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KINETIC STUDIES ON THE HELIX–COIL TRANSITION OF FLUORESCENT LABELED POLY(-L-LYSINE) BY THE TEMPERATURE-JUMP TECHNIQUE

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Fluorescent dansyl labels were covalently attached to poly (L-lysine) (poly(Lys)) with a degree of polymerization of 300 to 600. The degree of labeling was 0.01 to 0.085 (mol label to mol amino acid residues). From the decay of the anisotropy of fluorescence it was concluded that the labels were highly mobile both in the coiled and helical state. A decrease of fluorescence intensity accompanied the helix–coil transition. Identical pH induced transition curves were measured by circular dichroism and fluorescence. The midpoint of the transition was at pH 10.2. The kinetics of the transition were studied by temperature-jump relaxation using fluorescence detection. A single relaxation phase was observed. The relaxation time τ exhibited a distorted bell shaped dependence on the degree of helicity f with a maximum value $\tau_{max} = 15\ \mu s$ at $f = 0.3$ and 20°C. It was independent of polymer concentration and of the degree of labeling. A rate constant of helix propagation $k_F = 10^7\ s^{-1}$ was calculated from τ_{max} and published values of the nucleation parameter σ. The activation energy was 16 kJ/mol. The observed rate constant is comparable to that of poly(L-glutamic acid) but two orders of magnitude smaller than that found for polyamino acids with nonionizable side chains.

1. Introduction

The basic properties of the biologically important α-helix $\rightleftharpoons$ coil transition have been extensively studied with simple model compounds such as poly-α-L-amino acids. Equilibrium transition curves have been measured for many neutral and charged polyamino acids in various solvent systems as a function of external parameters like pH, temperature and solvent composition (for reviews see [1,2]). Most of the equilibrium properties are satisfactorily described by the linear Ising model which is commonly applied in the formalism as developed by Zimm and Bragg [3]. In its simplest form it involves two parameters, the cooperativity parameter σ and the equilibrium constant of helix propagations s. The cooperativity or nucleation parameter σ defines the ratio of the equilibrium constants of helix nucleation and helix propagation and is normally an intrinsic property of the polypeptide system. The equilibrium constant s is influenced by the external parameters temperature, pH, etc. [1,2]. The smaller the value of σ the larger is the cooperativity of the transition. Values of σ clustered about 3×10^{-4} have been reported for many poly-L-amino acids with neutral side chains [2]. Values of σ about 10 times larger were found for poly(L-lysine) [4,5] and poly(L-glutamic acid) [6,7] which are charged in the coiled form.

The kinetics of the α-helix $\rightleftharpoons$ coil transition has also attracted much attention [8]. The complete kinetic mechanism is rather complex but it was shown by Schwarz [9,10] that for very long chains a mean relaxation time τ^* is a function of σ, s (or the degree of helicity f) and the rate constant of helix propagation k_F only. The theory predicts a pronounced dependence of τ^* on the degree of conversion with a maxi-

mum value at the midpoint of the transition. This value is related to σ and k_F by the equation $\tau^*_{max} = (4\,\sigma k_F)^{-1}$. It was further shown that the relaxation spectrum consists of only four relaxation times in the case of long chains, for which end effects can be ignored [10]. Under most experimental conditions the amplitudes of three of these relaxation times are close to zero and only a single relaxation with $\tau = \tau^*$ is seen [10].

Experimentally the determination of τ by fast relaxation methods such as ultrasonic relaxation, dielectric methods and field jump techniques encountered much greater difficulties than the equilibrium studies. Somewhat controversial results were reported which have been reviewed by Zana [11]. More recent studies [12,13,14] added interesting data but did not resolve the controversies. In the case of polyamino acids which undergo side chain protolytic reactions and especially when ultrasonic relaxation was measured, it was difficult to distinguish between relaxation effects due to protolytic processes and those due to the coupled α-helix ⇌ coil transition [15,16]. Data on poly(L-glutamic acid) obtained by ultrasonic relaxation [17,18] and field jump experiments [13,14] revealed values of τ_{max} in the range of a microsecond and indicated that helix propagation may be slower ($k_F = (3$ to $8) \times 10^7$ s^{-1}) than for other polyamino acids with neutral side chains ($k_F \approx 10^{10}\ s^{-1}$) [11]. Ultrasonic data on poly(L-lysine) and poly(L-ornithine) [19] which seemed to indicate a faster transition were later reinterpreted as being due to protolytic processes [15].

Relaxation times slower than a microsecond can be measured by the classical and most direct temperature jump technique provided that a suitable optical signal is available. Following earlier work in which a fast phase due to the α-helix ⇌ coil transition was demonstrated but not resolved [20,21]. Sano et al. [22] were recently able to measure a τ_{max} of 3×10^{-6} s for poly(L-glutamic acid) by a temperature jump technique. These authors used polarimetric registration which responds very directly to the conformational change but which is instrumentally difficult to apply in the microsecond range.

In the present study a small number of dye molecules were covalently attached to the polymer chains with the expectation that their fluorescence or absorption would be sensitive to changes of the conformational state. The aim of the study was to obtain reliable kinetic data for poly(L-lysine) and poly(L-glutamic acid) and to prove the potential of the labeling technique to provide signals for studies of fast kinetic processes.

2. Materials and methods

2.1. Chemicals

Poly(L-lysine HBr) (poly(Lys)) was purchased from Miles-Yeda Ltd. and Serva and poly(L-glutamic acid) sodium salt (poly(Glu)) from Pilot Chemicals. Dansyl chloride and 7-chloro-4-nitrobenzofurazane (NBD-chloride) (Merck), N-[p-(benzoxazolyl)-phenyl] maleinimide and 4-dimethylamino-4-isothiocyanatostilbene (Serva), 4-aminosalicylic acid and 5-aminosalicylic acid (Fluka AG) were used without further purification. 4-Iodoacetamidosalicylic acid, 5-iodoacetamidosalicylic acid, 4-[2-(iodoacetamido)ethylamino] 1-naphthalenesulfonate and 1-aminochrysene were were generous gifts of Dr. A. Steinemann. All other chemicals were of reagent grade from Merck and Fluka.

2.2. Preparation of labelled poly(L-lysine)

A solution of 100 mg poly(Lys) and 50 mg 1,4-diaza-bicyclo[2,2,2] octane in 1 ml water was mixed with 1.5 ml of acetone. After addition of 5 mg dansyl chloride in 0.5 ml acetone the mixture was refluxed for 15 minutes. Then three drops of glacial acetic acid were added to keep the product water-soluble. The acetone was evaporated and the clear yellow solution was applied to a 35 × 1 cm Sephadex G10 column. A solution of 1 g/l NH_4Cl in water was used for equilibration and for elution. The fractions containing the labelled poly(Lys) were pooled and lyophilized. An average mole ratio of label to lysine residues of $p = 0.014$ was calculated using a molar extinction of bound dansyl of $3.4 \times 10^3\ M^{-1}\ cm^{-1}$ [23] and the known weight of the lyophilized dansyl-poly(Lys). About 50% of the total dansyl chloride was bound. Dansyl-poly(Lys) with $p = 0.046$ and 0.085 were synthesized in a similar way.

NBD-poly(Lys) was prepared essentially by the same procedure. A small filamentous precipitate was removed by centrifugation before gel filtration. Only

20% of the dye reacted giving an average mol ratio of label to residues of approximately 0.01. The water-soluble reactive dyes 4-iodoacetamidosalicylic acid, 5-iodoacetamidosalicylic acid, 5-[2-(iodoacetamido) ethylamino] 1-naphthylenesulfonate were reacted in 0.1 M sodium borate or sodium carbonate buffer, pH 10 containing 50% acetone or dimethylformamide.

2.3. Preparation of labelled poly(L-glutamic acid)

Dansyl and NBD-chloride were converted to their respective ethylenediamine monoamides. About ten percent of the glutamic acid residues were converted to the acyl chloride. The appropriate monoamides of the dyes were then added to the acyl chloride to yield the desired labelled polypeptides with degrees of labelling of $p = 0.01$ to 0.05.

2.4. Instruments and measurements

For measurements of absorption a Shimadzu spectrophotometer UV-200 and a Cary 14 were employed. Corrected excitation and emission spectra of fluorescence were measured with a Schoeffel spectrofluorimeter RRS 1000. The fluorescence life time and depolarisation of fluorescence were measured with a modified Ortec nanosecond fluorimeter. This instrument was operated on-line with a PDP 11-40 computer (Digital Equipment Co.). Sedimentation and diffusion coefficients were determined with a Beckmann Model E ultracentrifuge using the Schlieren optical system. Partial specific volumes were obtained with a digital densitometer DMA 02C (Anto Paar Co.). Optical rotation was measured in a Perkin Elmer polarimeter type 141. Spectra of circular dichroism were recorded with a Cary 61 spectropolarimeter. Thermostatted cells of 0.1 to 10 cm path length were employed.

Temperature-jump experiments were performed with two instruments of the same basic design [24]. A discharge voltage of 25 kV produced a temperature jump of about 7°C. The temperature before discharge was adjusted by an external water bath to various temperatures (±0.1°C). The kinetics was monitored by either fluorescence or by absorption. In the fluorescence mode, the recording of scattered incident light was prevented by a cut-off filter Schott 395 GG. In order to prevent photodegradation of the labels the samples were illuminated only for compensation and reading.

Kinetic traces were recorded by an oscilloscope and photographed or data were digitized by a Datalab DL 905 transient recorder and processed by a PDP 11-40 computer. In the latter case 5 to 15 single relaxation curves were averaged. In all experiments a filter time constant of 1 μs was used. Lyophilized samples of the labelled polymers were dissolved in distilled water and were allowed to stand at 4° for several days or sonified to disrupt possible aggregates. The solutions were mixed with buffer, passed through a Millipore filter, and extensively degassed before measurement. The pH was read after performing the temperature jump experiment.

3. Results

3.1. Characterization of the poly-L-amino acids

The molecular weights were determined from sedimentation and diffusion data. For poly(Lys) (Serva "MW 100,000") a diffusion coefficient of 2.7×10^{-7} $cm^2\ s^{-1}$ and a sedimentation coefficient of 1.55×10^{-13} s were determined in 0.1 M sodium borate buffer, pH 7 containing 0.3 M KCl. A partial specific volume of 0.751 ml/g was measured which yielded a molecular weight of $M = (4 \pm 0.2) \times 10^4$ and a degree of polymerization $n = 320$. The sharpness of this sedimentation profile indicated a rather narrow molecular weight distribution. For poly(Lys) (Miles) $M = 8 \times 10^4$ ($n = 640$) and for poly(Glu) $M = 8.5 \times 10^4$ ($n = 660$) were obtained in a similar way. After labelling, the products were freed completely from non-covalently bound dye by gel filtration. All labelled polymers were eluted in single symmetric peaks except for the NBD-derivatives. The attached NBD-groups seem to favour aggregation.

Information on the flexibility of the covalently attached dansyl groups was obtained from measurements of the rotational correlation time derived from the decay of the anisotropy of fluorescence [25]. Dansyl-poly(Lys) was studied in 0.1 M sodium borate buffer containing 0.6 M KCl. In both the coiled and α-helical states at pH 9 and pH 11 respectively, the rotational correlation time of the label was found to be less than the dead time of the apparatus which was

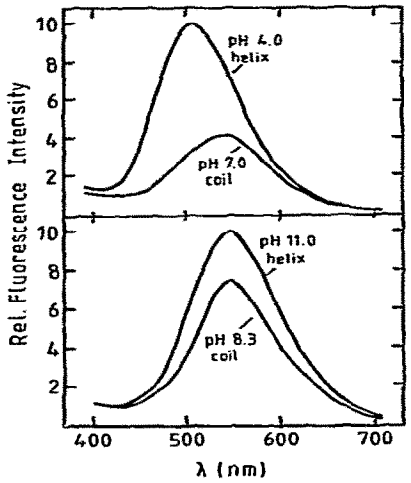

Fig. 1. Fluorescence emission spectra of dansyl-poly(Glu) (a) and dansyl-poly(Lys) (b) at 25°C. The excitation wavelength was 315 nm. Poly(Glu) (0.69 mM) with a degree of labeling $p = 0.014$ was measured in 0.1 M sodium acetate buffer, pH 4 or 7, containing 0.3 M KCl. Poly(Lys) (0.52 mM) with $p = 0.085$ was dissolved in 0.1 M sodium borate buffer, pH 11 or 8.3, containing 0.3 M KCl.

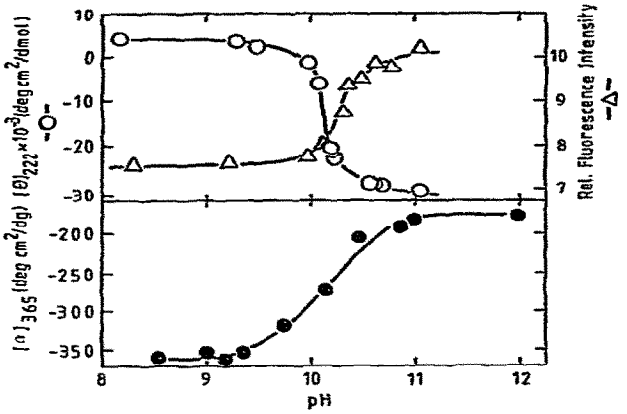

Fig. 2. The α-helix ⇌ coil transition of poly(Lys) as a function of pH. (a) The transition was monitored by molar residue ellipticity at 222 nm for unlabelled poly(Lys) (0.1 mg/ml) (—○—) and by fluorescence at 545 nm (excitation at 315 nm) for dansyl-poly(Lys) of $p = 0.085$ (0.067 mg/ml) (—△—). These comparative measurements were performed in 0.1 M sodium borate buffer containing 0.6 M KCl at 20°. The pH was adjusted by addition of a concentrated sodium hydroxyde solution. (b) Equilibrium transition curve measured in the solvent system employed for the kinetic measurements. Specific optical rotation at 365 nm was monitored for poly(Lys) (0.45 mg/ml) in 0.1 M sodium borate buffers of pH 8.5 to 12 containing 0.5 M KNO_3 at 22° (—●—).

2 ns. No slow phase which would be indicative of immobilization was detectable. Apparently the relatively long lysine side chains act as spacers and endow the labels with a high degree of flexibility. It also follows that possible contacts between labels in the polypeptide have lifetimes shorter than 2 ns.

3.2. Changes in absorption and fluorescence accompanying the helix ⇌ coil transition

Poly(Lys) undergoes a coil to α-helix conversion when the pH is changed from 9 to 11 [26]. The same type of transition takes place in poly(Glu) when the pH is decreased from 6 to 4 [27]. For an initial check of the signals provided by the various labels fluorescence and absorption spectra were recorded at these limiting pH-values. The fluorescence emission spectra of dansylated poly(Glu) and poly(Lys) are shown in fig. 1. There is a drastic increase in the fluorescence intensity and a blueshift of 32 nm when the pH is decreased to 4 for dansyl-PLGA. These very large changes are however partially due to aggregation. This was manifested by an increase in turbidity and by precipitation at higher concentrations and by the occurrence of slow and concentration dependent relaxation processes. An intrinsic tendency of poly(Glu) to aggregate at low pH was noticed earlier [28] and this aggregation tendency is probably increased by the introduction of hydrophobic labels.

No shift in the emission spectrum of dansylated poly(Lys) was observed, even at a high degree of labelling $p = 0.085$. The maximum of the emission for the coiled and helical state of dansyl-poly(Lys) is located at the same wavelength (545 nm) and approximately at the same position as that of dansyl-poly(Glu) in the coiled state (537 nm). It is concluded that the about 25% increase in fluorescence intensity observed for dansyl-poly(Lys) between pH 8.3 and 11 (fig. 1b) is not due to aggregation but may be associated with the coil to helix transition. The excitation maxima for coiled and helical dansyl-poly(Lys) were located at 331 nm and 329 nm respectively.

Large changes of fluorescence intensity were also

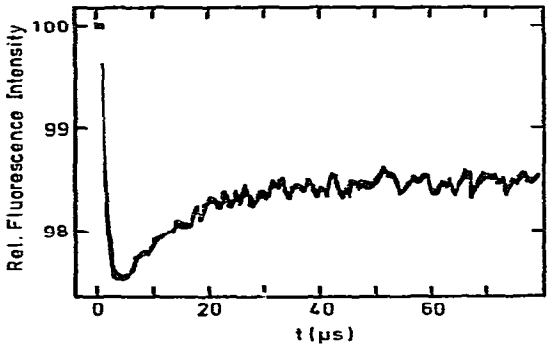

Fig. 3. Oscilloscope trace of the change in fluorescence intensity of dansyl-poly(Lys) (p = 0.014) in 0.1 M sodium borate buffer, pH 9.8 containing 0.5 M KNO_3 after a temperature jump from 15 to 22°C. The polymer concentration was 0.22 mg/ml (1.7×10^{-3} M lysine residues).

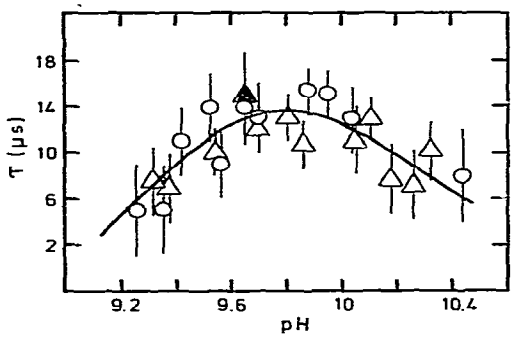

Fig. 4. Relaxation time as a function of pH for dansyl-poly-(Lys) (p = 0.046) in 0.1 M sodium borate buffer containing 0.5 M KNO_3 at 15°C. All values are averages of at least eight measurements. The bars indicate the calculated standard deviation. The dansyl-poly(Lys) concentration was 0.053 mg/ml (4.2×10^{-4} M) (▲), 0.269 mg/ml (2.1×10^{-3} M) (○) and 0.538 mg/ml (4.2×10^{-3} M) (△). Numbers in brackets stand for molar concentrations of lysine residues.

observed for NBD-poly(Lys). This derivative with p = 0.008 showed a large increase of absorption of 7.5% at 492 nm when the pH was changed from 8.5 to 11. Smaller changes in fluorescence or absorption were measured for all the other dyes mentioned in the materials section. No signals for the helix to coil conversion were observed for poly(Lys) labelled with 5-[2-(iodoacetamino)ethylamino] 1-naphthalenesulfonate. The fluorescence of this dye is known to be hardly influenced by its environment [23]. Dansyl-poly(Lys) and to some extent NBD-poly(Lys) were employed in the equilibrium and kinetic studies because of the convenient signals provided by these labels and because of the small association tendency of these polymers.

3.3. Equilibrium transition curves as monitored by fluorescence, circular dichroism and optical rotation

The large changes in the circular dichroism (CD) at 222 nm could be used for monitoring the coil ⇌ α-helix formation of poly(Lys) [29]. The transition curve which was obtained for our preparations (fig. 2a) agrees well with published data [4,29,30]. Most importantly fig. 2 shows that the change of fluorescence measured according to fig. 1b parallels the change of CD. Therefore the fluorescence signal could be used to monitor the coil ⇌ α-helix transition in fast kinetic measurement for which the change of CD was not applicable.

The transition curve which applies to the solvent conditions used in the temperature jump experiments is shown in fig. 2b. For the temperature jump experiments 0.5 M KNO_3 was added to the buffer to provide sufficient conductivity [31]. Since this solution had a very high optical density below 250 nm, the reference transition curve could not be measured by CD but was monitored by optical rotation at 365 nm. The change of specific optical rotation from −360 for the coil to −180 for the helix is related to a change from −130 to −40 which was observed at 546 nm by Greenfield and Fasman [30].

3.4. Temperature jump kinetics

Most kinetic experiments were performed with dansyl-poly(Lys) in the fluorescence mode. The best signal-to-noise ratio was obtained for the dansyl derivative with a degree of labelling p = 0.046 but measurements were also possible when the degree of labelling was only 0.014 (fig. 3). The excitation wavelength was 315 nm and the total fluorescence was observed.

An initial large decrease of fluorescence occurred upon raising the temperature within 5 μs (fig. 3). This decay time was close to the time resolution of the instrument and was therefore unresolved. The amplitude

of the fast phase corresponded in sign and magnitude to the temperature dependence of fluorescence which was measured in static experiments. For dansyl-poly(Lys) in the randomly coiled conformation at pH 7 the slope of the relative fluorescence was −0.003 deg^{-1} which accounts for an about 2% decrease of fluorescence at a temperature jump of 7 degrees. The fast phase was therefore assigned to the trivial decrease of fluorescence with increasing temperature which is observed for most chromophores. A similar fast phase was also observable for small model compounds such as dansylated amino acids. In a following kinetically resolved phase the decrease of the fluorescence signal was partially reversed. The direction of this change corresponds to an increase of helicity with increasing temperature which is in agreement with the positive enthalpy change which was found for this transition [32]. The exponential increase of fluorescence could be described by a single relaxation time τ which was 15 μs under the conditions of the experiment described in the legend of fig. 3.

The relaxation time τ (fig. 4) and the corresponding amplitude (not shown) were pH-dependent. Both showed a maximum near pH 9.9. Measurements were possible only in the pH range of 9.2 to 10.5. At these limits τ approached the resolution time of the instrument and outside this range only the fast unresolved phase was observed.

No dependence of τ on the concentration of the polyamino acid was found (fig. 4) at concentrations lower than 0.65 mg/ml. At higher polymer concentrations and at low temperatures an additional slower process was observed. Its relaxation time was in the millisecond range and was strongly dependent on concentration. This phase was attributed to aggregation of dansyl-poly(Lys) which gave rise to turbidity at high concentrations. Measurements were therefore restricted to the concentration range in which no slow relaxation phase was detectable. An activation energy of 16 ± 4 kJ/mol was estimated from the temperature dependence of τ at pH 9.9 and at a polymer concentration of 0.5 mg/ml.

Some supporting experiments were performed with NBD-poly(Lys) in the absorption mode at 492 nm. Similar values of τ were found with this label as for dansyl-poly(Lys) at pH values around 9.9. No systematic study was performed because of the low signal-to-noise ratio and because NBD-poly(Lys) showed a higher tendency to aggregate than dansyl-poly(Lys).

For comparison, temperature jump experiments of dansyl-poly(Glu) were performed. At pH values lower than 6.2 highly concentration dependent relaxation processes were observed in the millisecond and second range. These were interpreted as being due to the association of the polymer in the helical state (see preceding paragraph). At pH 6.2, at which the aggregation phenomena no longer overshadowed all other effects, a fast relaxation process was observed. Its relaxation time of less than 5 μs was poorly resolved. Because of these difficulties no further studies were performed with labelled poly(Glu).

4. Discussion

4.1. Comparison of experimental results with theoretical predictions for the α-helix ⇌ coil transition

For an interpretation of the kinetic results of the α-helix ⇌ coil transition of poly(L-lysine) we have to rely on published equilibrium data. From the chain length dependence of the transition curves the nucleation parameter was found to be $5.8 \times 10^{-4} \leqslant \sigma \leqslant 3.6 \times 10^{-3}$ [5] and from an analysis of titration data a value of $\sigma = (2.3 \pm 0.5) \times 10^{-3}$ was derived [4]. This corresponds to a cooperative length $N_0 = \sigma^{-1/2}$ of 17 to 40 residues. N_0 is the average length of uninterrupted helical sequences at the midpoint of the transition (degree of helicity $f = 0.5$), when the degree of polymerization $N \gg N_0$. The latter condition is well fulfilled for the two poly(Lys) samples of $N = 320$ and $N = 640$ which were used in the present study. This implies that the influence of end effects can be neglected [10]. Indeed identical results were obtained with $N = 320$ and $N = 640$. In the framework of the theoretical treatment derived by Schwarz [9,10] the dependence of τ on f is given by the simple expression valid for $N \gg N_0$

$$\tau = f(1-f)/\sigma k_F. \quad (1)$$

According to eq. (1) the maximum value

$$\tau_{max} = (4\sigma k_F)^{-1} \quad (2)$$

is predicted at the midpoint of the transition for $f = 0.5$.

This function is compared in fig. 5 with the experi-

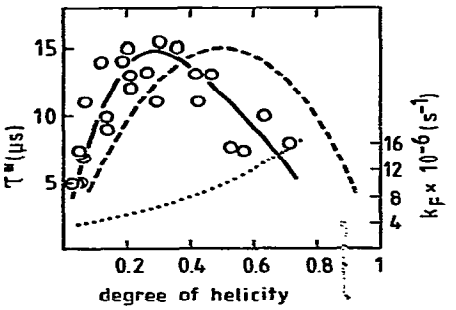

Fig. 5. Experimental relaxation times (o) as a function of the degree of helicity f. The solid curve is a fit to the data by eq. (1) with the pH-dependent k_F-values represented by the dotted curve. The dashed curve was drawn according to eq. (1) with a pH-independent k_F of 7.5×10^6 s^{-1}.

mental dependence which shows a maximum near $f = 0.3$. It must be recalled, however, that for the derivation of eq. (1) the assumption was made that the rate constant of helix propagation k_F is constant in the narrow transition range. This is however not necessarily true for the transition of poly(L-lysine) in which the state of ionization of the side chains is dramatically changed during the transition. The change of f from 0.05 to 0.7 occurs in a pH-range of 9.35 to 10.4 (see fig. 2b) and corresponds to a decrease of the degree of ionization α from approximately 0.5 to 0.1 [32]. Already a rather small dependence of k_F on pH may lead to a shift of the maximum of the τ versus F dependence. A dependence of k_F on pH was therefore introduced and evaluated by a best fit to the experimental data. When k_F is assumed to increase from 4×10^6 s^{-1} (at $f = 0.05$) to 15.5×10^6 s^{-1} (at $f = 0.7$) according to the dotted curve in fig. 5 a good fit of the experimental τ-data is obtained by eq. (1). For these calculations a value of $\sigma = 2.2 \times 10^{-3}$ was used as an average of the published data [4,5]. At the midpoint of the transition k_F is 10^7 s^{-1}. With the extreme values of σ reported in the literature, the range of the rate constant (at $f = 0.5$) is calculated to be $6 \times 10^6 \leqslant k_F \leqslant 3.8 \times 10^7$ s^{-1}.

4.2. Possible role of protolytic processes

The value of $k_F = 10^7$ s^{-1} is two orders of magnitude smaller than a value of 2.8×10^9 s^{-1} derived from ultrasonic absorption of poly(L-lysine) [33]. This disagreement supports the re-interpretation by Zana et al. [11,15] that the observed ultrasonic relaxation is related to side chain protolytic reactions rather than the α-helix $\rightleftharpoons$ coil transition. For a simple protolytic equilibrium $-NH_3^+ + OH^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} -NH_2 + H_2O$ in which the side chain NH_3^+-groups of poly(L-lysine) are assumed to react independently, a reciprocal relaxation time $\tau^{-1} = k_1(C_{NH_3^+} + C_{OH^-}) + k_2$ is expected [34]. With pK = 10.2 and $k_1 = 2 \times 10^{10}$ M^{-1} s^{-1} [34] the value of τ would be about 2×10^{-9} s for a polymer concentration of 4×10^{-3} M at pH 9.9. This is 500-fold smaller than the value experimentally observed by the temperature jump technique for the above conditions (see fig. 4). In addition the relaxation time of the protolytic reaction should strongly depend on polymer concentration in contrast to experimental observation. It is however clear that protolytic reactions in polymers with dissociable groups are more complex. The pK values of side chains depend on the conformation of the residues to which they are attached as well as on the state of dissociation and on the conformation of neighbouring residues. It was recently shown for poly(L-glutamic acid) [16] that proton transfer relaxation times may be expected in a wide time range. The observability of these relaxation times will however critically depend on the corresponding amplitudes and on the signal employed for monitoring the reaction.

4.3. The fluorescence signal and the α-helix ⇌ coil transition

The fluorescence signal employed in the present study probably reflects the conformational change of the polymer and is less sensitive to changes of the degree of dissociation α. The change in fluorescence closely follows the transition curve as measured by circular dichroism and optical rotation. For the spectroscopic origin of the fluorescence signal two possibilities may be discussed. The labels are probably statistically distributed along the polymer. For poly(Lys) with a degree of labelling $p = 0.048$ approximately every 20th side chain will be labelled. For a Förster type self-quenching between labels, the average over the sixth power of the reciprocal distances between labels has to be considered. This average is ex-

pected to be smaller in the coiled form of poly(Lys) than in the helical form since close encounters by loop formation are possible in a random coil only. The increase in the average distance between labels accompanying helix formation would therefore explain the observed increase in fluorescence intensity. Another effect may be seen in the changed microenvironment of the labels. The magnitude and direction of this effect is difficult to predict. It is apparent from measurements of depolarization of fluorescence that no immobilization of labels takes place in the coil or in the helix. Stacking between labels is considered to be of no or little importance because of the low stacking tendency of dansyl group, the small fraction of groups which is expected to occur in nearest neighbour positions, the absence of spectral shifts and the lack of immobilization. The large spectral shifts which accompany association of dansyl-poly-(Glu) are probably due to aggregation. This material was therefore not used for the kinetic studies.

4.4. Interpretation of k_F and comparison with other kinetic data on the α-helix ⇌ coil transition

The foregoing discussion and the agreement of the experimental data with the theoretical prediction for the α-helix coil transition supports the view that the observed τ corresponds to the α-helix ⇌ coil transition. Slow relaxation times due to intrinsic proton transfer reactions [16] may not be ruled out but more likely protolytic reactions will constitute fast pre-equilibria of the conformational change. In order to demonstrate the direction of the influence of such pre-equilibria it is assumed that only coiled residues with uncharged side chains (c) can convert to the helical state (h) and that only a single pK value governs the state of their protonation

$$\begin{array}{ccc} & c^+ & \\ & \uparrow & \\ K & \| & \\ & \downarrow k_F^0 & \\ c & \longrightarrow & h \\ + & & \\ H^+ & & \end{array} \qquad (3)$$

the rate constant of helix propagation depends on pH according to

$$k_F = \frac{10^{-pK}}{10^{-pK} + 10^{-pH}} k_F^0. \qquad (4)$$

Scheme (3) is too simple to account for the complex real situation. Repulsions between charged residues which prevent helix formation also depend on the state of the neighbouring residues. A charged coil residues can also convert to the helical state if no other charges surround it. Therefore the effect of pH is possibly overestimated by eq. (4). It is seen however that k_F will always increase with decreasing degree of dissociation of poly(Lys) in qualitative agreement with the experimental finding.

The data on the α-helix ⇌ coil transition in poly-(Lys) may be compared with kinetic data for poly(Glu) which resembles poly(Lys) in its polyelectrolyte nature. The main difference between the two transitions is that negative charges are removed in poly(Glu) whereas positive charges have to be removed in poly(Lys) in order to induce helix formation. Only a single relaxation time was observed for poly(Glu) and a remarkable agreement between the results of ultrasonic methods [17], temperature jump [22] and field jump [13] studies was obtained. In the two latter studies, polarimetric recording was employed which proves that the conformational transition was followed. Data vary by a factor of three only and the average values of τ_{max} and k_F are 1.6×10^{-6} s and 6×10^7 s^{-1} respectively.

The α-helix coil transition of poly(Glu) is only somewhat faster than that of poly(Lys). A striking difference exists between the kinetic constants derived for these two polyamino acids with ionic side chains at one hand and those of a number of polyamino acids with non-ionic side chains at the other. For poly-(γ-benzyl-L-glutamate) and poly-(γ-benzyl-L-aspartate) in non-aqueous solvents avlues of k_F of 1.3×10^{10} s^{-1} [35] and 3×10^{10} s^{-1} [26] were obtained. Recently a value of 5.6×10^9 s^{-1} was measured for poly(N^5-3-hydroxypropyl-L-glutamate) in a mixture of water and methanol [37]. The k_F-values for the last group of polyamino acids correspond to the upper limit of the rate constant which was estimated for helix propagation [9]. Retarding effects which are connected with the ionic side chains may be of the type shown by eq. (3). It is however not likely from the experimental data that the k_F-values obtained

by extrapolation to the neutral form of poly(L-lysine) will match with the high values measured with polyamino acids with non-ionic side chains. Another reason for the slower k_F-values may be sought in the hydration of the lysine or glutamic acid residues in the coild form. Water molecules may compete with hydrogen bonding groups participating in α-helix formation. The hydration and the fraction of residues which are not available for helix formation steps may be higher in polyelectrolytes as compared to neutral polyamino acids.

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