ΔW_{12} the interaction free energy change associated with the formation of a contact between polymer segment and solvent. In classical theory it is assumed that Z and ΔW_{12} are constants. Now if we assume that they could depend on the average chain conformation, *i.e.*, on the unperturbed dimensions, one has to write, in order to find the equilibrium value, that ΔG is minimum, and it is not absolutely necessary that this minimum is always obtained for the same value of $\overline{r_0^2}$.

This type of argument could be extended to solvent mixtures very easily. Changes in average conformation can change the interactions polymer–solvent 1, –solvent 2 and the number of contacts between solvent 1 and solvent 2. There are many more possibilities in this case than in the case of pure solvents; it is therefore not surprising that for these solvent mixtures one usually obtains larger effects.

At first sight, one does not see any reason to try to put the explanation of the observed phenomena in thermodynamic terms rather than to use a molecular description of the intermolecular forces of the type we have given at the beginning of this paper. But this point of view is strongly supported by our results on solvent mixtures. In this case we have shown that it is not only the interactions between solvent and polymer (χ_{13} and χ_{23}) which are responsible for the changes in K_Θ but also the interaction between solvent 1 and solvent 2. This way of presenting the results eliminates the effect of each solvent and shows the importance of the thermodynamic properties of the solvent mixture, associating ΔK_Θ to ΔG^{E}. The fact that we obtain the same function for PS and P2VP is somewhat surprising. It means that ZW_{12} is the same function of the unperturbed dimensions for these two polymers and that this quantity does not depend on the nature of the side groups. Since the geometry of two chains is very similar, this can perhaps be understood. It would be interesting to check if this relation can be generalized to other polymers.

Conclusion

In this paper we have tried to give a systematic review of K_Θ values for different polymers. It seems that this quantity is not always independent of the solvent but, especially when one is dealing with polar polymers or polar solvent, there is an influence of the polymer–solvent interaction on its value.

We have tried to give a qualitative explanation of these facts. At first sight, it seems to be difficult to correlate these results with classical thermodynamic properties of solutions, since one has to take into account specific interactions between polymer and solvent. But in the case of mixed solvents there seems to be a correlation between these effects and the free enthalpy of mixing of the solvents, indicating that this effect is not only due to interactions between polymer and solvent but also to the interactions between solvent molecules.

Recently, Vrij[31] has proposed to take into consideration interfacial energies between the coil and the surrounding solvent. This kind of argument would perhaps also explain our results, but more experimental data are needed in order to confirm the validity of this theory.

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Conformational Transitions of Poly(L-tyrosine) in Mixed Water–Ethanol Solvents[1]

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Received August 25, 1970

ABSTRACT: The conformation of poly(L-tyrosine) (PT) was investigated in mixed water–ethanol solvents as a function of the degree of ionization, the solvent composition, and the polymer concentration by means of ORD, ir, and potentiometric titration techniques. While a slow transition from coil to antiparallel β conformation is observed in water and in the prevailing aqueous region, the α helix is the only stable form for ethanol concentrations greater than 45%. It was found that in the region between 20 and 40% PT may assume either the α-helical or the β conformation, depending on kinetic factors. The effect of concentration, as well as the role of solvent on the conformational states of PT, is discussed and some related thermodynamic data are reported.

ORD and CD techniques alone cannot solve the problem of determining the conformation of poly(α-amino acids) bearing optically active chromophores in the side chain: typically, the CD and ORD spectra of the ordered conformation of poly(L-tyrosine) (PT) have been described by Fasman[2a] and Beychok,[2b] but their interpretation of the experimental curves in terms of the α-helical conformation was largely hypothetical, mainly because there is an extensive overlapping between peptide and side chain bands near 224 mμ.

On the other hand, a recent theoretical approach by Pao[3] was not successful in fitting the gross features of the dispersion curves. Recently,[4] we investigated the charge-induced conformational transition of PT in aqueous solution by means of potentiometric titrations, sedimentation velocity experiments, and infrared spectroscopy. An aggregation process was detected around the pH value at which the transition occurs and the ordered conformation was recognized as possessing an antiparallel β conformation. Further work was directly concerned with the factors responsible for the

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(1) This work was financially sponsored by Consiglio Nazionale delle Ricerche.

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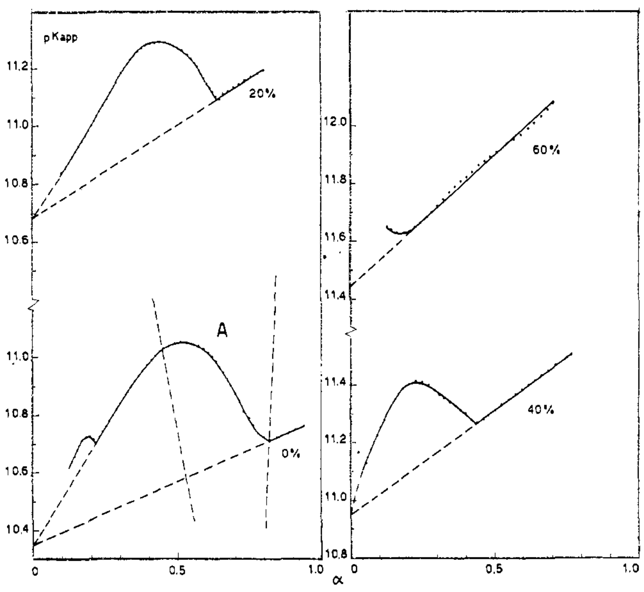

Figure 1. Potentiometric titrations of poly(L-tyrosine) in water–ethanol mixtures (containing 0.1 *M* KCl) at 25°: the corresponding amounts (per cent by volume) of ethanol are indicated.

stability of the β form, as well as the mechanism of the transition, and the results are reported in this paper.

First of all, the role of solvent composition has been investigated for water–ethanol mixtures. It clearly appears that not only is the stability of the β form strongly solvent dependent, but also that in alcohol-rich mixtures PT assumes a quite different conformation, which is recognized as α-helical. A very interesting situation exists at intermediate solvent compositions, where either the β- or α-helix forms may be observed, the actual situation depending mainly on kinetic factors.

Experimental Section

Materials and Methods. Materials and methods have already been described in the preceding paper.[4] Ir spectra of PT in the solid state were recorded from samples obtained by direct deposition of some drops of the solution on the CaF_2 window, followed by evaporation of the solvent in an inert gas flow. This resulted in an improved resolution with respect to the method previously reported, which involved the dispersion of a finely divided powder in a KBr matrix. It is, of course, necessary to start from solutions whose pH is in the acidic range in order to avoid the reionization of the polymer as a consequence of the increase of concentration. A sufficiently uniform deposition is obtained at pH 3–4, although in these conditions the polymer is completely precipitated from the solution.

For measurements in water–ethanol mixtures (40:60 v/v) in the region around 1550 cm^{-1} (amide II band), cells having an optical path length of 25 μ were used, while a path length of 50 μ was used for the experiments in D_2O–deuterioethanol, as reported previously. The smaller path length was required because of the strong absorption of the solvent mixture in this region. Using the 25-μ path length, however, the value of the transmittance for the solvent at 1550 cm^{-1} is about 35%; this is an acceptable value, which allows accurate solute spectra to be obtained by simply applying the routine procedure for differential determinations. The accuracy of the determinations is tested by considering the values of the frequency of the side chain bands at 1615, 1600, 1517, and 1500 cm^{-1}, respectively, which were found to coincide with those determined in D_2O solution and in the solid state.

Some difficulty arose when potentiometric titration experiments were carried out above 40% ethanol owing to the evaporation of alcohol; this may be detected by a small steady decrease of the pH when the addition of the titrant is stopped. This effect was eliminated by placing the thermostated beaker used for the titration in a closed vessel which was saturated with the same mixed solvent used in the experiment.

Results

Potentiometric Titration Curves. We have already noted that an aggregation process is observed for PT in 0.1 *M* KCl when the pH of the solution is lowered to 11.3. This affects the potentiometric titration curves in two ways: first, the shape of the curve is time dependent, owing to the time required to attain equilibrium aggregation: in the aggregation region, which is indicated as A in the pK_{app}/α plot reported in Figure 1, a very slow lowering of the pH is required in order to obtain reproduceable results. In the second place, the pH/α curve as well as the pK_{app}/α curve were found to be very steep around the aggregation point. The effect of ethanol concentration on the aggregation is shown in Figure 1, where the curves refer to very slow titration experiments, and must be regarded as corresponding to equilibrium situations. It immediately appears that the aggregation process becomes more unfavorable as the ethanol concentration increases. Consequently, the value of the degree of ionization, α, corresponding to incipient aggregation is shifted from 0.82 to 0.44 when the composition of the solvent mixture goes from 0 to 40% (v/v) ethanol. Experiments in 60% ethanol show no time dependence, and an almost linear variation of pK_{app} with α is obtained. Sedimentation velocity experiments confirm that molecular aggregates are no longer observed for ethanol concentrations near 45% at the same polymer concentration (*ca.* 0.6%) used in the titration experiments.

Optical Rotatory Dispersion. It is necessary to distinguish between the situation which is observed above *ca.* 45% ethanol, where the ORD spectra depend only on the degree of ionization, and that which predominates on the aqueous side of the composition, where two different kinds of spectra are obtained depending on α and on the time in which the actual value of α is reached.

Figure 2a refers to a solution in 20% ethanol slowly titrated from $\alpha = 1$ to $\alpha = 0.44$; the corresponding pH values are indicated. It appears that the typical random coil ORD curve (peak at 254 mμ, trough at 236 mμ) is observed down to pH *ca.* 11.4 ($\alpha = 0.66$) where the transition to the aggregated form begins.

In this region the same conformation transition as observed in water occurs, the ordered conformation having peaks at 286 and 254 mμ and a trough at 236 mμ. This conformation was recognized as an antiparallel β one on the basis of infrared spectra both in the solid state and D_2O solution,[4] and the same result is of course obtained in D_2O–deuterioethanol solution (see below). Alcohol greatly increases the rotation for both conformers, $[\alpha]_{254}$ being 2500 for the random coil in water and 5500 in 20% ethanol; the corresponding values for the β conformation are 3200 and 7200. The variation of α from 1 to 0.66, on the other hand, has a small effect on the rotation at 254 mμ. If the pH is lowered rapidly, another well-defined spectral change occurs. The peak at 286 mμ is no longer observed, while the rotation near 236 mμ becomes positive, and a trough appears at *ca.* 226 mμ (Figure 2b). Such a change occurs sharply in a narrow range of α and could be indicative of a charge-dependent conformational transition. This kind of dispersion curve becomes the only one observed around 50% ethanol, irrespective of the rate at which the polyion is discharged. The curves in Figure 3a refer to 60% ethanol. Here the spectral change is observed between pH 11.4 and pH 10.8. At this pH value the ORD

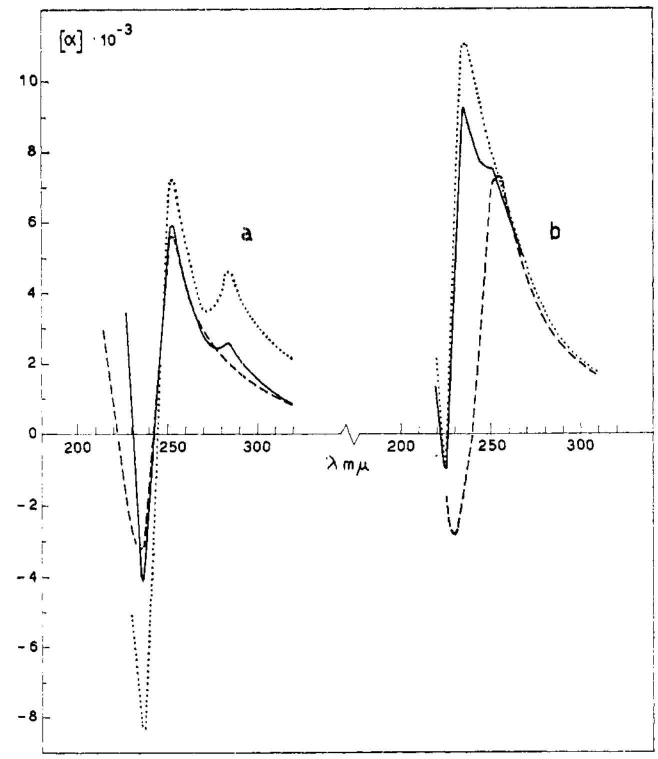

Figure 2. ORD curves of poly(L-tyrosine) in 20% ethanol (0.1 *M* KCl) at 25°: (a) slowly titrated solution at *c* 0.4%, pH 12.6, ---, pH 11.4, ——, pH 11.2, · · ·; (b) rapidly titrated solution pH 11.7, ---, pH 11.04, ——, pH 10.91, · · ·.

curve exhibits a peak at 236 mμ (having a very large rotation value) and a trough at 226 mμ. The rotation of the random coil at 254 mμ reaches a value as high as 8700, while it again appears unaffected by the degree of ionization. The highest ethanol concentration at which the β conformation may be obtained by slow titration is about 40%, where an incomplete coil to β transition is revealed by the ORD curves.

Our determinations have shown that the b_0 parameter of the Moffitt-Yang equation[5] is strongly solvent dependent: for example, it assumes a value of about +620 in 60% ethanol for the random coil at $\alpha = 1$; this value in water would correspond to a fully β conformation. This fact makes the parameter b_0 much less useful than the far-ultraviolet dispersion for conformational attributions.

Infrared Spectroscopy. Infrared spectra in mixed D_2O–deuterioethanol solvents are reported in Figure 4, where the pD values obtained by the relation $\text{pD} = \text{pH}_{\text{app}} + 0.4$[6] are indicated. Figure 4a refers to 20% deuterioethanol: this spectrum corresponds to the ionized, randomly coiled form, with a broad band at 1648 cm^{-1} (amide I′ band). Spectrum b refers to the conformation obtained by slow titration down to pD 11.6. Two sharp bands appear at ~1683 and ~1623 cm^{-1}, clearly related to the fully antiparallel β conformation. Only the 1648-cm^{-1} band appears in the spectrum c, which refers to a solution rapidly titrated down to pD 11.3; it exhibits the new ORD pattern, with a peak at *ca.* 236 mμ and a trough at 226 mμ. This supports the fact that under these experimental conditions PT cannot assume the β conformation. In 40% deuterioethanol and at pD 11.4 some amount of β conformation is still present if the titration is performed slowly (Figure 4d), while in 60% deuterioethanol and pD 11.5 (Figure 4e) only the *ca.* 1648-cm^{-1} band is observed. These results confirm the loss of stability of the β conformation with increasing ethanol concentration. No conformational indication may be drawn, on the other hand, from the ir spectra in D_2O–deuterioethanol mixtures under conditions where the unusual ORD pattern is observed; in the amide I′ region almost the same frequency is found for both the random coil and the α helix, and strong

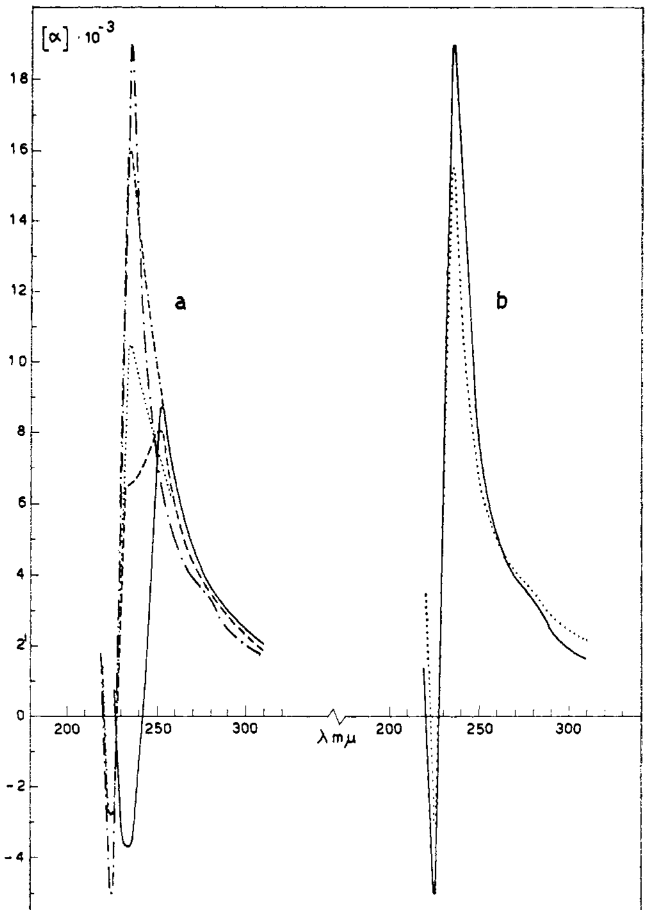

Figure 3. ORD curves of poly(L-tyrosine) at 25°: (a) in 60% ethanol (0.1 *M* KCl) pH 13.2, ——, pH 11.37, ----, pH 10.97, · · ·, pH 10.8, -·-·-, in trimethyl phosphate, -·-·-; (b) in 80% ethanol (0.1 *M* KCl) pH 8.85, · · ·, in trimethyl phosphate, ——.

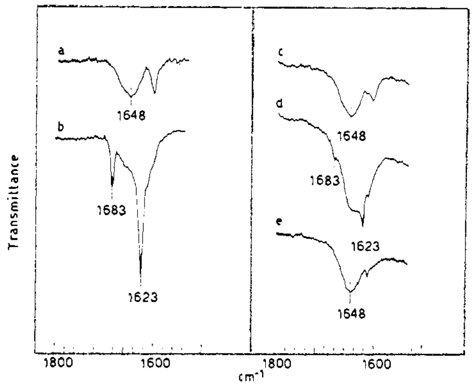

Figure 4. Infrared spectra of poly(L-tyrosine) in mixed D_2O–deuterioethanol solvents at *c* 0.6%: (a) 20% deuterioethanol, pD 13.0; (b) 20% deuterioethanol, pD 11.6 (slow titration); (c) 20% deuterioethanol, pD 11.3 (rapid titration); (d) 40% deuterioethanol, pD 11.4 (slow titration); (e) 60% deuterioethanol, pD 11.5.

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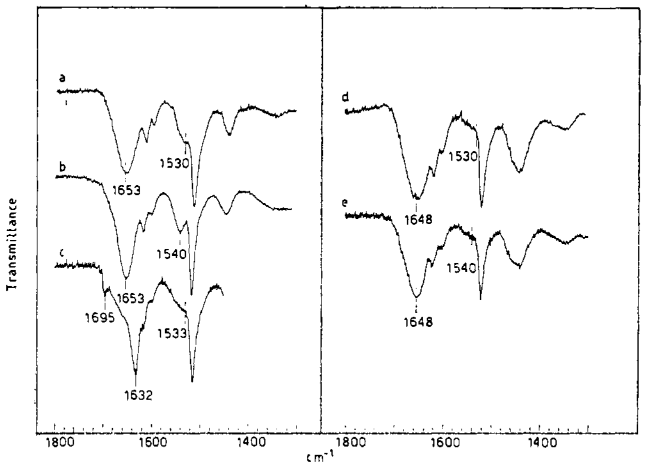

Figure 5. Infrared spectra (amide I and amide II regions) of poly(L-tyrosine) scanned on films: (a) film obtained by precipitation with concentrated HCl from aqueous solution at pH 12.5; (d) film obtained from D_2O, same conditions as for (a); (b, e) film obtained from 60% ethanol and 60% deuterioethanol, respectively; (c) sample in H_2O slowly titrated down to pH 11.2 and precipitated with concentrated HCl.

bands from deuterioethanol in the region around 1400 cm^{-1} prevent the amide II′ band from being observed in solution.

In order to investigate further the conformation of PT, determinations in both the solid state and in solution were carried out, the latter in mixed water–ethanol solution (40:60 v/v) in the region around 1550 cm^{-1} (amide II) where the more significant differences in absorption between the random coil and the α helix are to be expected. The ir spectra in the solid state were scanned on films directly cast on the CaF_2 windows, according to the procedure described above. We have already shown[4] that, as far as the coil → β transition is concerned, the abrupt addition of concentrated HCl to the solution causes the polymer to maintain in the solid state the conformation present in the original solution. In Figure 5, spectrum a refers to a sample precipitated from aqueous solution at pH 12.5; it shows a shoulder at ~1530 cm^{-1}, where the amide II band is expected to occur for the randomly coiled conformation. This band vanishes upon N-deuteration (Figure 5d). The β pattern is exhibited by a sample slowly titrated in water down to pH 11.2 (Figure 5c). Samples obtained from 60%, as well as from 20%, ethanol (at 20% ethanol, of course, a fast lowering of pH is required, while at high ethanol concentration only one kind of transition, which is rate independent, is observed) show small but significant variations in the amide II band (Figure 5b). We observe that the intensity of this band, which is now well separated from the side chain band at 1517 cm^{-1}, increases, while the frequency shifts up to 1540 cm^{-1}. This frequency is characteristic of the α-helical conformation.[7,8] This result strongly suggests that the α helix is present in 20% ethanol solution in the rapid titration experiments and that it is the only possible ordered conformation in the alcohol-rich mixtures. On the other hand, one cannot completely rule out that a conformational transition takes place when the solvent is removed, and that more direct evidence for the α-helical conformation in solution is needed. Such evidence is reported in Figure 6-1, where the ir spectra of PT in a water–ethanol mixture (40:60) are shown as a function of pH. At pH 13, where α = 1, the 1633-cm^{-1} band corresponding to the random coil is shown, while the α-helix band at 1643 cm^{-1} is shown to appear at a pH of 11.3. Finally the curve at pH 10.96 corresponds to the α-helical conformation.

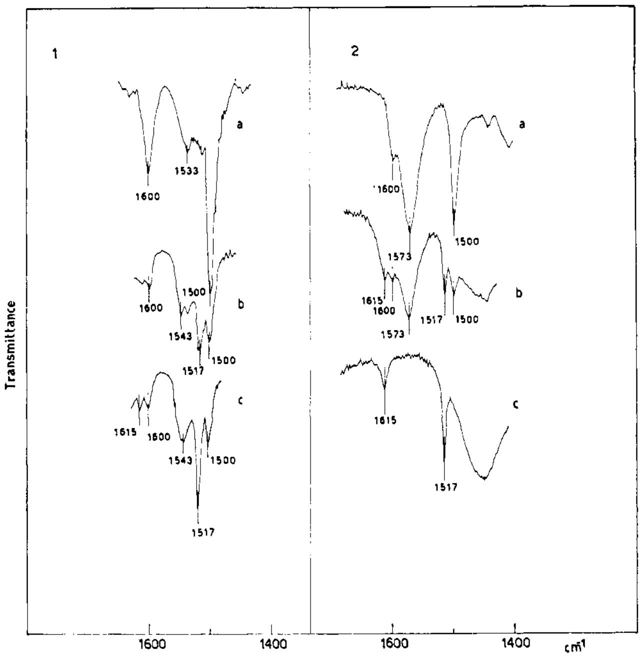

Figure 6. (1) Infrared spectra of poly(L-tyrosine) (amide I region) in 60% ethanol: (a) pH 13, (b) pH 11.3, (c) pH 10.96; (2) infrared spectra of L-tyrosine in D_2O: (a) pD 13.4, (b) pD 9.2, (c) pD 1.4.

If compared with the ORD data reported in Figure 3, these results show clearly that the sharp spectral variations observed in ultraviolet dispersion curves fall in the same pH range where the coil to α-helix transition occurs.

Other important spectral changes may be detected as a function of the degree of ionization in the ir spectra of PT; at 1600 and 1500 cm^{-1} two sharp bands are present for the fully ionized poly(α-amino acid). By lowering the degree of ionization they decrease in intensity, while two new bands appear at 1517 and 1615 cm^{-1}. They arise from the aromatic side chain and reflect only the effect of ionization. It may be seen on the basis of the spectra we have reported that they are completely insensitive to conformational changes and vary in a regular manner as a function of α alone. From Figure 6-2, where the spectra of L-tyrosine in D_2O at different pH's are reported, it is apparent that these bands are independent of the main chain and consequently of its conformation. The strong band at 1573 arises from the carboxylate ion and cannot, of course, be detected in the high molecular weight PT. This measurement was made in order to eliminate the possibility that such spectral changes could be related to some specific arrangement of the side chain in a given conformation.

Recently, Quadrifoglio, *et al.*,[9] have reported the ir spectra of films of PT cast from trimethyl phosphate (TMP) and directly in trimethyl phosphate solution which agree well with ours. These authors have concluded that PT assumes the α-helical conformation in this solvent, and were able to support this interpretation by the analysis of partially oriented

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samples in polarized radiation. The ultraviolet ORD curve of PT in TMP is shown in Figure 3. It is very close to the curve in 60% ethanol, although in the latter solvent some broadening of the 236-mμ peak results (Figure 3a). ORD determinations carried out in 80% ethanol at low charge almost coincide with those obtained in TMP (Figure 3b) with some difference in the $[\alpha]$ value ($\sim$15%) near 236 mμ, probably reflecting specific solvent effects on the electronic transitions.

Thus, the ORD curves reported in Figure 3b must be related to the existence of the fully α-helical conformation of PT in a low state of charge in mainly organic media, while the complex spectral variations reported in Figure 2b and 3a arise from the charge-induced coil to helix transition. It must be pointed out that both the conformational transition and the decrease of the degree of ionization α may contribute to the ultraviolet dispersion spectra in a way which cannot be deduced by the analysis of ORD data alone. The observed independence of the ORD curves for the random coil of changes of α over an extended range allows us to conclude that the spectacular change of the rotation near 236 mμ which, on the contrary, occurs in a narrow α range, essentially reflects the pure conformational contributions.

The Concentration Dependence of the Coil to β Transition. Our interest in investigating how polymer concentration affects the coil to β transition arises chiefly from the fact that an intermolecular nucleation process should become less favored at high dilution, while an intramolecular one should not be affected at all. In water, our search for such concentration dependence was unsuccessful for values of the concentration down to 0.04%. This fact, however, was not considered as definitely supporting the intramolecular mechanism, because even at such a low concentration the polymer was completely aggregated at the end point of the transition. On the other hand, in water–alcohol mixtures, the β form is less stable, owing probably to a decreased strength of the side chain–side chain interactions, facilitating the detection of a concentration dependence in an experimentally accessible range of compositions.

The results reported in Figure 7 refer to slowly titrated solutions down to pH 11.25 in 20% ethanol, at different polymer concentrations ranging from 0.04 to 0.41%.

It is seen that concentration strongly affects the conformation attained by this procedure. At 0.04% no peak at 286 mμ is found, while in the 236-mμ region the curve shows the presence of some α-helical conformation.

The effect of increasing concentration causes the β pattern to appear at 0.08%, while a fully β conformation is attained for concentrations higher than 0.2%.

This result clearly points to an intermolecular mechanism of transition. It is probably of a kinetic nature, because it is experimentally difficult, at so low a concentration and in solvent conditions where the β form is considerably less stable than in water, to perform the titrations at a sufficiently slow rate in order to attain an equilibrium condition. As a matter of fact, once the full β conformation has been obtained at high concentration in 20% ethanol, it may be diluted down to 0.08% without any appreciable change in the ORD spectra (on letting the solution stand several days). This seems to support the stability of the β form at concentrations lower than is indicated by the titration experiments. Although further investigation of the concentration dependence of the coil to β transition will be of interest, the discussion of the thermodynamic results reported below requires only that the intermolecular nature of the transition be taken into account.

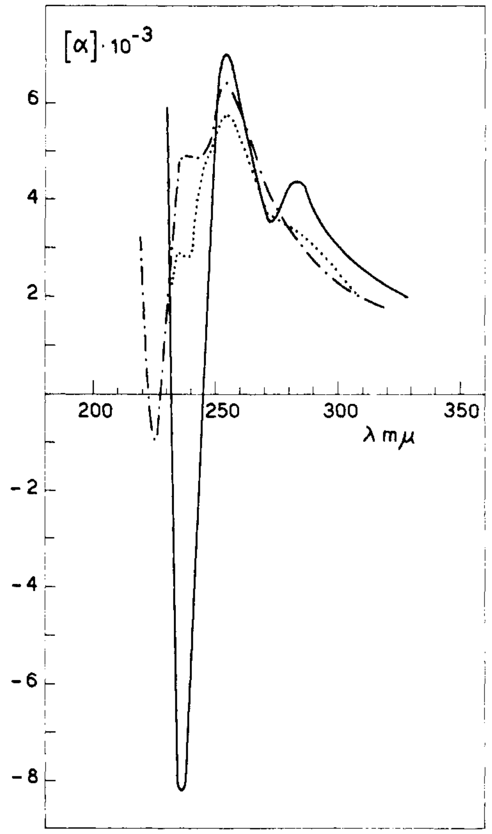

Figure 7. Poly(l-tyrosine) in 20% ethanol (0.1 *M* KCl) slowly titrated down to pH 11.25 at different concentrations: *c* 0.04%, -·-·-·-; *c* 0.08%, ···; *c* 0.41%, ——.

Discussion

We have already pointed out that alcohol destabilizes the β form. This effect is quite similar to the solvent-induced native → denaturated form of globular proteins[10,11] and indicates that the β form of PT posses a "micellar" structure, with a predominant contribution of hydrophobic interactions, side chain–side chain stacking probably being involved. It is clear that the disruption of such interactions may be obtained by the addition of alcohols, trimethyl phosphate, and several other organic solvents, and therefore in these prevailing organic media PT may assume a different conformation. Such a conformation is the PT α helix (which has been reported by Applequist and Mahr,[12] on the basis of dielectric dispersion determinations, to have the left-handed α-helical conformation in quinoline). It must be remarked that, as a rule, the denaturated form of globular proteins in water–organic solvent mixtures often possesses a high α-helical content and that, on the other hand, the ability of alcohols to enhance the stability of the α helix in simple poly(α-amino acids) is well known, and has been extensively investigated.[13–15]

No matter how effective alcohol is in promoting the α-helical conformation, the stability of this form for PT, too, increases in going from water to alcohol-rich mixtures. In a very schematic way, we may now represent the free energy per

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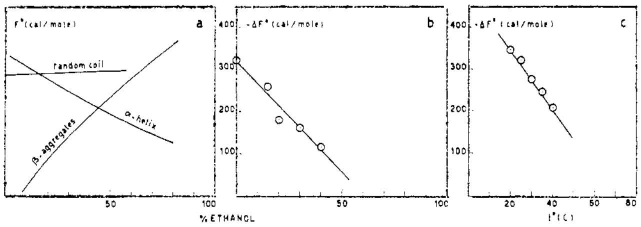

Figure 8. (a) Schematic diagram representing the dependence of the free energy, F°, for uncharged conformers on solvent composition; (b) free energy, ΔF°, of formation on the uncharged β aggregates from the uncharged random coil at 25° as a function of ethanol per cent; (c) free energy of formation of the uncharged β aggregates from the uncharged random coil in 0.1 M KCl as a function of the temperature.

mole of residue of uncharged conformers, F^0, as a function of ethanol content in Figure 8a. In water the β conformation is more stable than either the random coil or the α helix. If, by a rapid lowering of pH, the slowly occurring coil to β transition is by-passed, the random coil is observed at every value of the charge. At a certain alcohol concentration, between 0 and 20% we have $F^0_{\mathrm{coil}} > F^0_{\mathrm{helix}}$, so that the uncharged α helix becomes more stable than the random coil, and the coil → α-helix transition is encountered in the rapid titration experiments.

Above the composition corresponding to the crossing of the F^0_{helix} and F^0_β curves, the α helix is the stable conformer, and for higher values of ethanol concentration the coil → α-helix transition occurs independently from kinetic factors.

We may now attempt to give a more quantitative interpretation of our results, considering that the $\mathrm{p}K_{\mathrm{app}}/\alpha$ curve which we have reported here and in the preceding paper allows us to evaluate the free energy of transition between uncharged random coil and β form as a function of alcohol content and of the temperature.

The area between the extrapolated titration curve of the random coil and the β aggregates, titration curve is, of course, a measure of such a free energy change, according to the relationship[16,17] $\Delta F^0 = -RT\int \alpha \mathrm{d} \ln a_{\mathrm{H}}$. It represents, in other words, the free energy of formation of uncharged equilibrium β aggregates from uncharged random coil, and is, therefore, in the hypothesis of an intermolecular mechanism, the really meaningful quantity. It must be remarked that the experimental values may be affected by a rather large error, owing to the very narrow range of α in which the titration of the random coil occurs in several cases, and to the fact that the point of incipient transition is often not easily detectable. The values of the free energy of formation of the uncharged β form from the uncharged random coil, ΔF^0, at 25° and at 0, 15, 20, 30, 40% ethanol are reported as a function of the solvent composition in Figure 8b. This plot yields a value of about 60% for the ethanol composition at which $\Delta F^0 = 0$. Such points cannot of course be experimentally observed, because above 45% ethanol the α helix becomes the uncharged stable conformer. Finally, values of ΔF^0 in 0.1 M KCl are reported as a function of the temperature in Figure 8c. From this plot the values of ΔH^0 and ΔS^0 are obtained, which are equal to -2400 cal/mol and to *ca.* -7 eu, respectively. They probably include the contribution of side chain–side chain interactions, as seems to be indicated by their strong negative values as compared with the experimental values of the enthalpy and entropy of hydrophobic bond formation for tyrosyl residues reported by Brandts.[18]

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A Study of the Effect of Impurities in Living Anionic Polymerization

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Received October 26, 1970

ABSTRACT: Kinetics of homopolymerizations of polystyrylcesium were investigated in tetrahydrofuran at 25° by using solvents of various degrees of purity, and those of polystyryllithium were also briefly studied in 2-methyltetrahydrofuran. The apparent propagation constant k_{p} was greatly decreased by using seed polymer solutions, the purity of which was not sufficiently high. From conductivity measurements, it was demonstrated that the decrease in k_{p} was due to that in the free-ion rate constant k_{p}''. The value of k_{p}'' was 1.3×10^5 M^{-1} sec^{-1} when the system was most elaborately purified, and decreased to 4×10^4 M^{-1} sec^{-1} in the presence of impurities.

The anionic polymerizations of styrene in ethereal solvents have been studied by several authors.[1–5] However, the agreement among kinetic data reported is not always satisfactory. The cause of the disagreement has been discussed by the Syracuse and Mainz groups.[6–10]

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