Thermodynamic Parameters for the Intramolecular Disordered-to-β Transition of Poly(L-tyrosine) in Aqueous Solution[1]

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ABSTRACT: We have examined the disordered-to-β transition of two samples of high molecular weight poly(L-tyrosine), a polypeptide with an ionizing side chain, by potentiometric titration of solutions of this polymer in aqueous buffer. The degree of ionization was determined spectrophotometrically from the absorption of the tyrosinate anion at 293.5 nm. By using polymer solutions of high dilution the region of the conformational transition was separated from that of aggregation. As a consequence it was possible to obtain titration data for the intramolecular transition which we believe are free of interference from aggregation. For this transition we found that $\Delta H^\circ = -1330$ cal/mol of residue. The two samples gave values of -3.42 ± 0.14 and -3.85 ± 0.10 u for the change in entropy. Possible artifactual origins for this distinction have been examined and may be discounted. Rather, it probably reflects a difference in the β structure formed which depends on molecular weight.

In most organic solvents, poly(L-tyrosine) assumes the α-helical conformation.[2-13] However, in aqueous media, a disordered-to-β transition occurs as the degree of ionization (α) decreases.[11,12,14-18] Potentiometric titrations on polyelectrolytes which undergo conformational transitions as a function of α can be applied to obtain values of the thermodynamic parameters for the transformation.[19-23] In the case of poly(L-tyrosine), however, at the relatively high concentrations required for titration experiments the β conformation readily undergoes aggregation, so that the data cannot be analyzed to yield values of ΔH° and ΔS° for the conformational transition which are independent of the aggregation phenomenon.[12,15,16]

The ionizing hydroxyl group of poly(tyrosine), however, is part of a chromophore so that the ionization may be followed in very dilute solution through the use of spectrophotometry. The transition can therefore be induced and followed in solutions which are dilute enough that the polymer remains unaggregated until α reaches a value about 0.15 unit below its value at the conformational transition point. This extends sufficiently the range in α prior to the onset of aggregation that a reasonable extrapolation of the titration data for the ordered form to pK_0 can be made, and the thermodynamic parameters for the nonaggregating system can be evaluated.

We have carried out such potentiometric–spectrophotometric titrations on two samples of poly(L-tyrosine) whose molecular weights differ by a factor of 3 in order to evaluate the thermodynamic parameters for the intramolecular disordered-to-β transition, uncomplicated by simultaneous aggregation. Our two samples show significantly different behavior, and we believe this to be due to a difference in the β sheets formed in the transition.

Previous studies[17] have shown that solutions of poly(L-tyrosine) at concentrations of 2×10^{-4} residue molar remain nonaggregated at 0.1 pH unit below the transition point in sodium phosphate buffer containing 0.1 M NaCl salt. This concentration was therefore adopted for the titration studies reported below. Furthermore, a somewhat lower ionic strength (ca. 0.03 M Na^+) was employed in order to inhibit precipitation at low α[24] by increasing the electrostatic repulsion between polymer molecules.

Experimental Section

Materials. Two samples of poly(L-tyrosine) were obtained from commercial suppliers. That obtained from Pilot Chemical (Lot No. T-52) was purified before use by dissolution in aqueous alkali and reprecipitation by acid. The precipitate was collected by centrifugation, washed several times to remove salts, and lyophilized. The polymer obtained from Sigma (Lot No. 31 C 5140) was purified by dialysis against aqueous alkali before acid precipitation, collection, washing, and lyophilizing.

Molecular weights of the purified samples were determined by equilibrium ultracentrifugation using a Beckman Model E Analytical Ultracentrifuge equipped with a photoelectric scanner. The Pilot sample showed an average value of 99 700 ± 3 400; the Sigma sample, 27 300 ± 750.

The standard pH buffers were purchased from Mallinckrodt (pH 10.00 at 25 °C) and E. Merck (pH 11.00 and pH 12.00 at 20 °C). The Merck buffers were supplied in concentrated form and were made up in a nitrogen atmosphere to minimize CO_2 dissolution in the buffers.

The titrant used in the titration experiments was Fisher Certified Grade HCl (1.0 ± 0.0001 N).

Buffer for the polymer solutions was made up from reagent grade materials and distilled, deionized water. Dibasic sodium hydrogen phosphate ($Na_2HPO_4 \cdot 7H_2O$) was weighed out and dissolved in water, then the solution was titrated to pH 12.2 using concentrated sodium hydroxide solution. This stock buffer (0.01 M in PO_4) was used for all experiments.

The concentration of all sample solutions was determined spectrophotometrically at 293.5 nm after dilution with 1 N NaOH in a 1:1 ratio. A value of ϵ 2250 cm^{-1} mol^{-1} was used for the extinction coefficient.[25]

Description of Apparatus. A Cary 16 Spectrophotometer was fitted with a thermostatable holder for a rectangular, 1 cm path length, quartz cell. A combination glass–calomel electrode was introduced through the Teflon cap into the spectrophotometer cell above the light path to allow simultaneous measurement of optical density and pH of the solution in the cell. The conformational transition was monitored at 293.5 nm, where the disappearance of the tyrosinate anion with decreasing pH was followed.

Serial titration of the sample was done using 1 M HCl in a thermostated vessel external to the spectrophotometer cell compartment. Titrant was added to the sample solution using a micrometer syringe-buret. Transfer of the sample from the titration vessel to the spectrophotometer cell was accomplished using a manual syringe-pump and a three-way stopcock valve. About 3 to 5 min were allowed for equilibration of the sample solution in the cell compartment before readings were taken.

To minimize evaporation of the solution over the 5- to 6-h period of the titrations, the titration vessel was flushed continuously with nitrogen which had been bubbled through distilled water prior to admission to the vessel. To prevent changes in pH due to absorption of atmospheric CO_2, the spectrophotometer cell compartment was flushed with dry nitrogen.

Measurement of pH. All pH measurements were made with a Radiometer pHM26 meter equipped with a Sargent-Welch microcombination electrode (No. S-30070-10). The pH readings were made on the meter's expanded scale allowing pH values to be read to ±0.002 pH units. The sodium error for this combination electrode is less than 0.01 H unit under our experimental conditions.

The range of pH covered in our titrations is approximately 10.5 to

12.2. Since the Iso-pH setting (electronic zero) of the meter could not be set to a value higher than 10, and the transition occurs above pH 11, we found it necessary to correct the experimentally determined pH values for the changing sensitivity of the electrode with increasing pH. To make these corrections, we employed three buffer solutions whose pH values and temperature coefficients are known. Buffer A has a pH value of 11.00 at 20 °C, buffer B is 12.00 at 20 °C, and buffer C is 10.00 at 25 °C.

pH Meter Calibration. A rigorous calibration was carried out at the inception of the titration experiments. The meter was first adjusted to read 11.00 using buffer A at 20 °C. The electrode sensitivity and the buffer null controls were then varied until a minimum discrepancy was obtained between the observed and standard values for buffer B. Since these two controls interact unless the Iso-pH setting is the same as one of the buffer values, these adjustments had to be made by successive approximation and repeated readjustment until the desired readings were obtained. The final settings reduced the pH range of 11 to 12 to a difference in meter readings of 1.1 pH units. After we had obtained the proper setting of the electrode sensitivity by this method, it remained fixed at this value for the remainder of the work.

We then carried out a calibration run at 25 °C using pH buffers covering the range 11 to 12 by 0.1 unit steps. The meter was standardized with buffer A, the value of buffer B recorded, and the values of the other buffers then read from the meter. The resulting plot of true pH vs. apparent pH fit well to the straight line between the points for buffers A and B. Likewise, several buffers between pH 10 and 11 were found to fit reasonably well to the straight line between the points for buffers A and C.

Before each experimental run, the meter was standardized with buffer A at the temperature of that run. Apparent values for buffers B and C were then recorded, along with the known true pH of these buffers at that temperature. The true pH value of each experiment point was found by linear interpolation between the observed values for the two buffers which bracketed that experimental value.

Calculation of α and pK_a. The tyrosinate anion has an absorption maximum at 293.5 nm and an extinction coefficient of 2250 when in a polymer chain.[25] Since the tyrosine moiety has an extinction coefficient of 25 at this wavelength, the degree of ionization of the polymer in solution may be found using the formula

$$\alpha = (\epsilon_{293.5} - 25)/2225 \quad (1)$$

The apparent equilibrium constant of the transition, K_a, may be found using the equation[20-22]

$$pK_a = pH - \log \frac{\alpha}{1 - \alpha} \quad (2)$$

A Zimm–Rice plot[19] of the apparent pK vs. α can therefore be constructed for analysis of the data.

Results

Potentiometric–Spectrophotometric Titrations. Examples of the Zimm–Rice plots for our samples are shown in Figure 1. The range in α over which data points were taken is limited on the upper end by our aim of working at low overall ionic strength (see the introductory section). On the lower end precipitation of the polypeptide obscured the absorption measurements. The temperature range studied was similarly limited. Even at the low concentrations used in our studies, precipitation could not be prevented below about 30% ionization. The degree of ionization at which this occurs is strongly temperature dependent, and below 10 °C precipitation begins before the titration reaches the region following the conformational transition (i.e., precipitation occurs shortly after the "peak" of the curve; see Figure 1). For this reason, experiments below 10 °C could not be evaluated even if carried out. Furthermore, as may be noted from Figure 1, the high-ionization "tail" of the curve becomes progressively shorter as the temperature is raised. It is not possible experimentally to increase the ionization further without increasing the ionic strength as well and thus possibly introducing other artifacts into the data. Above 41 °C it becomes impossible to determine where the bottom of the trough should be, and experiments could not be performed above this temperature.

The appearance of the curves in Figure 1 is characteristic of that observed in systems undergoing typical order–disorder

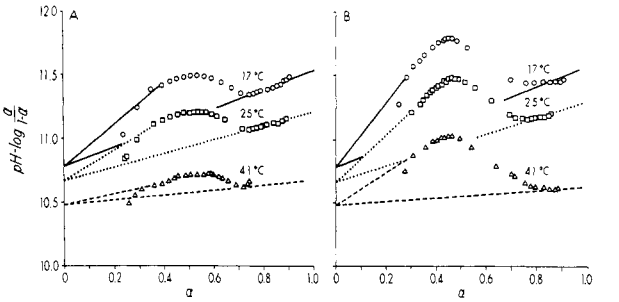

Figure 1. Zimm–Rice plots of experimental titration data on poly(L-tyrosine). Panel A is the Sigma sample (mol wt = 27 300); Panel B is the Pilot sample (mol wt = 99 700).

transitions.[12,14-16,19-24,26] A region at high α which is nearly linear corresponds to the titration of the disordered form of the polymer. A region at low α, also approximately linear, corresponds to the titration of the ordered form. Finally, a region between the other two is found in which the points pass from one titration curve to the other. This is assumed to arise from the conformational transition between the two limiting states. Infrared studies confirm that the disordered-to-β transition in our samples of $(Tyr)_n$ is occurring in the same region as the transition in the titration curves (R. P. McKnight and H. E. Auer, unpublished results). There can therefore be no question that the latter feature of the curves in Figure 1 is attributable to the conformational transition in question.

Cosani et al.[16] have demonstrated that the disordered-to-β transition of $(Tyr)_n$ observed spectrophotometrically is the same one as is observed potentiometrically. Figure 1 of their paper shows that the spectrophotometric points (as far as they could be followed prior to the onset of precipitation) and the potentiometric points fall on the same curve in the region of the transition.

To further check the validity of using the optical density to monitor changes in ionization which occur during the transition, we carried out a titration in which we followed the change in the absorption spectrum from 260 to 320 nm using a Cary 118 spectrophotometer. Because of the solubility characteristics of the poly(L-tyrosine), our titrations were always carried out from high pH to low, and thus also from high values of α to low. The resulting spectra for the Pilot sample at 25 °C (Figure 2) show two sets of isosbestic points.

The first set of isosbestics (A in Figure 2) is observed as the ionization decreases from 90 to 70%. It will be noted from Figure 1B that this range corresponds to the titration of the disordered form before the conformational transition begins. At about 70 to 75% ionization, the spectra shift to a second set of isosbestics at 269 and 284 nm (B in Figure 2) and continue to pass through these points until precipitation begins at about 35% ionization. The persistence of this second set of isosbestics throughout the range in α over which the conformational transition occurs, together with the results of Cosani et al.,[16] shows that the optical density is monitoring only the state of ionization of the side chains and is independent of the conformation over this range in α. Only in the disordered region are differences found in the spectrophotometric and potentiometric results.

Determination of $\Delta G°$ and pK_0. Using the Zimm–Rice treatment, Hermans[22] has shown that for titrations of proteins and polypeptides the change in free energy ($\Delta G°$) for the transition from an uncharged coil form to an uncharged ordered form may be found using the equation

$$\Delta G° = 2.3RT \int_0^1 (pK_{ad} - pK_{ae})\, d\alpha \quad (3)$$

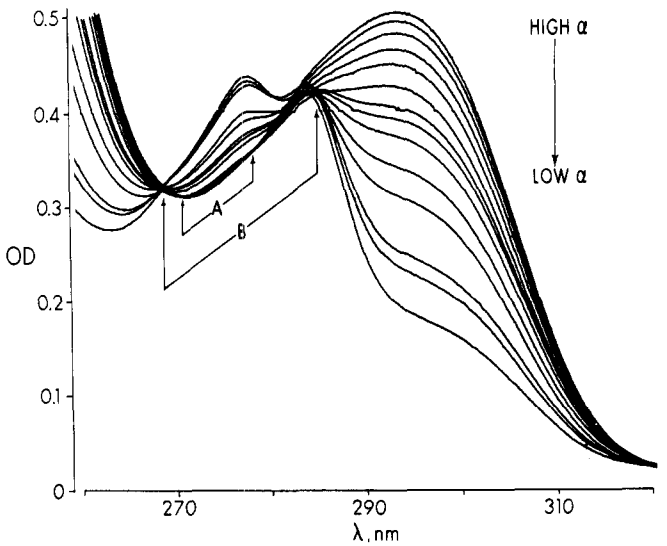

Figure 2. Spectra of the poly(tyrosine) sample from Pilot Chemical during sequential titration. Isosbestic set A is at high pH; set B is present from the onset of the conformational transition to the point at which aggregation occurs. In the final curve (minimum α) precipitation has begun.

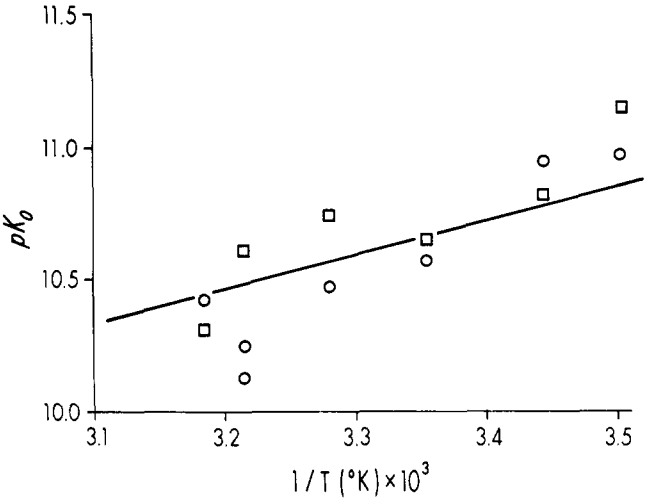

Figure 3. Plot of rough pK_0 vs. $1/T$: (○) Pilot sample; (□) Sigma sample. The line is calculated using the value $\Delta H°_{ion}$ = 6.0 kcal/mol of residue (see text).

where pK_{ae} is the experimental value of pK_a (eq 2) and pK_{ad} is the value of pK_a calculated from eq 2 for the hypothetical disordered form at the value of α. In Figure 1, the experimental points and their extrapolation to $\alpha = 0$ form the upper boundary of this area, while the extrapolation of the data at high α to pK_0 forms the lower boundary. The value of $\Delta G°$ is then proportional to the enclosed area.

Theory[20,23] predicts that the extrapolated curves are not straight lines, but rather exhibit some curvature. In practice, however, it has been found that the curvature is so slight that no significant difference in the resulting value of $\Delta G°$ (eq 3) occurs by using linear extrapolations.[22,23,26] We have therefore used the traditional method of linear extrapolation in this work.

As is obvious from the plots in Figure 1, the area determined depends on the value of pK_0 used for the extrapolations. In theory, the value of pK_0 for the ordered form should be identical with that of the disordered state.[27] This has in fact been shown to be the case for several different homopolypeptides.[12,14,16,23,24,26] In addition it may be assumed that, for long chains, pK_0 is independent of chain length. Since we found experimental difficulties with the extrapolations from both ends of the titration (see below), we have taken pK_0 to be identical for both samples of $(Tyr)_n$, in both the ordered and disordered forms, at a given temperature.

The problem of precipitation involved two competing effects. Most often, as the polymer began to precipitate, the solution became turbid, resulting in anomalously high optical density readings. This is clearly shown in Figure 1 where the experimental points below $\alpha = 0.30$ fall below and to the right of their anticipated location. Further addition of acid resulted in calculated values of α which increase, contrary to all expectations, in an acid titration. The second effect which occasionally predominated was that as the polymer precipitated, absorbing material was removed from the solution in the light beam. The optical density then dropped sharply, resulting in a "bump" in the Zimm–Rice plot which is similar to the precipitation "bump" in other potentiometric titrations.[12,13] The combined effect of these problems made the inclusion in the extrapolation of points below 30% ionization extremely tenuous, and all such points were excluded from consideration in the work reported here. Sedimentation velocity experiments confirm that aggregation is not observed above the pH value corresponding approximately to α = 0.3 (R. P. McKnight and H. E. Auer, unpublished results), well below the value of α at which the conformational transition is concluded.

At the highly ionized end of the transition, another problem arises. We mentioned above the shift during the titration from one set of isosbestics to another. This behavior indicates a breakdown of the two-state model at the high-ionization end of the transition. The data presented by Cosani et al.[16] also show anomalous behavior in that, at high ionization, the points determined optically fall below those determined from the pure potentiometric titration. Thus, whereas the absorption of the system in the region of the disordered-to-β transition is linearly related to α (see above), the unique set of isosbestic points in the disordered region (high α) characterizes a state in which this is apparently no longer true. It is not surprising, therefore, that in most titrations the region above 75% ionization contained no linear portion from which to make the extrapolation to $\alpha = 0$.

The procedure we adopted for determining pK_0 involved making an extrapolation from the data between 40 and 30% ionization to get initial values of pK_0 for each run. We then plotted these rough values of pK_0 against $1/T$ and fitted the points using a least-squares procedure (Figure 3).

The slope of the line in a pK_0 vs. $1/T$ plot depends on the heat of ionization (ΔH_{ion}) of a tyrosine residue. ΔH_{ion} is known to be 6.0 kcal/mol for the tyrosine monomer,[28] and a brief review of the literature[29] shows that 21 substituted phenolic compounds also have ΔH_{ion} values of from 5.2 to 6.4 kcal/mol. In addition, we find that a van't Hoff plot of available values of pK_0[12,15,16] for poly(L-tyrosine) (determined potentiometrically in 0.1 M KCl) yields a value for ΔH_{ion} of 4.90 ± 1.25 kcal/mol. Further evidence is drawn from the agreement in values of ΔH_{ion} for poly(L-glutamic acid) and acetic acid[23] and for poly(L-lysine), lysine, and ϵ-amino caproic acid.[30] It may therefore be concluded that ΔH_{ion} for the side chain of an ionizable polypeptide is well represented by that of an appropriate model compound.

We have consequently used the monomer value of ΔH_{ion}, 6.0 kcal/mol, as the slope of the least-squares line in our plot of rough pK_0 values vs. $1/T$. The equation of the line shown in Figure 3 was then determined by optimizing the intercept value. Optimal values for pK_0 at each experimental temperature were calculated from this equation, and the extrapolations to $\alpha = 0$ in the pK_a vs. α plots (Fig. 1) were made to these values of pK_0. Since the titration data originally used to de-

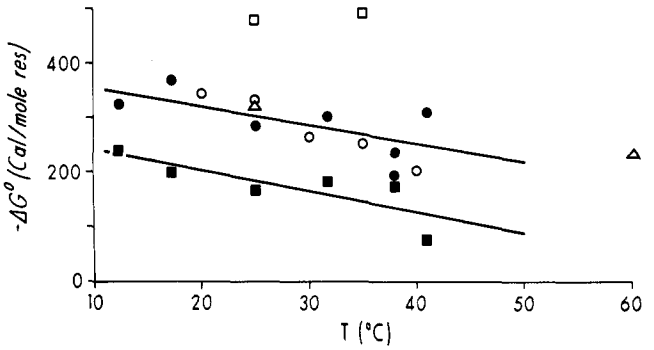

Figure 4. $\Delta G°$ values for the disordered-to-β transition of poly(L-tyrosine) plotted as a function of temperature. Experimental values: (●) from the Pilot sample; (■) from the Sigma sample. Literature values: (○) ref 12; (△) ref 16; (□) ref 15 (see ref 38).

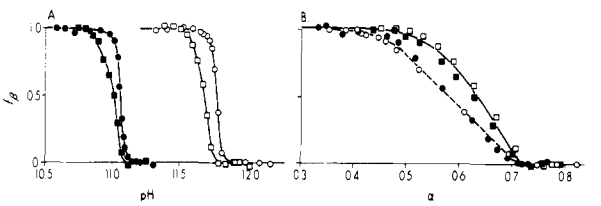

Figure 5. Plot of fraction of poly(L-tyrosine) in the β form vs. thermodynamic parameters: (A) f_β vs. pH, (B) f_β vs. α; (○) Pilot sample at 17 °C; (●) Pilot sample at 38 °C; (□) Sigma sample at 17 °C; (■) Sigma sample at 38 °C.

Table I
Values of $\Delta pK_a/\Delta\alpha$ for Poly(L-tyrosine) in the β Conformation at 25 °C

Origin	$\Delta pK_a/\Delta\alpha$
Patrone et al.[a]	1.82
Cosani et al.[b]	1.63
Senior et al.[c]	2.75
Pilot[d]	1.75
Sigma[d]	1.11

[a] Data taken from ref 12. [b] Data taken from ref 16. [c] Data taken from ref 15. [d] Data from Figure 1, this work.

termine the rough pK_0 values were those from low ionization, one would expect the fit of these data to the final extrapolations to be good, and this is indeed seen to be the case in Figure 1. No line from pK_0 to the disordered region produces a good fit to all of the data because of the curvature of the points in this region, as noted above. We have therefore chosen to draw the line through the minimum in the trough at high α. Where the trough is broad, the center of the level portion is taken as its minimum.

As can be seen from Figure 3, the rough pK_0 values for the two samples show no consistent trends with respect to differences from the averages, thus verifying the assumption that pK_0 does not vary with chain length.

Values of $\Delta G°$, $\Delta H°$, and $\Delta S°$. Figure 4 shows the temperature dependence of $\Delta G°$ for our two samples of poly(L-tyrosine) as well as the literature values from other potentiometric studies. Note that with the lower molecular weight sample, the values of $\Delta G°$ are only about two-thirds of those determined for the higher molecular weight sample. We believe this difference is significant, since the lines determined for the two samples lie further than one standard deviation from each other. These lines were calculated using a simple least-squares method. The values of $\Delta H°$ derived from these calculations, -1326 ± 40 cal/mol of residue for the Pilot sample and -1333 ± 28 cal/mol of residue for the Sigma sample, are the same for both samples within the calculated errors. The entropy changes were derived from point-wise evaluation using the value $\Delta H° = -1330$ cal/mol residue and the experimental values of $\Delta G°$. The averages are -3.42 ± 0.14 eu for the Pilot sample and -3.85 ± 0.10 eu for the Sigma sample. The difference in the $\Delta S°$ values is clearly more than twice the standard deviation of either, and it is this parameter which is apparently responsible for the differences in the experimental values of $\Delta G°$ between the samples shown in Figure 4.

Differences between the Samples. The titration results presented in Figure 1 contain a number of indications of differences between our two samples. First, it is evident to the eye that the areas required for evaluating $\Delta G°$ are smaller for the Sigma sample than for the Pilot sample, regardless of the precise value adopted for pK_0. This is the basis for the finding of the preceding section that the values of $\Delta S°$ differ. Second, consider the slopes of the titration curves for a given conformation. The value of $\Delta pK_a/\Delta\alpha$ is proportional to the electrostatic potential of the polyion.[19,20] Whereas the value of this slope does not differ significantly between samples in the disordered form, in the β conformation the slope for the Pilot sample is 1.58 times larger than that of the Sigma sample (Figure 1 and Table I). Third, the transitions in the two samples manifest different breadths under identical conditions. The fractional conversion in the order–disorder transition may be obtained from the titration data in the central region where the points pass from one limiting form to the other.[21] In Figure 5A, the fraction of the sample titrating as the β form, f_β, is plotted vs pH. It is seen that the conformational transition of the Sigma sample is broader than that of the Pilot sample. Alternatively, Figure 5B shows that the disordered-to-β transition for the Pilot sample is broader on the α scale than is the Sigma sample.

In auxiliary experiments we have sought to eliminate any artifactual causes of these differences between the two samples. Close examination of the absorption spectra shows no evidence of the finger-like absorption peaks of the CBZ group, and we conclude therefore that the deprotection of the side-chain hydroxyls is at least 99% complete in both samples. Likewise, circular dichroism studies of the two polymers in the ionized, disordered state yield molar ellipticity values which agree with the literature value for poly(L-tyrosine).[8,17] A third sample of poly(tyrosine) on which we had done some preliminary studies was in fact eliminated from further consideration because it consistently gave molar ellipticities 5% below those displayed by the other samples.

Both our samples of poly(tyrosine) were extremely resistant to hydrolysis. We found no detectable hydrolysis products after 48 h at 110 °C in 12 N HCl, nor after treatment with a chymotrypsin/pronase mixture at pH 8.0 and 38 °C for 12 h. Treatment with subtilisin at pH 10.0 and 38 °C for 12 h resulted in about 10% hydrolysis of the tyrosine polymer, which was not sufficient for any meaningful analysis of chemical or optical purity. However, from previous studies with shorter poly(tyrosine) chains,[31] it seems safe to conclude that the material we used, made by similar procedures, is pure poly(L-tyrosine). As a consequence it may be concluded that the distinctions in properties of the two samples in the ordered state reflect a difference in the structure of the β conformation. A similar finding has been reported for poly(*S*-carboxymethyl-L-cysteine),[32] in which the value of $\Delta G°$ for the disordered-to-β transition at 25 °C depends on the molecular weight.

Discussion

We have used spectrophotometric determination of α in the potentiometric titration of poly(L-tyrosine), in order to

evaluate the thermodynamic parameters for the ionization-dependent disordered-to-β transition of this polypeptide. By working in very dilute solutions of polymer[17] and by employing a relatively low counterion concentration,[24] we have succeeded in resolving the conformational transition from the complicating effects of aggregation and precipitation. In contrast to previous potentiometric titration studies of poly(L-tyrosine),[12,14-16] which were necessarily conducted at much higher concentrations, there is no question that our results refer to the intramolecular transition. Interesting differences in properties were discovered between the two samples studied in this work, which are attributed to their differing molecular weights.

In a previous paper,[17] two simple hydrodynamic models for the β structure of poly(L-tyrosine) were considered and compared to the sedimentation results obtained with a sample of molecular weight 60 700. The simplest model of a single-layered square sheet was found to be untenable, because the experimental value of the frictional ratio was too small. A model in which the sheet was folded over on itself to form a double-layered particle of half the width and twice the thickness of the single-layered square sheet was in better accord with the results.

It is reasonable to suppose that our high molecular weight sample forms the same sort of folded-sheet β conformation as was proposed for the sample in the previous paper. Furthermore, our 27 000 molecular weight sample might reasonably form a sheet too small to fold over on itself readily, and therefore be expected to form a single-layered sheet in solution. Recent results indicate differences in the circular dichroism and sedimentation properties of the two samples (R. P. McKnight and H. E. Auer, unpublished results), which are consistent with the models proposed. As outlined below, the distinctions observed in the present work also fit this pattern.

Thermodynamic Parameters. The difference in $\Delta S°$ values which we find for our two samples cannot be explained by a difference in configurational entropy between the proposed open-sheet and folded-sheet structures, for this should be negligible. Rather, the observed differences between the samples are most likely due to hydrophobic interactions between the layers of the intramolecular folded conformation which are absent in the open-sheet model. The strongly hydrophobic nature of the tyrosine side chain[33] makes this assignment reasonable. Frank and Evans[34] showed that the driving force behind hydrophobic bonding is entropic in origin, and Kauzmann[35] has noted that transfer of an aromatic compound (such as the tyrosine side chain) from an apolar environment to an aqueous phase is nearly athermal, but is accompanied by a large decrease in the entropy. The hydration of proteins and polypeptides has been thoroughly analyzed;[36] Kuntz has obtained evidence for desorption of water from poly(L-tyrosine) at −35 to −45 °C, upon lowering the pH from 12 to 11.3.[37] The folding process which we propose for the longer-chain molecule creates a nonpolar environment for the residues in the interior of the fold, which should lead to expulsion of all adsorbed water from this region. We would thus expect little change in the overall enthalpy of the transition to result from the folding,[34,35] but a positive contribution to the overall entropy change should arise. The identical values of $\Delta H°$ found for the two samples and the small increase in the value of $\Delta S°$ (+0.4 eu) for the longer-chain polymer are therefore consistent with the model proposed above.

The values of $\Delta G°$ which we find for the higher molecular weight polymer agree well with those of Conio et al.[14] and Cosani et al.[16] as can be seen in Figure 4. The values for the data of Senior et al.[15] depend on the method of analysis.[38] One may think of our folded-sheet ordered conformation as a prototype, where the folding is an intramolecular analogue of aggregation. Our supposed open-sheet system (the shorter-chain polymer) might then be expected to give lower magnitudes for $\Delta G°$. The values obtained are only two-thirds of those found for the longer-chain polymer, and lie well below any of the literature values (Figure 4).

Slopes of the Modified Titration Curves. As may be seen from eq 4, the slope of a curve of pK_a vs. α,

$$pK_a = pK_0 - 0.434\frac{\epsilon\psi}{kT} \tag{4}$$

$\partial pK_a/\partial\alpha$ (Figure 1, Table I) is related to the dependence of ψ, the shielded electrostatic potential in the region of the electrical double layer, on α[20] (ϵ is the electronic charge). The value of this slope for the high molecular weight sample is similar to two of the three values which we evaluated from the literature (Table I), while the value for the low molecular weight sample is far smaller than any of the others. For the samples of this work, the values depend on the way in which pK_0 is obtained, but for any reasonable value of pK_0 used, the *relative* difference in the values is preserved. At any given value of α, the surface charge density for an intramolecular folded β sheet is double that for an open sheet, since we assume no ionized residues remain in the hydrophobic interior of the folded form. This should produce a stronger dependence of ψ on α in the former case than in the latter. The relative slopes of the Pilot and Sigma samples are consistent with this interpretation (Table I). The results suggest that inthe experiments taken from the literature, the poly(L-tyrosine) samples form either as intramolecularly folded or an intermolecularly aggregated β structure, but not an open sheet structure. This too is in accord with the available information concerning the molecular weights.[12,15,16]

Breadth of the Transitions. When an order–disorder transition is presented as a function of an environmental thermodynamic parameter (e.g., pH, see Figure 5A), the breadth decreases as the cooperativity increases. A linear array of residues undergoing nearest-neighbor interactions cannot have a first-order phase transition.[39] It is easily demonstrated that in an open-sheet β structure the enumeration of states (i.e., whether a given residue is hydrogen bonded in the conformation or not) yields a linear array of elements even though the structure forms an extended sheet. This clearly is not possible with the folded-sheet model, since more than one dimension is needed for the enumeration. Therefore it is predicted that the open-sheet model have a less cooperative, or broader, transition than the folded-sheet model. When plotting the fractional conversion vs. an intrinsic thermodynamic parameter (e.g., α, see Figure 5B) the transition would appear broader for the folded-sheet model than for the open-sheet one. As noted above, the value of ψ at a given α is greater for the former model than for the latter. This means that in order to preserve the same value of ψ in the ordered form which prevailed in the disordered state the folded sheet must pass to a lower overall value of α than the open sheet.

Both ways of presenting the results for the fractional content of the β conformation (Figure 5) are consistent with these predictions. The transition of the high molecular weight sample, for which the folded sheet is proposed, is narrower on the pH scale and broader on the α scale than that of the low molecular weight sample, which is hypothesized to be in the open-sheet form. This result is essentially unaffected by the values of pK_0 used in other portions of this communication. An alternative interpretation could claim that our low molecular weight sample exhibits lower cooperativity (Figure 5A) because its molecular weight is less than the size of the cooperative unit defined for the α helix by v^{-1} [40] while that of the Pilot sample exceeds this value but retains an open-sheet β structure. In such a model the dependence of ψ on α should

be similar for both samples, so that no gross distinctions should be apparent in Table I and Figure 5B. This contradicts the results obtained, so the model must be dismissed.

It should be noted that the model we use to explain our data differs significantly from that which Senior et al. propose in ref 15. We attribute the free energies obtained from the modified titration curves to a single-step transition of the polymer from the disordered form to a compact β sheet as the pH is lowered. In the model of Senior et al., the titration curv is explained using a two-step process. First, portions of the molecule fold into hairpin-like β structures, a process for which $\Delta G°$ is thought to be "essentially zero." These structures then aggregate, and the observed free energy change is attributed to this aggregation step. Their conclusion that $\Delta G° = 0$ for the intramolecular disordered-to-β transition is open to question, for it is based on the values of pK_a obtained over a very narrow range of α. It may be seen from eq 3 above that data over the entire range of α from 0 to 1 must be employed to evaluate this quantity.

Work presently under way in this laboratory should help to resolve the discrepancy between the models. These findings will be reported in a later publication, along with further work characterizing the chain-length dependence of the β conformation.

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References and Notes

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(38) The points shown in Figure 4 for the data from ref 15 were obtained using the values of pK_0 provided in that reference. By extrapolating to $\alpha = 0$ from the *ordered* form we obtain values of pK_0 of 10.88 and 10.68 for 25 and 35 °C, respectively, from the data of ref 15. Using these values for pK_0, we get for $\Delta G°$ about −250 cal/mol of residue at 25 °C and −270 cal/mole of residue at 35 °C which are in excellent agreement with the values reported in ref 14 and 16. This latter procedure is identical with the method we use in the analysis of our own data.
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