The Effect of Aliphatic Alcohols on the Helix-Coil Transition of Poly-L-ornithine and Poly-L-glutamic Acid*

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SUMMARY

The helical stability of poly-L-glutamic acid and poly-L-ornithine in aqueous alcohol solutions was studied by optical rotatory dispersion and potentiometric titration determinations. It was found that aliphatic alcohols enhance the stability of the helix according to the series methanol < ethanol < 1-propanol < 1-butanol. The role of secondary and tertiary alcohols was also investigated. Thermodynamic analysis of the thermal transition of uncharged poly-L-ornithine showed that the greater stability of the helix in aqueous alcohol solutions with respect to that in water arises from a more favorable value of the entropy change of the transition $\Delta S^\circ$. A similar effect of alcohols on the thermodynamics of the coil-helix transition in aqueous solution has already been observed for poly-L-glutamic acid.

It is suggested that solvation of the peptide amide group in the randomly coiled conformation may be responsible for the observed trend of the variation of the thermodynamic quantities.

In a previous paper (1) we have investigated the transition to the uncharged helix from the uncharged random coil of poly-L-glutamic acid in water-ethanol solutions and we have found that the stabilization of the helical form by the alcohol has an entropic origin. In this work we wish to report the effect of other aliphatic alcohols on the helical conformation of polyglutamic acid, as well as the thermodynamic parameters obtained from the analysis of the thermal transition of poly-L-ornithine in water-alcohol mixtures.

Polyornithine was chosen for several reasons, primarily because the thermal transition of the uncharged helix from the uncharged random coil falls in a temperature range which can be experimentally explored. Furthermore, a comparison of the data obtained for polyornithine and for polyglutamic acid allows the influence of the side chain and the nature of the ionizable group on the thermodynamic parameters of the coil-helix transformation to be investigated.

The results presented in the literature show that for several proteins (2-5) and poly-$\alpha$-amino acids (6-9) the stability of the $\alpha$-helix conformation is enhanced by addition of alcohols or other organic solvents (for globular proteins this step follows the breakdown of a well defined tertiary structure). We now wish to demonstrate that the effect of alcohols on the thermodynamic parameters of coil-helix reaction is the same for poly-$\alpha$-amino acids as different as polyglutamic acid and polyornithine.

EXPERIMENTAL PROCEDURE

Materials—The sodium salt of poly-L-glutamic acid (Lot G-92) was obtained from Pilot Chemicals, Inc., Watertown, Massachusetts, and its nominal degree of polymerization was 570. Poly-L-ornithine hydrobromide (Lot O-21) with a reported molecular weight of 70,000 was also obtained from Pilot Chemicals. All alcohols were of reagent grade.

Titrations—The titrations were carried out at 25° ($\pm$0.1) as previously described (10) with a Radiometer pHM4 meter. Experiments were performed with solutions containing 40 to 60 mg of polymer in 10 ml of solutions 0.1 M with respect to KCl.

Optical Rotatory Dispersion—The instrument used was the JASCO ORD/UV-5 recording spectropolarimeter, with fused quartz cells of 1-, 5-, and 10-mm path length. Polymer concentrations were varied between 0.3 and 10 mg per ml. Rotations were expressed in terms of reduced mean residue rotation, $[\phi R]_\lambda$, in degrees square centimeter per deci mole, given by (11)

$$[\phi R]_\lambda = \frac{3}{\bar{n}^2 + 2} \left( \frac{MWR}{100} \right) [\alpha]_\lambda$$

We assumed the refractive index, $n$, of the alcohol-water mixtures to be the same as that of pure water, and the refractive index dispersion was estimated from Table III reported by Fasman (12) or from the International Critical Tables (13). The wave length range was 350 to 600 nm, fitted with the Moffit equation (14) and $\lambda_0$ was taken to be 212 nm in calculating $b_0$. All solutions were prepared by first dissolving the polyornithine in water at pH ~3, and then raising the pH to 12.3 and finally adding the required amount of alcohols.

RESULTS

The dependence of $[\ϕ R]_\lambda$ for uncharged polyornithine in water-alcohol mixtures at pH 12.3 on alcohol content is shown in Fig. 1, A and B. A different ability of alcohols in inducing the helical conformation at 25° is immediately displayed from these plots. Thus about 40% (v/v = 9.9 M) methanol is required to obtain a value of $[\ϕ R]_\lambda = -14,000$, while about the same value is reached at about 23% (=3.9 M) ethanol and 15% M...
**Table I**

Values of \(b_0\) and [\(\psi\)] for polyornithine in water-alcohol mixtures at different compositions and temperatures, at pH 12.3

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(t)</th>
<th>(b_0)</th>
<th>[(\psi)]</th>
<th>Helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>4°</td>
<td>-440</td>
<td>-11,000</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-100</td>
<td>-5,200</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>39.5</td>
<td>-110</td>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>40% (v/v = 9.9 M) methanol</td>
<td>4</td>
<td>-640</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-600</td>
<td>-14,100</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>39.5</td>
<td>-550</td>
<td>-12,200</td>
<td>84%</td>
</tr>
<tr>
<td>40% (v = 8.85 M) ethanol</td>
<td>4</td>
<td>-620</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-580</td>
<td>-13,900</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>39.5</td>
<td>-550</td>
<td></td>
<td>87%</td>
</tr>
<tr>
<td>40% (v = 5.4 M) 1-propanol</td>
<td>10</td>
<td>-600</td>
<td></td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-560</td>
<td>13,700</td>
<td>89%</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>-510</td>
<td></td>
<td>81%</td>
</tr>
</tbody>
</table>

For 1-butanol the transition should be complete at about 10% (=1.1 M), but this value of the composition is not obtainable at 25°. The order of alcohols according to increasing enhancement of the helical conformation is: methanol < ethanol < 1-propanol < 1-butanol. The effect of secondary and tertiary alcohols was also investigated and the results are reported in Fig. 1B. It is evident that at 25° no alcohol concentration can be found at which the polymer is in the completely helical conformation. In all of the systems investigated, the maximum value of [\(\psi\)] is about -14,000, corresponding to 90% of helix, with the empirical equation (15):

\[
\text{Fraction of helix} = \frac{[\psi]}{[-14,000]} 
\]

Moreover, we observed that at lower temperatures, depending on the type of alcohol and mixtures composition, polyornithine displays a value of [\(\psi\)] corresponding to 100% helix. Owing to the effect of possible aggregation of the polymer on [\(\psi\)] (15), we have also estimated the helical content of polyornithine by determining the \(b_0\) of the Moffitt equation (14), which is only slightly affected by the presence of aggregation (16, 17). For comparison, the estimated helix amounts from both [\(\psi\)] and \(b_0\) are reported in Table I. The agreement between \(b_0\) and [\(\psi\)] is satisfactory and allows the use of [\(\psi\)] which can be obtained with a less laborious procedure, with some confidence.

The order of alcohols for increasing helix stability was independently confirmed on polyglutamic acid, by performing potentiometric titrations in 0.1 M KCl-alcohol mixtures. In Fig. 2 the plots of \(pK_{app} = pH - \log \alpha/(1 - \alpha)\) are reported as a function of the degree of ionization, \(\alpha\), curves for polyglutamic acid in water-alcohol mixtures (containing 0.1 M KCl) at 25°. The corresponding concentrations (moles per liter) of alcohol are indicated.

The fact that not only does the stabilizing effect of alcohols extend to both polyornithine and polyglutamic acid with side chains of different length but also that the same series of alcohols has been found for both suggests that this effect is quite general for a class of poly-\(\alpha\)-amino acids.
A more fundamental analysis of the role of alcohols is found by separating the enthalpic and the entropic contributions to the free energy term. For polyglutamic acid we have already shown (1) that the stabilizing effect of ethanol with increasing alcohol content has an entropic origin. The enthalpy, $\Delta H_0$, becomes less negative, i.e. less favorable for helix, while the entropy, $\Delta S_h$, increases and the melting temperature, $T_m^*$, for uncharged helix greatly increases. The two separate contributions cannot be obtained for polyornithine by potentiometric titrations, because very small areas are obtained in the $pK_a$ vs. plot, even at high ethanol concentration and low temperatures (9).

The thermal transition for the uncharged polyornithine, on the contrary, may be very easily investigated by studying the variation of $[\eta]^\infty_{233}$ with temperature. Such determinations were carried out at pH 12.3, at which the NH$_2$ groups of the side chains are uncharged under all experimental conditions. The variation of $[\eta]^\infty_{233}$ with temperature for polyornithine in aqueous mixtures of methanol, ethanol, and 1-propanol is reported in Fig. 4, A, B, and C. The data were treated according to Zimm and Bragg (21, 22); at the mid-point of the transition, the slope of the straight line resulting from a plot of ln $K$ against $1/T$ gives the values of $\Delta H_0/RT$. The apparent equilibrium constant $K$ was defined as the ratio, (random coil)/(α-helix), and was obtained from the data in Fig. 4, with a value of $[\eta]^\infty_{233} = -15,000$ for the fully helical form and $[\eta]^\infty_{233} = -2,000$ for the fully randomly coiled form. From Fig. 4A, it is evident that polyornithine is completely helical at about 10°C in 6.19 M methanol.

Because both $\Delta H_0$ and $\sigma$ determine the sharpness of thermal transition of poly-$\alpha$-amino acids and the assumption of the independence of $\sigma$ on the nature of the solvent is probably unrealistic (for example, a solvent effect on $\sigma$ has been shown (23) for poly-(γ-benzyl-l-glutamate) in dichloroacetic acid-1,2-dichloroethane mixtures) caution should be exercised when this method is used to obtain information about the energetics of the process.

The potentiometric titration data for polyglutamic acid in water-

![Fig. 3. Values of $\Delta F_0$, the free energy of formation of the uncharged helix from the uncharged coil, as a function of alcohol concentration for polyglutamic acid at 25°C. ○, methanol; ●, ethanol; ♦, 1-propanol; □, 1-butanol. O, this value corresponds to measurement in 0.1 M KCl (19, 20).](image1)

![Fig. 4. The mean residue rotation at 233 nm as a function of temperature for polyornithine in water-alcohol mixtures. A, measurements in methanol: ○, 1.98 M; ●, 2.23 M; □, 6.19 M; ●, 12.38 M. The values corresponding to water are indicated with (●). B, measurements in ethanol: ○, 0.01 M; ●, 1.37 M; □, 2.91 M; ●, 2.91 M. C, measurements in 1-propanol: ○, 0.20 M; ●, 0.27 M; ○, 0.67 M; ●, 1.07 M; □, 1.14 M; ■, 1.74 M.](image2)

![Fig. 5. Experimental values of $\sigma$ for polyglutamic acid at different molarity of alcohols and different temperatures.](image3)
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Fig. 6. Values of the enthalpy, entropy, and free energy of formation of the uncharged helix, \( \Delta H_0 \), \( \Delta S_0 \), and \( \Delta F_0 \), respectively, and melting temperature, \( T_m \), for polyornithine as a function of alcohol concentration. ○, methanol; ●, ethanol; ○, 1-propanol.

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Fig. 7. Increments of the thermodynamic quantities of helix formation with respect to water per methylene's molarity as a function of methylene's molarity, for polyornithine and polyglutamic acid. ○, polyornithine in methanol; ●, polyornithine in ethanol; ○, polyornithine in 1-propanol. □, polyglutamic acid in methanol; ■, polyglutamic acid in ethanol; □, polyglutamic acid in 1-propanol; ●, polyglutamic acid in 1-butanol.

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alcohols mixtures which we have collected in the present and in a preceding work (1) offer the possibility of evaluating the solvent effect on \( \sigma \) for a system which has already been extensively investigated. For an ionizable poly-amino acid, \( \sigma \) is obtained from the slope of \( \ln ((1/f_n) - 1) \) as a function of \( 1 - s' \) near the transition point (\( s' = 1 \)) (24). The main uncertainty of this method is connected with the difficulty of obtaining the correct pH/\( \alpha \) curves for both the pure helix and random coil in the transition region. We have assumed that the \( \text{pK}_{\alpha}/\alpha \) curve for the pure conformers is quite linear: the validity of this choice has already been discussed (1). The result is that \( \sigma \) is independent of solvent composition in the range investigated, i.e. from 0.87 to 4.95 M alcohol, as well as of temperature, as shown in Fig. 5. The mean value obtained from eight determinations is \( 1.0 \pm 0.3 \times 10^{-1} \). This value was used in the analysis of the thermal transition of polyornithine over the entire solvent composition range. We observe that our value is intermediate between the values already reported in literature (24–26). However, because we are interested in the trend of the variation of the thermodynamic quantities for the coil-helix transition, rather than in their absolute values, the validity of our results will be unaffected by the value selected.

The thermodynamic parameters for the formation of the uncharged helix from the uncharged random coil for polyornithine, together with the corresponding melting temperature, are shown in Fig. 6 (\( \Delta F_0 \) values were calculated at 25°C for every solvent mixture, assuming both \( \Delta H_0 \) and \( \Delta S_0 \) to be temperature-independent). The value of \( \Delta F_0 \) at 25°C in water is +24 cal per mole. If \( \sigma \) is taken to be equal to 2.10 \times 10^{-4}, \( \Delta F_0 \) should be about +10 cal per mole.

The most significant information drawn from these results is that \( \Delta S_0 \) increases with alcohol content, causing the observed increasing of \( T_m \). It is very important to note that this behavior is shown by a system for which both the ordered and disordered conformers are uncharged under the experimental conditions investigated. Indeed, potentiometric titrations, according to the treatment of Zimm and Rice (18), give the free energy of formation for the uncharged helix from the uncharged random coil, without any contribution of the electrostatic free energy, but, experimentally, one deals with the charged conformers, and therefore any secondary effect of the charge on the polymer-solvent interaction is included in the values of \( \Delta F_0 \). We may therefore conclude, from the determinations carried out on uncharged polyornithine, that strong electrostatic effects on the solvent-polymer interactions are not included in the mechanism of the \( \alpha \)-helical form stabilization induced by alcohols.

Rather than in terms of alcohol molar concentration, our experimental data may be treated, according to von Hippel and Wong (27), by considering the dependence on methylene's molarity. For globular proteins, the series methanol, ethanol, 1-propanol, 1-butanol corresponds to the order by which these alcohols are effective, on a molar scale, in depressing the melting temperature of the native form, but, if the molarity of methylenes in solution is assumed as variable, the melting temperature will be an almost linear function of it, irrespectively, of the alcohol used. The quantities here considered are the increments of the thermodynamic parameters of formation of the helix in the mixed solvent with respect to water per unit of methylene molarity;
values of $\Delta(\Delta F_\circ)/$methylene molarity for both polyglutamic acid and polyornithine, together with the values of $\Delta T_\circ$/methylene molarity and $\Delta(\Delta S_\circ)/$methylene molarity for polyornithine, are reported as a function of methylene’s molarity in Fig. 7. It may be seen that, within the experimental error, constant values are obtained for these quantities on the entire range of solvent composition: they are $\Delta(\Delta F_\circ)/$methylene molarity $\simeq -13$; $\Delta T_\circ$/methylene molarity $\simeq +1.5$, and $\Delta(\Delta S_\circ)/$methylene molarity $\simeq +0.25$.

The same value of $\Delta(\Delta F_\circ)/$methylene molarity may be considered to fit the experimental values for polyornithine and polyglutamic acid: this finding greatly substantiates the observation that the effect of the alcohols on the helix-coil transition is the same for both the poly-$\alpha$-amino acids, although their side chains have a very different nature. The ability of alcohols both in depressing the melting temperature of the native form of globular proteins (27–29) and in enhancing the $\alpha$-helical conformation for some poly-$\alpha$-amino acids seems to depend only, at least up to 6 M alcohol, on the molarity of methylene groups; such dependence, in spite of the opposite effect which is exerted on the stability of the two kinds of ordered conformations, could be considered as an indication that the intramolecular interactions are in both the cases related to the same properties of the solvent mixture.

DISCUSSION

A reasonable model for interpreting the effect of alcohols on the $\alpha$-helical conformation of poly-$\alpha$-amino acids ought to explain why an order for the stabilization of the helix for aliphatic alcohols exists, as well as the entropic origin of this stabilization. It is generally accepted that side chain effects and backbone hydrogen bonds stabilize the helical form in an aqueous solvent. An interpretation, in terms of solvent effects on side chain interactions, however, is easily ruled out. Hydrophobic interactions between the side chains are weakened in the presence of alcohols. This should lead, for water-alcohol mixtures, to a lower helical stability with respect to water. The trend of the variation of the entropy of transition, $\Delta S_\circ$, with alcohol content is, too, well indicative, because $\Delta S_\circ$ is close to zero for polyglutamic acid in 20% (3.42 M) ethanol-water mixtures (1) and this limit value is approached also by polyornithine, for which we have found $\Delta S_\circ \simeq 0.8$ e.u. in 1.39 M 1-propanol, which must be compared with $\Delta S_\circ \simeq -1.65$ e.u. found in water. This observation rules out the possibility that alcohol enhances the stability of the $\alpha$-helix by increasing the number of intramolecularly hydrogen-bonded side chains, because, as a consequence of the loss of rotational freedom, a decrease of the entropy of transition, rather than a large increase, should result. Alcohols, furthermore, increase the solubility of the single, uncharged helix of polyglutamic acid (1) from the precipitate. This indicates that in the case of the helical array the side chains are still able to interact readily with the solvent, and therefore that the difference with respect to the situation in the random coil, in which the side chains are completely exposed to the solvent, must not be large. The main contribution arises from the removal of the peptide groups from the solvent with the formation of intramolecular hydrogen bonds.

It could be suggested that alcohols or other organic solvents which possess nonpolar groups “strengthen” intramolecular hydrogen bonds on account of their ability to decrease the dielectric constant of the solvent medium. However, if a dielectric constant effect were predominant in water-alcohol mixtures, a contribution of the enthalpy to the stability of the helix should be observed experimentally. Klotz et al. (30–32) have investigated the stability of the hydrogen bond in an aqueous and nonpolar environment: the free energy of transfer of a nonhydrogen bonded amide group from water to CCl$_4$ is positive (4.1 kcal per mole) while the related entropy and enthalpy terms are equal to +1 2 e.u. and +7.8 kcal per mole, respectively. Because the transfer from water to CCl$_4$ of bonded NH···O=C involves no change in the free energy, the thermodynamic standard quantities corresponding to the formation of a hydrogen bond in water and in CCl$_4$ are $\Delta F_\circ = +3.1$ and $-2.4$ kcal per mole, $\Delta S_\circ = -10$ and $-9$ e.u., $\Delta H_\circ = 0$ and $-5.1$ kcal per mole, respectively. These data show essentially that the greater ability of the amide group to form the hydrogen bond in a nonpolar solvent has an enthalpic rather than entropic nature. In our case, on the contrary, both $\Delta H_\circ$ and $\Delta S_\circ$ increase with alcohol content (both $\Delta(\Delta S_\circ)$ and $\Delta(\Delta H_\circ)$ per unit of methylene molarity are positive); i.e. the intramolecularly hydrogen bonded $\alpha$-helix becomes more stable owing to an entropic reason. If we still assume that the value of the intrinsic interaction between donor and acceptor for the hydrogen-bonded peptide group is unaffected by solvent environment (31) the observed increase in $\Delta S_\circ$ formally requires a decrease of the entropy of mixing of the unbonded peptide group with the solvent. There are, in connection with this point, some thermodynamic properties of the water-alcohol solutions which are pertinent to mention here because they offer the set of conditions according to which any significant model of interpretation must be discussed. Water-alcohol solutions show very large deviations from ideality and possess unique structural features. Mixing of water with alcohols leads to large negative values of the entropy and, furthermore, alcohols are the only known solutes together with some aliphatic amines (33) which raise the temperature of maximum density of water (34). Apart from the uncertainty connected with the type of structure involved, dilute solutions of alcohols in water (in a region in which water is still thought to retain its structural features) must therefore be regarded as possessing less liquid character than water itself (34). An additional interesting feature is that the maximum observed (for the values of the elevation of the temperature of maximum density) as a function of alcohol content is shifted toward lower molar fractions in going from methanol to 1-propanol. On a molar scale, therefore, the ability of structure promoting in water increases from methanol to 1-propanol in evident connection with the methylene’s molarity in solution. The question, therefore, may be properly formulated asking how the solvation of the peptide group depends on the degree of structuration of water in the mixed solvent. That a decrease of the entropy of mixing of the peptide group with water should result with increasing the degree of structuration of the latter is not an unreasonable assumption, in terms of a lattice treatment of liquid mixtures, and we put it forward here as a working hypothesis, although of course very little is known about the specific interactions between amide group and water, even from the simple thermodynamics standpoint. This statement, however, is susceptible to an experimental control, because, in terms of the classical theory of dilute polymer solutions, a decrease of the entropy parameter $\psi$ (35) for the randomly coiled conformation should result with increasing alcohol concentration, and a complete thermodynamic analysis of the solution properties of poly-$\alpha$-amino acids as random coil could provide the necessary evidences.
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