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Effect of Salts and Dioxane on the Coiled Conformation of Poly-L-glutamic Acid in Aqueous Solution*

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ABSTRACT: The conformation of poly-L-glutamic acid at pH 7.3 was studied in LiBr, NaCl, KF, and CsCl solutions by viscometry and optical rotatory dispersion (ORD). The intrinsic viscosity, $[\eta]$, of the polyion decreased with increasing salt concentrations, indicating contraction of the polypeptide chains. Above 6 M salt, $[\eta]$ began to drop more steeply, suggesting further change in conformation, possibly owing to aggregation. Visible rotatory dispersion in all cases obeyed a one-term Drude equation; the dispersion constant, λ_c , varied between 204 $m\mu$ (in low salt) and 223 $m\mu$ (up to 8 M LiBr), whereas the levorotations dropped markedly with increasing salt concentrations. Correspondingly, the b_0 values of the Moffitt equation (with $\lambda_0 = 212 m\mu$) changed from +50 in water to -50

in high salt solutions.

Similar studies of poly-L-glutamic acid in dioxane-water solutions at pH 7.3 (apparent) showed that both the viscosities and levorotations in the visible region also reduced with increasing dioxane concentrations. But at 40% dioxane (v/v) the ORD no longer obeyed a one-term Drude equation; at 50% dioxane the conformation was essentially helical (b_0 became -670). Similar drastic changes were observed in the ultraviolet region, where the Cotton effects revealed a coil-to-helix transition. These conclusions were further corroborated by measurements of rotations as a function of pH. In all cases a sharp transition was observed, but the transition pH depended on the salt and dioxane concentrations used.

The helix-coil transition of poly-L-glutamic acid in aqueous solutions has been demonstrated by several physical techniques such as viscometry, optical rotation, and infrared spectroscopy (Doty *et al.*, 1957), and more recently also by dilatometry and refractometry (Noguchi and Yang, 1963). In 0.2 M NaCl-dioxane (2:1,

v/v) the helical conformation did not break up unless more than 40% of the carboxyl groups were ionized through change in pH and temperature, thus implying the stability of these helices against a substantial degree of electrostatic repulsion among the side groups (Doty *et al.*, 1957). Wada (1960) has since shown that the transition point can be shifted by the addition of NaCl. This is attributed to the increase in the apparent dissociation constant of the -COOH groups with ionic strength in spite of the reduction in electrostatic repulsion in the presence of counter ions. Earlier, the levorotation of poly-L-glutamic acid in its coiled conformation was found to undergo a sharp drop with increasing salt concentration (Yang, 1962). In this paper we attempt to present a more detailed study of the effect

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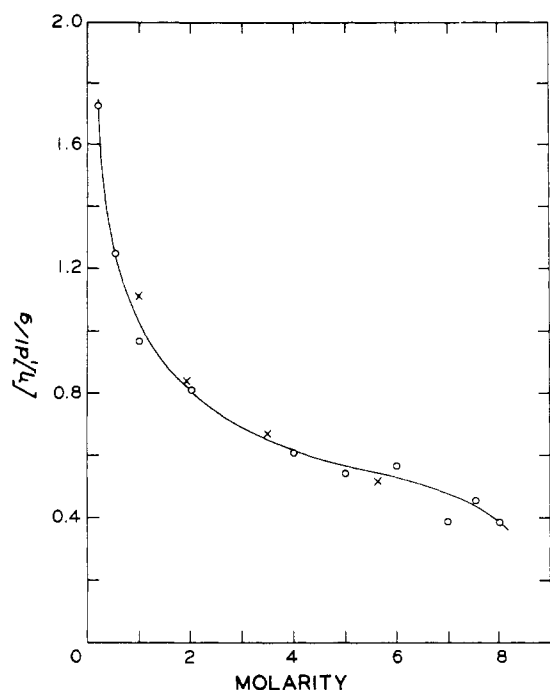


FIGURE 1: Intrinsic viscosity of poly-L-glutamic acid in its coiled form (pH 7.3) at various salt concentrations. O, LiBr; x, KF.

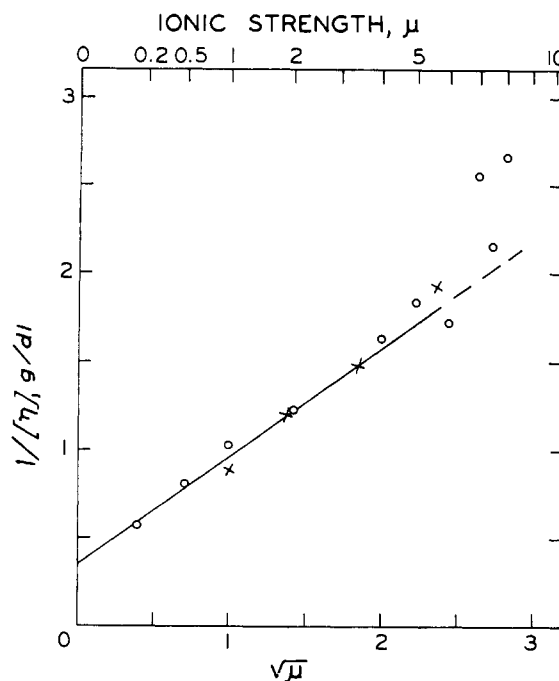


FIGURE 2: The reciprocal of the intrinsic viscosity of poly-L-glutamic acid (pH 7.3) as a function of the square root of ionic strength. Symbols same as in Figure 1.

of alkali salts and also of dioxane on the conformation of poly-L-glutamic acid in its coiled form by viscometry and ORD.

The carboxyl groups of an acid are known to have a smaller apparent pK in dilute salt solutions than in pure water. On the other hand, the electrostatic free energy of a polyion becomes insignificant because of the swamping of the counterions. In concentrated salt solutions, the activity of the solvent (water) will be greatly reduced. In the case of dioxane-water mixtures, on the one hand the electrostatic repulsion of a polyion will be enhanced because of the decrease in dielectric constant, but, on the other hand, the apparent pK of the carboxyl groups will be shifted toward a higher pH with increasing dioxane concentration. Likewise, with a sufficient amount of dioxane the activity of water will again be lowered. These factors are expected to influence the polypeptide conformation and its stability in a rather complicated fashion, and at present it is difficult to treat all their contributions in quantitative terms. Thus, only a phenomenological approach will be adopted in this study. Poly-L-glutamic acid was chosen because of its structural simplicity and also its well-studied conformations under various conditions. We used only alkali salts in this study, since Jacobson (1964) has investigated in some detail the effect of divalent ions such as Mg^{2+} on the conformation of poly-L-glutamic acid. She has shown that some of these divalent ions enhance the stability of the helical conformation against electrostatic repulsion.

Experimental Procedures

Materials. Two samples of the sodium salt of poly-L-glutamic acid were used: one (no. 1) was purchased from Pilot Chemicals, Inc., Watertown, Mass., and the other (no. 2) was prepared in this laboratory. The weight-average molecular weights of the preparations were about 79,000 for no. 1 and 93,000 for no. 2. The polymer was dissolved in double-distilled water and thoroughly dialyzed against water; its concentration was determined by micro-Kjeldahl analyses. The stock solutions were then mixed with appropriate salt solutions or dioxane-water mixtures to make up the desired ionic strengths or dioxane concentrations; the apparent pH of the solutions was finally adjusted to 7.3. The solutions were clarified by being filtered through a medium sintered-glass filter. All the chemicals used were of reagent grade.

Methods. Viscosities were measured in the Ubbelohde-type viscometers at 25.0° . Flow times for the solvents were kept at more than 100 seconds in most cases, and at least over 50 seconds for a few solutions that were highly viscous. In high salt concentrations the slope of the Huggins plot for determining the intrinsic viscosities was found to be close to zero.

ORD¹ was measured at 27° with a Cary Model 60 recording spectropolarimeter, the calibration of which has been described elsewhere (Iizuka and Yang, 1964). The

¹ Abbreviation used in this work: ORD, optical rotatory dispersion.

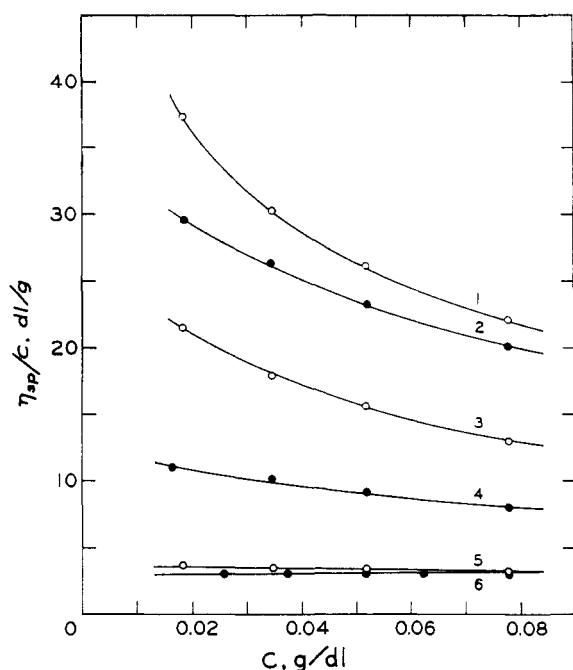


FIGURE 3: The reduced viscosities of poly-L-glutamic acid in dioxane-water mixed solvents at pH 7.3. Volume per cent of dioxane: (1) 0, (2) 10, (3) 30, (4) 40, and (5) 50

concentrations of the poly-L-glutamic acid solutions were 0.5% or less. Cells of path lengths 100, 10, 1, and 0.1 mm were used. In measuring the Cotton effects below 240 mμ, the absorbance of the solutions was always kept well below 2 to avoid the complications of artifacts.

Results

Intrinsic Viscosities. (1) **EFFECT OF SALT.** It is practical to say that all the carboxyl groups on the side chains of poly-L-glutamic acid are ionized at pH 7.3 and the polypeptide chain is expected to behave as a typical polyelectrolyte. The results of sample no. 2 in Figure 1 clearly show a gradual contraction of the polymer chains with increasing salt concentration. As a further test, we plot in Figure 2 the reciprocal of the intrinsic viscosity against the square root of the salt concentration, according to an empirical equation of Fuoss and Strauss (1948):

$$[\eta] = A/(1 + B\sqrt{C}) \quad (1)$$

Rather surprisingly, we obtained a straight line even up to about 6 M salt (Figure 2). At still higher salt concentrations, $[\eta]$ actually began to drop even more steeply, a fact indicative of an additional change in conformation, although precipitation of poly-L-glutamic acid above 8 or 9 M salt prevented any measurements at these salt concentrations. Note also that no significant difference in $[\eta]$ could be detected among the alkali

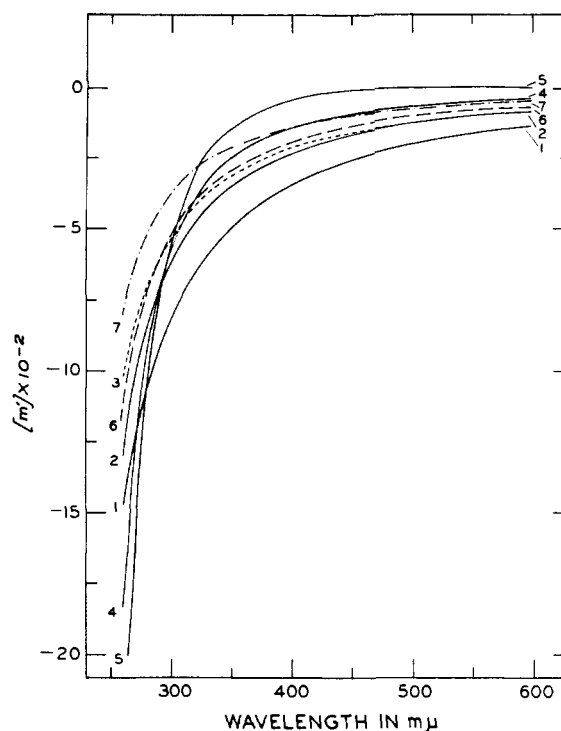


FIGURE 4: Visible and near-ultraviolet rotatory dispersion of poly-L-glutamic acid in salt and dioxane solutions at pH 7.3. (1) Water, (2) 30% dioxane, (3) 30% dioxane with 0.2 M KF, (4) 40% dioxane, (5) 50% dioxane, (6) 6 M KF, and (7) 8 M LiBr.

salts used (KF and LiBr in the present study and also NaCl in an early study [Yang, 1962]) in the range of concentrations where they overlapped one another.

(2) **EFFECT OF DIOXANE.** Figure 3 shows the viscosities of poly-L-glutamic acid (sample no. 1) which drop drastically in the presence of dioxane. At low dioxane concentrations, however, poly-L-glutamic acid still behaves as a polyelectrolyte, as reflected by the marked increase in the reduced viscosity with decreasing concentration of the polymer. We will demonstrate from ORD study that it underwent a coil-to-helix transition above 40% and became almost completely helical at about 50% dioxane.

Addition of salts to the dioxane solutions reduced the viscosities which gave linear Huggins plots; for example, with 0.2 M KF, $[\eta]$ was 1.3 in 10% dioxane and 0.8 in 30% dioxane (poly-L-glutamic acid precipitated when the dioxane concentration was over 30% in the presence of 0.2 M KF).

Optical Rotatory Dispersion. Figures 4 and 5 summarize some of the representative ORD of poly-L-glutamic acid in both salt and dioxane solutions. The reduced mean residue rotation, $[m']$, in the visible region obeyed a one-term Drude equation (between 270 and 600 mμ):

$$[m'] = k/(\lambda^2 - \lambda_c^2) \quad (2) \quad 1251$$

TABLE I: Parameters of the One-Term Drude and Moffitt Equations for Poly-L-glutamic Acid (pH 7.3) above 270 mμ.

Solvent	λ_c (mμ)	$k \times 10^{-6}$	b_o	a_o
KF (moles/liter)				
0	208	-37.5	+50	-820
0.2	204	-34.2	+50	-750
0.5	205	-32.9	+50	-730
1.0	205	-32.1	+40	-710
2.0	208	-29.1	+20	-650
3.0	211	-25.9	+10	-580
4.0	219	-22.6	-20	-510
6.0	223	-20.3	-50	-460
LiBr (moles/liter)				
2.0	206	-28.7	+40	-620
4.0	206	-24.7	+30	-550
6.0	211	-20.4	0	-450
7.5	217	-15.6	-20	-350
8.0	221	-15.4	-40	-340
Dioxane (% v/v)				
10	203	-34.7	+30	-770
20	211	-32.0	-30	-700
30	221	-25.4	-60	-560
40	Nonlinear		-340	-230
50	Nonlinear		-670	+140
10 (+0.2 M KF)	203	-31.6	+50	-700

except in 40% or more dioxane. In all cases the data (Figure 4) can be fitted with the Moffitt equation (Moffitt and Yang, 1956):

$$[m'] = a_o \lambda_o^2 / (\lambda^2 - \lambda_o^2) + b_o \lambda_o^4 / (\lambda^2 - \lambda_o^2)^2$$

$$(\lambda_o = 212 \text{ m}\mu) \quad (3)$$

The parameters, k , λ_c , a_o , and b_o of poly-L-glutamic acid under various conditions are listed in Table I. The obvious feature of our results is the significant decrease in levorotation with increasing salt or dioxane concentration, even though the dispersion constant, λ_c , changed very slowly (except above 30% dioxane). This is more clearly seen from Figure 6, where the rotations at a single wavelength are plotted against the solvent composition. The b_o values based on equation (3) varied ± 50 (except in high dioxane concentrations); the small positive values were attributed to the contribution of the nonhelical b_o , as can be seen from the modified Moffitt equation (Yang, 1962):

$$[m'] = a_1^R \lambda_1^2 / (\lambda^2 - \lambda_1^2) + f a_o^H \lambda_o^2 / (\lambda^2 - \lambda_o^2)$$

$$+ f b_o^H \lambda_o^4 / (\lambda^2 - \lambda_o^2)^2 \quad (4)$$

or

$$[m'] \cong (a_1^R \lambda_1^2 + f a_o^H \lambda_o^2) / (\lambda^2 - \lambda_o^2) + [f b_o^H \lambda_o^4$$

$$+ a_1^R \lambda_1^2 (\lambda_1^2 - \lambda_o^2)] / (\lambda^2 - \lambda_o^2)^2 \quad (5)$$

where a_1^R approximates the sum of all partial rotations other than those due to the helical backbone, f is the per cent helicity, and b_o^H is currently taken as $-630^\circ \text{ cm}^2 \text{ decimole}^{-1}$ for a right-handed α -helix (with λ_o preset at 212 mμ). The small negative b_o values (0 to -50) could be the result of variation in λ_1 (if $\lambda_1 \geq \lambda_o$) in different solvents, or of the presence of partial helices (less than 10%). The precision of the current ORD treatment does not warrant an accurate estimate of helicity good to the first integer; we are therefore led to believe that poly-L-glutamic acid retains its coiled conformation in up to 8 M salts or about 30% dioxane. This conclusion is substantiated by the Cotton effect measurements and also helix-coil transition studies to be described. In high dioxane concentrations (above 30%) the coiled form of poly-L-glutamic acid began to be converted to the helical conformation. At 50% dioxane it was essentially helical, as witnessed by the b_o and a_o values in Table I. The fact that the magnitude of the levorotations declined with increasing salt or dioxane concentration indicates that the extended coiled form of poly-L-glutamic acid in pure water has more restricted freedom of rotation than that of the contracted molecules.

The results in Figure 5 fully support the foregoing conclusions. The profile of the Cotton effects for poly-L-glutamic acid at 50% dioxane is characteristic of that of an α -helix with a trough and a peak at 233 and 198 mμ, and $[m']_{233} \cong -16,000$ and $[m']_{198} \cong +80,000$ (see Blout *et al.*, 1962; Yang and McCabe, 1965); at

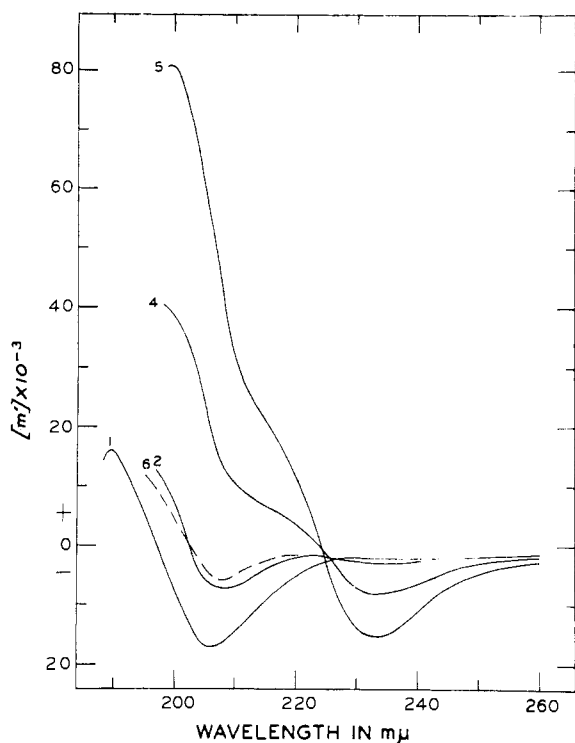


FIGURE 5: Ultraviolet rotatory dispersion of poly-L-glutamic acid in salt and dioxane solutions at pH 7.3. Symbols same as in Figure 4.

40% dioxane poly-L-glutamic acid is only partially helical. All the other curves in the figure resemble those observed for the coiled conformation, but with increasing salt concentration the 204-m μ trough undergoes a red shift, and the other small Cotton effect at higher wavelength undergoes a blue shift. Such changes will of course influence the rotatory behavior in the visible region.

To ascertain further the conformational changes of poly-L-glutamic acid in solutions we followed the helix-coil transition by measuring $[m']_{233}$ in various salt solutions (Figures 7 and 8) and in dioxane-water mixture (Figure 9). (Because of strong absorption of LiBr solutions at 233 m μ , we chose the data at 240 m μ instead for Figure 8.) In all cases except those above 30% dioxane, poly-L-glutamic acid remained in the coiled form at the apparent pH 7.3. In 30% dioxane poly-L-glutamic acid was partially helical, whereas in 50% dioxane it was almost completely helical at pH 7.3. In general, the addition of alkali salts such as LiBr, NaCl, and KCl lowers the transition pH, although the nature of the salt may also affect the transition point as illustrated by the primed curves in Figure 7, for example, 3 M KCl (3') versus 3 M NaCl (3''). Of interest is the finding that poly-L-glutamic acid in 8 M LiBr remained in the coiled form throughout the pH range studied (Figure 8). That LiBr does not enhance the formation of α -helix could have some important bearing on our understanding of, say, the effect of 8 M LiBr on the conforma-

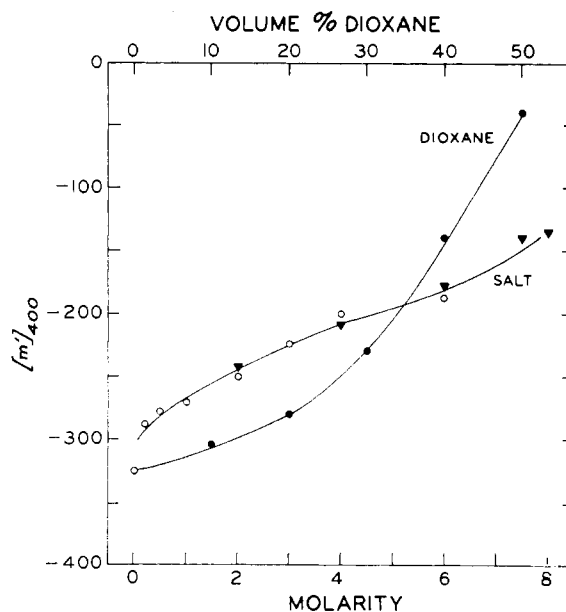


FIGURE 6: Optical rotation of poly-L-glutamic acid at 400 m μ in different salt and dioxane concentrations at pH 7.3. O, KF; ∇ , LiBr; \bullet , dioxane. (N.B. The $[m']$'s showed small differences among various salts having the same molarity [see Table I]).

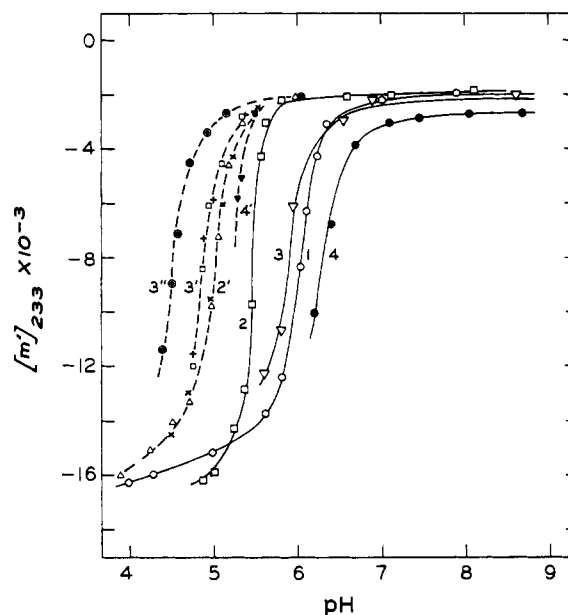


FIGURE 7: Helix-coil transition of poly-L-glutamic acid in various salt solutions. (1) Water, (2) 0.2 M KF, (2') 0.2 M NaCl or CsCl, (3) 3 M KF, (3') 3 M KCl or CsCl, (3'') 3 M NaCl, (4) 6 M KF, and (4') 6 M CsCl.

tional stability of proteins (see Discussion). KF and CsCl appeared to be two exceptions among the alkali salts studied. The transition pH was lower in 0.2 M KF than in pure water, while the trend was reversed at high

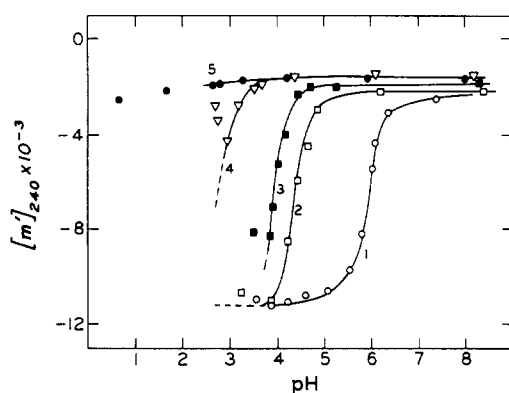


FIGURE 8: Helix-coil transition of poly-L-glutamic acid in various LiBr solutions. (1) Zero, (2) 2 M, (3) 4 M, (4) 6 M, and (5) 7.5 M. Note that the wavelength used differs from that in Figure 7.

KF concentrations; for example, in 3 M KF the transition pH was still slightly more acidic than that in water, but in 6 M KF it definitely shifted toward a higher pH. The same phenomenon was also true to some extent for CsCl solutions.

The effect of dioxane on the conformation of poly-L-glutamic acid is just opposite to that of salt; the transition pH (apparent), with increasing dioxane concentration, shifted toward a higher pH than in pure water. In 30% dioxane (pH 7.3) poly-L-glutamic acid was already partially helical, while in 50% dioxane at the same pH it was essentially helical. These findings substantiated the Cotton-effect curves in Figure 5. A simple explanation for these results in Figures 7–9 can be found from the changes in the degree of dissociation of the carboxyl side groups with the addition of salt or dioxane. It is well known that the apparent pK of a weak acid becomes larger in the presence of dioxane. For example, the apparent pK of acetic acid increased from 4.6 in water to 8.1 in 50% dioxane. The same trend should appear in the side groups of a polypeptide chain. The situation was somewhat more complicated in the case of alkali salts. For acetic acid in LiBr and, to a small extent, in NaCl solutions, we did find that the apparent pK was gradually lowered with increasing salt concentration, thus partly explaining the acid shift of the transition pH in these cases. In CsCl and, to a small extent, in KCl solutions, however, the apparent pK was first lowered with increasing salt concentration, but reversed in higher salt concentrations. This phenomenon must also have occurred more drastically for poly-L-glutamic acid in KF solutions, although we did not make any titration studies in this case because of the expected damage of the glass electrodes by HF. That the change in the apparent pK cannot account for the entire shift in the helix-coil transition can be illustrated by the following examples. The pK of acetic acid was lowered from 4.62 in water to about 3.1 in 8 M LiBr, and yet Figure 8 shows that the transition does not take place even at pH 1; on the other hand, the pK dropped only slightly in 4 M NaCl,

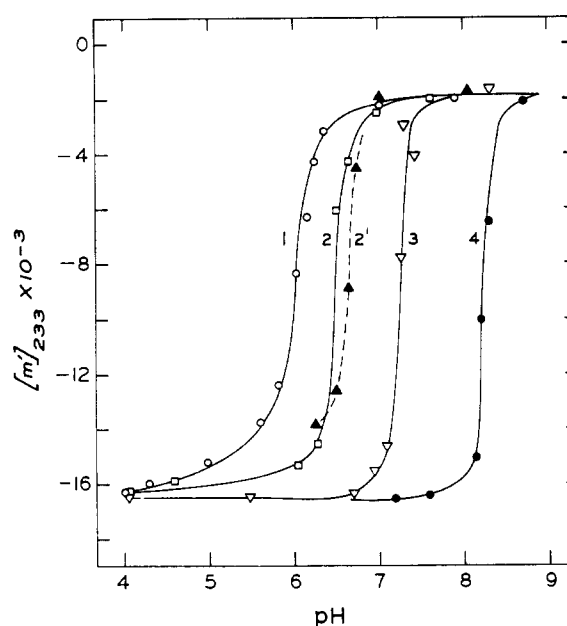


FIGURE 9: Helix-coil transition of poly-L-glutamic acid in dioxane-water solutions. Volume per cent of dioxane: (1) zero, (2) 10, (2') 10 with 0.2 M KF, (3) 30, and (4) 50.

but we can see a significant shift in the transition pH from Figure 8. Perhaps specific interactions between the ions and polypeptide chains might have occurred in salt solutions, e.g., the binding of Li^+ , which, if true, could restrict the winding of the polypeptide chains into the helical form. This problem, however, must await future experimentation to prove or disprove such a hypothesis.

Discussion

The principal finding in this study is that polypeptide chains contract in the presence of salt or dioxane. The carboxyl groups of poly-L-glutamic acid in aqueous solutions (pH 7.3) are completely ionized, and the molecules behave like those of a typical polyelectrolyte except in high dioxane solutions (above 30 volume per cent), where the apparent pK shifts toward the alkaline side. The viscosities of the solutions drop drastically when a small amount of salt is added to the aqueous solution, e.g., 0.2 M NaCl, because of the reduction in electrostatic repulsion due to the swamping of counterions. This change in gross conformation is accompanied by a decrease in the magnitude of rotations (in the visible region), indicating that polypeptide chains are less extended and have more freedom of rotation in the presence of salt or dioxane. Electrostatic interactions, however, are not the only factor that accounts for the contraction of the polypeptide chains. In general, the addition of 0.2–1 M salt to the aqueous solution should be sufficient to suppress the electrostatic repulsion, and yet we have seen from Figures 1 and 6 that both viscosity and levorotation drop continuously with increasing salt concentration. In dioxane solutions, where the di-

electric constant is low, we would have expected an enhancement of the electrostatic interaction, but actually the polypeptide chains contract with increasing dioxane concentration. Thus, other factors must be taken into consideration and a plausible explanation found elsewhere.

The effects of neutral salts on the stability of macromolecular conformations have aroused considerable interest in recent years, although the mechanism or mechanisms involved are still far from defined. Bigelow and Geschwind (1961) referred to such alteration of conformations simply as a "general medium effect." A comparative study of the stability against neutral salts of macromolecules as diverse as ribonuclease, collagen, DNA, and myosin permitted Von Hippel and Wong (1964) to rule out certain possibilities that correlate these salt effects with simple electrostatic charge-shielding, or the lowering of the activity of water, or specific chemical or conformational properties of the individual macromolecules. They further suggested that in the absence of any specific ion-macromolecule interactions, these salt effects are a consequence of very general effects of the various ions on the structure of solvent, which in turn modify solvent-macromolecule interactions involved in the stabilization of the native structures. The same interpretation can be applied equally to a simple polypeptide such as poly-L-glutamic acid, and we postulate that dehydration of the polypeptide chains causes the contraction of the molecules in salt solutions. This effect is more predominant in higher salt concentrations, where solvated ions leave little water of hydration for the polypeptide chains. This explanation, although oversimplified, can account for the reduction in both the viscosities and levorotations as observed in this study. In high LiBr concentrations (above 6 M), we see from Figure 1 that the intrinsic viscosity of poly-L-glutamic acid drops more steeply, thus indicating an additional change in the overall conformation. However, we can reasonably rule out the possibility of a coil-to-helix conversion and the formation of partial helices in concentrated LiBr solutions, since the data in Figure 8 clearly show no such transition at neutral pH. Neither can we detect in Figure 6 any drastic change in rotations for poly-L-glutamic acid in LiBr solutions, as contrasted with the sharp curvature in the case of dioxane-water solutions. Rather we suspect that the aggregation of poly-L-glutamic acid in high salt concentrations in some way further reduces the hydrodynamic volume of the molecules, thus causing the downward curvature in the viscosity curve of Figure 1 (poly-L-glutamic acid began to precipitate at about 9 M LiBr).

Although any conclusion drawn from the study of a simple model polypeptide cannot be directly applicable to the more complicated globular proteins, our optical rotation results do resemble those obtained for the unfolded states of such proteins as oxidized ribonuclease (Bigelow and Geschwind, 1961). They also reaffirm the contention that a decrease in levorotation does not imply any hyperfolding of the molecules such as the formation of an α -helix, especially when the solvent composition has varied significantly. (This point will be

further elaborated in a later section.) This conclusion is equally true for the native proteins. Thus, a sharp decrease in levorotation of ribonuclease in 3 M LiBr (Harrington and Schellman, 1957) by no means points toward a helix-coil transition. As a matter of fact Mandelkern and Roberts (1961) have now shown a lowering of the transition temperature of ribonuclease in the presence of increasing quantities of LiBr. As far as our viscosity and rotation studies are concerned, we are unable to detect any peculiar properties of LiBr which distinguish it from other alkali salts.

We have already mentioned that electrostatic interaction alone cannot explain the continuous drop in the intrinsic viscosity of poly-L-glutamic acid in dioxane-water solutions. A plausible explanation for this phenomenon is that dioxane is a hydrophobic agent which "dislikes" the polar side groups (carboxylate ions) of the polypeptide chains and vice versa. As such, the polyions would contract to minimize their exposure to the solvent medium. The fact that coiled molecules of poly-L-glutamic acid can be converted into the helical conformation in high dioxane concentrations has already been explained in terms of the increase in pK of the carboxyl groups with increasing dioxane concentrations.

ORD Analyses. We have treated the ORD data in the visible region with the one-term Drude and Moffitt equations, which are adequate to explain the conformational changes of poly-L-glutamic acid as a result of contraction of the polypeptide chains and of the conversion from coils into helices such as in 30% or more dioxane solutions. To complete our ORD analyses, however, we should mention the recent renewed interest in the use of the two-term Drude equation for such studies (Imahori, 1963; Yamaoka, 1964; Shechter and Blout, 1964):

$$[m'] = A_1\lambda_1^2/(\lambda^2 - \lambda_1^2) + A_2\lambda_2^2/(\lambda^2 - \lambda_2^2) \quad (6)$$

The graphic solution of equation (6) can easily be done by plotting $[m'](\lambda^2/\lambda_1^2 - 1)$ against $1/(\lambda^2/\lambda_2^2 - 1)$ (Shechter and Blout, 1964) or, better, against $(\lambda^2/\lambda_1^2 - 1)/(\lambda^2/\lambda_2^2 - 1)$ (Imahori, 1963; Yang, 1965) for trial values of λ_1 and λ_2 until a straight line is obtained. In a series of papers Shechter and Blout chose 193 and 225 $m\mu$ for λ_1 and λ_2 . From the ORD of poly-L-glutamic acid in water at pH 4 (helical) and 7 (coiled) they observed that $A_{193}^H = 2900$, $A_{225}^H = -2050$, $A_{193}^C = -750$, and $A_{225}^C = -60$ (the letters H and C here representing the helical and coiled conformations). These authors then proposed the estimate of helicity in proteins by the following relationship:

$$\text{per cent helix} = (A_{193} + 750)/(29.0 + 7.50)$$

$$\text{or} \quad (7)$$

$$\text{per cent helix} = -(A_{225} + 60)/(20.5 - 0.6)$$

Following the procedure of Shechter and Blout, we determined the A -parameters of poly-L-glutamic acid in salt and dioxane solutions, some of which are illus-

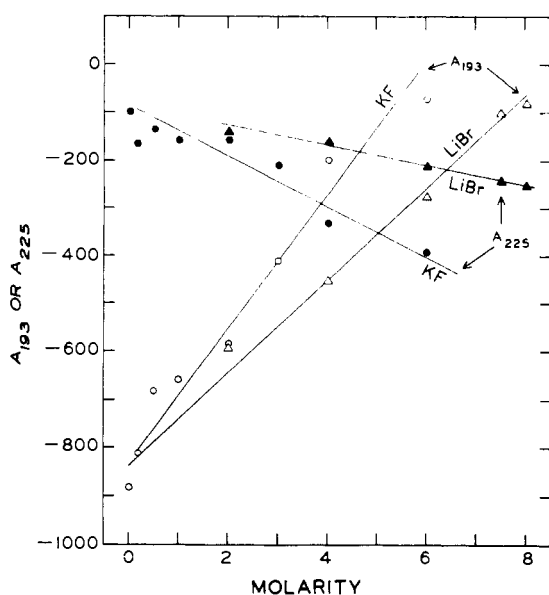


FIGURE 10: The A -parameters of the two-term Drude equation for poly-L-glutamic acid in various salt solutions at pH 7.3. See text for detail.

trated in Figure 10. (Our values of $A_{193}^0 = -880$ and $A_{225}^0 = -100$ in pure water differed slightly from those reported by Shechter and Blout [1964], but this will not affect the discussion here.) In both cases A_{193} increases and A_{225} decreases with increasing salt or dioxane concentrations. The problem then lies in the uncertainties of the reference values for the coiled form for equation (7). If we substitute the experimental values of Figure 10 into equation (7), we are led to believe that poly-L-glutamic acid in KF or LiBr at pH 7.3 formed partial helices, the amount of which gradually increased with increasing salt concentrations, a conclusion which is difficult to accept in view of the observed sharp helix-coil transition in Figures 7 and 8. We point this out to caution against overenthusiasm toward the use of any phenomenological equation, whether the Moffitt equation or the two-term Drude equation. In retrospect we might mention that the two-term Drude equation was first used for poly- γ -benzyl-L-glutamate in organic solvents (Yang and Doty, 1957) prior to the introduction of equation (3). The advantage of using the latter is to reduce four parameters, A_1 , A_2 , λ_1 , and λ_2 in equation (6) to three, a_0 , b_0 , and λ_0 in equation (3). Once λ_0 is fixed experimentally ($= 212 \text{ m}\mu$), the solution of equation (3) is unique. On the other hand, the limited precision of the current experimental data leads to many graphical solutions of equation (6) with different sets of λ_1 and λ_2 . With each pair of λ_1 and λ_2 , the A -parameters in equation (3) will vary accordingly. Some of the problems that arise in the use of equation (6) were discussed in a separate communication (Yang, 1965).

We recognize that the current ORD treatments have certain limitations. If we use the phenomenological equations too literally, an alternate explanation for our

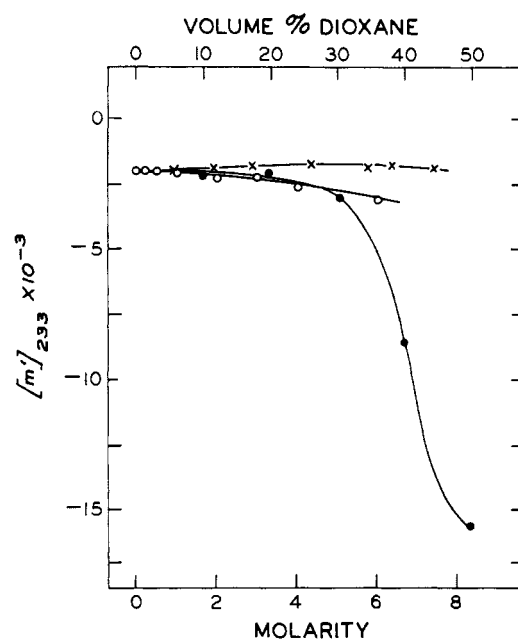


FIGURE 11: Optical rotation of poly-L-glutamic acid at $233 \text{ m}\mu$ in different salt and dioxane concentrations at pH 7.3. Symbols: O, KF; x, LiBr; and ●, dioxane; cf. Figure 6.

results (suggested by an anonymous referee) can be put forward. For example, poly-L-glutamic acid in 6 M KF had a $b_0 = -50$ as compared with $+50$ in pure water or 1 M KF (Table I). If the latter were assumed to represent the nonhelical b_0 in equation (5), we would have concluded a 15% helical content in 6 M KF. Then $A_{193} = -70$ and $A_{225} = -390$ (read from Figure 10) would have indicated a 19–17% helical content according to equation (7). In addition, we could even argue that $[m']_{233}$ was more negative in 6 M KF than in water (Figure 11), again seemingly supporting the formation of partial helices. In view of the uncertainty of current ORD analyses, probably $\pm 10\%$ helical content, such a possibility of course cannot be excluded completely. But if this were true, we must assume a constant helicity of more than 10% for poly-L-glutamic acid in 6 M KF above pH 7 (Figure 7), which then increased sharply in the helix-coil transition region. By the same token, the rotatory behavior of poly-L-glutamic acid in 8 M LiBr would have been even more strange; its b_0 of -40 as compared with $+50$ in water would again have suggested a helicity of more than 10%. Substitution of $A_{193} = -80$ and $A_{225} = -250$ (read from Figure 10) into equation (7) would have given a helical content of 18 and 10%, respectively. But from the data in Figure 8 we had to accept the conclusion that the partial helices, if they existed in 8 M LiBr, would remain constant throughout the entire pH range studied, since no helix-coil transition occurred even at pH 1. Furthermore, Figure 11 actually shows a slightly smaller levorotation at $233 \text{ m}\mu$ for poly-L-glutamic acid in LiBr solutions than in water. If any increase in levorotatory $[m']_{233}$

must reflect an increase in helical content, we would have been compelled to conclude that even poly-L-glutamic acid in water (at pH 7.3) might have some helical segments, at least more so than in, say, 4 M LiBr solution. Such possibilities are indeed very difficult to visualize, although they are not entirely improbable. We are, however, not convinced that poly-L-glutamic acid in water must have the same $[\alpha]_{233}^{\circ}$ as that in 6 M KF or 8 M LiBr, had there been no helicity present. How the nature of the side groups and the solvents will affect the magnitude of the Cotton effects for both the helical and coiled conformations remains to be investigated (Yang, 1965; Yang and McCabe, 1965). Neither can we accept the notion that the residue (or background) rotations in equation (4) must be the same for poly-L-glutamic acid in water and in high salt concentrations. In comparison, we note that the Cotton effect profile of poly-L-glutamic acid in 30% dioxane does resemble that in 6 M KF (Figure 5), but they are not identical. From Figure 9 poly-L-glutamic acid in 30% dioxane lies in a region which is the beginning of a helix-coil transition, whereas that in 6 M KF or 8 M LiBr (Figures 7 and 8) is located in the plateau away from the transition. Unless future evidence proves otherwise, we have therefore presented a qualitative picture that poly-L-glutamic acid in its coiled form contracts with increasing salt or dioxane concentrations, and it undergoes a helix-coil transition only in dioxane solutions (near and above 30%) when the apparent pK of the carboxyl groups is raised so high that the polyion is essentially un-ionized at pH 7.3 (apparent). Our results of both viscosities and optical rotations of poly-L-glutamic acid are in accord with this explanation.

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during the initial phase of this investigation (Yang, 1962).

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