rameter functions gives numerical support for the two-parameter theory of polymer solutions.[34] The form of the best function is a perturbed gaussian, $\exp(-ar^2 - br - c)$, with c a normalizing factor. Because the numerical Monte Carlo distribution is observed for a model with explicit long-range correlation, there is no need to resort to the usual series expansion[35] for $\langle r^2\rangle/\langle r^2\rangle_0$ in terms of the parameter $z = (c_2/\pi)^{1/2}\beta$. The major analytical problem with such an expansion arises from the slow convergence of the series, and implies validity of the expansion only near the θ point.

The form of eq 2 also suggests that a should be a gaussian-like parameter which pertains to the molecular geometry, and b a perturbation parameter which contains the "hardness" of the excluded-volume effect. In fact, data from Table IV obey the relation

$$1/a_{\min} = 0.645N^{4/3}$$

Thus, the best-fit values of the parameter a are proportional to $N^{4/3}$, which is the known N dependence of first-order perturbation theory.

Acknowledgments. We acknowledge the National Science Foundation for a Graduate Traineeship for one of us (S. D. S.). We thank the AEC Computing Center at the Courant Institute of Mathematical Sciences at New York University for a grant of computer time.

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A Comparison of the Effects of Salt and Temperature on Charged and Uncharged Polypeptides in Water[1]

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ABSTRACT: The hydrodynamic properties of poly(N^5-ω-hydroxyethyl-L-glutamine) and ionized poly(L-glutamic acid) in water differ in a manner which reflects the polyelectrolyte properties of poly(L-glutamic acid). However, the circular dichroism spectra of these two polypeptides are affected in a similar manner by increasing temperature and salt concentration. The ability of increasing temperature and increasing potassium chloride concentration to reduce the intensity of the positive circular dichroism band are cumulative for both poly(N^5-ω-hydroxyethyl-L-glutamine) and ionized poly(L-glutamic acid). On the other hand, in the helix–coil transition of poly(N^5-ω-hydroxypropyl-L-glutamine), the effects of increasing temperature and increasing potassium chloride concentration offset each other. It is concluded that the effects of salts on the circular dichroism of charged polypeptides cannot be accounted for by the formation of α helices and that the salt effect must be more complex than a simple shielding of electrostatic charges on the side chains.

The conformational properties of fully ionized homopolypeptides, notably poly(L-glutamic acid) and poly(L-lysine), have been of interest recently because of the suggestions that they may become partially ordered in dilute aqueous solution when the concentration of added electrolyte is properly chosen.[2–7]

On theoretical grounds Krimm and Mark[2] predicted that, in the absence of added salt, the repulsions between the charges in the side chains would lead to the formation of short ordered regions in the polypeptide backbone with values of the torsion angles ϕ and ψ[8] similar to those observed for collagen[9,10] and poly(L-proline) II in the solid state.[11] In the absence of added salt, the circular dichroism of fully ionized poly(L-glutamic acid) and poly(L-lysine), as well as native collagen and poly(L-proline) with all peptide bonds in the trans configuration, exhibits a weak positive band followed by a strong negative band about 20 nm toward shorter wavelengths.[3–5] In the presence of sufficiently high salt concentrations, the circular dichroism exhibits only a single moderately strong negative band near 200 nm.[3–5] The salt was believed to reduce the electrostatic interaction of the side chains, leading to the formation of a true random coil and the simultaneous loss of the positive circular dichroism band.[3–5] Recently electron micrographs of poly(L-glutamic acid) precipitated from calcium acetate have been interpreted as being consistent with the extended helical conformation proposed by Krimm and coworkers.[12]

Myer[6] and Dearborn and Wetlaufer[7] have observed similar effects of salts on the circular dichroism of ionized homopolypeptides, but they have offered a different interpretation. Curve fitting could hypothetically account for the

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(1) This work was supported by a contract with the Division of Biology and Medicine, Atomic Energy Commission (L. M.), a Public Health Service Postdoctoral Fellowship from the National Institute of General Medical Sciences (W. L. M.), and a Frederick Gardner Cottrell grant in aid (W. L. M.).

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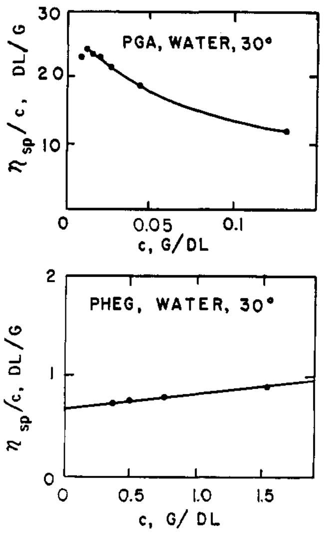

Figure 1. Reduced viscosity as a function of concentration for poly(L-glutamic acid) (PGA) as the sodium salt and poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG) in water at 30°.

circular dichroism spectra by postulating the existence of a "disordered"[6] or "unordered"[7] conformation in the absence of added salt and the formation of progressively more segments of α helix upon the addition of salt. These workers disagree with Krimm and coworkers in two respects: the definition of the ordered structure involved (α helix or a helix similar to poly(L-proline) II) and the effect of salt on the ordered structure (stabilizing or destabilizing).

New insight into this problem has been obtained by comparing the effect of salt and temperature on the circular dichroism and hydrodynamic properties of ionized poly(L-glutamic acid) and two uncharged water-soluble analogs, poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-ω-hydroxyethyl-L-glutamine). The optical rotatory dispersion of poly(N^5-ω-hydroxyethyl-L-glutamine) in water reveals no transition to an ordered state in the temperature range 0–70°.[13] Its characteristic ratio, $[r^2]_0/n_pl_p^2$,[14] at 30° in water is independent of molecular weight and has the value 10 ± 1,[15] which is the result to be expected for a polypeptide with a $-CH_2R$ side chain which is in a statistical conformation (random coil).[16,17] Poly(N^5-ω-hydroxyethyl-L-glutamine) is partially helical in water at 0° and becomes less helical upon heating.[18–20]

Materials and Methods

Materials. Poly(L-glutamic acid) was obtained from Pilot (Lot 6-72). Poly(N^5-ω-hydroxypropyl-L-glutamine) was obtained from New England Nuclear (Lot M-62-C). Poly(N^5-ω-hydroxyethyl-L-glutamine) was prepared from poly(γ-benzyl L-glutamate) using a slight modification of the procedure used by Lotan, *et al.*,[18] to prepare poly(N^5-ω-hydroxypropyl-L-glutamine). All polypeptides were dialyzed against distilled water and recovered by lyophilization before use. Polypeptide solutions were prepared in volumetric flasks using samples which had been dried under vacuum using a Dry Ice–diethylene glycol monobutyl ether trap. The pH was measured at ambient temperature using a Radiometer Model 28 pH meter. Salts were reagent grade. The salt solutions were prepared by the dilution of saturated stock solutions of known concentration.

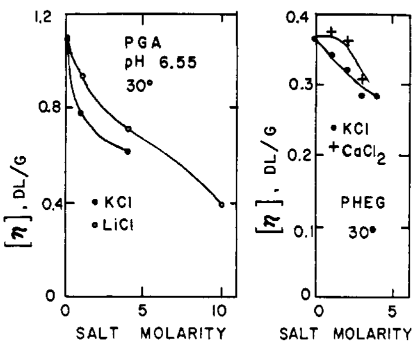

Figure 2. Effect of salts on the intrinsic viscosities of poly(L-glutamic acid) at pH 6.55 (PGA) and poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG) at 30°. The intrinsic viscosity of PGA in the absence of any added salt is much higher than indicated by this figure; the data point at lowest salt concentration is for an isoionic dilution with 0.025 *M* potassium chloride. See text for a more complete explanation.

Viscosity. Viscosity measurements were made in the manner previously described.[21]

Circular Dichroism. The circular dichroism of poly(L-glutamic acid) was obtained using a Durrum-Jasco Model ORD/UV-5 recording spectropolarimeter equipped with a circular dichroism attachment. The results with poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(N^5-ω-hydroxypropyl-L-glutamine) were obtained with a Durrum-Jasco Model J-20 recording spectropolarimeter. All results are based upon a calibration of $[\theta]_{291}$ = 7260 (deg cm^2)/dmol for camphorsulfonic-*10-d* acid in water.[22] Temperature control was achieved by circulating water from a constant temperature bath through a hollow metal cell holder. The relationship between the temperature of the bath and the temperature of the cell was determined with a thermocouple. Cell light paths ranged from 0.1 to 10 mm.

Results

Viscosity. The reduced viscosities of samples of the sodium salt of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30° are shown in Figure 1. The reduced viscosity of the sodium salt of poly(L-glutamic acid) decreases sharply with increasing concentration, a behavior typical of polyelectrolytes in water.[23] The reduced viscosity of poly(N^5-ω-hydroxyethyl-L-glutamine) in water, on the other hand, increases slightly with increasing concentration in a linear fashion. The Huggins constant of 0.32 is typical for a linear random coil which is not charged.[24]

The effects of certain salts on the intrinsic viscosities of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) at 30° are shown in Figure 2. The data point at lowest salt concentration represents the intrinsic viscosity in water for poly(N^5-ω-hydroxyethyl-L-glutamine). In the case of poly(L-glutamic acid) the lowest salt concentration represents an isoionic dilution in 0.025 *M* potassium chloride. The extrapolation to zero concentration is much simpler for the isoionic dilution with 0.025 *M* potassium chloride than in

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TABLE I

COMPARISON OF THE CIRCULAR DICHROISM OF POLY(L-GLUTAMIC ACID) AND POLY(N^5-ω-HYDROXYETHYL-L-GLUTAMINE) IN WATER

Poly(L-glutamic acid)				Poly(N^5-ω-hydroxyethyl-L-glutamine)			
pH 7.4, 22° [a]		pH 6.55, 30° [b]		22° [a]		30° [b]	
λ	[θ]	λ	[θ]	λ	[θ]	λ	[θ]
237	−300	232	−1,000	233	−800	231	−800
232	0	228	0	227	0	222	0
217	6,200	218	2,800	217	2,200	216	1,400
212	0	213	0	212	0	210	0
197	−37,000	198	−38,000	198	−26,000	198	−22,000

[a] A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.*, **90**, 4736 (1968). [b] This work.

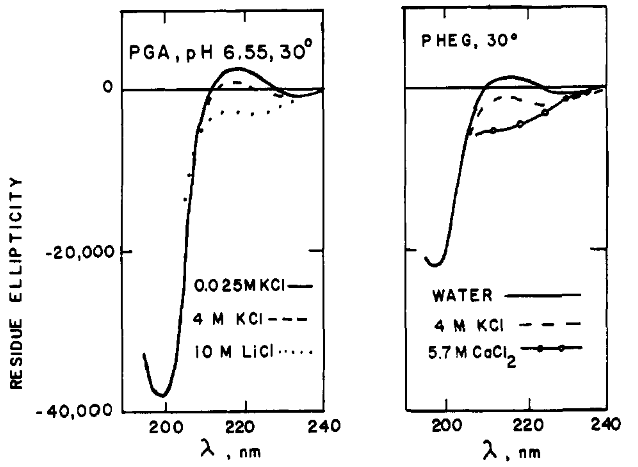

Figure 3. Effect of salts at constant temperature upon the circular dichroism of poly(L-glutamic acid) (PGA) and poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG).

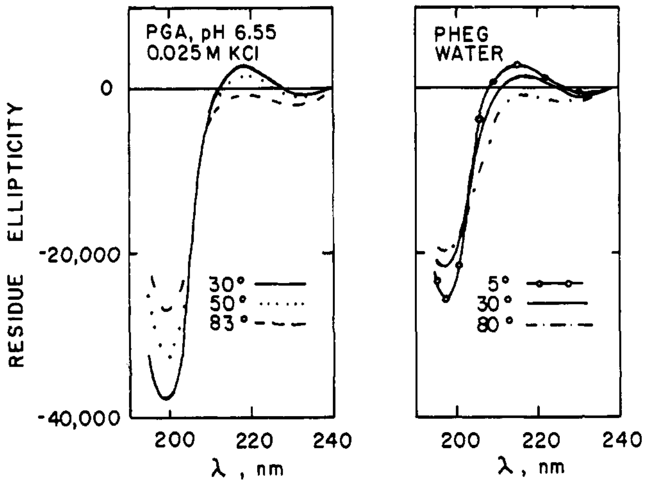

Figure 4. Effect of temperature on the circular dichroism of poly(L-glutamic acid) (PGA) and poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG).

the dilution with water because the reduced viscosity increases linearly with concentration for the isoionic dilution. However, the intrinsic viscosity of poly(L-glutamic acid) in 0.025 *M* potassium chloride is very much less than it is in the absence of any added salt. The ability of potassium chloride to reduce the intrinsic viscosity of poly(L-glutamic acid) is therefore much greater than is implied by Figure 2. Potassium chloride also decreases the intrinsic viscosity of poly(N^5-ω-hydroxyethyl-L-glutamine), but the change is not as marked as in the case of poly(L-glutamic acid). The intrinsic viscosity of these two polypeptides is reduced by salts other than potassium chloride, as is shown in Figure 2. Lithium bromide and potassium fluoride have previously been shown to markedly reduce the intrinsic viscosity of poly(L-glutamic acid) at pH 7.3.[25]

Circular Dichroism. The effects of certain salts on the circular dichroism of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) at 30° is shown in Figure 3. The circular dichroism spectra for poly(N^5-ω-hydroxyethyl-L-glutamine) in water and poly(L-glutamic acid) at pH 6.55 in 0.025 *M* potassium chloride are qualitatively similar, both exhibiting a large negative band at 198 nm, a smaller positive band at 216–218 nm, and a still smaller negative band at 231–233 nm. Quantitative differences are apparent, with the bands at 198 and 216–218 nm being larger in the case of poly(L-glutamic acid). Adler, *et al.*,[26] have noted a similar comparison at 22° in water between poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid) at pH 7.5. The main features in the spectra obtained by Adler, *et al.*,[26] are presented in Table I along with the results at 30° from Figure 3.

The data for the concentrated salt solutions in Figure 3 do not extend to low wavelengths owing to the absorption of light by chloride ions. In agreement with previous work on charged homopolypeptides,[3–7] the isothermal addition of salt causes a reduction (4 *M* potassium chloride) or disappearance (10 *M* lithium chloride) of the positive component in the circular dichroism of poly(L-glutamic acid). Figure 3 shows that salts bring about the same changes in the circular dichroism of poly(N^5-ω- hydroxyethyl-L-glutamine) as are observed with charged poly(L-glutamic acid). The largest change observed with poly(N^5-ω-hydroxyethyl-L-glutamine) was observed in 5.7 *M* calcium chloride. In the case of poly(L-glutamic acid), the largest change was seen in 10 *M* lithium chloride. (The circular dichroism of poly(L-glutamic acid) cannot be measured in 5.7 *M* calcium chloride due to solubility problems.)[4]

The effect of temperature upon the circular dichroism of poly(L-glutamic acid) in 0.025 *M* potassium chloride and poly(N^5-ω-hydroxyethyl-L-glutamine) in water is shown in Figure 4. The poly(L-glutamic acid) solution had a pH of 6.55 at ambient temperature. In both cases heating decreases the intensity of the negative band at 198 nm, increases the negative circular dichroism intensity at 231–233 nm, and reduces the strength of the positive component in the circular dichroism at 216–218 nm. At sufficiently high temperature no positive circular dichroism is observed with either polypeptide.

Inspection of Figures 3 and 4 suggests that increases in the temperature and potassium chloride concentration cause

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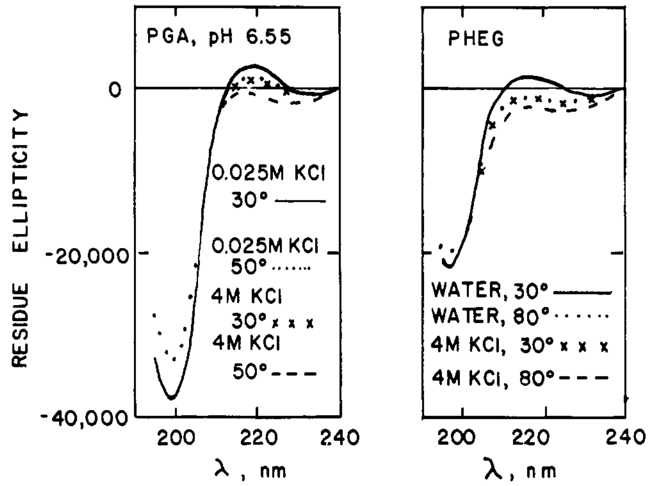

Figure 5. Demonstration of the cumulative effects of increasing temperature and salt concentration on the circular dichroism of poly(L-glutamic acid) (PGA) and poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG).

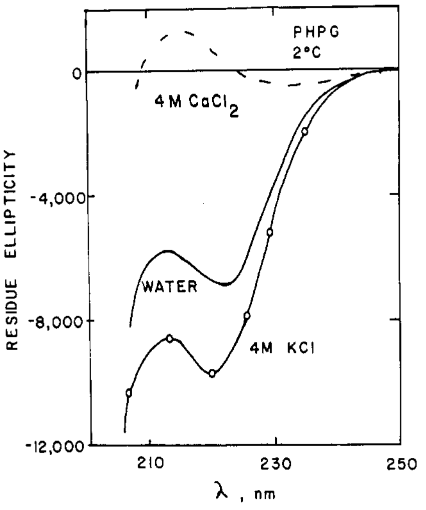

Figure 6. Circular dichroism spectrum of poly(N^5-ω-hydroxypropyl-L-glutamine) (PHPG) in water, 4 M potassium chloride, and 4 M calcium chloride at 2°.

similar changes in the circular dichroism spectra of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine). The spectra in Figure 5 show that the effects of increasing temperature and potassium chloride concentration are indeed cumulative. The solid line in the left portion of Figure 5 presents the spectrum obtained with poly(L-glutamic acid) at pH 6.55 in 0.025 M potassium chloride at 30°. Nearly the same change in the spectral region where chloride ion does not absorb prohibitively can be obtained by maintaining the salt concentration at 0.025 M potassium chloride and increasing the temperature to 50° or by maintaining a temperature of 30° while increasing the salt concentration to 4 M potassium chloride. Simultaneous raising of the temperature to 50° and the salt concentration to 4 M potassium chloride produces a larger change than either action taken individually. The right-hand part of Figure 5 shows that similar changes are produced in the circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) in water by either raising the temperature to 80° or by isothermally changing the solvent to 4 M potassium chloride. A larger change is observed by simultaneously adding potassium chloride and raising the temperature to 80°.

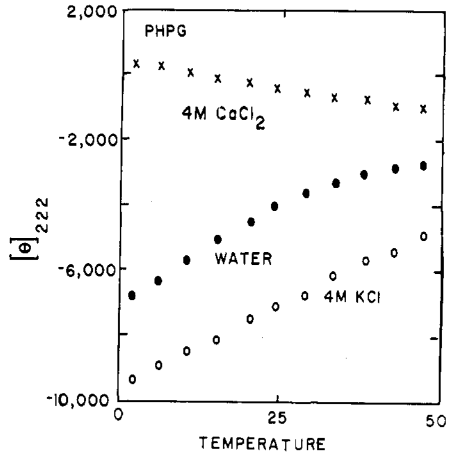

Figure 7. Effect of temperature on the residue ellipticity at 222 nm for poly(N^5-ω-hydroxypropyl-L-glutamine) (PHPG) in water, 4 M potassium chloride, and 4 M calcium chloride.

Figure 6 shows the circular dichroism observed with poly(N^5-ω-hydroxypropyl-L-glutamine) at 2° in water, 4 M potassium chloride, and 4 M calcium chloride. Poly(N^5-ω-hydroxypropyl-L-glutamine) has been shown to undergo a helix–coil transition in water near 0°.[13,18–20] The circular dichroism spectrum at 2° in water shows a large negative band at 222 nm due to the n–π* transition of those residues in the polymer which are in the α-helical conformation.[27] This band is located at 220 nm in the spectrum obtained in 4 M KCl and is slightly more intense. In 4 M calcium chloride there is no trace of the negative n–π* band of the α helix. The spectrum in 4 M calcium chloride at 2° shows the small positive band near 216 nm observed with random-coil poly(N^5-ω-hydroxyethyl-L-glutamine) at low temperature in water (Figure 4).

The residue ellipticities observed with poly(N^5-ω-hydroxypropyl-L-glutamine) at 222 nm in water, 4 M calcium chloride, and 4 M potassium chloride are presented as a function of temperature in Figure 7. Heating poly(N^5-ω-hydroxypropyl-L-glutamine) in 4 M calcium chloride leads to the same qualitative changes in the circular dichroism spectra as are seen upon heating poly(N^5-ω-hydroxyethyl-L-glutamine) in a solution of constant ionic strength (see Figures 4 and 5). The positive band near 215 nm becomes less intense and eventually disappears upon heating, with the result at 222 nm that the circular dichroism becomes more negative with increasing temperature. On the other hand, heating of poly(N^5-ω-hydroxypropyl-L-glutamine) in water and in 4 M potassium chloride decreases the strength of the negative dichroism band at 222 nm, as would be anticipated for the thermal denaturation of the α-helical conformation.[13,18–20]

A comparison of the effects of temperature and potassium chloride on the circular dichroism of poly(N^5-ω-hydroxypropyl-L-glutamine) is shown in Figure 8. Increasing the temperature from 2 to 28.5° in the absence of salt decreases the intensity of the circular dichroism. The isothermal change of solvent to 4 M potassium chloride has the opposite effect on the circular dichroism spectrum. The simultaneous change from water at 2° to 4 M potassium chloride at 28.5° leads to little net change in the circular dichroism spectrum. Therefore, the effects of increasing tem-

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perature and potassium chloride concentration are not cumulative, but rather oppose each other, when poly(N^5-ω-hydroxyethyl-L-glutamine) is undergoing the transition from α helix to random coil.

Discussion

Poly(N^5-ω-hydroxyethyl-L-glutamine) is in a statistical conformation in water as shown by optical rotatory dispersion[13] and by a characteristic ratio of 10 ± 1 at molecular weights of 17,000–71,000 in water at 30°.[15] Differences in the hydrodynamic behavior of poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid) are readily apparent in the attempt to obtain an intrinsic viscosity in the absence of any added salt and in the manner in which the intrinsic viscosity in the absence of any added salt and in the manner in which the intrinsic viscosity depends upon salt concentration at low ionic strength. The intrinsic viscosity of poly(N^5-ω-hydroxyethyl-L-glutamine) shows variations with salt concentration, but the effect is not nearly as dramatic as in the case of poly(L-glutamic acid). The hydrodynamic properties of poly(L-glutamic acid) are those anticipated for a statistically coiling polyelectrolyte.[23,24,28]

In contrast to the hydrodynamic properties, the optical effects presented in Figures 3–5 are very similar for the two polypeptides. Differences in intensities are apparent only if attention is restricted to a specific temperature and solvent. If instead attention is focused on the number of bands, their location, the effect of increasing salt concentration, the effect of increasing temperature, and the cumulative effect of increasing salt concentration and temperature, poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid) are seen to have similar optical properties.

While the effect of variation of the pH of the poly(L-glutamic acid) sample was not investigated in this study, data in the literature show similar effects of salt at pH values both higher and lower than those used here. At pH 5.7 the maximum intensity of the positive band in the circular dichroism of poly(L-glutamic acid) changes from $\Delta\epsilon = +1.0$ cm^2/mmol in water to $\Delta\epsilon = -0.4$ cm^2/mmol in 1.2 M sodium chloride,[4] while at pH 8.45 and 22° the maximum intensity of the positive band changes from $[\theta] = +5300$ (deg cm^2)/dmol in water to $[\theta] = +3200$ (deg cm^2)/dmol in 0.937 M potassium chloride.[6] Therefore, the ability of salts to reduce the intensity of the maximum in the circular dichroism of poly(L-glutamic acid) can be observed 0.85 pH unit lower and 1.9 pH units higher than the conditions used here.

According to Krimm and coworkers,[2–5] the positive band in the circular dichroism of ionized poly(L-glutamic acid) in the absence of salt arises from a helical backbone conformation, similar to that adopted by poly(L-proline) II in the solid state,[11] which minimizes the electrostatic interaction of the charged side chains. Addition of salt decreases the intensity of the positive band by reducing the repulsive interaction between charged side chains, according to this hypothesis. It is very difficult to rationalize the results reported here in terms of the work of Krimm and coworkers.[2–5] The measured characteristic ratio for poly(N^5-ω-hydroxyethyl-L-glutamine) in water[15] is in accord with the theoretical conformational energy maps of Brant and Flory.[17] Consequently, the ϕ, ψ rotational states for this polypeptide under these conditions are distributed according to the laws of statistical mechanics. Therefore, the presence of a positive circular dichroism peak does not require the existence of an ordered molecular conformation. Rather the observed optical properties are a manifestation of the distribution of rotational states. A similar situation is also found for poly(L-proline).[29]

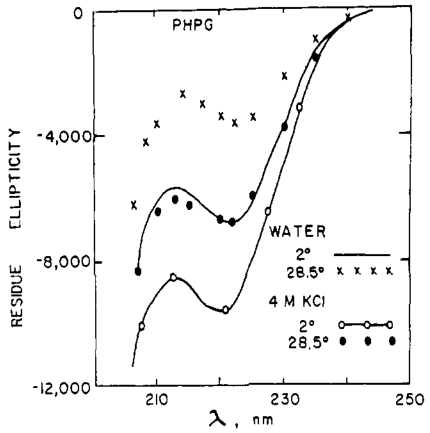

Figure 8. Demonstration of the opposite effects of increasing temperature and increasing potassium chloride concentration on the circular dichroism of poly(N^5-ω-hydroxypropyl-L-glutamine) (PHPG).

Myer[6] and Dearborn and Wetlaufer[7] proposed that the optical effects seen when salts are added to ionized homopolypeptides could be accounted for by the existence of a "disordered"[6] or "unordered"[7] conformation in the absence of salt and the formation of segments of α helix in the presence of salt. Figures 3–5 show that the effects of salt and heat upon the circular dichroism of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) are similar and cumulative. If the explanation offered by Myer[6] and Dearborn and Wetlaufer[7] for the optical effects is correct, the implication is that poly(N^5-ω-hydroxyethyl-L-glutamine) in water tends to become more α helical upon heating. The Moffitt b_0[30] remains between +40 and 0 (deg cm^2)/dmol over the range 0–70° for poly(N^5-ω-hydroxyethyl-L-glutamine) in water, which is inconsistent with the formation of any appreciable amount of α helix.[13] Poly(N^5-ω-hydroxybutyl-L-glutamine) and poly(N^5-ω-hydroxypropyl-L-glutamine) undergo helix–coil transitions at +40 and −5°, respectively, in water, with the helix being the stable form below the melting temperature.[13] Extrapolation from the hydroxybutyl and hydroxypropyl derivatives to the hydroxyethyl derivative leads to the conclusion that the melting temperature for poly(N^5-ω-hydroxyethyl L-glutamine) in water is considerably below 0° and that the helix is destabilized by increasing the temperature. It is difficult to see how the temperature effects on the circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) can be explained by the assumption that increasing the temperature induces the formation of segments of α helix. Since the effects of increasing temperature and salt concentration are cumulative, it also seems unlikely that the salt induces the formation of α helix.

In the case of poly(N^5-ω-hydroxypropyl-L-glutamine), salts can either stabilize or destabilize the α helix, as is shown in Figures 6 and 7. The α helix is more stable in the presence of 4 M potassium chloride than in water, but it is still destabilized by increasing the temperature. In 4 M calcium chloride there is no evidence of α helix even at 2°. When a polypeptide is undergoing an α helix to random-coil transition,

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(28) H. Morawetz, "Macromolecules in Solution," Interscience, New York, N. Y., 1965, Chapter VII.

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the effects of increasing temperature and potassium chloride concentration oppose each other (Figure 8). The opposite situation is encountered with poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid), with the effects of increasing temperature and potassium chloride concentration being cumulative (Figure 5). This observation constitutes further evidence that the effect of salts on the circular dichroism of ionized polypeptides cannot be due to the formation of segments of α helix in the presence of the salts.

The suggestion that the isothermal addition of salts leads to the formation of α helix in charged homopolypeptides has led to conclusions which are inconsistent with other properties of polypeptides. Based on the assumption that ionized poly(L-lysine) is in an unordered conformation in the absence of added salt and becomes increasingly α helical when salt is added, curve fitting of the circular dichroism from 212 to 228 nm led to estimates of 14.7% helix in 3 M calcium chloride and 32.3% helix in 6 M calcium chloride.[7] The common experience of other investigators has been that calcium chloride destabilizes the ordered structures formed by proteins, as has been documented with ribonuclease,[31] collagen,[32,33] elastoidin fibers,[34] and glycerinated muscle fibers.[35] The positive band observed in the circular dichroism of poly(L-proline)[21,36] and poly(γ-hydroxy-L-proline)[37] in water is eliminated by the isothermal addition of calcium chloride. It is impossible, for steric reasons, that the results with poly(L-proline) and poly(γ-hydroxy-L-proline) could reflect an increase in the amount of α helix in the presence of calcium chloride.[38] Finally, Iizuka and Yang have shown that the pH at the midpoint of the helix–coil transition of poly(L-glutamic acid) is shifted to lower pH values in the presence of salts, with the exception of high concentrations of potassium fluoride.[25] This observation is not consistent with the formation of additional α helix when salt is added at constant pH.

The data presented here are rather more effective in showing what the effect of salt is not than they are in defining the mechanism of action of the salts. It can be concluded, however, that the circular dichroism spectra of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) reported here are not due to changes in the content of α helix and do not support the proposal that the interaction of the charged side chains in ionized polypeptides leads to a locally ordered structure.

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Unperturbed Dimensions of Poly(N^5-ω-hydroxyethyl-L-glutamine) in Water[1]

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ABSTRACT: The characteristic ratio of poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30° is found to be 10 ± 1, in agreement with results obtained by Brant and Flory for four other polypeptides with $-CH_2R$ side chains. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) under these conditions, where the polypeptide is in a statistical conformation, exhibits a positive band at 216 nm. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) and ionized poly(L-glutamic acid) are similar but not identical under conditions where they have the same characteristic ratio. The possibility that this observation could arise from the presence of short left-handed helices in the ionized poly(L-glutamic acid) is *considered* and found to be improbable.

The Moffitt[2] parameter b_0 for poly(N^5-ω-hydroxyethyl-L-glutamine) in water is between 0 and 40 (deg cm^2)/dmol over the temperature range 0 to 70°.[3] Consequently, poly(N^5-ω-hydroxyethyl-L-glutamine) has been used as a model for a random-coil water-soluble polypeptide with a $-CH_2R$ side chain.[4,5] Poly(N^5-ω-hydroxyethyl-L-glutamine) is not a polyelectrolyte, in contrast to two other frequently used models for random-coil polypeptides, poly(L-glutamic acid) and poly(L-lysine) at neutral pH.[6-14] Recently, suggestions based upon calculations and circular dichroism measurements have indicated that ionized homopolypeptides such as poly(L-

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