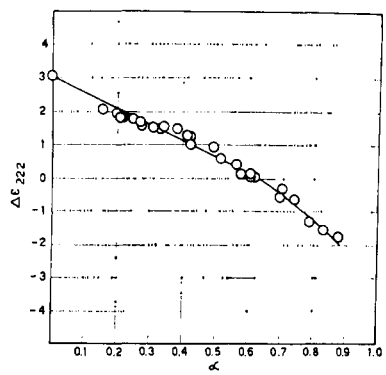

Figure 4. Δε values of PLH at 222 nm reported as a function of the degree of ionization α.

protonation. In other words, the CD spectrum in the 222-nm region seems to be scarcely sensitive to conformational changes of the peptide backbone, and should therefore contain major contributions from the side-chain chromophores.

The question now arises as to what the conformational states of PLH are at $\alpha = 0$ and 1. According to Norland, *et al.*,[1] the polymer should be in the random-coil form when the imidazole side chains are completely protonated ($\alpha = 0$). However, Beychok, *et al.*,[2] pointed out that the imidazolinium band at 222 nm is present only in the polymer, while the free amino acid does not show appreciable rotational strength in the 220-nm region. This behavior, which has been also confirmed by other authors,[16] could suggest that PLH is

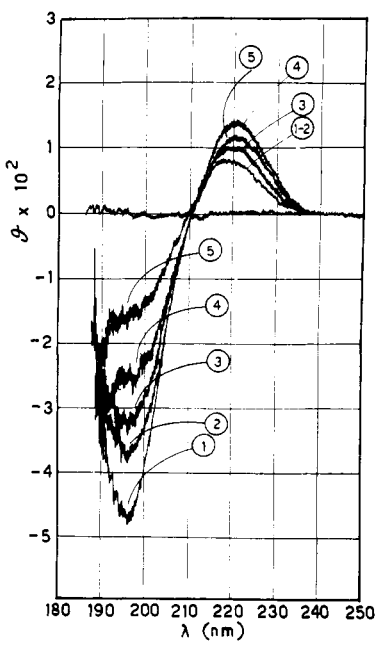

Figure 5. Some original CD spectra of completely protonated histidine–lysine random copolymers in aqueous solution: (1) copolymer no. 1 (0.2215 g/l.), (2) copolymer no. 2 (0.2063 g/l.), (3) copolymer no. 3 (0.2193 g/l.), (4) copolymer no. 4 (0.2445 g/l.), and (5) copolymer no. 5 (0.2002 g/l.). All spectra recorded in a 1-mm cell. The ordinate scale corresponds to the 0.1 sensitivity of the instrument.

(16) D. W. Urry and H. Eyring, *J. Amer. Chem. Soc.*, **86**, 4574 (1964).

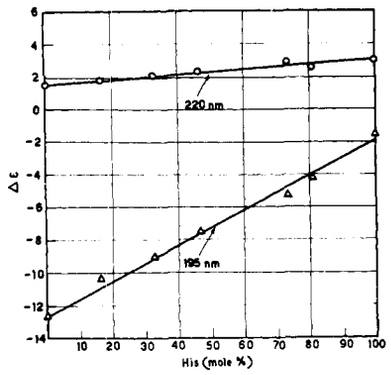

Figure 6. $\Delta\epsilon$ values at 195 and 220 nm of completely protonated lysine–histidine copolymers and of protonated PLL and PLH, plotted as a function of the histidine cotent (mole per cent) of the peptide chains.

not in the random-coil conformation at high degrees of protonation.

In order to clarify this important point, we have studied the CD spectra of random copolymers of lysine and histidine of various composition, at pH conditions at which all the basic side-chain groups (ϵ-NH_2 and imidazole) are completely protonated ($\alpha = 0$). The results of such studies are shown in Figures 5 and 6 and in Table III, where the CD data are reported as a function of the copolymer composition. The intensity of the CD spectra at two different wavelengths, *i.e.*, at 220 and 195 nm, are linearly proportional to the histidine content of the copolymers (Figure 6). Such a result seems to indicate that the progressive introduction of protonated histidine residues into the peptide chains does not alter the random-coil conformation of protonated PLL. Therefore, it can be concluded that the conformation of charged PLH is that of a random coil. Furthermore, the linearity of the graph of Figure 6 indicates that the contributions to the optical activity from the imidazolinium side-chain chromophores are additive. It is also interesting to note that the imidazolinium band at 222 nm is optically active *in the coiled polymer* and not in the free amino acid.[2,16] This means that, for a given histidine residue, the presence of neighboring histidine residues is sufficient to create a dissymmetric environment which causes optical activity.

Similar copolymer studies have been carried out in an attempt to elucidate the conformation of PLH in the region of low degrees of protonation ($\alpha \simeq 0.90$). In this case it

TABLE III

$\Delta\epsilon$ VALUES OF PLL, PLH, AND OF RANDOM COPOLYMERS OF LYSINE AND HISTIDINE MEASURED AT 195 AND 220 NM[a]

Sample	His content, mol %	$\Delta\epsilon_{195}$	$\Delta\epsilon_{220}$
PLL	0	−12.5	1.6
Copolymer no. 1	16.55	−10.4	1.82
Copolymer no. 2	32.8	−9.1	2.10
Copolymer no. 3	46.5	−7.55	2.36
Copolymer no. 4	73.5	−5.30	2.90
Copolymer no. 5	81.0	−4.26	2.58
PLH	100	−1.75	3.00

[a] All measurements were carried out in the presence of an excess of the HCl necessary to protonate both amino and imidazole group side chains.

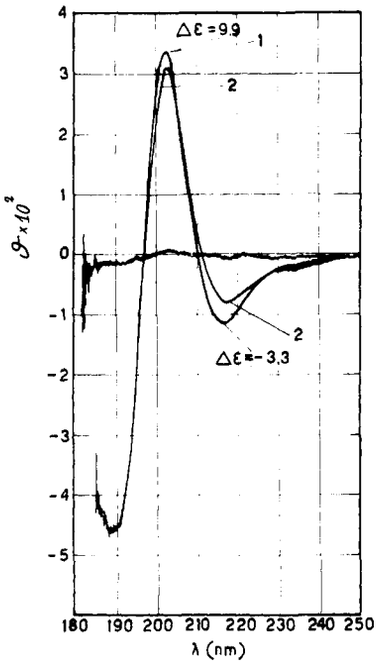

Figure 7. Original CD spectrum of PLH at $\alpha = 0.879$ (12.1% protonation) in methanol–water solvent containing 92% (by volume) of methanol (curve 1). The spectrum recorded in water and at the same value of α is also shown for comparison (curve 2). The polymer concentration was 2.02×10^{-3} *M*; a 0.5-mm cell was used. The ordinate scale corresponds to the 0.1 sensitivity of the instrument.

was not possible to carry out CD measurements on random copolymers of lysine and histidine and on PLH as well, in water, at pH >11, at which both lysine and histidine side chains are completely deprotonated. In fact, at pH >6.0 PLH and histidine-rich copolymers separate from the solution. This difficulty was overcome by carrying out the measurements in a methanol–water solvent mixture containing 92% (v/v) methanol. In such a solvent, as is known, completely protonated PLL assumes the right-handed α-helical form.[17] The substantial difference in base strength between the ϵ-amino groups of lysine and imidazole groups makes it possible to perform CD measurements on random copolymers of lysine and histidine in which the lysine side chains are completely protonated and the imidazole groups are not. In this particular solvent mixture we have also found (Figure 7) that PLH at $\alpha \simeq 0.9$ assumes the same conformation as in water. In fact, the CD pattern is exactly the same; only the intensities of the bands are slightly higher in 92% methanol (the $\Delta\epsilon$ values are −3.3 and 9.9 at 217 and 203 nm, respectively). This could probably be due either to a solvent effect, to a higher proportion of PLH in the ordered form, or to both. The results of CD measurements carried out in 92% methanol on PLL, PLH, and on random copolymers of lysine and histidine are shown in Table IV and in Figures 8 and 9. In all cases, the amount of HCl required to protonate the ϵ-amino groups of lysine was added. Table IV and Figure 9 report the $\Delta\epsilon$ values at 222 and 203 nm as a function of the histidine content of the copolymers. These wavelengths have been chosen since at 222 nm there is a

(17) R. F. Epand and H. A. Scheraga, *Biopolymers*, 6, 1383 (1968).

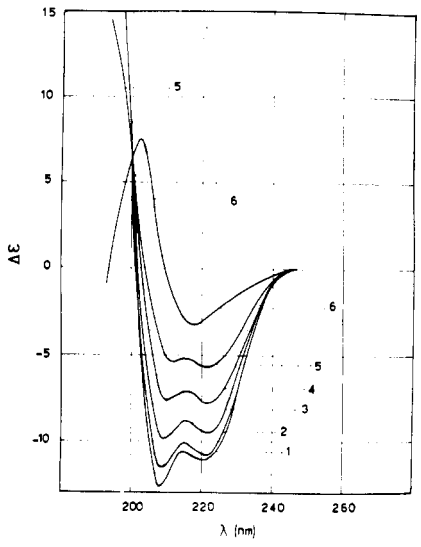

Figure 8. CD spectra of some histidine–lysine random copolymers in 92% methanol solution, and in the presence of the stoichiometric amount of 0.1 *N* HCl necessary to protonate only the ε-amino groups of lysine residues: (1) copolymer no. 1, (2) copolymer no. 2, (3) copolymer no, 3, (4) copolymer no. 4, (5) copolymer no. 5, and (6) copolymer no. 6.

CD band dependent on the conformation of lysine residues, and at 203 nm there is a CD band dependent on the conformation of histidine residues. If the conformation of the ordered form of charge-free PLH were that of a right-handed α helix, we should find a linear or monotonic variation of the CD pattern on increasing the histidine content of the peptide chains from 0 (pure PLL) to 100% (pure PLH).[3-6] Figure 9 unambiguously shows that this is not the case. The Δε values at 203 and 222 nm increase linearly with the histidine content only until ~70% histidine (on molar basis); between 70 and 90% histidine there is a sharp variation of the copolymer conformation from the right-handed α-helical form to a new ordered structure. These findings lead to the conclusion that *the ordered conformation of charge-free PLH is not that of a right-handed α helix.* The results of our copolymer studies contradict the conclusion drawn by previous workers,[2] based on the presence of a negative CD band in the 220-nm region.

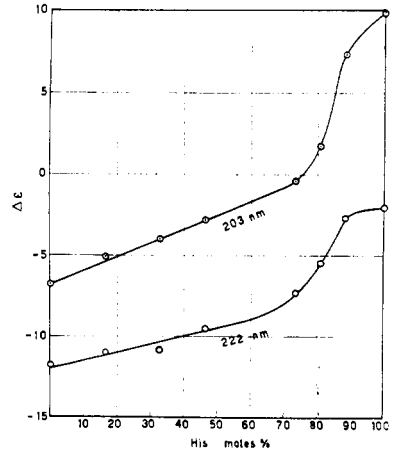

Figure 9. Δε values at 203 and 222 nm (from Figure 8 and Table IV) reported as a function of the copolymer composition.

TABLE IV
Δε VALUES MEASURED AT 222 AND 203 NM FOR RANDOM COPOLYMERS OF LYSINE AND HISTIDINE[a]

Sample	His content, mol %	$\Delta\epsilon_{222}$	$\Delta\epsilon_{203}$
PLL	0	−11.80	−6.82
Copolymer no. 1	16.6	−11.06	−5.10
Copolymer no. 2	32.8	−10.80	−4.05
Copolymer no. 3	46.5	−9.55	−2.83
Copolymer no. 4	73.5	−7.35	−0.40
Copolymer no. 5	81.0	−5.55	1.75
Copolymer no. 6	88.3	−2.73	7.43
PLH[b]	100.0	−2.06	9.90

[a] In 92% methanol and in the presence of the stoichiometric amount of HCl necessary to protonate *only* the ε-amino groups of lysine residues. [b] From Figure 7.

Very recently Zundel and coworkers[18] carried out ir studies on PLH films at various extents of protonation. They report that at 0 and 50% protonation PLH occurs as an α helix. According to the discussion of Miyazawa, *et al.*,[19] their results seem consistent with a *right-handed* α helix. It is well known that the conformation in the solid state, besides other factors, depends primarily on the "history" of the sample. For this reason it is always possible that the conformation in solution is different from that in the solid state. Zundel and coworkers prepared samples at 0 and 50% protonation by treating a 100% protonated film with KOH. We are studying the solid-state conformation of deprotonated PLH samples obtained by slow precipitation with KOH from a solution of PLH hydrochloride.

Under these conditions it should be possible to obtain solid samples in which the polymer molecules have the same conformation as in solution. In such a way we should be able to investigate by X-ray and ir techniques the conformation in the solid state of deprotonated PLH having the same conformation as in solution. As shown by our results this conformation *is not* that of a right-handed α-helix.

Conclusion

The results presented in this paper show that protonated PLH is in the random-coil conformation. On varying the degree of ionization of the side chains, PLH undergoes a conformational transition from the coiled form to an ordered structure stable at $\alpha = 0.9$. The CD spectrum in the 220-nm region seems to be scarcely dependent on the polymer conformation and should contain contributions mainly from the side-chain electronic transitions.

The complete CD pattern of charge-free PLH does not allow any safe conclusion on the possible conformation. However, the copolymer studies presented in this work rule out the possibility that deprotonated PLH exists in aqueous solution as a right-handed α helix.

Extensive ir and X-ray diffraction studies are required in order to establish the exact polymer conformation at low

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degrees of ionization. The results of these studies will be reported elsewhere.[20]

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(20) Just before submitting our manuscript for publication, a paper by Myer and Barnard appeared: Y. P. Myer and E. A. Barnard, *Arch. Biochem. Biophys.*, **143**, 116 (1971). These authors determined CD spectra of a commercial sample of PLH at various pH values and found a pH-induced conformational transition. A direct comparison of their data with our data cannot be made since these authors did not report spectra as a function of the significant variable, that is, the degree of protonation of the polymer side chains. However, the CD pattern reported in our work at α near 0.9 is very much the same as that reported by Myer and Barnard at pH near 6 (where deprotonation should be essentially complete) except for band intensity: the CD bands reported in our work are much more intense than those measured by the above-mentioned authors. We have observed (see the Experimental Section) that spectra with low band intensities are obtained when the PLH sample does contain substantial amounts of low molecular weight material. After exhaustive dialysis, which eliminates the low molecular weight fraction, normal spectra are obtained.

The results obtained by us in the 222-nm region are not in accord with those of the above-mentioned authors. We have carried out a number of measurements (see Figure 5) using 5-mm path-length cells in order to obtain large CD signals and minimize the errors. No clear transition appeared on varying the degree of protonation of the polymer side chains, and we therefore concluded that the variation of the spectrum in this region is mainly sensitive to changes of the protonation extent of the side chains.

From the CD spectral characteristics, the above-mentioned authors infer that PLH assumes the β form at a low degree of protonation of the imidazole side chains. This statement is not in conflict with our copolymer studies which exclude only the existence of the right-handed α helix. However, we stress the point that the presence of the side-chain chromophores prevents a safe interpretation of the CD pattern in terms of conformation. The fact that in the solid state PLH has been found in the β form does not necessarily mean that the same conformation exists in solution. As previously discussed, the solid-state conformation is strongly dependent on the "history" of the solid sample. For instance, Zundel and coworkers[18] found the α-helical form on films of PLH.

In conclusion, we believe that additional studies are needed in order to establish *unambiguously* the exact conformation of charge-free PLH in solution.

Polymer–Solvent Interactions for Homopolypeptides in Aqueous Solution

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ABSTRACT: A polymer–solvent interaction model, based upon the concept of a hydration shell, is used to evaluate the free energy of solvation of many different homopolypeptides in aqueous solution. The solvation free energy is roughly proportional to the solubility of the macromolecule, and the interactions of polar groups with aqueous solvent are more stabilizing than the interactions of hydrophobic groups with aqueous solvent are destabilizing. Polymer–solvent interactions and intrachain–side chain interactions make the major energetic contributions to the helix $\rightleftharpoons$ coil transition. The aqueous solution–homopolypeptide interactions are moderately sensitive to conformation and the right-handed α helix has the least favorable polymer–solvent free energy interaction.

The conformation of a macromolecule is dependent upon the medium in which it exists. For example, the conformation of a macromolecule in the solid state is dependent upon how it packs with other macromolecules in the crystal. In fact, the intermolecular configurational energy which results from all the pairwise interactions of atoms belonging to different macromolecules can be the same magnitude as the intramolecular conformational energy which results from all the pairwise interactions of the nonbonded atoms within a single macromolecule.[1] In a similar way one would expect to observe strong polymer–solvent interactions for certain polymers in certain solvents. That this is the case follows from experiments in which variations in solvent composition (*i.e.*, changes in pH, salt concentration, polarity, and binary solvent mixtures)[2–6] have been shown to induce specific conformational changes in macromolecules.

A major criticism of theoretical conformational calculations has been the neglect of these polymer–solvent interactions in the computations. In the past, the implicit assumption has been made that a stable conformation in a vacuum would also be stable in any of a number of solvents. In view of experimental data, this assumption apparently oversimplifies the true situation. The purpose of this paper is to (i) report the development of a polymer–solvent interaction model in which the parameters of the model have been evaluated for aqueous solution at room temperature, (ii) elucidate the role of polymer–solvent interactions on the conformational stability of several different types of homopolypeptides in aqueous solution, (iii) provide an energetic explanation of the helix $\rightleftharpoons$ coil transitions observed in some homopolypeptides which possess ionizable side chains.

Polymer–polymer interactions were not included in the calculations. Thus, the results reported in this paper should only be valid for very dilute aqueous solutions and/or dilute "neutral solvent" solutions in which the solvent molecules are nearly spherical and have small effective volumes. Since polymer–solvent interactions depend upon the shape of the solvent molecule as well as its effective polarity, care should be taken in any attempt to rationalize the behavior of various nonaqueous solvents in terms of the data presented in this paper.

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