route to its measurement. Because of the additive nature of the potential energy, the distribution function of a chain is factorable into a product of bond length distributions, bond angle distributions, and torsional angle distributions. Each is proportional to $\exp(-v/k_BT)$ with the appropriate v. Closing the chain into a ring actually ruins this factorizability, but the effect is small for a large ring, and will be ignored. Let there be given a set of observed values of a stochastic variable, x, which is distributed according to a probability density $f(x,T)$, with T a parameter. An estimate of T can be obtained by maximum likelihood estimation[22] (which is better than a moment technique). In this way a temperature for the bond length and bond angle distributions was determined. After short initial aging the estimate is quite stable. As an example, consider the simulation for potential A at a temperature 330.4 K, imposed by the strength of the random force according to eq I.6. Over 512 550 time steps of 5×10^{-6} ns, the temperature of the bond lengths is found to be 323.0 K and that of the bond angles 329.4 K. As expected, the result for the bond lengths is the worse because the ratio of the time step to the shortest vibration time is greatest. The temperature for the torsion angles was not determined, but it should be very good because these are the slowest modes.

References and Notes

(1) K. Matsuo, K. F. Kuhlmann, H. W.-H. Yang, F. Gëny, W. H. Stockmayer, and A. A. Jones, *J. Polym. Sci., Polym. Phys. Ed.* **15**, 1347 (1977), and references cited therein.
(2) T.-P. Liao, Y. Okamoto, and H. Morawetz, *Macromolecules*, **12**, 535 (1979).
(3) M. A. Cochran, L. H. Dunbar, A. M. North, and R. A. Pethrick, *J. Chem. Soc., Faraday Trans. 2*, **70**, 215 (1974).
(4) T. F. Schatzki, *J. Polym. Sci.*, **57**, 496 (1962); *Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem.*, **6**, 646 (1965).
(5) L. Monnerie and F. Gëny, *J. Chim. Phys. Phys.-Chim. Biol.*, **66**, 1691 (1969).
(6) J. C. Keck, *Discuss. Faraday Soc.*, **33**, 173 (1962).
(7) C. Bennett, *ACS Symp. Ser.*, **No. 46** (1977).
(8) J. Skolnick and E. Helfand, *J. Chem. Phys.*, to be published.
(9) J. H. Weiner and M. R. Pear, *Macromolecules*, **10**, 317 (1977).
(10) E. Helfand, *J. Chem. Phys.*, **69**, 1010 (1978). Equation 3.2 of that reference is corrected by eq III.4 of the present paper.
(11) M. Fixman, *J. Chem. Phys.*, **69**, 1527 (1978).
(12) M. R. Pear and J. H. Weiner, *J. Chem. Phys.*, **71**, 212 (1979).
(13) (a) R. M. Levy, M. Karplus, and J. A. McCammon, *Chem. Phys. Lett.*, to be published; (b) G. T. Evans and D. C. Knauss, to be published.
(14) D. Chandler, Report of the Workshop on Stochastic Molecular Dynamics, July 1978, National Resource for Computations in Chemistry, Lawrence Berkeley Laboratory, Berkeley, CA.
(15) J. P. Ryckaert and A. Bellemans, *Chem. Phys. Lett.*, **30**, 123 (1975); *Faraday Discuss.*, to be published.
(16) T. A. Weber, *J. Chem. Phys.*, **69**, 2347 (1978); **70**, 4277 (1979).
(17) T. A. Weber and E. Helfand, *J. Chem. Phys.*, **71**, 4760 (1979).
(18) E. Helfand, Z. R. Wasserman, and T. A. Weber, *J. Chem. Phys.*, **70**, 2016 (1979).
(19) R. Zbinden, "Infrared Spectroscopy of High Polymers", Academic Press, New York, 1964.
(20) M. Fixman, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3050 (1974); E. Helfand, *J. Chem. Phys.*, **71**, 5000 (1979), and references cited therein.
(21) E. Helfand, *Bell Syst. Tech. J.*, **58**, 2289 (1979).
(22) N. R. Mann, R. E. Schafer, and N. D. Singpurwalla, "Methods for Statistical Analysis of Reliability and Life Data", Wiley, New York, 1974.
(23) H. A. Kramers, *Physica (Utrecht)*, **7**, 284 (1940).
(24) E. Helfand, *J. Chem. Phys.*, **54**, 4651 (1971).

Conformational Properties of Poly(L-proline) and Poly(γ-hydroxy-L-proline). 2. Salt-Induced Isomerization‡

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ABSTRACT: The quantitative determination of the extent of imide bond isomerization in poly(L-proline) and poly(γ-hydroxy-L-proline) in aqueous salt solutions is reported. The results are quite different for the two polymers, and this major conformational change does not correlate with the measured intrinsic viscosity as a function of salt concentration. A generalized salt binding mechanism is proposed which allows for the repopulation of the conformational energy state in the vicinity of $\Psi = -50°$. A major difference between the two chains is caused by the intramolecular hydroxyl group interaction which makes cis isomerization energetically unfavorable and also retards the population of the other trans state. When these factors are taken into account, very good agreement is obtained between the intrinsic viscosity and the total salt induced conformational change. These results also allow for a general understanding of salt effects in polypeptides which do not undergo isomerization about the amide group.

In the previous paper of this series, we have shown that in pure solvents the all-trans poly(L-proline), poly(Pro), and poly(γ-hydroxy-L-proline), poly(Hyp), chains possess conformational energy minima in the vicinity of $\Psi = 160°$ as well as at $\Psi = -50°$.[1] Both of these conformational energy states need to be populated to explain the characteristic ratios of the polymers in the different solvents. In addition it was found that, while a small fraction of the residues of both polymers are in the cis configuration in D_2O,[2,3] this configuration is absent for poly(Pro) in trifluoroethanol.[1]

Certain classes of salts, in aqueous solutions of polypeptides and polyimides, cause major changes in optical properties, such as the optical rotatory dispersion and circular dichroism, and in the chain conformation, as is reflected in hydrodynamic properties.[4,5] Although these salts affect all classes of polypeptides, polyimides, and the globular and fibrous proteins in this manner, the mechanism of their action has not as yet been universally demonstrated. However, Torchia and Bovey[6] and Dorman et al.[7] have demonstrated the presence of cis imide bonds in concentrated, aqueous salt solutions of poly(Pro) by both 220 MHz ^1H NMR and 15 and 25 MHz ^{13}C NMR. The detailed functional relation between the salt concentration, or activity, and the extent of isomerization has not, how-

‡ It is our pleasure and privilege to dedicate this paper to Professor Flory on the occasion of his 70th birthday celebration. His scientific accomplishments and incisive contributions to all aspects of polymer science need not be amplified by us. However, we take the opportunity on this occasion to wish this gentleman and scholar many more years of pioneering and exciting contributions to the science which he has pioneered and loves so dearly.

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ever, been reported. In contrast to the results for poly(Pro), it was originally reported from ^{13}C NMR studies that poly(Hyp) does not undergo trans → cis isomerization in 6 M LiBr.[8] The strong implication was made that the behavior of this polyimide was different from that of poly(Pro). We have, however, recently reported the ^{13}C NMR spectra of poly(Hyp) in 8 M LiBr.[1] Here it can be clearly demonstrated that isomerization does indeed take place. The previous conclusion with respect to poly(Hyp) was not correct and can probably be attributed to the very low signal-to-noise ratio which probably obscured the cis resonances.

In the present paper, we report the quantitative results of a systematic study of the dependence of the extent of isomerization of poly(Pro) and poly(Hyp) on salt concentration. The conformational changes that occur, as expressed by the intrinsic viscosity, are reported and compared for the two polymers for a given state of isomerization. From these results a binding model can be postulated which allows for a quantitative explanation of the present results and which can be extended to other polypeptides which show changes in other properties but for which isomerization has not been established.

Experimental Section

The poly(Pro) and poly(Hyp) samples used in this work were the same as those studied previously[1] and were purified in a similar manner. The salts used in this work—$CaCl_2$, LiCl, LiBr, and $LiClO_4$—were of reagent grade and used without any further purification. Solutions were prepared from salt which had been dried several hours under vacuum. The concentrations of the chloride salt solutions were checked by titration with silver nitrate. The concentration determined in this way was found to agree within 5% with that determined from the weight of the added dried salt.

The mean activities, $a_\pm$, were calculated as $\gamma_\pm m$ for 1–1 salts and as $2^{2/3}\gamma_\pm m$ for 1–2 salts, where $\gamma_\pm$ is the mean activity coefficient and m is the molality. The salt molality was calculated from the molar concentration and the tabulated densities at 20 °C.[9] The mean activity coefficients for $CaCl_2$ at 25 °C were taken from Lewis and Randall.[10] For the other salts below 6m the $\gamma_\pm$ at 25 °C were taken from Robinson and Stokes.[11] Above 6m the data of Robinson and Stokes[11] were extrapolated by using the following relationship:

$$\log \gamma_\pm = -0.5115(m^{1/2}/(1 + m^{1/2})) + Bm + Cm^2$$

The constants B and C were graphically fit to the tabulated data between 3 and 6m. The equation above represents the experimental data in this region to better than 0.5%. The largest extrapolation of $\gamma_\pm$, and hence the greatest error in $a_\pm$, is with the higher concentrations of LiCl and LiBr, i.e., greater than about 8 M.

The flow times for the viscosity measurements were obtained by the methods previously described[1] by using Cannon–Ubbelohde semi-microdilution viscometers. The ^{13}C NMR spectra were obtained by using a Bruker HX-270 spectrometer with quadrature detection operating at 67.905 MHz. Field/frequency stabilization was provided by a deuterium lock. A 90° pulse width of approximately 54 μs was used. The procedures and precautions that we have previously described[1] were used to minimize temperature gradients along the sample tube when a high concentration of salt was present.

Results and Discussion

The salts chosen for this study were $CaCl_2$, $LiClO_4$, LiBr, and LiCl. They were selected because of their strong disruptive ability on the ordered structure of polypeptides and proteins and their influence on the optical properties of such molecules even when in the disordered state.[12-20,29]

The ^1H and ^{13}C spectra of poly(Pro) in these and similar aqueous salt solutions have already been published,[6,7] and hence there is no need to repeat them here. In Figure 1, however, we illustrate some typical ^{13}C spectra of poly(Hyp) in several aqueous salt solutions. The cis resonances, for the β and γ carbons assigned from previous work,[3,6,7] are clearly discernible in all of the spectra. These results give further confirmation that isomerization occurs in poly(Hyp) under these conditions and further refutes the previous generalization.[8] The resonant lines in Figure 1a for 5 M $LiClO_4$ are relatively narrow. There is a slight broadening of the spectrum in Figure 1b with an unassigned weak line appearing upfield from the C_γ resonance. A similar situation has been reported previously.[3] The resonances are observed to be broader at higher $CaCl_2$ concentrations, as is seen in Figure 1c. This broadening is similar to that which has been found for the proton resonance of poly(Pro) under similar conditions.[7] Elastic and quasielastic light-scattering experiments[21] on aqueous solutions of poly(Pro) suggest that the salt additives influence both the conformational characteristics of the polymer and its state of aggregation. Salts, such as guanidinium·HCl at 4 M $NaClO_4$, induce aggregate dissociation; however, 4 M $CaCl_2$ results in enhanced aggregation. Since the carbon resonances of poly(Pro) or poly(Hyp) do not broaden even at high salt concentrations of $LiClO_4$, the results imply that the resonance broadening

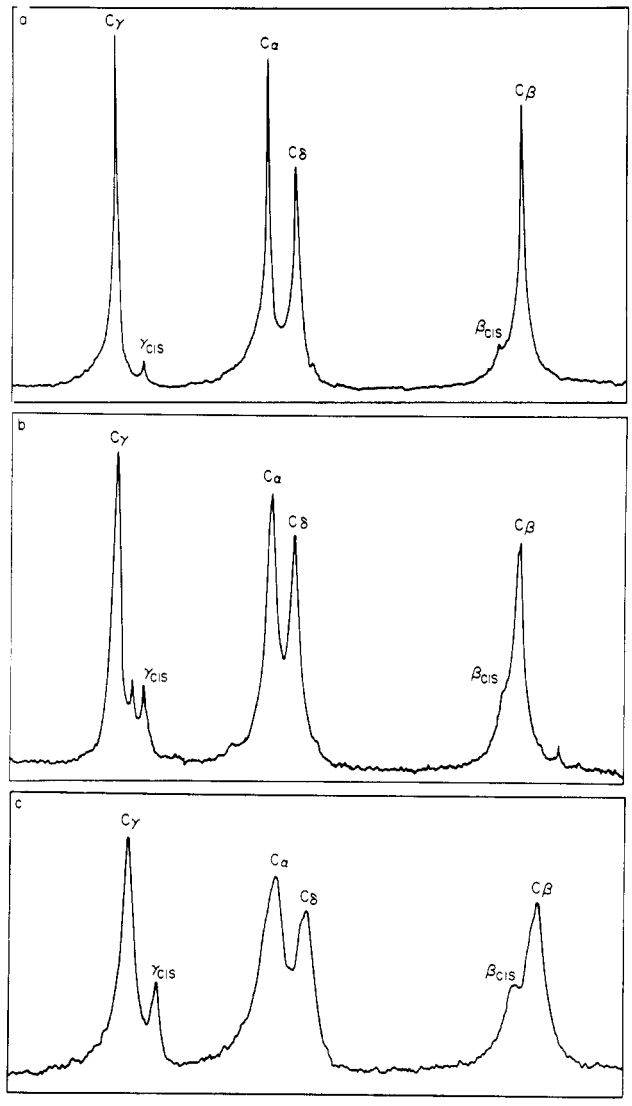


Figure 1. Partial carbon-13 spectra at 67.9 MHz of poly(γ-hydroxy-L-proline) for indicated salts in D_2O solutions. The spectra were obtained at ambient temperature, and each consisted of 20 000 scans with a delay of 1.7 s between 90° pulses. The cis resonance assignments are indicated: (a) in 5 M $LiClO_4$; (b) in 12 M LiBr; (c) in 5.5 M $CaCl_2$.

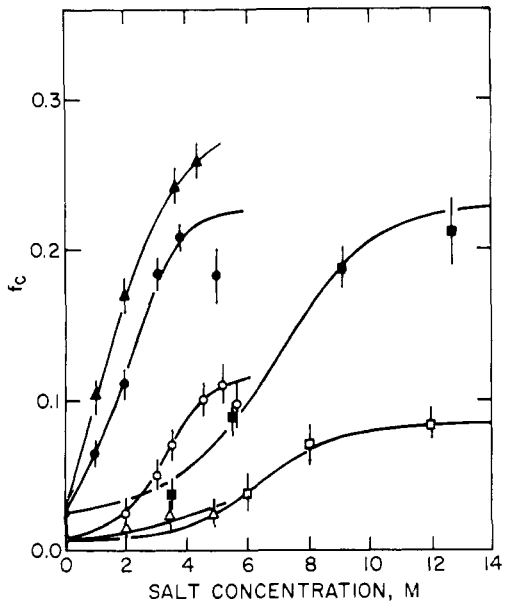


Figure 2. Plot of the fraction of cis residues, f_c, as determined from the ^{13}C NMR spectra as a function of salt concentration in molarity M, poly(Pro) filled symbols and poly(Hyp) open symbols: $CaCl_2$ (●, ○); $LiClO_4$ (▲, △); LiCl (■); LiBr (□).

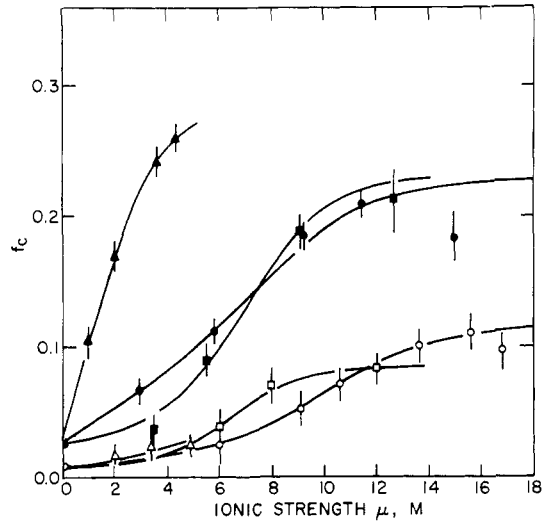


Figure 3. Plot of the fraction of cis residues, f_c, against ionic strength μ in molarity. The symbols have the same meanings as those described for Figure 2. The data are the same as those given for Figure 2.

is due to polymer association.

The fraction of cis residues was directly calculated from the areas of the cis and trans resonances of the γ_{cis} peaks.[3] The nuclear Overhauser enhancement for the cis and trans resonances has been shown to be the same for poly(Pro) in D_2O.[3] We assume that this condition also holds for poly(Pro) and poly(Hyp) in the aqueous salt solutions.

Figure 2 is a plot of the fraction of cis residues, f_c, as a function of salt concentration (in molarity, M), while in Figure 3 the same data are plotted against ionic strength. The filled symbols are for poly(Pro), and the open ones are for poly(Hyp) for the indicated salts. The experimental error, represented by the bars in these graphs, is about twice as great (~20%) at the higher concentrations of $CaCl_2$ and LiCl than at the lower salt concentrations. It cannot be established, therefore, whether the apparent very small decrease in f_c in both polymers at near saturating concentrations of $CaCl_2$ is real or whether it is due to the associating conditions.

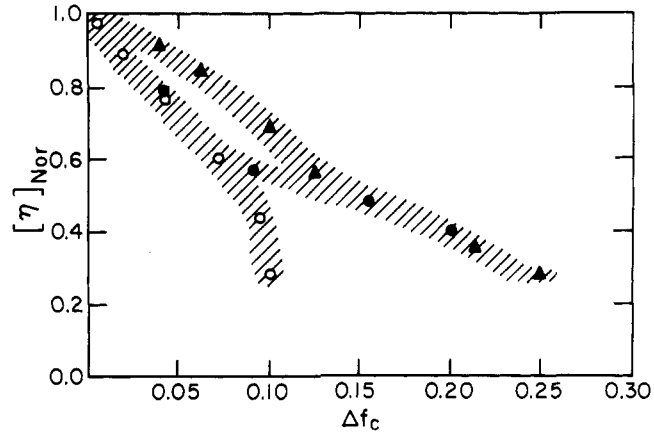


Figure 4. Variation of normalized intrinsic viscosity, $[\eta]_{nor}$, against fractional change in the cis content. Poly(Pro) and poly(Hyp) in $CaCl_2$ and poly(Pro) in $LiClO_4$. The symbols have the same meanings as those described for Figure 2. Data in $CaCl_2$ are taken from ref 16.

With the apparent exception of poly(Pro) in $LiClO_4$, the fraction of cis residues reaches a limiting value at the high salt concentrations. This limiting fraction is approximately twice as great for poly(Pro) as for poly(Hyp). For poly(Pro) the fraction of cis residues approaches the same level at high concentrations of $CaCl_2$ and LiCl but is greater in $LiClO_4$. It appears to be dependent on the anion present. This anion effect is just the opposite for poly(Hyp). Here, in contrast, $CaCl_2$ has the greater effect and $LiClO_4$ the least. These differences will be discussed in more detail subsequently. The character of the curves in Figure 3 makes it evident that the mechanism for the salt-induced isomerization cannot be attributed simply to a solvent or to an electrostatic effect. The set of curves for each polymer would then be superimposeable, or very similar, if this were the case. Although the curves for poly(Pro) in $CaCl_2$ and LiCl are similar, the curve for poly(Pro) in $LiClO_4$ is quite different.

The intrinsic viscosity can serve as a measure of the conformational change caused by the added salt. If upon the introduction of salt the formation of the cis isomer were the only structural change which occurred in the predominantly trans polyimide chain, then there should be a correlation between the intrinsic viscosity and the fraction of cis residues for both polymers with the different salts. Following this suggestion, the normalized intrinsic viscosities, $[\eta]_{nor} \equiv [\eta]_{salt}/[\eta]_{H_2O}$, are plotted in Figure 4 against the fraction of cis change, $\Delta f_c = f_c^{salt} - f_c^{H_2O}$, for poly(Hyp) and poly(Pro) in $CaCl_2$ and poly(Pro) in $LiClO_4$. All the polymer samples had approximately the same molecular weight. The hashed marks represent the error in the determination of the cis fraction. Even within this experimental error, the curves for poly(Pro) deviate from the curve for poly(Hyp) for Δf_c greater than 0.1. Although the fraction of cis residues for poly(Hyp) levels off at the higher salt concentrations (Figure 3), its intrinsic viscosity, in contrast to poly(Pro), continues to decrease. For Δf_c less than 0.1, $[\eta]$ is lower for poly(Pro) and poly(Hyp) in $CaCl_2$ than it is for poly(Pro) in $LiClO_4$, despite the fact that a higher fraction of cis residue is attained for the latter case. Thus the surmise that there is a direct correlation between the fraction of cis residues and the intrinsic viscosity is not substantiated. Other factors must be involved. The possibility exists that another conformational state, besides the cis conformer, is influenced by the salt. As we have shown previously,[1] there is a conformational state in the vicinity of $\Psi = -50°$ which can reduce the dimensions for a predominantly all-trans imide bond chain. Hence

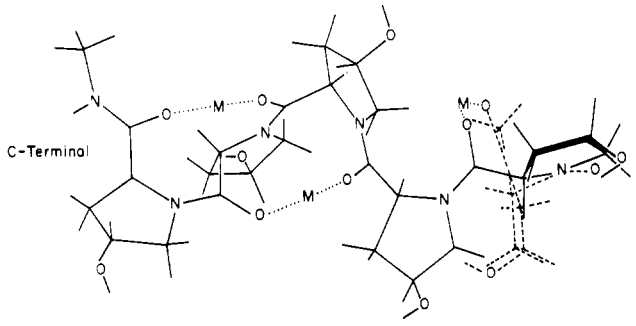


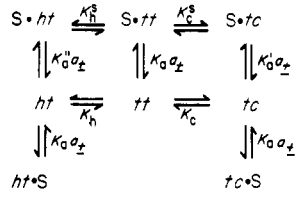
Figure 5. Section of the poly(Hyp) chain with the sequence *tcthtct* showing three metal cation complexes. The metal cations, M, are shown complexed between the first, from the *N*-terminal end, and third; the fourth and sixth; and the fifth and seventh carbonyl oxygen atoms representing the complexed sequences *tct*, *htc*, and *tct*.

it becomes important to ascertain whether the population of this state is affected by the addition of salt and, if so, whether it can resolve the anomalies presented by Figure 4.

It has been suggested that there may be direct salt binding to the amide group of peptides[22-27] and polyacrylamide gels.[28] Preferential interactions between the salt and polymer are necessary consequences of the laws of thermodynamics to explain the cooperative conformational transitions that are widely observed.[22,29] We try now to develop a more specific model. The carbonyl oxygen atoms in amides are known to interact with cations in the complexes that are formed between them and certain salts.[30-32] These results make it desirable to investigate a model that takes into account salt binding in the analysis of the conformational changes of poly(Pro) and poly(Hyp). Certain assumptions must by necessity be made. The most important ones that are made here are as follows. (i) The imide group is taken to be the site of salt binding. For simplicity and ease of understanding we consider first cation binding to the carbonyl oxygen atom. The role played by the anion will be considered later. (ii) Each of the individual binding sites are independent of one another. (iii) There are three different association constants for the cation which depend on the configurational states of an internal dimer sequence. Two of these constants, K_a' and K_a'', are for multi-residue complexes and the third, K_a, is for single-residue binding. We let *t* denote the trans conformer with Ψ near 160°, *h* the trans conformer with Ψ near −50°, and *c* the cis imide conformer. K_a' is the association constant for the trans imide group in the internal sequences *tc* or *ct*. K_a'' is the association constant for the trans imide group of *h* in the sequences *ht* or *th*. K_a is the association constant for the trans imide group of *t* in the sequences *tt*, *th*, or *ht*, or for a cis imide group in any dimer sequence.[33] (iv) The theoretical limiting salt-induced conformational change is a polypeptide chain with 50% *t* residues. The remaining 50% of the *X* residues will be comprised of *h* and *c* residues. At the theoretical limit of maximum bound salt, the polypeptide chain will have the sequence *txtxtx*... in the absence of other specific intramolecular interactions.

These last two assumptions are based on the suggestion by Torchia and Bovey[6] and on examination of CPK spacefilling models. It was proposed that a multi-residue cation complex could be formed with sequences of the type *tct* or *tcc*. In such sequences the cation can bind between the first and third residue carbonyl oxygen atoms. By examining CPK models it also becomes evident that in addition a similar complex could be formed by the sequences *htt* or *htc*. Examples of three cationic complexes, *tct*, *htc*, and again *tct*, are shown in Figure 5 which represents a section of the poly(Hyp) chain with the sequence *tcthtct*. Poly(Pro) can also form similar complexes. Every carbonyl oxygen atom in the figure is in an appropriate position to pair with another oxygen atom two residues removed. The only exception to this is the second carbonyl oxygen atom from the *N*-terminal end, which belongs to a *c* residue and which could then pair with a preceding *h* residue, if it existed. It is easily seen from the figure how the sequence *txtxtx* maximizes the number of such multi-residue complexes and becomes the theoretical limiting sequence at maximum bound salt, corresponding to assumption iv.

Scheme I



With these assumptions the salt binding equilibrium can be represented in its most general form by Scheme I, where the S·*xy* dimer unit represents either the sequence S·*xy* or *yx*·S with the salt, S, bound to the imide group of residue *x*. K_c, K_h, K_c^s, and K_h^s are the equilibrium constants for a *t* residue converting to either a *c* residue or an *h* residue in the absence or presence of bound salt, respectively, while $a_\pm$ represents the mean salt activity. In this scheme it follows that:

$$K_c K_a' = K_c^s K_a \tag{1}$$

$$K_h K_a'' = K_h^s K_a \tag{2}$$

We define R_c as the ratio of the total concentration of cis peptide bonds to the total concentration of all-trans peptide bonds (ca. both *h* and *t* residues). This ratio can be expressed as:

$$R_c = \frac{[tc] + [S{\cdot}ct] + [S{\cdot}tc]}{[tt] + [S{\cdot}tt] + [ht] + [S{\cdot}th] + [S{\cdot}ht]} \tag{3}$$

The square brackets denote species concentration. This equation can be represented in terms of the equilibrium constants K_c, K_h, K_c^s, K_h^s, and K_a as

$$R_c = \frac{K_c + (K_c + K_c^s)K_a' a_\pm}{1 + K_h + (1 + K_h + K_h^s)K_a a_\pm} \tag{4}$$

Equation 4 could have been written in terms of K_a' and K_a'', using eq 1 and 2. However, it will be more convenient in its present form. The fraction of cis residues, f_c, can be expressed in terms of R_c by

$$f_c = \frac{R_c}{1 + R_c} \tag{5}$$

From assumption iv we have

$$[c] + [h] = [t] \tag{6a}$$

which leads because of our model to

$$[S{\cdot}ct] + [S{\cdot}tc] + [S{\cdot}th] + [S{\cdot}ht] = [S{\cdot}tt] \tag{6b}$$

Since there is a common *t* in each of the terms above, the term [S·*tt*] is required to satisfy assumption iv.

In terms of the equilibrium constants, eq 6 becomes

$$(K_c + K_c^s) + (K_h + K_h^s) = 1 \tag{7a}$$

or

$$K_h^s = 1 - K_h - K_c - K_c^s \tag{7b}$$

Table I
Best Fit Parameters for Salt Binding

polymer	salt	K_c	K_h	K_c^s	K_h^s	K_a	ΔG^{OH} [a]	f_c^{lim}	f_h^{lim}	K_a (exptl)
Pro	$CaCl_2$	0.026	0.031	0.43	0.51	0.16		0.23	0.27	0.054,[b] 0.10,[c] 0.22[d]
Pro	LiCl	0.026	0.031	0.43	0.51	0.12		0.23	0.27	0.013,[b] 0.048[c]
Pro	$LiClO_4$	0.026	0.031	0.55	0.39	0.23		0.29	0.21	0.078[b]
Hyp	$CaCl_2$	0.0079	0.017	0.18	0.32	0.047	0.28	0.12	0.22	0.054,[b] 0.10,[c] 0.22[d]
Hyp	LiBr	0.0079	0.017	0.11	0.22	0.012	0.44	0.085	0.17	0.039,[b] 0.41[d]
Hyp	$LiClO_4$	0.0079	0.017	0.038	0.10	0.068	0.75	0.039	0.18	0.078[b]

[a] In kcal/mol at 25 °C. [b] Salt binding to polyacrylamide gels, ref 28. [c] Salt binding to collagen, ref 22. [d] Salt binding to acetyltetraglycine ethyl ester, ref 23.

For each polymer (ca. poly(Pro) or poly(Hyp)), K_c and K_h are independent of the salt studied, since in the absence of salt eq 4 reduces to

$$(R_c) = K_c/(1 + K_h) \tag{8}$$

An additional equation is still needed in order to uniquely describe Figures 2 and 3, i.e., the dependence of the fraction of cis residues on salt concentration.[34] We thus make the apparently arbitrary assumption that

$$K_h = K_c(K_h^s/K_c^s)_{Cl^-} \tag{9}$$

The subscript Cl^- denotes that the poly(Pro) system was first analyzed for $CaCl_2$ and then for LiCl. As has been indicated, the fraction of cis residues at high salt concentration is dependent on the relative binding strength of the anion and is independent of the cation. Since the chloride anion binds weakly to amides, it was assumed that the ratio K_h^s/K_c^s would more closely represent the ratio K_h/K_c for these salts. Substituting the right-hand side of eq 7b for K_h^s in eq 9 and rearranging gives

$$K_h = \frac{K_c(1 - K_c - K_c^s)}{K_c + K_c^s} \tag{10}$$

Substituting eq 7b and eq 10 for K_h^s and K_h, respectively, in eq 4 yields

$$R_c = \frac{K_c(K_c + K_c^s) + (K_c + K_c^s)^2 K_a a_\pm}{(1 - K_c)(K_c + K_c^s) + K_c + (K_c + K_c^s)(2 - K_c - K_c^s)K_a a_\pm} \tag{11}$$

This equation, which involves the one necessary arbitrary mathematical assumption, expresses the influence of the general binding mechanism on the bond orientations. Three parameters are involved, K_c, K_a, and K_c^s.

We first analyze the results for poly(Pro). Equation 11 was used to obtain a non-linear least-squares fit of the experimental points for poly(Pro) in $CaCl_2$ and LiCl. All three parameters were used for this analysis which yielded the same values for K_c and K_c^s for both salts. The value of K_c, thus obtained, was then used to analyze the poly(Pro)–$LiClO_4$ data. Thus only the parameters K_c^s and K_a were needed to be fit for this latter system. The curves drawn through the experimental points in Figures 2 and 3 for poly(Pro) are those calculated by this method, using the best fit parameters which are given in Table I, columns 3-7. These curves clearly represent the experimental points quite well. The association constants, K_a, column 7, for poly(Pro) determined from these salt-induced isomerization experiments can be compared with the association constants determined from other works, as is indicated in the last column of the table. In general, the association constants can be considered to be in relatively good agreement; the deductions from the isomerization experiments for $CaCl_2$ and LiCl are in the same range as the results for other similar systems and different methods.

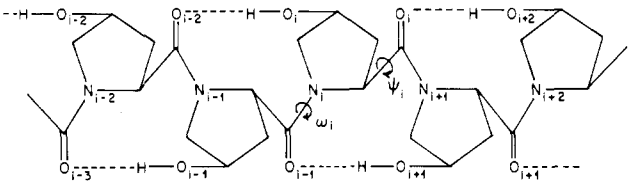


Figure 6. Diagrammatic representation of a section of the poly(Hyp) chain illustrating the dependence of the hydroxyl proton–carbonyl oxygen interaction on the ω and Ψ rotational angles. Rotation about Ψ_i disrupts the interaction between the $i + 1$ hydroxyl proton and the $i - 1$ carbonyl oxygen. Rotation about ω_i disrupts both this interaction and the one between the i hydroxyl proton and the $i - 2$ carbonyl oxygen.

Since only one other binding study has been reported for $LiClO_4$,[28] the agreement with this salt is not quite as good.

The ninth and tenth columns of Table I list the theoretical limiting fraction, f_c^{lim} and f_h^{lim}, of the cis and h residues, respectively. In the theoretical limit of maximum bound salt, eq 4 reduces to

$$R_c^{lim} = \frac{K_c + K_c^s}{1 + K_h + K_h^s} \tag{12}$$

A similar equation can be derived for R_h^{lim}. Because of assumption iv, the sum of f_c^{lim} and f_h^{lim} must be 0.5. This condition is satisfied for poly(Pro). It can also be seen from the table that f_c^{lim}, and hence f_h^{lim}, is dependent on the anion and not on the cation present in solution. The value of f_c^{lim} determined for poly(Pro) is the same for $CaCl_2$ as it is for LiCl, but it is slightly higher for $LiClO_4$.

In the analysis of the poly(Hyp) data, attention must also be given to the possibility of an additional interaction involving the hydroxyl group. The thermal stability of collagen[35,36] and collagen-like copolypeptides[38,39] has long been known to increase when Hyp is substituted for Pro. The hydroxyl group has been implicated as the source of the added stability. Its exact role has not been clearly demonstrated. It has been suggested that the hydroxyl group forms an intermolecular hydrogen bond with a carbonyl group from a neighboring chain.[40,41] This interaction cannot play a role in stabilizing poly(Hyp) in solution.[42] An intramolecular hydrogen bond involving the hydroxyl group has been suggested and was first implicated in the structure for the sequential copolymer poly(Hyp-Gly).[43] This intramolecular interaction was later suggested to be present in poly(Hyp)[44] and was found to be energetically favorable for trans peptide bonds with Ψ near 110°.[45]

Figure 6 schematically illustrates this particular hydroxyl interaction for poly(Hyp). A study of the figure indicates that when Ψ is rotated from 110° to near −50°, one such hydroxyl interaction is broken. However, when ω is rotated from 180 to 0°, i.e., when trans–cis isomerization occurs, two interactions are broken. Therefore, with the acceptance of this interaction, a rotation about Ψ will be about twice as favorable as a rotation about ω. To take this factor

into account, we define ΔG^{OH} as the difference between the Gibbs free energy, $G_B{}^{OH}$, of the hydroxyl group in the bound, intramolecular-interacting state and $G_F{}^{OH}$, the free, nonintramolecular-interacting state. Thus,

$$\Delta G^{OH} = G_B{}^{OH} - G_F{}^{OH}$$

The relationships between $K_c{}^s$ and $K_h{}^s$ for poly(Hyp) and poly(Pro), for a particular salt, are then given by

$$(K_h{}^s)_{Hyp} = (K_h{}^s)_{Pro} \exp(-\Delta G^{OH}/RT) = (K_h{}^s)_{Pro}(F^{OH}) \quad (13)$$

and

$$(K_c{}^s)_{Hyp} = (K_c{}^s)_{Pro} \exp(-2\Delta G^{OH}/RT) = (K_c{}^s)_{Pro}(F^{OH})^2 \quad (14)$$

Similar relationships for K_c and K_h for poly(Hyp) can be written as

$$(K_c)_{Hyp} = (K_c)_{Pro}(f_c{}^{Hyp}/f_c{}^{Pro})_{H_2O} \quad (15)$$

and

$$(K_h)_{Hyp} = (K_h)_{Pro}(f_c{}^{Hyp}/f_c{}^{Pro})^{1/2}_{H_2O} \quad (16)$$

In the initial analysis of the poly(Hyp) data, it was found that the empirical relation

$$(K_a)_{Hyp} = (K_a)_{Pro}(f_c{}^{Hyp}/f_c{}^{Pro})_{H_2O} \quad (17)$$

was applicable. This expression was used throughout the subsequent numerical analysis of the poly(Hyp) data. We are then left with only one unknown in eq 4, namely (F^{OH}). The data were again fit by a least-squares procedure.[46]

The curves drawn through the experimental points for poly(Hyp) in Figures 2 and 3 are calculated by using the parameters determined by the procedure described and listed in columns three–seven of Table I. The curves again represent the experimental points quite well. As was found for poly(Pro), $f_c{}^{lim}$ and $f_h{}^{lim}$ are dependent on the anion present (columns nine and ten). In contrast, however, $f_c{}^{lim}$ for poly(Hyp) is greater for the Cl^- anion than for the ClO_4^- anion. In column 8 of Table I, ΔG^{OH} is not a constant, which is a reflection of the fact that $f_c{}^{lim}$ is different for the various salts.

For poly(Hyp) the sum of $f_c{}^{lim}$ and $f_h{}^{lim}$ does not yield 0.5 as was found for poly(Pro). The reason is that the hydroxyl interaction stabilizes the t state relative to the c and h states and thus lowers the values of the equilibrium constants. In turn, lower values are found for $f_c{}^{lim}$ and $f_h{}^{lim}$ relative to poly(Pro). The value of $f_c{}^{lim}$ is severely reduced, and assumption iv is no longer applicable.

By infrared analysis Taylor and Kuntz have determined the equilibrium binding quotients, K_1, of 1:1 complexes of a number of anions which bind to phenol in organic solvents.[47] Although the absolute magnitudes of the equilibrium quotients are sensitive to the choice of solvent, the relative values are not. In Figure 7 there is a plot of ΔG^{OH} for poly(Hyp) as deduced here, against ln K_1, for the three salts studied. The correlation found is very good, particularly when consideration is given to the 25-fold change in the relative magnitudes of K_1 for the different salts. The ClO_4^- anion is bound weakest to the phenol proton since it has the lowest K_1 of the three anions and thus the highest ΔG^{OH}. On the other hand, the Cl^- anion has the highest degree of phenol association and the lowest ΔG^{OH}. These results can be explained by the anion's ability to compete for the hydroxyl proton. The less the interaction between the hydroxyl proton and the carbonyl oxygen, the lower is ΔG^{OH}. Thus, on this basis we would expect, as is observed, that the limiting fraction of cis residues for poly(Hyp) would be much lower in $LiClO_4$ than it is in

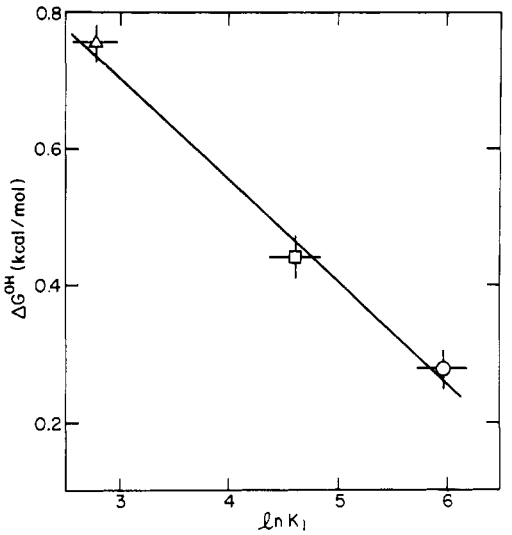


Figure 7. The dependence of the Gibbs free energy of the hydroxyl interaction, ΔG^{OH}, on the natural logarithm of the anion binding constant for a 1:1 complex to the phenol proton, K_1, for the perchlorate (△), bromide (□), and chloride (○) anions. K_1 values are taken from ref 47.

$CaCl_2$. For poly(Pro) we have seen from Figures 2 and 3 and Table I that the trend in $f_c{}^{lim}$ is just the reverse. The reason for the differences is that the major controlling factor which limits salt-induced isomerization in poly(Hyp) is the hydroxyl interaction. The stronger the interaction, the less the isomerization taking place. This interaction is modified by added reagents, but the limiting fraction of cis residues is still substantially less than that for poly(Pro). Since this interaction is not present with poly(Pro), the major anion influence is probably its ability to bind to the imide group by a charge-dipole mechanism. Poorly hydrated anions, such as ClO_4^-, interact more strongly with the imide because either they have a small hydration sphere or the interaction involves dehydration which would be easier with those which are poorly hydrated. Since part of the energy difference between a cis and a trans imide bond involves an electrostatic term, this energy difference will decrease with increasing charge neutralization. Hence, for poly(Pro) the fraction of cis residues should be greater at high salt concentration of $LiClO_4$ than in high concentrations of $CaCl_2$ or LiCl.

Since the interactions are different for the two polymers, the fraction of h as well as the fraction of cis residues should be affected by the added reagent. The ratio of the concentration of h residues to the concentration of c and t residues, R_h, is given by

$$R_h = \frac{K_h + (K_h + K_h{}^s)K_a a_\pm}{1 + K_c + (1 + K_c + K_c{}^s)K_a a_\pm} \quad (18)$$

in analogy to eq 4. The fraction of h residues, f_h, can be calculated from

$$f_h = \frac{R_h}{1 + R_h} \quad (19)$$

Using these equations and the parameters listed in Table I, we calculated theoretical curves of the f_h against salt concentration for the various salts studied; the results are given in Figure 8. The general appearance of these curves is similar to the corresponding curves for f_c. However, the limiting fraction of h for poly(Hyp), for a given salt, approximates more closely the limiting fraction of h for poly(Pro) as compared to the limiting fractions of cis residues for the two polymers. The reason for this is that,

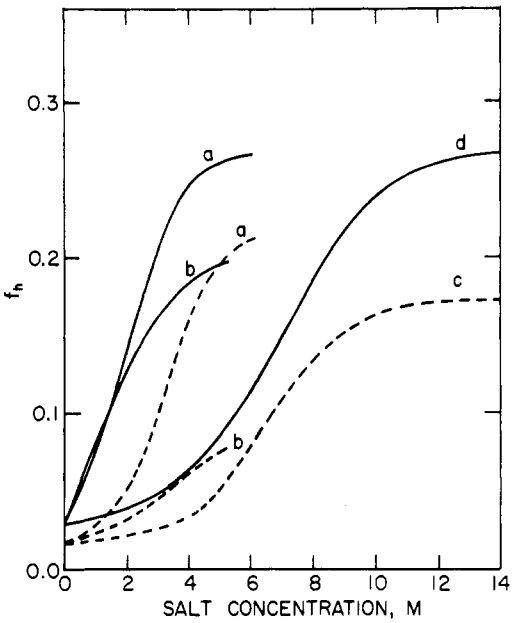


Figure 8. Theoretical curves showing the dependence of the fraction of *h* residues, f_h, on the salt concentration in molarity, M, for poly(Pro) (solid lines) and poly(Hyp) (dashed lines) in (a) $CaCl_2$, (b) $LiClO_4$, (c) LiBr, and (d) LiCl.

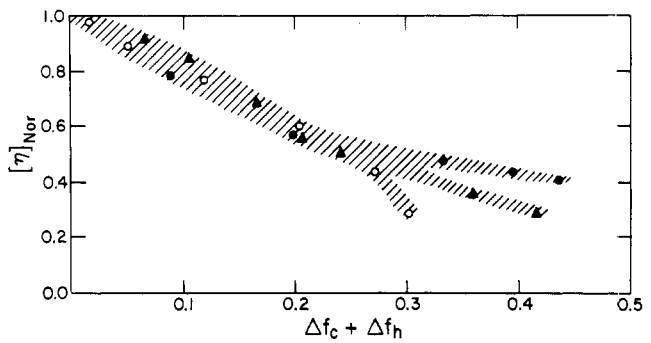


Figure 9. Plot of the normalized intrinsic viscosity, $[\eta]_{nor}$, against the total fractional conformational change, $\Delta f_c + \Delta f_h$, for poly(Pro) and poly(Hyp) in $CaCl_2$ and poly(Pro) in $LiClO_4$. The symbols have the same meanings as those described for Figure 4. The viscosity data are the same as those given for Figure 4.

in going from a *t* to an *h* state in poly(Hyp), one hydroxyl interaction is broken whereas two are broken in going from a *t* to a *c* state.

We can now reexamine the relationship, and the apparent anomaly that was previously observed, between the intrinsic viscosity and the conformational changes caused by added salt. Consideration has to be given to the fact that, not only is the fraction of cis residues increasing with added salt, but the concentration of the *h* state is enhanced as well. Both of these states favor more compact conformations and thus will influence the intrinsic viscosity in the same way. Consequently, in Figure 9 we have plotted the normalized, intrinsic viscosity against the sum of Δf_h and Δf_c for the same polymer–salt systems that were presented in Figure 4. The hashed lines again represent the experimental error in the determination of the fraction of cis residues. The results for the three systems are now close to one another, particularly when compared with the previous analysis (Figure 4) where Δf_c was the only conformational variable considered. Within experimental error, the three curves are superimposeable with one another and are linear for values of $\Delta f_h + \Delta f_c$ equal to or less than about 0.30. At higher values of $\Delta f_h + \Delta f_c$, i.e., at higher salt concentrations, the curves deviate slightly from one another. The model that we have used, which introduces naturally the importance of the population of *h* states, adequately explains the changes in the intrinsic viscosity and thus the molecular conformations for poly(Pro) and poly(Hyp). The apparent differences between the two polymers can also be reconciled in a consistent manner.

Although the salt binding scheme that we have postulated is formulated in a quite general way, several approximations had to be made to arrive at tractable expressions. Despite this shortcoming, not only can the present results be explained, but several apparently unrelated and unexplained observations can now be given a consistent interpretation. One such experiment is the early dilution experiment by Steinberg et al.[48] for poly(Pro) in solutions of 10 M LiBr and 5 M KSCN. They observed essentially an instantaneous optical rotatory change from $[\alpha]_D$ −250° to $[\alpha]_D$ −400°. This initial fast change was followed by a slower change leading to a final $[\alpha]_D$ −540°. The slow change yielded an enthalpy of activation of ΔH^* = 20.6 kcal/mol per Pro residue. We now know that this latter change must be due to cis-trans isomerization. Since new resonances, other than the cis resonances, do not appear in the NMR spectra of these polymers, even at the higher salt concentrations, we must assume that the *h* to *t* transition is fast on the NMR time scale. Madison[49] calculated an *h* to *t* energy barrier of about 5 kcal/mol for $AcProMe_2A$, while Venkatachalam et al.[50] calculated a barrier for this same transition of about 15 kcal/mol for an internal Pro dimer. Therefore the fast change observed by Steinberg et al.[48] is most probably due to such a change. If one makes an estimate of the fraction conformational changes, *f*, observed in these dilution studies by assuming that *f* is a linear function of $[\alpha]_D$,[51] then initially *f* = 0.49, and upon dilution it immediately decreases to 0.24. This calculation implies that f_c = 0.24 and f_h = 0.25 for high salt concentrations of LiBr or KSCN. These values are in excellent agreement with the values of f_c = 0.25 and f_h = 0.24[46] which we would predict for poly(Pro) in 10 M LiBr.

A very important generalization which describes salt-induced effects, other than merely electrostatic ones, for other polypeptides and proteins immediately follows from the present model and analysis. From these conclusions, the conformational changes induced by these salts for non-imino acid residues in polypeptides which do not undergo trans-cis isomerization would involve a repopulation of rotational states to ϕ, Ψ near −60°, −50°, for the *h* state, and to ϕ, Ψ near −60°, 160°, for the *t* state. These would be nearly equally populated at the high salt concentrations. For example, when the polypeptide in the absence of salt is a random coil, at high salt concentration it would develop an equal mixture of both *h* and *t* residues. It would still be a random coil but with a different population of states. The resultant chain would have the general conformational sequence of ...*hththt*.... Although the dihedral angles representing the *h* state are in the α-helical region, this sequence is not helical. The salt binding to the polypeptide would be such that a redistribution of *h* and *t* states takes place. In addition, the *h* and *t* states have broad minima, so that the net result is still a random coil polypeptide. There are many salt effects which can be explained on the basis of these conformational changes.

Early optical studies of several proteins[48,52-57] in particular ribonuclease showed a decrease in levorotation upon the addition of LiBr at a fixed temperature. Based upon the correlations that were obtained between the molecular chain conformation and optical rotation for simple homopolypeptides (not involving the salts),[58,59] it was deduced[53]

that the helical structures were stabilized by the action of LiBr. However, it was shown[60] that in fact LiBr destabilized the native structure of ribonuclease and hence other proteins. The failure of the single-temperature optical-rotation studies to correctly predict the action of LiBr was attributed to specific solvent or medium effects.[60] Our model offers another explanation. As LiBr is added to protein solutions, an increase in the population of the *h* state results. This increase in the *h* population gives the appearance of increasing the α-helical content and hence causes a decrease in the levorotation of the native structure. The reason for this is that the conformational coordinates of a unit in the *h* state and in the α-helix are close to one another. Thus qualitatively similar optical properties will result. The action of LiBr on the optical rotation of proteins can be accounted for by actual structural changes, but not α-helical formation.

The un-ionized water-soluble polymer, poly(ϵ-*N*-hydroxyethyl-L-glutamine) (PHEG), is a statistical random coil in aqueous solution.[61] The general features of its circular dichroism spectrum, CD, between 200 and 240 nm in water[18,62] are similar to the predominantly trans CD spectrum of poly(Pro).[12,17] Both systems have a weak long-wavelength positive band followed by a much more intense short-wavelength negative band. Upon the addition of the salts of interest, the weak positive band of both CD spectra diminishes, leaving only a less intense red-shifted negative band.[12,17,18,63] Since no evidence for a trans to cis isomerization has been detected for PHEG, a *t* to *h* transition could be responsible for its salt-induced CD effect. The CD spectrum of the α-helix[64,65] is negative in the general region where the CD spectrum of PHEG is positive. Although the contribution to the CD spectrum of an isolated *h* residue would not be expected to be the same as the α-helix CD, they should share certain general features. In particular, they should both display a long-wavelength negative band followed by a more intense, short-wavelength positive band.[66-68] Therefore, as the population of the *h* conformer increases with added salt, the positive CD band of PHEG will diminish until it is completely compensated for by the negative band of the *h* conformer. These expectations are confirmed experimentally, and the resultant spectrum reveals only one negative band between 200 and 240 nm. In fact Dearborn and Wetlaufer[69] have had fair success in reproducing the CD spectra of poly(L-lysine), poly(Lys), in various salts, including concentrated $CaCl_2$, by assuming a linear combination of the α-helical poly(Lys) (pH 11.4) CD and the unordered poly(Lys) (pH 3.7) CD. The poly(Lys) unordered CD, like PHEG, has characteristics which are very similar to the predominantly *trans*-poly(Pro) CD.

Poly(Pro) is well known to undergo mutarotation. The trans-cis isomerization usually is accomplished by the addition of a poorer solvent. On the other hand, a similar mutarotation for poly(Hyp) has not as yet been reported. The hydroxyl-group interaction which has been shown to be so important in the salt-induced isomerization should also play an important role here, the poorer solvents favoring the interaction and thus making energetically less favorable the rotations necessary for isomerization.

The salt binding model that we have presented and its attendant influence on the population of the allowed conformational states is in excellent agreement with the changes that are observed upon the addition of salts to poly(Pro) and poly(Hyp). The intramolecular hydroxyl interaction that has been suggested for poly(Hyp) receives indirect support from the results reported here. The nature of this interaction appears to be the reason for the differences in intrinsic viscosity and extent of isomerization between these two polymers. In addition, the principles that have been developed unify many of the spectral and conformational observations that have been observed in other polypeptides and proteins where the interpretation has been subject to many different viewpoints.

More elaborate salt binding schemes, with their attendant complexity, can easily be developed. They would not alter the important characteristics of the present model, whose essential feature is to allow by appropriate bond rotations two carbonyl oxygen atoms to be in sufficient proximity for a multi-residue cation complex to be formed. This complexation is apparently influenced by anion interactions with the peptide bond. Further studies to elucidate the anion role are needed.

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References and Notes

(1) Clark, D. S.; Dechter, J. J.; Mandelkern, L. *Macromolecules* **1979**, *12*, 626.
(2) Wu, C. C.; Komoroski, R. A.; Mandelkern, L. *Macromolecules* **1975**, *8*, 635.
(3) Dechter, J. J.; Clark, D. S.; Mandelkern, L. *Macromolecules* **1978**, *11*, 274.
(4) Mandelkern, L.; Mattice, W. L. *Jerusalem Sym. Quantum Chem. Biochem.* **1973**, *5*, 121.
(5) Mandelkern, L. In "Poly α-Amino Acids"; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967; p 675.
(6) Torchia, D. A.; Bovey, F. A. *Macromolecules* **1971**, *4*, 246.
(7) Dorman, D. E.; Torchia, D. A.; Bovey, F. A. *Macromolecules* **1973**, *6*, 80.
(8) Chien, J. C. W.; Wise, W. B. *Biochemistry* **1973**, *12*, 3418.
(9) Landolt-Börnstein In "New Series"; Hellwege, K.-H., Ed.; Springer-Verlag: Berlin, 1977; Vol. IV.
(10) Lewis, G. N.; Randall, M. In "Thermodynamics", 3rd ed.; revised by Pitzer, K. S.; Brewer, L.; McGraw-Hill: New York, 1961; p 653.
(11) Robinson, R. A.; Stokes, R. H. "Electrolyte Solutions", 2nd ed., revised; Butterworths: London, 1970; p 491.
(12) Tiffany, M. L.; Krimm, S. *Biopolymers* **1968**, *6*, 1767.
(13) Iizuka, E.; Yang, J. T. *Biochemistry* **1965**, *4*, 1249.
(14) Tiffany, M. L.; Krimm, S. *Biopolymers* **1968**, *6*, 1379.
(15) Tiffany, M. L.; Krimm, S. *Biopolymers* **1969**, *8*, 347.
(16) Mattice, W. L.; Mandelkern, L. *Macromolecules* **1970**, *3*, 199.
(17) Mattice, W. L.; Mandelkern, L. *Biochemistry* **1970**, *9*, 1049.
(18) Mattice, W. L.; Lo, J.-T.; Mandelkern, L. *Macromolecules* **1972**, *5*, 729.
(19) Mattice, W. L.; *J. Am. Chem. Soc.* **1973**, *95*, 5800.
(20) Mattice, W. L. *Biopolymers* **1974**, *13*, 169.
(21) Schleich, T. *Biopolymers* **1973**, *12*, 993.
(22) Mandelkern, L.; Stewart, W. E. *Biochemistry* **1964**, *3*, 1135.
(23) Robinson, D. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 2470.
(24) Kurtz, J.; Harrington, W. F. *J. Mol. Biol.* **1966**, *17*, 440.
(25) Mandelkern, L.; Halpin, J. C.; DiOrio, A. F.; Posner, A. S. *J. Am. Chem. Soc.* **1962**, *84*, 1383.
(26) Schleich, T.; Von Hippel, P. H. *Biopolymers* **1969**, *7*, 861.
(27) Lo, J. T.; Mattice, W. L. *Biopolymers* **1976**, *15*, 15.
(28) Von Hippel, P. H.; Peticolas, V.; Schack, L.; Karlson, L. *Biochemistry* **1973**, *12*, 1256.
(29) Von Hippel, P. H.; Schleich, T. "Structure and Stability of Biological Macromolecules"; Timasheff, S. N., Fasman, G. D., Eds.; Marcel Dekker: New York, 1973; p 417.
(30) Bello, J.; Haas, D.; Bello, H. R. *Biochemistry* **1966**, *5*, 2539.
(31) Pinkerton, M.; Steinrauf, L. K.; Dawkius, P. *Biochem. Biophys. Res. Commun.* **1969**, *35*, 512.
(32) Balasubramanian, D.; Shadely, R. *Biopolymers* **1973**, *12*, 1639.
(33) The internal dimer sequence *hc* is not allowed because of severe steric contacts between the imide group of *c* and the pyrrolidine ring of *h*.
(34) Calculation of the corresponding curves for poly(Hyp) requires a somewhat different treatment which will be described shortly.
(35) Dehm, P.; Prockop, D. J. *Biochim. Biophys. Acta* **1972**, *264*, 375.
(36) Berg, R. A.; Prockop, D. J. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 115.
(37) Sakakibara, S.; Inouye, K.; Shudo, K.; Kishida, Y.; Kobayashi, Y.; Prockop, D. J. *Biochim. Biophys. Acta* **1973**, *303*, 198.

(38) Kobayashi, Y.; Sakai, R.; Kakiuchi, K.; Isemura, T. *Biopolymers* **1970**, *9*, 415.
(39) Berg, R. A.; Olsen, B. R.; Prockop, D. J. *J. Biol. Chem.* **1970**, *245*, 5759.
(40) Traub, W. *Isr. J. Chem.* **1974**, *12*, 435.
(41) Ramachandran, G. N.; Bansal, M.; Bhatnager, R. S. *Biochim. Biophys. Acta* **1973**, *322*, 166.
(42) Clark, D. S.; Mattice, W. L. *Macromolecules* **1977**, *10*, 369.
(43) Mattice, W. L.; Mandelkern, L. *Biochemistry* **1971**, *10*, 1926.
(44) Torchia, D. A. *Macromolecules* **1972**, *5*, 566.
(45) Clark, D. S.; Ph.D. Dissertation, The Louisiana State University, 1976.
(46) Since we do not have data for poly(Pro) in LiBr, we must make an estimate of K_c^s, K_h^s, and K_a for this system. We have assumed that the K_a for poly(Pro) in LiBr is the same as that given by Von Hippel et al.[28] We have estimated f_c^{lim} for this system by making a linear interpolation between the f_c^{lim} of poly(Pro) in $CaCl_2$ (or LiCl) and the f_c^{lim} of poly(Pro) in $LiClO_4$ and the "single anion" binding constants for these salts to polyacrylamide gels.[28] Knowing f_c^{lim}, and hence R_c^{lim}, we have calculated K_c^s and K_h^s, using eq 7 and 10.
(47) Taylor, R. P.; Kuntz, Jr., J. D. *J. Am. Chem. Soc.* **1972**, *94*, 7963.
(48) Steinberg, I. Z.; Harrington, W. F.; Berger, A.; Sela, M.; Katchalski, E. *J. Am. Chem. Soc.* **1960**, *82*, 5263.
(49) Madison, V. *Biopolymers* **1977**, *16*, 2671.
(50) Venkatachalam, C. M.; Price, B. J.; Krimm, S. *Biopolymers* **1975**, *14*, 1121.
(51) Here f was calculated as $f = (\alpha_D - \alpha_D^{II})/(\alpha_D^{I} - \alpha_D^{II})$, where α_D^{II} and α_D^{I} are the specific rotations at the sodium D line of the predominately trans and cis forms, respectively, of poly(Pro).[48]
(52) Carpenter, D. C.; Lovelace, F. E. *J. Am. Chem. Soc.* **1935**, *57*, 2337.
(53) Harrington, W. F.; Schellman, J. A. *C. R. Trav. Lab. Carlsberg, Ser. Chim.* **1957**, *30*, 167.
(54) Harrington, W. F. *Nature (London)* **1958**, *181*, 997.
(55) Harrington, W. F.; Sela, M. *Biochim. Biophys. Acta* **1959**, *31*, 427.
(56) Perlmann, G. E. "Proceedings of the Fourth International Congress of Biochemistry"; Pergamon Press: New York, 1959; Vol. IX, p 32.
(57) Bigelow, C. C.; Geschwind, I. I. *C. R. Trav. Lab. Carlsberg, Ser. Chim.* **1961**, *32*, 89.
(58) Yang, J. T.; Doty, P. *J. Am. Chem. Soc.* **1957**, *79*, 761.
(59) Doty, P. *Rev. Mod. Phys.* **1959**, *31*, 107.
(60) Mandelkern, L.; Roberts, D. E. *J. Am. Chem. Soc.* **1961**, *83*, 4292.
(61) Mattice, W. L.; Lo, J.-T. *Macromolecules* **1972**, *5*, 734.
(62) Adler, A. J.; Hoving, R.; Potter, J.; Wells, M.; Fasman, G. D. *J. Am. Chem. Soc.* **1968**, *90*, 4736.
(63) Tiffany, L. *Physiol. Chem. Phys.* **1975**, *7*, 191.
(64) Holzwarth, G.; Doty, P. *J. Am. Chem. Soc.* **1965**, *87*, 218.
(65) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 408.
(66) Tinoco, Jr., I.; Woody, R. W. *J. Chem. Phys.* **1963**, *38*, 1317.
(67) Zubkov, V. A.; Birshtein, T. M.; Milevkaya, I. S.; Volkenstein, M. V. *Biopolymers* **1971**, *10*, 2051.
(68) Aebersold, D.; Pysh, E. S. *J. Chem. Phys.* **1970**, *53*, 2156.
(69) Dearborn, D. G.; Wetlaufer, D. B. *Biochem. Biophys. Res. Commun.* **1970**, *39*, 314.

Conformational Analysis of Poly(thiopropylene)

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ABSTRACT: Conformational energies associated with poly(thiopropylene) (PTP) chains were calculated by using semiempirical potential energy functions. Reliability of these functions was tested against the known values of conformational energies of various simple alkyl sulfides bearing somewhat related structures. The magnitude of the gauche sulfur effect associated with the $S-CH_2-CH(CH_3)-S$ moiety was estimated from the rotational isomeric state analysis of the experimental values of the unperturbed dimension, dipole moment, and their temperature coefficients observed for atactic samples of PTP. The value obtained for the gauche conformation (α) with the articulated methyl group trans to the preceding sulfur atom indicates that the gauche effect involved in such polymer systems is slightly repulsive in contrast to that found for the gauche oxygen effect of the poly(oxypropylene) (POP) chain, the oxygen analogue of PTP. The gauche sulfur effect was estimated to be zero or possibly positive for the conformation (β) in which the preceding sulfur atom is syn to both the following sulfur and the methyl group. Using the conformational energy parameters thus estimated, we were able to calculate the characteristic ratio $\langle r^2\rangle_0/nl^2$, the dipole moment ratio $\langle\mu^2\rangle/nm^2$, and their temperature coefficients for the isotactic and syndiotactic chains as well. The results compared favorably with the existing experimental data for the isotactic chain.

Conformational analyses on a series of polyoxide chains such as $(-CH_2CH(R)O-)_x$, where R = H, CH_3, CH_2CH_3, $CH(CH_3)_2$, and $C(CH_3)_3$, have been reported in our previous papers.[1-3] Extra stabilization energies associated with the gauche conformation about the skeletal C–C bonds were estimated by taking the difference between the conformational energies (E_{calcd}) calculated by using semiempirical expressions and those (E_{exptl}) derived from the analysis of the experimental data such as the unperturbed dimensions, dipole moments, and bond conformations. The gauche oxygen effect ($\Delta E = E_{calcd} - E_{exptl}$) estimated in this manner amounts to ca. 1 kcal mol^{-1} for poly(oxyethylene) (POE) and ca. 0.7 kcal mol^{-1} for the gauche α conformation of poly(oxypropylene) (POP) in which the pendant CH_3 group is situated trans to the preceding oxygen atom of the skeletal chain. A value of $\Delta E = 0.2$ kcal mol^{-1} was obtained for the gauche conformation (β) of the latter polymer. These gauche effects play very important roles in the aforementioned polymer systems by enhancing fractions of the gauche conformation about the C–C bonds and thus rendering conformational flexibility to the polymer chain.[1-3]

A similar treatment has been extended to poly(thiopropylene) (PTP), the sulfur analogue of POP.[4] The PTP may be obtained in a highly isotactic or an atactic form depending upon the catalyst system employed in the polymerization of propylene sulfide.[5] The PTP chain differs considerably from POP in its structural features: (1) the C–S bond length is larger by ca. 30% than C–O;[6-8] (2) the bond angle CSC is less by ca. 10° than that of COC;[6-8] and (3) the van der Waals radius of the sulfur atom is larger by ca. 20% than that of oxygen.[9] These differences should manifest themselves in the conformational characteristics: e.g., it is suggested from spectroscopic studies[8,10-12] on

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