Notes

Helix–Coil Transition of Poly(L-tyrosine) in Aqueous Ethanol

ELIZABETH PATTON and HENRY E. AUER*

Department of Biochemistry and Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, New York 14642.
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Poly(L-tyrosine), $(\text{Tyr})_n$, takes on an α-helical conformation in numerous organic liquids.[1–7] In aqueous solution $(\text{Tyr})_n$ assumes the antiparallel β conformation at low ionization of the phenolic hydroxyl groups.[8–11] As the proportion of ethanol in mixed aqueous–ethanol solvents increases a transformation from the β structure to the α helix is observed[9,12] between 20 and 40% ethanol. Potentiometric titrations of $(\text{Tyr})_n$ in water manifest large values of ΔG^0, the standard free energy change per residue for the conformational transition of the un-ionized polypeptide,[8–10] when plots of $\text{p}K_{\text{app}}$ vs. α, the degree of ionization,[13] are made. This observation has been attributed to the formation of the aggregated β structure.[8,10] As the ethanol content increases and the transformation becomes a helix–coil transition, the value of ΔG^0 diminishes, until in 60% ethanol the Zimm–Rice plot appears linear.[9] This could indicate either that there is a transformation whose ΔG^0 under the conditions examined is essentially zero, or that no conformational transition of any kind occurs as the titration proceeds. As examples of the second possibility, both neutral and fully charged poly(L-lysine) are α helical above 90% methanol in water[14] and poly(L-glutamic acid) and its sodium salt are α helical at ethanol contents greater than 50%.[15]

Infrared spectroscopic studies have shown that in mixed aqueous–organic solvents $(\text{Tyr})_n$ generates the α helix,[7,9,11] and that at high ionization a disordered conformation is found.[9] In this article we examine the transition between these states using circular dichroism (CD) spectroscopy and sedimentation velocity experiments in 50% (v/v) aqueous ethanol. In addition, a hydrodynamic model for the helical state is proposed.

Experimental Section

$(\text{Tyr})_n$ was obtained from Sigma Chemicals (St. Louis, Mo.) and Pilot Chemicals (Watertown, Mass.). Solutions were made up in 50% (v/v) ethanol, 0.05 M NaCl, and 0.0125 M Na_2HPO_4. Concentrations were determined spectrophotometrically in 0.5 M NaOH.[16] pH was measured with combination electrodes having low sodium ion corrections. CD spectra were recorded at 27 °C on a Cary 60 spectropolarimeter equipped with the 6002 CD accessory. Ultracentrifugal studies were done at 25 °C using a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner. Polymer concentrations in both types of experiment were 7×10^{-4} M residue or less. Because of the high dilution, observed values of the sedimentation coefficient are identified as the values at infinite dilution.

Results and Discussion

CD spectra of $(\text{Tyr})_n$ over a broad range of pH values in 50% ethanol are presented in Figure 1. At pH_{app} above 13 there is a broad maximum at 245 nm, about the same wavelength at which L-tyrosinate[17] and poly(L-tyrosinate) have absorption maxima[17,18] and CD extrema[11,19] in water. At pH_{app} 11, where α is quite low,[9] the band at 245 nm has virtually disappeared, and a sharp band at 230 nm, near absorption bands found for the neutral forms of L-tyr[17] and $(\text{Tyr})_n$,[18] develops instead. This spectrum is evidently due to the α-helical form[7,9] of $(\text{Tyr})_n$. It agrees with numerous CD spectra of the helical form,[2,4–6,12] although the intensity at 230 nm is slightly lower. There clearly is no isoelliptic point common to all the spectra in Figure 1. The occurrence of such a phenomenon would have been expected, for example, in the case of a titration of the chromophoric side chains in the absence of a conformational transition. Thus a transformation which affects the optical activity of the side chains apparently occurs concomitantly with the titration.

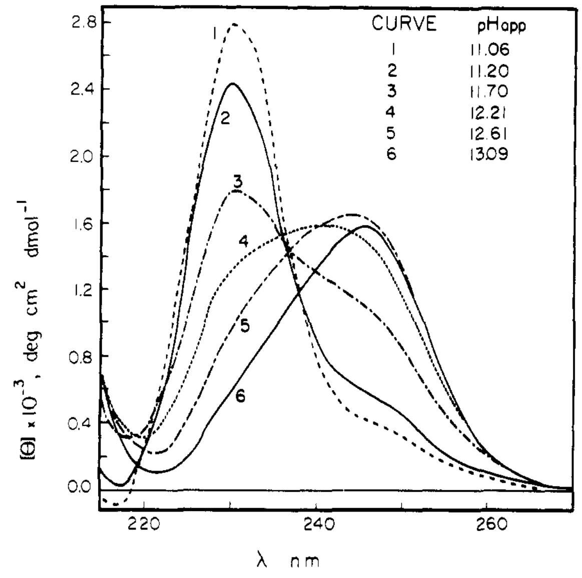

Figure 1. CD spectra of $(\text{Tyr})_n$ in 50% (v/v) ethanol at various pH_{app} values.

The pH dependence of the CD amplitudes at 230 and 245 nm is shown in Figure 2a. The molar ellipticities at both wavelengths exhibit broad, essentially monotonic changes with pH with no apparent inflection points. The fractional changes monitored at the two bands do not have similar pH profiles, especially above pH 11.5. This, and the finding of absorption hypochromicity at 193 nm in α-helical $(\text{Tyr})_n$,[2] suggest the occurrence of electronic excitation interactions in the ordered form.[20]

Values of $s_{20,\text{w}}$ as a function of pH are shown for two samples of $(\text{Tyr})_n$ in Figure 2b. Both samples follow the same pattern. A linear decrease in $s_{20,\text{w}}$ is found as the pH_{app} falls from 12.6 to 11.4. Below pH_{app} 11.1 two sedimenting components were observed. The rapid component is attributed to aggregated α-helical[7,9] $(\text{Tyr})_n$, and the slow fraction to the nonaggregated species. Between pH_{app} 11.1 and 11.4 single sedimenting boundaries were observed having values of $s_{20,\text{w}}$ higher than those just above pH_{app} 11.4. This probably arises from incipient aggregation whose dynamics are rapid on the sedimentation time scale. Our interest in this work remains expressly with the nonaggregated species at low ionization.

Reasonable estimates of the effects of counterion binding on M_2, the polymer molecular weight, and on $\bar{v}_2$, the partial specific volume, were made,[11] employing the conclusions of Alexandrowicz and co-workers.[21,22] Approximating α from the titrations of Conio et al.,[9] it is found that the factor $M_2(1 - \bar{v}_2\rho)$ decreases by about 15% between pH 12.7 and

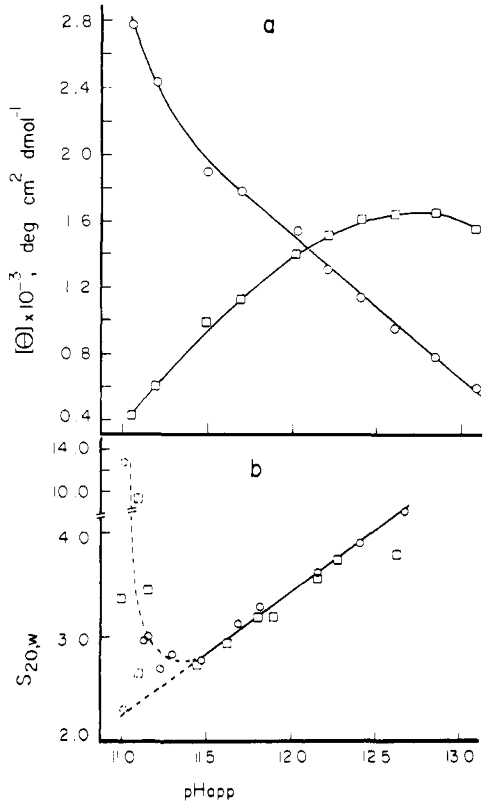

Figure 2. (a) pH dependence of $[\theta]_{230}$ (○) and $[\theta]_{245}$ (□) for $(Tyr)_n$ in 50% (v/v) ethanol. (b) pH dependence of $s_{20,w}$ for $(Tyr)_n$ in 50% (v/v) ethanol: (○) $(Tyr)_n$ from Sigma; (□) $(Tyr)_n$ from Pilot. Dashed curves are used in the region where aggregation is thought to occur. Dashed symbols indicate $s_{20,w}$ values calculated for each component in cases where two boundaries were observed.

11.4. It is likely, in addition, that $(Tyr)_n$ adsorbs ethanol preferentially in the solvent system used here.[23] The amount bound may be expected to respond in a limited way to changes in ionization, but significant desorption is considered likely upon passing from the disordered chain to the α helix. If the degree of preferential binding were to decrease from 0.8 to 0.5 g of ethanol per gram of polymer in this process, the buoyant mass of the hydrodynamic particle[24] would increase by 24%, thus counteracting the putative effect of the change in counterion binding just considered. These potential effects are therefore deemed unlikely to account for the observed large decrease of $s_{20,w}$ over the pH range shown in Figure 2b. It is more likely due to the occurrence of a broad transition between a relatively compact disordered state at elevated pH_{app} and an asymmetric helical form at low pH_{app}. This contrasts with the sharp discontinuities in curves of s vs. pH reported for the helix-coil transition in poly(L-lysine)[25] and for the intramolecular β-coil transition in $(Tyr)_n$ in aqueous solution.[11]

The absence of an isoelliptic point in the CD spectra and the large change in the value of $s_{20,w}$ support the view that $(Tyr)_n$ undergoes an ionization-dependent α-helix-disordered chain transition in 50% ethanol, which had been hinted at in earlier infrared and potentiometric titration studies.[9] The transformation occurs over a range of about 2 pH units, which is approximately as broad as the titration curve illustrated[9] for 60% ethanol. This is consistent with the observation that ΔG^0 in the latter solvent is zero.

Let us evaluate the experimental frictional ratio f/f_{min}[26] at low ionization to see whether it is consistent with a hydrodynamic model for an α-helical rod. The molecular weight of the Sigma sample was determined by equilibrium ultracentrifugation; the result is 78 500 daltons using a value for $\bar{v}_2$ of 0.71 cm^3/g.[27] The value of $s_{20,w}$ at pH_{app} 11.48 is 2.78 S, leading to $f/f_{min} = 2.57$. It is evident from Figure 2b that as the state of zero ionization is approached, the limiting value of $s_{20,w}$ for the nonaggregated helix could be 20% or more lower than the value just quoted, so that the value of f/f_{min} would be proportionately increased. If it is assumed that 0.5 g of ethanol is bound per gram of polymer,[23] the correction for the volume of this adsorbed solvent reduces the contribution of asymmetry to about 81% of the observed value of f/f_{min}.[26] This is compensated by an augmented contribution from asymmetry of about 13%, which arises upon correction for the increased diameter of the cylindrical particle due to the adsorbed ethanol, assuming the latter forms a uniform shell about the polymer. Thus the two effects approximately cancel each other.

A simple hydrodynamic model for a cylindrical α-helical rod of $(Tyr)_n$ is the corresponding prolate ellipsoid of revolution.[4,26] Using the molecular weight, the axial displacement per residue in the α helix of 1.50 Å and a helical diameter of 16.0 Å estimated from a Corey-Pauling-Koltun model, we get for f/f_0, the frictional ratio due to asymmetry alone,[26] the value 2.57. Alternatively, using an experimental value for the diameter of 13.2 Å,[4] f/f_0 is 2.80. These values are reasonably consistent with our determination of f/f_{min}, keeping in mind the experimental uncertainties for the latter quantity discussed above. Thus at low ionization a good model for α helical (Tyr) in 50% ethanol is a stiff cylindrical rod. Other plausible hydrodynamic models are of course not excluded by the treatment presented here.

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