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KINETICS OF THE DISORDERED CHAIN-TO-β TRANSFORMATION OF POLY(L-TYROSINE) IN AQUEOUS SOLUTION

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The kinetics of the transformation of poly(L-tyrosine) from the disordered chain to the intramolecular β structure in aqueous solution has been studied. The reaction is induced by an isothermal pH jump and is followed by conventional circular dichroism methods. Upon application of curve-fitting procedures, it is found that the kinetics are poorly represented by a single first-order process, but a two-step sequential first-order equation is adequate. Sharp pH-dependent maxima in the phenomenological rate constants and in the fractional amplitude of the rapid step were found. It is proposed to attribute these phenomena to a transition in initial states which is shown to occur over the same pH range within the domain of the disordered-to-β transition. No sigmoid transient curves were observed, indicating that no slow nucleation events are discernible in this system. These observations contrast strikingly with the mechanism elaborated for β formation in $(Lys)_n$ [R. Hartman et al., J. Mol. Biol. 90 (1974) 415].

1. Introduction

Polyamino acids have been extensively studied as model systems for elucidating the structural principles governing the conformational stability of native proteins. The properties of α-helical polyamino acids are quite thoroughly understood as a result of this effort, while the β conformation has received less attention. This is due at least in part to the limited tractability of many polypeptide-solvent systems in which the β structure is generated. The results of recent crystallographic analyses of globular proteins demonstrate that this deficiency is of some consequence. β-Pleated sheet stuctures occur in a number of proteins to considerable extents, and even traverse intersubunit boundaries in two oligomeric proteins, liver alcohol dehydrogenase [1] and concanavalin A [2]. Further investigation of β-forming polyamino acids is thus merited.

Poly(L-lysine), one of the most extensively studied polyamino acids, adopts the antiparallel β conformation at low ionization and elevated temperature in aqueous solution, while below room temperature the α-helix is thermodynamically the most stable state [3, 4]. The kinetics of the formation of the β structure in $(Lys)_n$, induced by large temperature changes, are first-order and independent of concentration at very high dilution [5,6]. This was interpreted as evidence that the rate-limiting step leading to the β structure is intramolecular. In a recent re-examination of this problem, the conversion to the β form was induced by an isothermal pH jump [7]. The rate constants observed vary by a factor of more than one thousand at various pH values and temperatures. A slow initiation step was found to occur at the outset of the reaction; at longer times the kinetics are transformed to an apparent first-order process. This was interpreted in terms of a slow intramolecular β nucleation step, followed by rapid propagation steps involving intermolecular condensation between β aggregates and disordered or α-helical single polymer molecules. The low probability of nucleation events, and the consequent limited number of β aggregates, is proposed [7] as the origin of the pseudo-first order rate law observed in the earlier studies [5,6].

Poly(N^{ϵ}-methyl-L-lysine) and poly(N^{δ}-ethyl-L-ornithine) are α-helical at low ionization and room temperature in aqueous solution, and poly(N^{δ}-methyl-L-ornithine) is partially helical [8]. Upon heating, the

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first two substances are transformed to the β structure, just as is $(\mathrm{Lys})_n$, but the third is simply disordered at elevated temperature [9]. It was found that the rate of conversion from the α-helix to the β form under comparable conditions increased in the order $[\mathrm{Lys(Me)}]_n$, $[\mathrm{Orn(Et)}]_n$, $(\mathrm{Lys})_n$. At concentrations on the order of 70 μg/mℓ the pseudo-order of the reaction is 1.0, and the authors conclude that the process is generating intramolecular β forms in both cases.

In aqueous solution poly(L-tyrosine) passes from a disordered state at high side chain ionization to an antiparallel β form as the fixed ionic charges are neutralized [10–13]. Potentiometric titrations, sedimentation velocity and light-scattering experiments on this transformation encountered aggregation of the β structure as a complicating factor at the relatively high polymer concentrations employed in these investigations [10–12]. In a recent study of the disordered-β transformation in $(\mathrm{Tyr})_n$ it was shown that only an intramolecular β structure occurs below about 30 μg/mℓ in a limited domain of ionization [13]. The transition occurs over a narrow range on the pH scale, about 0.02 unit wide. In addition the conformation of a metastable state of $(\mathrm{Tyr})_n$ generated upon rapid acidification to pH values below 11 has tentatively been assigned as nonordered [13], although circular dichroism (CD) spectra acquired under similar conditions resemble those of α-helical $(\mathrm{Tyr})_n$ in organic solvents [14]. In this regard, rapid acidification of $(\mathrm{Tyr})_n$ in 20% ethanol leads to the α-helical state, while equilibrium titration in the same medium generates the β structure [11].

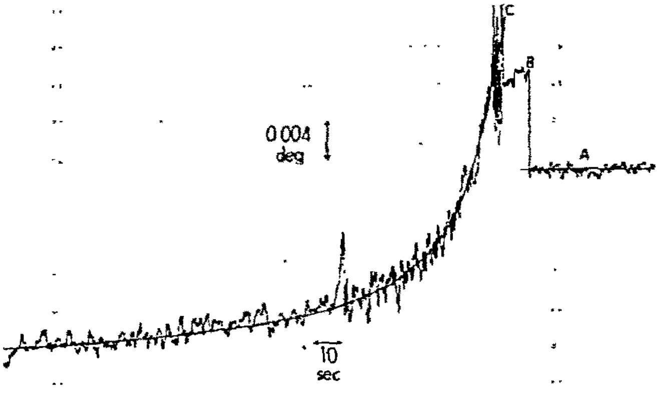

Fig. 1. CD kinetic trace for the conversion of $(\mathrm{Tyr})_n$ from the coil to the β form. Potassium buffer, 25.0°C, pH 11.262, solute concentration 60 μg/mℓ. See text for explanation. The spike at 57 sec is due to a change in time constant setting from 0.3 to 1 sec.

We have investigated the kinetics of the transformation from the disordered state to the intramolecular β form in $(\mathrm{Tyr})_n$. The transition was induced by rapid manual mixing to achieve an isothermal pH change, and the rate was followed by conventional circular dichroism instrumentation. Our procedures required operation of the detection method near the limits of resolution in both time and amplitude. The results to date, presented in this paper, show that this system is considerably different from the corresponding transformation in $(\mathrm{Lys})_n$.

2. Experimental section

2.1. Materials

$(\mathrm{Tyr})_n$ from New England Nuclear Corp. (Lot T-52) was acid precipitated from an alkaline solution prior to use. Stock solutions were prepared in the buffer medium used in this work, 0.10 M KCl, 0.025 M $\mathrm{K_2HPO_4}$.

2.2. CD measurements

Circular dichroism transients were recorded with a Cary 6003 CD accessory to the Cary 60 spectropolarimeter using a spectral bandwidth of 1.5 nm. The chart was driven by the synchronous scan, initially with the 10X lever engaged. When appropriate the lever was dropped back to the 1X setting. A jacketed fused silica cylindrical cell of 5.00 mm optical path and total volume 0.60 ml was firmly held to the sample trough of the CD optical platform. Since we desired to work at low solute concentration, the two highest sensitivity settings were required because the ration of optical activity to absorbance in $(\mathrm{Tyr})_n$ is relatively unfavorable. Time Constant settings (nominal time for 63% pen response) of 0.3 and 1 sec were used. The temperature of all vessels was regulated by means of a circulating water bath.

2.3. Procedure

Kinetic runs were executed as follows. Buffer solu-

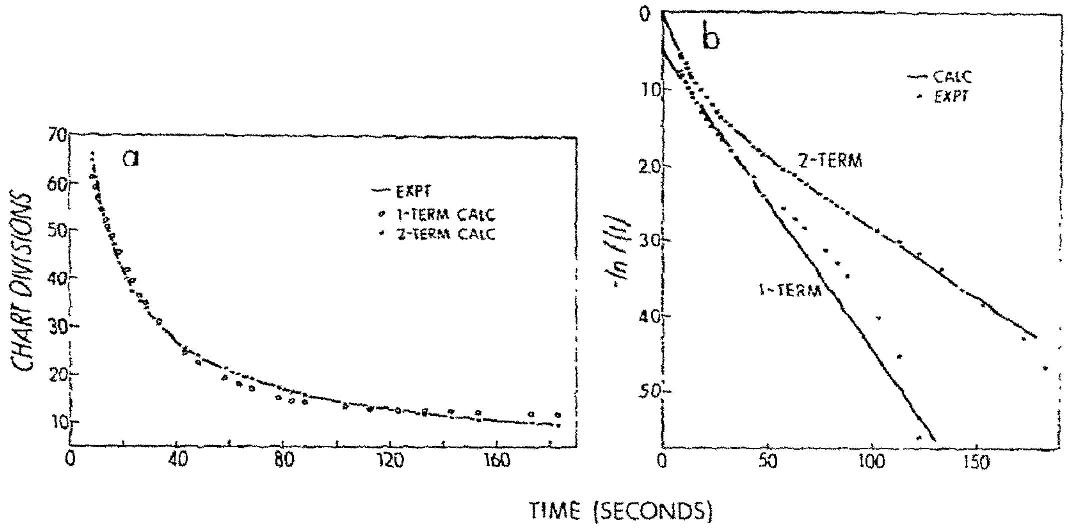

Fig. 2. Comparison of results of the data-fitting procedure with the experimental data for the run shown in fig. 1. θ, θ_0 and θ_∞ are given as the percent of full chart deflection. For eq. (1), $\theta_0 = 80.6$, $\theta_\infty = 12.4$ and $k_1 = 3.94 \times 10^{-2}$ sec^{-1}. For eq. (2), $\theta_0 = 104.6$, $\theta_\infty = 8.8$, $f_1 = 0.619$, $k_1 = 0.104$ sec^{-1} and $k_2 = 1.85 \times 10^{-2}$ sec^{-1}.

a) θ versus time. The solid line is the experimental curve. The points are calculated from (○) eq. (1), and (×) eq. (2).

b) semilog plot versus time. The solid lines show $f(t)$ equal to the rhs of eq. (1) and eq. (2). The points show the experimental data represented as $f(t)$ equal to the lhs of eq. (1) and eq. (2). The plot for the 1-term equation is offset by −0.50 unit along the ordinate.

tion adjusted roughly to the value desired after the pH jump was allowed to equilibrate thermally in the cell, and a baseline was recorded (A, fig. 1). A stock solution of ionized disordered $(\mathrm{Tyr})_n$ was measured into the lumen of a Luer-fitted Teflon tube, which was threaded through the lid of the CD sample compartment and into the cell. With the cell in the optical beam the chart was engaged at the monitoring wavelength, usually 227 nm (B, fig. 1). Buffer and $(\mathrm{Tyr})_n$ were quickly withdrawn into the barrel of a Hamilton gas-tight syringe; the meniscus falling through the light beam caused a sharp pen spike (C, fig. 1) marking time zero. The mixture was immediately expelled back into the cell and the Teflon tube withdrawn. The earliest reliable readings could be taken at about 6 to 7 sec. A portion of a typical recording is shown in fig. 1. The final pH was read with a Radiometer PHM 26 meter after the run was completed, with an estimated reproducibility of ±0.01 unit. The concentration of $(\mathrm{Tyr})_n$ in the sample was determined spectrophotometrically [15].

2.4. *Data analysis*

In order to analyze the data, the experimental traces were smoothed with the aid of French curves (fig. 1). Attempts to fit the data to a single first-order decay process, eq. (1),

$$(\theta - \theta_\infty)/(\theta_0 - \theta_\infty) = \exp(-k_1 t), \tag{1}$$

in which θ_0 and θ_∞, the initial and final ellipticities, and k_1, the phenomenological rate constant, were parameters to be optimized using a nonlinear least squares data-fitting procedure, in general led to poor agreement with observation (fig. 2). A two-term exponential relaxation curve, eq. (2),

$$(\theta - \theta_\infty)/(\theta_0 - \theta_\infty) = f_1 \exp(-k_1 t) + (1 - f_1)\exp(-k_2 t), \tag{2}$$

in which values of θ_0, θ_∞, k_1, k_2 and f_1, the amplitude of the rapid process, were determined by the curve-fitting program, gave quite satisfactory agreement with the data (fig. 2).

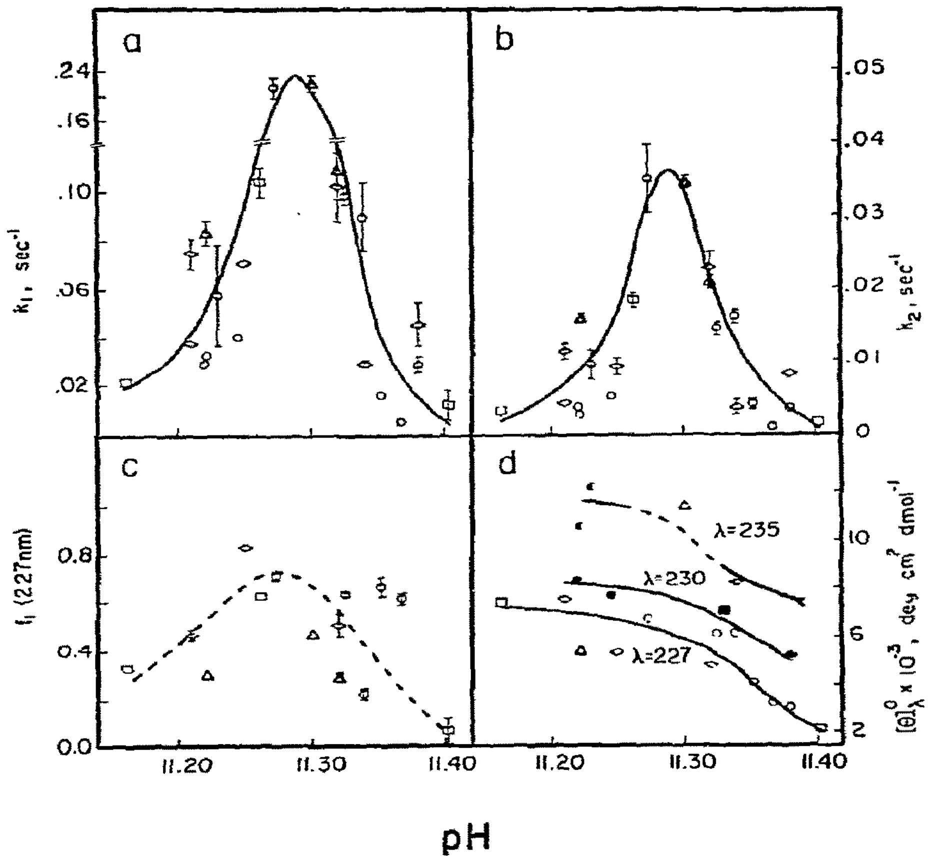

Fig. 3. pH dependence of fitted parameters of eq. (2) for the coil-to-β transformation of $(Tyr)_n$ in potassium medium at 25.0°C. Concentration ranges are (△), 26 to 31 μg/mℓ; (□), 37 to 45 μg/mℓ; (◇), 48 to 56 μg/mℓ; (○) 58 to 66 μg/mℓ. Error bars denote ± standard deviation and are omitted if the latter is smaller than the size of the symbol. In d) open symbols represent 227 nm, closed symbols represent 230 nm and half-filled symbols represent 235 nm.

3. Results

3.1. An intramolecular transformation

It has been shown that in sodium medium at 25°C, the equilibrium coil-β transition is centered at pH 11.32 [13]. The β form of $(Tyr)_n$ is intramolecular below a concentration of 30 μg/mℓ to pH values at least as low as 11.1. At concentrations up to at least 100 μg/mℓ sedimentation velocity experiments suggest that no higher aggregates than dimers are generated at these pH values. In potassium medium the corresponding transition, as determined by CD titration and sedimentation velocity experiments, is centered at pH 11.40. The curve of $s_{20,w}$ versus pH determined at a concentration of 60 μg/mℓ follows closely that shown [13] for $(Tyr)_n$ in sodium medium at 25 μg/mℓ in both shape and magnitude. No evidence of aggregation was found. This suggests that under the conditions employed in this work, the β form of $(Tyr)_n$ is intramolecular.

Kinetic runs were conducted in potassium medium and in the corresponding sodium medium. Variations in the concentration of $(Tyr)_n$ by factors of 2 (potassium, fig. 3) and 5 (sodium, not shown) had no effect on the rates of the transformation. As noted in section 2, the transients are adequately represented by a two-term exponential decay equation (fig. 2). Had a single exponential decay sufficed to fit the data, a fractional lifetime analysis should have shown a reaction order of 1. In fact, nonintegral values between 1 and 2 were obtained, which is consistent with the requirement for two decay terms. Strictly speaking, the fractional lifetime method is not applicable to such a situation. The failure to observe an order of 2 is in agreement with the absence of any dependence of the rate constants on initial solute concentration. These results support the view that it is an intramolecular process which is under observation in this investigation.

3.2. Absence of sigmoid reaction curves

Experiments were performed in which the time required to approach equilibrium varied from less than 100 sec to greater than 2000 sec, depending on pH. In no case was an inflection point apparent in the kinetic traces.

3.3. pH-Dependence of the fitted parameters

The pH dependences of the phenomenological rate constants k_1 and k_2 for the fast and slow processes, obtained in potassium medium at 25°C by fitting the data to eq. (2), are displayed in figs. 3a and 3b, respectively. Polymer concentrations used in these runs were 66 μg/mℓ or less. Low velocities occur at pH 11.40, the midpoint of the equilibrium transition. As the pH falls, a dramatic increase in both k_1 and k_2 occurs, reaching maximum values at pH about 11.29. Over the same pH range the degree of ionization at equilibrium in 0.1 M KCl falls from 0.61 to 0.35 [12]. Further decrease in pH produces a profound reduction in the rate constants. Thus within 0.25 pH unit, the values of k_1 and k_2 increase by factors of about 20, then fall back to their original values.

The values of f_1 [eq. (2)] are shown as a function of pH for the same experiments in fig. 3c. Although the scatter of the points is large, a maximum is discernible roughly at pH 11.27, about the same pH at which the values of k_1 and k_2 pass through maxima.

From the optimized values of θ_0 [eq. (2)] and the position of the buffer baseline (A, fig. 1) $[\theta]^0$, the molar ellipticies at time zero could be calculated. These are shown in fig. 3d. As the pH decreases from 11.40 the curve of $[\theta]^0_{227}$ versus pH undergoes an apparent sigmoid increase in value until pH about 11.28, the same pH at which maxima in k_1, k_2 and f_1 occur. At pH values below this, a further gentle increase is observed.

The value of $[\theta]^0_{227}$ at pH 11.402, 2150 deg cm^2 dmol^{-1}, is not much larger than the equilibrium values of $[\theta]_{227}$ for the ionized disordered form of (Tyr)$_n$ found at pH values higher than that at which the coil-β transition occurs (sodium [13], potassium [18]). At pH values between 11.16 and 11.28, the value of $[\theta]^0_{227}$ is about 7000 deg cm^2 dmol^{-1}. This is close to that observed at pH 10.7 to 10.9 for the rapidly-titrated species of (Tyr)$_n$ [13]. This suggests that the increase in the values of $[\theta]^0_{227}$ upon going from pH 11.402 to 11.28 arises from a transition in initial states.

In order to examine this possibility further, the spectral dependence of $[\theta]^0_\lambda$ was examined. The points obtained at 230 nm and 235 nm are shown in fig. 3d, and corroborate the pH dependence already established for $[\theta]^0_{227}$. The spectral dependence of $[\theta]^0_\lambda$ at the three pH values examined is shown in fig. 4, superimposed on the circular dichroism spectra reported previously in sodium medium [13]. A transformation in the zero-time CD spectra is indeed apparent. The high pH limit is almost certainly that of the disordered state. The low pH limit is not as clear from the data at hand; it is important to note that there is a difference in pH of about 0.5 unit between the value at which the spectrum of the rapidly-titrated species was recorded and the lowest pH at which $[\theta]^0_\lambda$ was determined. In out view, it is likely that the zero-time kinetic CD spectrum is approaching that of the rapidly-titrated species at low pH.

No pH-dependence or other unanticipated observations were made with the values of $[\theta]^\infty_\lambda$. They agree with the values expected for the β form [13].

4. Discussion

Our results on the kinetics of the disordered-to-β transformation in (Tyr)$_n$, reported here, are of considerable interst due to the striking contrast with the analogous process in (Lys)$_n$. In the concentration range examined here, the rate-limiting steps are concerned with formation of an intramolecular β structure in (Tyr)$_n$. In sodium medium, no distinction in kinetic behavior was observed when the solute concentration

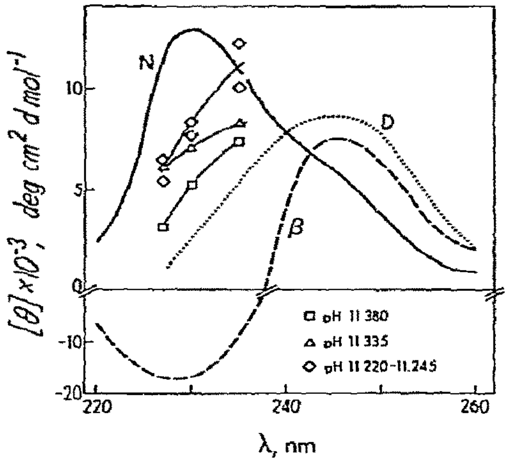

Fig. 4. CD spectra of $(Tyr)_n$. Continuous curves from fig. 1, ref. [13]. N, rapidly-titrated compact form obtained at pH < 11.0, probably nonordered. D, ionized disordered form. β, β form. Zero-time kinetic spectra, $[\theta]_\lambda^0$, are shown by the points.

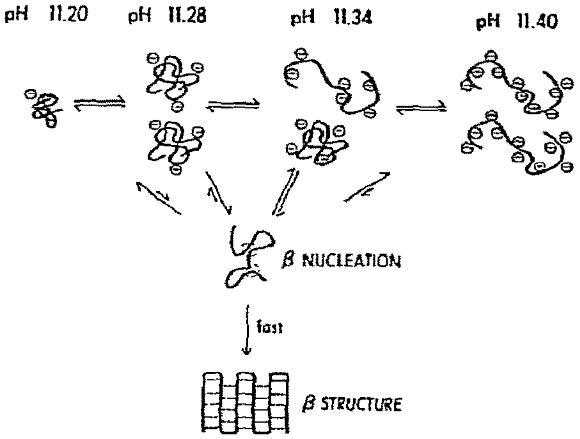

Fig. 5. Model proposed to explain pH dependence of k_1 and k_2 (see text). Dotted circles are intended to suggest potential β-nucleation events.

was varied below and above the value [13] at which the rapidly-sedimenting (dimeric) species is observed. In potassium medium all our work was done at concentrations which are conducive to the formation of only nonaggregated β $(Tyr)_n$. These results are in accord with conclusions voiced earlier [10,12,13] that the first product in the coil-to-β transformation in $(Tyr)_n$ is the intramolecular species, and that subsequent aggregation occurs probably between these preformed β molecules. This is in contrast to the mechanism proposed for β structure formation in $(Lys)_n$ [7], in which disordered or helical molecules are thought to crystallize in the β form as they condense onto large β aggregates.

Coil-to-β conversions were observed over a range of pH values and at three different temperatures. In no case was a sigmoid transient curve evident. This is expected if β-nucleation is rate-limiting and intramolecular propagation occurs much more rapidly. This observation is again in contrast to the kinetics of β-aggregation of $(Lys)_n$ studied by Hartman et al. [7]. They found sigmoid curves in all experiments which was attributed to slow nucleation of the first β polymer molecule and rapid condensation rates for the addition of successive chains to the growing aggregate.

A transition in initial states has been identified within the pH domain of the coil-to-β transformation whose existence was not known before. Its narrowness on the pH scale is consistent with a high degree of cooperativity in the process. The available evidence suggests that the initial species at pH below 11.28 is similar to that obtained upon rapid acidification of $(Tyr)_n$ to pH values below 11 and is most probably nonordered [13,14]. Below pH 11 this species adopts an essentially compact spherical shape [13].

In fig. 3 it is apparent that the values of k_1 and k_2 increase to a maximum over the same pH range in which the transition of initial states to the supposed nonordered form occurs. A model to explain this coincidence assumes that the transition is responsible for the pH effect on the k's. In the disordered polyelectrolyte form (pH ⩾ 11.40), the polymer chain is considerably extended, as is evident from the low value of $s_{20,w}$ [13], so that distant neighbors along the chain have little contact with each other (fig. 5). As the pH falls and the transition to the proposed compact nonordered species proceeds, it may be supposed that the juxtaposition of distant neighbors becomes more and more possible, thereby providing a progressively higher frequency of potential nucleation events for the generation of the β structure (fig. 5). High values of the rate constants are attained by pH 11.28, the value at which this transition is apparently completed. Further lowering of the pH serves to increase the thermodynamic stability of the compact species because of the continued decrease in side chain ionization. This increases the activation energy required for β nucleation and leads to decreased rate constants. This model hopefully provides a basic understanding of the competing effects which underlie the observed phenomenon, and should lead to further work for corroboration.

The fact that two first-order relaxation processes were observed indicates that two unimolecular interconversions between three states are occurring. It is not clear at present what the third state might be. In the simplest case, the sequence of reacting species is linear rather than cyclic [16,17]. In this case, the four microscopic rate constants describing these interconversions could in principle be determined if the reverse reaction, the intramolecular β-to-coil transformation, were to be studied. In a corollary way the pH dependences of k_1 and k_2 are to be related to the variation of the microscopic constants with pH. This may turn out not to be possible, for it is our experience that at all pH values studied in this work the transformation to the β form is complete.

In continuing our studies, the following topics will be examined. First, it is important to extend observation to shorter times in order to seek evidence for a discernible nucleation step. Second, the transition in initial states must be more thoroughly characterized. And third, the reverse reaction is to be investigated to gain additional information about the interconversions observed. Results of these studies will be forthcoming.

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References

[1] C.-I. Branden, H. Eklund, B. Nordstrom, T. Boiwe, G. Soderland, E. Zeppezauer, I. Ohlsson and A. Akeson, Proc. Natl. Acad. Sci. 70 (1973) 2439.
[2] K.D. Hardman and C.F. Ainsworth, Biochemistry 11 (1972) 4910.
[3] B. Davidson and G.D. Fasman, Biochemistry 6 (1967) 1616.
[4] D. Pedersen, D. Gabriel and J. Hermans Jr., Biopolymers 10 (1971) 2133.
[5] S.-Y.C. Wooley and G. Holzwarth, Biochemistry 9 (1970) 3604.
[6] C.R. Snell and G.D. Fasman, Biochemistry 12 (1973) 1017.
[7] R. Hartman, R.C. Schwaner and J. Hermans Jr., J. Mol. Biol. 90 (1974) 415.
[8] H. Yamamoto and J.T. Yang, Biopolymers 13 (1974) 1093.
[9] H. Yamamoto and J.T. Yang, Biopolymers 13 (1974) 1109.
[10] E. Patrone, G. Conio and S. Brighetti, Biopolymers 9 (1970) 897.
[11] G. Conio, E. Patrone and F. Salaris, Macromolecules 4 (1971) 283.
[12] M.B. Senior, S.L.H. Gorrell and E. Hamori, Biopolymers 10 (1971) 2387.
[13] E. Patton and H.E. Auer, Biopolymers 14 (1975) 849.
[14] S. Friedman and P.O.P. Ts'o, Biochem. Biophys. Res. Commun. 42 (1971) 510.
[15] E. Katchalski and M. Sela, J. Amer. Chem. Soc. 75 (1953) 5284.
[16] A. Ikai and C. Tanford, J. Mol. Biol. 73 (1973) 145.
[17] G.H. Czerlinski, Chemical Relaxation (Marcel Dekker, New York, 1966).
[18] E. Patton and H.E. Auer, unpublished results.