concentration range by 1:2 and 1:3 complexes. The different physical measurements used in detecting their presence are, however, not equally sensitive to both species because of the characteristic times of the various methods.

(4) The biophysical activity of these compounds in promoting denaturation depends upon competing interactions with polymers. The competitions proposed are

$$\begin{aligned} H_2O + B^{+0-} &\rightleftharpoons H_2O\cdot B^{+0-} \\ M^+,X^- + B^{+0-} &\rightleftharpoons M^+,X^-\cdot B^0 \\ A + B^{+0-} &\rightleftharpoons A\cdot B^{+0-} \end{aligned} \tag{9}$$

where B^{+0-} represents the polymer in positively charged, uncharged, and negatively charged states, M^+, X^- are counterions, and A represents the solute which interacts with the polymer and with water. The assumption is that the conformation of B^{+0-} is stabilized or destabilized depending upon the proportions and nature of A.

Acknowledgments. This work was supported by Grant OSW-14-01-0001-1700 from the U. S. Department of the Interior, Office of Saline Water, and N. S. F. Grant GB-8468 from the National Science Foundation, Molecular Biology Division.

Participation of Water in Conformational Changes of Biopolymers as Studied by Deuteron Magnetic Relaxation

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Abstract: Deuteron magnetic spin–lattice relaxation experiments performed on D_2O solutions of various macromolecules are described and discussed. This technique allows the interaction of water with various conformations of biopolymers to be measured quantitatively. The molecules studied are: poly(L-glutamic acid), poly(L-lysine), poly(adenylic acid), poly(uridylic acid), poly(methacrylic acid), poly(vinyloxazolidinone methyl), and poly(vinylpyrrolidone). General classes of interactions are proposed on the basis of the results, and the effect of geometrical fluctuations of polymer conformation upon polymer–water interactions are discussed. It is concluded that the competing effects of counterion–polymer interaction (electrostatic) and polymer–water interaction (hydrogen bond) are of vital importance in mediating polymer conformation.

The phenomena associated with conformational transitions in biopolymers, such as polypeptides and polynucleotides containing ionizable groups, have been intensively studied. This is especially true of those such as poly(L-glutamic acid), poly(L-lysine), and poly(adenylic acid) which undergo helix–coil transitions in aqueous solutions. Various qualitative and quantitative explanations of these phenomena have been proposed[1] although most, for lack of information, neglect effects of solvent–polymer interaction.

In this paper experiments on D_2O solutions of the above-mentioned polymers and polymers I, II, III, and VII (see Figure 1), using the method of deuteron magnetic spin–lattice relaxation, are described.[2] The purpose of this is to provide information on the role of water structure in the stabilization or destabilization of the various polymeric tertiary structures encountered. This role has often been invoked to explain the properties of the polymers in solution. Unfortunately, it has been little investigated due to the scarcity or inapplicability of experimental methods. As previously described, nuclear magnetic resonance (nmr) relaxation methods in systems where quadrupole induced relaxation is dominant provide a powerful tool for such investigations.[3,4] The fundamental theory is based upon the fact that the spin–lattice relaxation time, T_1, for deuterons in D_2O is governed completely by the rotational reorientation time, τ, of the individual D_2O molecules (intramolecular relaxation). This is not true of proton relaxation. T_1 for pure D_2O is of the order of 0.5 sec at room temperature. The results in the following discussion are given in terms of the reciprocal of this, *i.e.*, $1/T_1$, which is the relaxation rate and is directly proportional to τ. For bulk D_2O it is therefore equal to about

$$1/T_1 = K\tau \tag{1}$$

2 sec^{-1} at room temperature. When another species such as an ion, polymer, enzyme, or colloid is present in a solution of D_2O the molecules of bulk water collide with the species, spend an average lifetime, t_b, associated with it, and then return to the bulk phase. During their association with the dissolved species the rotational reorientation time of the associated D_2O molecules is no longer that of bulk water. It may be either longer or shorter than for bulk water. In the former case we say that the water interacting with the species is more "structured" than in bulk water, and in the latter case, "less structured." This is in analogy to the relative reorientation times in liquid water and in ice.

Special note should be taken of the difference between this and previous work on relaxation rates of protons, deuterons, and oxygen-17 in aqueous solutions of

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(1) T. M. Birshstein and O. B. Ptitsyn, "Conformations of Macromolecules," Interscience Publishers, New York, N. Y., 1966.

(2) J. A. Glasel, *Nature*, **220**, 1124 (1968).

(3) J. A. Glasel, *Proc. Nat. Acad. Sci. U. S.*, **58**, 27 (1967).

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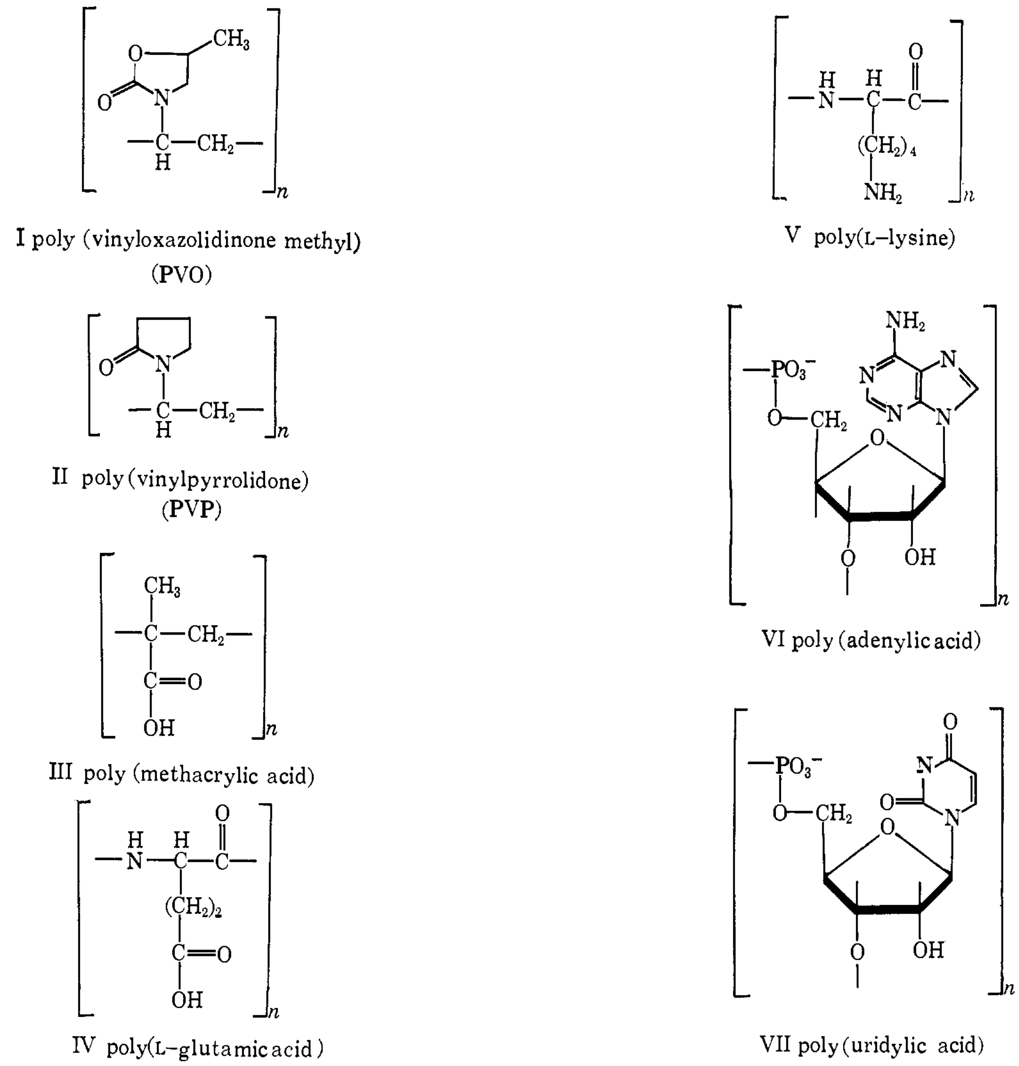

I poly (vinyloxazolidinone methyl) (PVO)

II poly (vinylpyrrolidone) (PVP)

III poly (methacrylic acid)

IV poly(L-glutamic acid)

V poly(L-lysine)

VI poly (adenylic acid)

VII poly (uridylic acid)

Figure 1. Molecular structures and abbreviations of polymers used in this study.

simple electrolytes and small organic molecules.[5-7] The observable effects are comparable in magnitude but the solute concentrations (on a monomer basis) used in the present studies are more than an order of magnitude smaller than those used in the studies on simpler molecules. In itself this is a demonstration of the effect of the association of water with some polymers. In further proof, experiments with polymers at a given concentration were performed also with solutions of monomeric units under the same conditions. No observable effects were encountered in the latter experiments and this is shown in the figures presented under Results.

The polymers studied have certain superficial resemblances to each other as far as their physical properties are concerned. All are very soluble in water. Polymers I and II undergo cloud-point transitions at high temperatures and/or high pH. The temperature or pH at which the transitions take place are affected by agents such as urea and ammonium sulfate in a manner analogous to protein cloud points. The polymers have been discussed as model systems for protein–solvent interactions.[8] Polymer III undergoes a pH-dependent conformational change from a state in which it is an isotropic light scatterer (low pH) to an extended system at high pH.[9] Polymers IV, V, and VI are, of course, well known because of their ability to undergo order–disorder transitions as a function of pH. Polymer VII does not undergo any known conformational changes as a function of pH.

Despite some of these similarities with respect to their conformations in solution as a function of pH, salt concentration, and concentration of ordering and denaturing agents, any *internal* system or hydrogen bonds holding the polymers together in a rigid conformation cannot be of the same general type. For example, I and II have no internal hydrogen bonds which might be broken during denaturation. The arrangement of the H bonds holding the helix of IV and V together is along the helical axis while for VI it is perpendicular to the axis. This leads to the idea that the interaction of solvent structure with these polymers may be a mediating property with respect to their conformations in solution.

Experimental Section

The following materials were used: **poly(vinyloxazolidinone methyl)**; the original source was Dow Chemical Corp., Midland, Mich., lot No. 411070, obtained through the courtesy of Professor I. M. Klotz; mol wt = 40,000 (viscosity). This was used directly.

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(5) G. Engel and H. G. Hertz, *Ber. Bunsenges. Phys. Chem.*, **72**, 808 (1968).

(6) L. Endom, H. G. Hertz, B. Thul, and M. D. Zeidler, *ibid.*, **71**, 1008 (1967).

(7) F. Fister and H. G. Hertz, *ibid.*, **71**, 1032 (1967).

(8) I. M. Klotz, *Fed. Proc.*, **24**, S24 (1965).

(9) A. Oth and P. A. Doty, *J. Phys. Chem.*, **56**, 43 (1952).

Poly(vinylpyrrolidone) was obtained from Sigma Chemical Co., pharmaceutical grade; mol wt = 360,000, 40,000, 10,000 (viscosity). This was used directly.

Poly(methacrylic acid) was synthesized and fractionated by Professor A. Krasna of this department; mol wt = 312,000 (viscosity). This was used after lyophilizing from HCl solution.

Poly(L-glutamic acid). Sample 1 was obtained from Sigma Chemical Co., grade III, sodium salt, lot No. 126-B-0390-1, mol wt = 105,000; sample 2 from Miles-Yeda, Ltd., lot No. GL89A, mol wt = 5400. These were used directly.

Poly(L-lysine). Sample 1 was obtained from Miles-Yeda, Ltd., lot No. LY120, mol wt = 75,000; sample 2 from Miles-Yeda, Ltd., lot No. LY124, mol wt = 3090. These were obtained as the hydrobromide and used directly.

Poly(adenylic acid) was obtained from Miles Chemical Co., control No. 111531, s = 4.23. This was obtained as the sodium salt and used directly.

Poly(uridylic acid) was obtained from Miles Chemical Co., control No. 411541, s = 2.22. This was obtained as the ammonium salt and used directly.

The D_2O solutions were made up from high isotopic purity commercial D_2O, DCl, and NaOD. Salts were all reagent grade.

Deuteron relaxation times were all measured using the adiabatic fast-passage attachment previously described[10] fixed to a Varian DA-60EL spectrometer operating in the wideline mode with a V-4210A variable-frequency transceiver. The sample size was 3 ml and the sample temperature was 31 ± 1°. Each relaxation time derived is the average of three independent null point experiments on separate photographs. Reproducibility between measurements averaged ±3%.

Titrations were performed with the aid of a Radiometer Model TTT1a pH meter with expanded scale. No correction is given in the following figures to convert pH to pD, and the results are given as apparent pH (pH_a). However, the currently accepted equation is pD = pH + 0.4.[11] T_1 measurements at various pH_a's were made by dropping concentrated DCl or NaOD into the nmr sample tube (accompanied by agitation of the sample) until an approximate pH_a was obtained. The T_1 measurement was then made after thermal equilibrium was attained in the nmr probe space (about 15 min). Then the pH_a was measured accurately, and the process continued. The dilution due to the added acid or base was negligible. Titrations were performed in the usual way with 3% wt/v solutions of the polymers and 0.1 N NaOD or DCl.

Viscosities were measured with appropriate Cannon-Manning semimicro viscometers at 31 ± 1°.

Theoretical

As in previous work,[4] it is assumed that the lifetime of a water molecule associated with a polymer is less than 0.1 sec. In this case the two-phase equation for the observed relaxation rate may be used in its fast-exchange approximation.[12] This is

$$\begin{aligned}(1/T_1)_{\text{obsd}} &= (1/T_1)_{\text{free}} + CW(1/T_1)_{\text{a}} \\ &= (1/T_1)_{\text{free}} + CWK\tau_{\text{a}} \qquad (2)\end{aligned}$$

where $(1/T_1)_{\text{obsd}}$ is the measured relaxation rate for the solution, $(1/T_1)_{\text{free}}$ is the relaxation rate of the pure solvent, C is the concentration of the polymer in grams of dry weight/gram of solvent, W is the time-independent weight of water associated with the polymer per gram of polymer, $(1/T_1)_{\text{a}}$ is the relaxation rate for the associated water, K is the quadrupole coupling term, and τ_{a} is the rotational reorientational time of the water molecules associated with the polymer. It is assumed that the K for associated and free water are equal. This is certainly valid since the K for deuterons in water and ice are almost equal.[13] Equation 2 predicts that a plot of $(1/T_1)_{\text{obsd}} - (1/T_1)_{\text{free}}$ *vs.* C should yield a straight line for a given polymer. This has been shown to hold true for several proteins.

In general, unless the extreme narrowing approximation $\omega_o^2\tau_a^2 \approx 4\omega_o^2\tau_a \ll 1$[14] holds up for $(1/T_f)_a$ (it always does for $(1/T_1)_{\text{free}}$) eq 2 is frequency dependent as

$$(1/T_1)_{\text{obsd}} = (1/T_1)_{\text{free}} + \frac{KCW}{5}\left[\frac{\tau_a}{1 + \omega_o^2\tau_a^2} + \frac{4\tau_a}{1 + 4\omega_o^2\tau_a^2}\right] \qquad (3)$$

where ω_o is the nmr frequency and the usual angular-autocorrelation function is assumed. In the extreme narrowing approximation eq 3 becomes 2. Equation 3 has been modified in order to take into account the possibility that relaxation of the second-phase species (here, the associated water molecules) may not be characterized by a single rotational reorientation time. Thus, the frequency-dependent terms may be replaced by

$$(1/T_1)_{\text{obsd}} = (1/T_1)_{\text{free}} + \frac{KCW}{5}\left[\int_{-\infty}^{\infty}\frac{\tau G(S)\mathrm{d}S}{1 + \omega_o^2\tau^2} + 4\int_{-\infty}^{\infty}\frac{\tau G(S)\mathrm{d}S}{1 + 4\omega_o^2\tau^2}\right] \qquad (4)$$

where

$$\int_{-\infty}^{\infty} G(S)\mathrm{d}S = 1$$

$$S = \ln\,(\tau/\tau_{a,m}) \qquad (5)$$

and $G(S)$ is the distribution function for the τ. $\tau_{a,m}$ is the mean reorientation time for the distribution of τ. Several distribution functions have been discussed.[15] However, the most realistic one with respect to the present discussion is the Gaussian distribution

$$G(S) = \sqrt{\beta/\pi}\, e^{-\beta^2S^2} \qquad (6)$$

where β is the parameter characterizing the width of the distribution of the τ. A small value of β indicates a narrow range of reorientation times distributed about the mean. Zero β is the limiting case for a single reorientation time. Values of the integrals appearing in eq 4 cannot be obtained in closed form using the distribution of eq 6 but may be derived from the appropriate numerical tabulation.[16]

Results

Figure 2 shows a plot of relaxation rate *vs.* concentration and relative viscosity *vs.* concentration ($\eta_{\text{rel}} = \eta_{\text{soln}}/\eta_{\text{solvent}}$) for polymer I of a single molecular weight and three, widely differing molecular weights of polymer II. The observed relaxation rate of these polymer solutions did not change when the cloud-point transition was induced by raising the pH. The extremely low slope of the plot of relaxation rate *vs.* concentration indicates vanishingly small interaction of D_2O with these polymers. To be precise, either W is near zero, or $(1/T_1)_{\text{a}}$ is essentially that of bulk water. Thus, there is no nmr evidence of interaction of water with these polymers. The results are clearly independent of bulk viscosity as has been observed before in other systems.[2]

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[15] T. M. Connor, *Trans. Faraday Soc.*, **60**, 1574 (1964).

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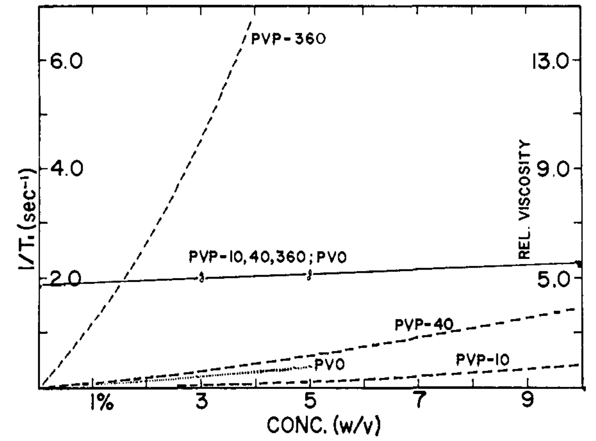

Figure 2. Relaxation rate and relative viscosity *vs.* wt/v concentration for I and II (mol wt = 360,000, 40,000, 10,000) in salt-free D_2O: solid line, relaxation rate; dashed lines, relative viscosities.

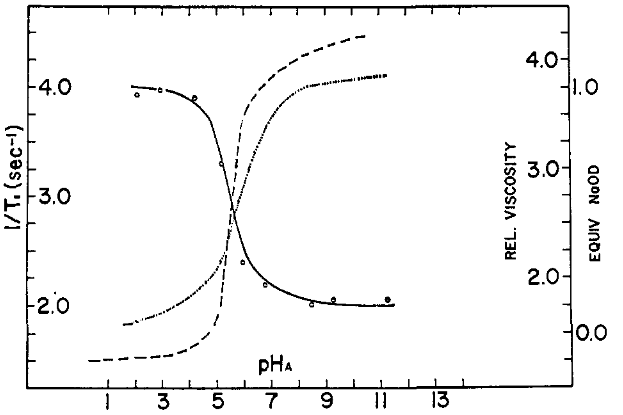

Figure 3. Relaxation rate and relative viscosity *vs.* apparent pH and titration curve of a 3% solution of poly(methacrylic acid) (mol wt = 300,000 in D_2O): solid line, relaxation rate; dashed line, viscosity; dotted line, titration curve.

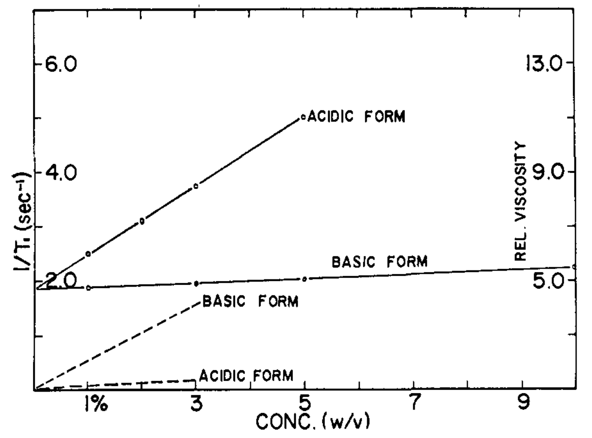

Figure 4. Relaxation rate and relative viscosity *vs.* wt/v concentration for acidic (pH_a 1) and basic (pH_a 11) forms of poly(methacrylic acid) (mol wt = 300,000 in D_2O): solid lines, relaxation rates; dashed lines, relative viscosities.

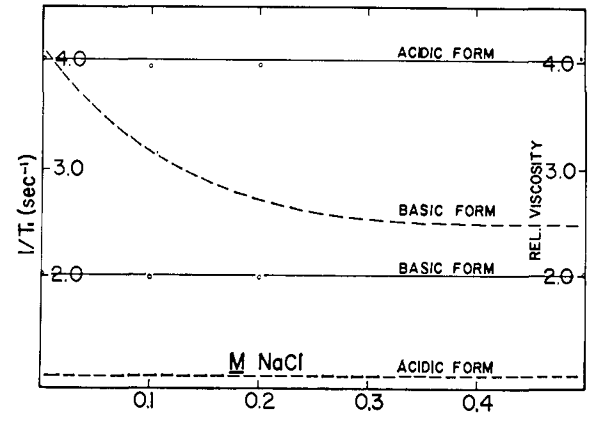

Figure 5. Relaxation rate and relative viscosity for acidic (pH_a 1) and basic (pH_a 11) forms of poly(methacrylic acid) *vs.* salt concentration in 3% wt/v D_2O solutions: solid lines, relaxation rates; dashed lines, relative viscosities.

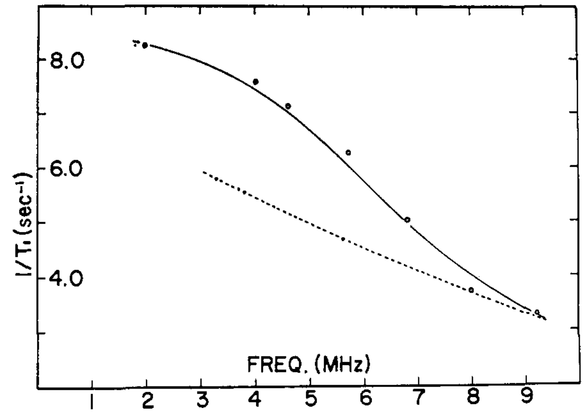

Figure 6. Relaxation rate *vs.* nmr frequency for a 1.5% wt/v solution of poly(methacrylic acid) (mol wt = 300,000) in acidic form (pH_a 1) in D_2O: solid line, calculated for Gaussian distribution of reorientation times with $\tau_{m,a} = 1.6 \times 10^{-8}$ sec and $\beta = 0$; dotted line, calculated for Gaussian distribution of reorientation times with $\tau_{m,a} = 1.0 \times 10^{-8}$ sec and $\beta = 0$.

Figure 3 contrasts the relative viscosity and observed relaxation rate for polymer III (mol wt = 360,000) as a function of pH. D_2O strongly interacts with this polymer in its uncharged, isotropically scattering form. In the extended form, whose solution has a higher relative viscosity, the water does not recognize the existence of the polymer in solution by this measure. Contrasts in the concentration-dependent properties of the acidic and basic forms of polymer III are shown in Figure 4 where $(1/T_1)_{obsd}$ and η_{rel} are plotted *vs* concentration. It is clear that the linear relationship predicted by eq 2 holds. Figure 5 shows the dependence of relaxation rate and viscosity upon salt concentration for the acidic and basic forms of polymer III. The relaxation rates are independent of this variable.

Figure 6 presents the measured frequency dependence of $(1/T_1)_{obsd}$, within the frequency range currently attainable in this laboratory, for a 1.5% wt/v solution of polymer III (mol wt = 300,000) in its acidic form. The form of the curve to which the experimental points have been fitted in this figure will be discussed later.

Figure 7 shows the values of relaxation rates for solutions of L-glutamic acid and poly(L-glutamic acid) of differing molecular weights *vs.* pH_a. In these experiments severe aggregation was observed below pH_a 4.5. However, as found with gels and denatured proteins,[2,4] the presence of aggregates does not change the relaxation rates observed unless the aggregates have different microscopic interactions with water than before aggregation. This figure shows that, in contrast with III, there is interaction with water in the (negatively)

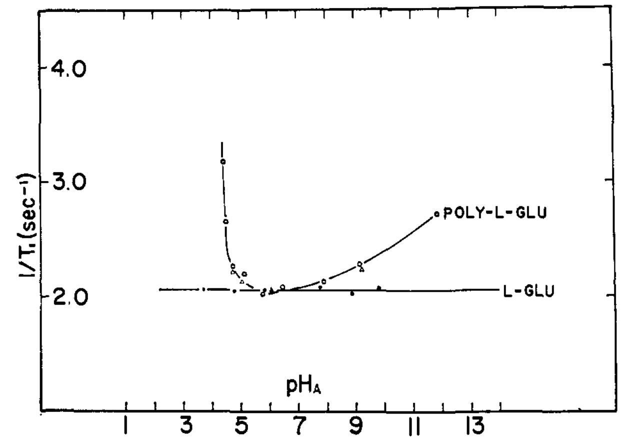

Figure 7. Relaxation rate *vs.* apparent pH for 3% wt/v solutions of poly(L-glutamic acid) and glutamic acid, 0.1 *N* NaCl in D_2O: O, mol wt = 100,000; Δ, mol wt = 3000.

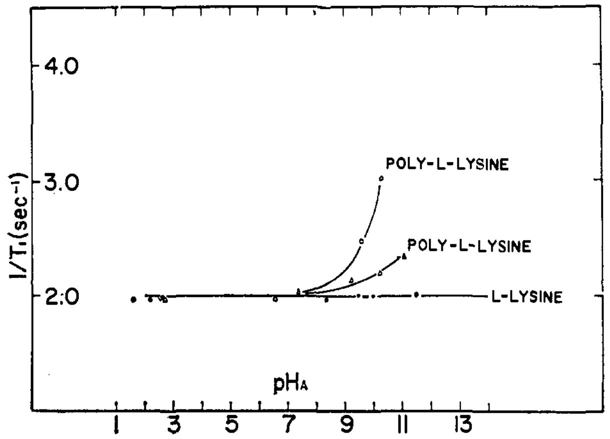

Figure 8. Relaxation rate *vs.* apparent pH for 3% wt/v solutions of poly(L-lysine) and L-lysine, 1 *M* KBr in D_2O: O, mol wt = 75,000; Δ, mol wt = 5000.

charged state. The observed strength of the interaction is molecular weight independent. The interaction decreases as the γ-carboxyl group is neutralized, becoming zero interaction in the middle of the helix-coil transition region,[17] and then increases very rapidly as the polymer is fully neutralized.

Figure 8 shows an analogous plot for L-lysine and poly(L-lysine) of differing molecular weights. Here there is a definite molecular weight dependence and zero interaction in the charged form. There is strong interaction with water in the polymer's uncharged form. The helix-coil transition for this polymer has also been studied by optical methods.[17]

Figure 9 shows the curves for relaxation rate *vs.* pH_a for poly(uridylic acid) and poly(adenylic acid). As mentioned before poly U does not undergo a pH-dependent order-disorder transition. It is disordered throughout the pH range. This figure shows that there is no interaction with water at any pH regardless of the charge on the polymer. In contrast, poly A behaves in a manner very similar to poly(L-glutamic acid).

Discussion

From the results given above one major conclusion may be drawn immediately. That is, in order to have an observable interaction with water in these experiments it is necessary to have a proton donor on the polymer which may hydrogen bond to a water molecule. This is a necessary but not a sufficient condition.

Polymers I and II do not possess proton donors, and consequently interact weakly, if at all, with water. Thus, proposed interactions of water with carbonyl groups, for example, are not experimentally borne out. The cloud-point phenomena in these polymer systems, observed at concentrations approaching 1 *M* hydroxide ion (or at high temperatures), might be explained by changes in the interaction of molecular dipole moments due to changes in the microscopic dielectric constant of the medium.

For the class of polymers that do interact with water the dominant interactions take place with the uncharged species. This is illustrated in the case of poly(methacrylic acid). It is of interest to know whether this interaction with the uncharged species is specific or nonspecific; that is, whether or not it is characterized by a single reorientation time for the associated water molecules. As explained in the Theoretical section, information on this question can be derived from the frequency dependence of the relaxation rate. With reference to Figure 6, the experimental points were fitted to a theoretical curve consisting of eq 6 substituted into eq 4. This was with the help of a FOCAL program written for a PDP-8/I computer. The solid line in this figure shows the best fit determined visually after varying both τ and β. This is found for a mean reorientation time of 1.6×10^{-8} sec and a width parameter, β, equal to zero. The conclusion is therefore that the interaction is specific and characterized by a single reorientation time. Given a τ, and using absolute reaction rate theory[18] which, crude as it is, describes these relaxation processes well, it is possible without temperature-dependent studies to derive an *estimate* for the free energy of activation corresponding to the reorientation processes accompanying rotation of the associated molecules. The value obtained is 6.9 kcal/mol as opposed to 2.3 kcal/mol obtained in the same way for reorientation of a D_2O molecule in pure heavy water. Experiments are in progress to derive similar information on other polymeric systems.

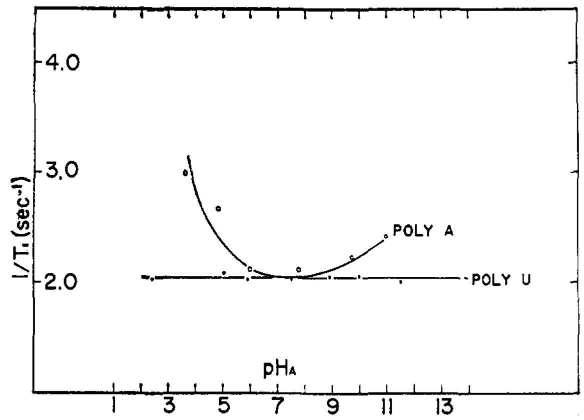

Figure 9. Relaxation rate *vs.* apparent pH for 3% wt/v solutions of poly(adenylic acid), *S* = 4.23, and poly(uridylic acid), *S* = 2.22; 2 *M* NaCl in D_2O.

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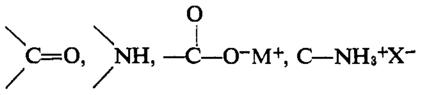
(18) S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p 548 ff.

In its extended form poly(methacrylic acid) displays the contraction expected of a "simple" polyelectrolyte upon increasing counterion concentration (Figure 5). There is no effect of salt concentration upon the observed interaction of water with either form of the polymer. In addition, as shown in Figures 2 and 3, the observed viscosity has no relation to the water-interaction phenomena discussed here. The acidic form of poly(methacrylic acid) follows eq 2 quite well.

The following are phenomenological rules which are being proposed to explain these observations.

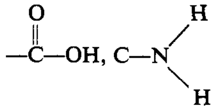
(1)



$$\rangle C{=}O,\ \rangle NH,\ -\overset{\overset{O}{\|}}{C}-O^-M^+,\ C-NH_3^+X^-$$

do *not* form strong interactions with water, and where M^+ are counterions

(2)



$$-\overset{\overset{O}{\|}}{C}-OH,\ C-N\begin{smallmatrix}H\\H\end{smallmatrix}$$

do form strong interactions with water.

(3) Strong interactions of water with polymers bearing a formal charge take place only when the charges are intra- or intermolecularly wholly or partly neutralized; that is, when counterion effects are eliminated by polymer–polymer interactions.

(4) When intramolecular topological fluctuations are large, and have characteristic times of the order of 10^{-3} sec, the water interaction with polymers is destroyed.

These rules may be discussed and justified individually. Rule 1. Evidence for this is based on the behavior of polymers I, II, III (basic), V (acidic), and VII. This and rule 2 point out clearly the importance of counterions in shielding polymers from water interaction. When counterion shielding is present conformational changes in the polymer do not effect water–polymer interactions because there are no interactions. This is illustrated by the behavior of poly(methacrylic acid) as shown in Figure 5. Similar conclusions on the basis of partial molal volume changes have been made for this polymer.[19] The results obtained from Figure 6 indicate *specific* interaction of water molecules with at least this one polymer. For poly U, where the pyrimidine p*K* is of the order of 9.5, neither the charged nor the uncharged form shows any interaction with water. This and the similar absence of interaction for the acid form of poly(L-lysine) indicate the weakness of imide–water interaction. In addition, all of the polymers studied having simple carbonyl functions display no observable interaction. To summarize: in order to display interaction there must be a proton donor without counterion shielding.

Rule 2. The coverage of rule 1 is supported by the observations on III (acidic), IV, V, and VI. All of these have labile proton donor groups which may hydrogen bond to water in their uncharged form.

Rule 3. The interaction of water with poly A in acid solution and poly(L-glutamic acid) in basic solution remains to be explained. The fact that the acid (NH^+) form of poly A is the ordered form is an exception to the simple notion that charge repulsion is the fundamental cause of order–disorder transitions. In this case, however, X-ray evidence shows clearly that in the charged state the NH^+ is internally neutralized by the phosphate which is negatively charged throughout the region studied here.[20] Hence, again water interacts strongly with an uncharged species. A similar zwitterion configuration does not exist for poly(L-glutamic acid). However, there are several structures possible whereby the carboxyl ion can interact with, for example, an NH group with subsequent delocalization of charge. If this is great enough the competing processes of water and counterion interaction may favor water. The proposed rule therefore predicts that despite the absence of helical content in the basic form of poly(L-glutamic acid), order is still present in the system. The independence of the interaction from molecular weight changes is consistent with a self-association phenomenon. Clear evidence that this is the case has been presented recently on the basis of circular dichroism spectra of poly(L-glutamic acid) in basic solution.[21]

Rule 4. The observation that the interaction for both poly A and poly(L-glutamic acid) goes to zero in the middle of the helix–coil transition region is one of the most interesting aspects of these experiments. It is known, at least for poly(L-glutamic acid), that in this region the polymer undergoes a contraction as shown by a drop in intrinsic viscosity,[22] and in the dielectric relaxation time.[23] In the same region dynamic theories of the helix–coil transition predict that the structural relaxation time should reach a maximum and in any given region of the polymer the relative amounts of helix and coil contents should fluctuate rapidly.[24,25] Evidence obtained from high-resolution nmr experiments on polymers undergoing helix–coil transitions[26–28] suggests that such fluctuations exist and that their lifetimes are 10^{-3}–10^{-2} sec. The experiments described here indicate that these fluctuations destroy the interaction of water with poly A and poly(L-glutamic acid). The interaction of water with polymers is, in those polymers where it exists, remarkably stable as long as the topology of the polymer is stable. In the case of poly(L-lysine) the effect is not noticed because there is no interaction with the charged form and, hence, no minima can be observed.

Conclusions

These experiments provide evidence for some phenomenological rules concerning the interaction of water with polymers on a microscopic scale. These are presented in the Discussion. Contrary to expectation no evidence is found for the interaction of water with imide or carbonyl functions. No " hydrophobic" interactions are found even where expected. For other groups which can hydrogen bond to water the governing principle seems to be that such binding is very sensitive to counter-

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ion shielding. The stability of the water–polymer interactions observed indicates that they must have an important mediating effect upon the structural possibilities in polymer conformation. It is clear that more experimental work remains to be done in this area.

Acknowledgments. The author is indebted to Professor M. Printz of Rockefeller University for helpful discussions. He also wishes to thank Professors I. M. Klotz and A. Krasna for generous gifts of materials. Some of the measurements were made with the technical assistance of Mrs. J. M. Glasel. This work was supported by Grants No OSW-14-01-0001-1700 and GB-8468 from the U. S. Department of the Interior, Office of Saline Water, and the National Science Foundation, Molecular Biology Division, respectively.

Conformational Studies on Poly-L-cyclohexylalanine and on Poly-L-phenylalanine in Water–Strong Acid Mixtures[1]

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Abstract: The conformation of poly-L-cyclohexylalanine (PCHA) and poly-L-phenylalanine (PLP) in water–methanesulfonic acid mixtures has been studied by circular dichroism (CD) in the far-ultraviolet region. It was found that PCHA assumes the right-handed α-helical conformation in solvent mixtures containing less than 86% acid. This result is in favor of the hypothesis, based on previous investigations, that PLP might also assume the α-helical form. The ordered forms of PCHA and PLP exhibit different conformational stabilities. This result is tentatively explained in terms of different contributions from noncovalent bonds of the side chains.

It is well known that poly-L-phenylalanine (PLP) in solution exhibits some peculiar optical rotatory properties. The CD spectrum of this polymer is different from all known spectra of polypeptides either in the ordered form or in the coiled form.[2–5]

The interpretation of the CD spectrum in terms of conformation is not unequivocal, since possible contributions from the aromatic chromophores can overlap those from the peptide chromophore with consequent complication of the CD pattern.

In a previous investigation carried out on copolymers of L-phenylalanine (Phe) and ϵ-carbobenzoxy-L-lysine (Z-Lys) in tetrahydrofuran (THF) solution, it was found that the progressive introduction of phenylalanine (Phe) residues in the peptide chain perturbs the CD pattern of polycarbobenzoxy-L-lysine, but apparently does not alter the helical sense.[6] No transition appeared to be involved on varying the copolymer composition. On this basis it was tentatively concluded that PLP in THF solution has the same helical conformation as poly-ϵ-carbobenzoxy-L-lysine (PCBL), that is, the right-handed α helix, the actual CD spectrum arising from overlapping of contributions to the optical activity both from the side-chain chromophores and from the peptide chromophores.

Support for this conclusion might be obtained from conformational studies in solution of poly-L-cyclohexylalanine (PCHA) (polyhexahydro-L-phenylalanine). Cyclohexane side chains of PCHA do not absorb in the region of the peptide transitions and the interpretation of the CD spectrum of the polymer in terms of conformation is unequivocal. If it is found that PCHA in solution, in spite of the enhanced bulkness of the side chains (chair or boat conformations of the nonplanar cyclohexane ring are possible), is in the α-helical form, it will be reasonable to assign the same conformation to PLP.

Optical rotatory properties of PCHA in the far-uv have not yet been reported, mainly because the polymer is not soluble in organic solvent transparent in this spectral region.

Very recently it has been found that concentrated strong acids like H_2SO_4 and CH_3SO_3H are good and transparent solvents for polypeptides.[7] In most cases the polymer solutions are stable enough to permit measurements to be made (polymer degradation occurs only after several hours). In these solvents polypeptides are usually in the form of random coils. Addition of water to the acidic solutions causes sharp coil-to-helix transitions in the case of poly-γ-ethyl-L-glutamate[7] and poly-L-glutamic acid.[8]

The present work describes CD and uv properties of PCHA and PLP in CH_3SO_3H–H_2O mixtures. In addition a block copolymer, [γ-benzyl-DL-glutamate]$_{200}$-[L-cyclohexylalanine]$_{35}$, has been prepared, and the optical rotatory properties of the L-cyclohexylalanine sequence in organic solvents are also reported.

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