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KINETICS OF THE HELIX–COIL TRANSITION OF A POLYPEPTIDE WITH NON-IONIC SIDE GROUPS, DERIVED FROM ULTRASONIC RELAXATION MEASUREMENTS

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Ultrasonic absorption and velocity dispersion curves have been measured in the temperature induced helix-coil transition range of poly-N^5-(3-hydroxypropyl)-L-glutamine in a methanol/water mixture. The results clearly reflect an effect due to the kinetics of the conformational conversion. A practically single relaxation time is observed which passes through a maximum when plotted versus the degree of transition. This maximum occurs at definitely less than 50% helix as predicted for by the theory for the comparatively short chain length involved here. The results are discussed in relation to previous theoretical and experimental findings.

1. Introduction

The α-helix-coil transition of polyamino acids in solution is a basic model for cooperative conformational transitions of biopolymers. Its thermodynamics has been extensively studied [1,2]. The corresponding kinetics [3] is not only more complicated to describe theoretically but also rather difficult to measure because of technical difficulties. As an apparent consequence some controversial results have been reported in the literature [4,5,6]. This is especially the case for the ultrasonic relaxation method, which was first proposed as a suitable experimental means in view of the expected very fast reaction rate [7,8]. Unfortunately it so far proved to be of little use because the ordinarily investigated polypeptides undergo side chain protolytic reactions whose ultrasonic relaxation masks that of the α-helix-coil transition reaction [5].

We have now been able to avoid the interfering effect of protolysis by choice of poly-N^5-(3-hydroxypropyl)-L-glutamine (PHPG) [9], which has no dissociable side groups. Experimental data on this system will be reported in this article, analyzed in terms of the previously developed theory and the results discussed together with pertinent work of other authors.

2. Fundamental theory

The thermodynamics of helix-coil transitions has been commonly described on the basis of the linear Ising model, in particular by means of the matrix method as first applied by Zimm and Bragg [10]. This yields, however, comparatively simple relations only for the limiting cases of either infinitely long or very short chains. A complete treatment for the general case of any chain length becomes somewhat involved. It was developed in an earlier paper [11] and is used for the interpretation of the experimental data presented below.

For helix-coil transitions the essential observable quantity is the fraction of chain segments (corresponding to individual peptide bonds) in the helix state, the

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so-called degree of transition, θ. Under the assumption of only nearest neighbour interaction it has been explicitly given [11] as a function of five parameters, namely

$$\theta = f(s, \sigma, \beta', \beta'', N) . \qquad (1)$$

The parameter s denotes the equilibrium constant of the growth reaction, i.e. the conversion of a coiled to a helical segment when just one of the contiguous neighbours is already in the helix state. The equilibrium constant for the nucleation reaction, i.e. the formation of a helical segment not immediately neighboured by helix segments, is expressed as σs which defines the cooperativity (or nucleation) parameter σ. On the other hand, β' and β'' describe the special cooperative properties of the two ends of the linear chain. The symbol N stands for the total number of segments per chain.

This general formulation will be important for chains, where

$$N \lesssim 1/\sqrt{\sigma} = N_0 \qquad (2)$$

(N_0 being the so-called cooperative length of the system, which is usually $\gg 1$). For $N \gg N_0$ the degree of transition becomes independent of the chain length, i.e. the influence of the end effects vanishes. In this case of "infinite" chain length eq. (1) degenerates into

$$\theta = \tfrac{1}{2}\{1 + (s-1)/\sqrt{(s-1)^2 + 4\sigma s}\} \qquad (3)$$

which always yields $\theta = 0.5$ (midpoint of transition) for $s = 1$ independent of σ.

In accordance with the equilibrium treatment reviewed so far the kinetics has also been worked out [3,7,8,11,12,13]. The chemical relaxation of the conformational change at slight perturbance of equilibrium is in general shown to be described by $4N - 5$ individual relaxation modes. For extreme chain lengths, however, substantial simplifications of this relaxation spectrum apply [3,13]:

(i) Very short chains ($N \ll N_0$) exhibit an "all or none" transition between practically all helix or all coil. The kinetics is of first order implying only one relaxation time.

(ii) For very long chains ($N \gg N_0$) the number of relaxation modes is in principle reduced to four, but just one occurs with appreciable amplitude (provided $N_0 \gg 1$).

There is no apparent simplification for $N \approx N_0$. At any rate a mean relaxation time τ^* has been defined [14], which can here be expressed in general as

$$\tau^* = \varphi^*(\theta, \sigma, \beta', \beta'', N) \cdot \tau^*_{\max} \qquad (4)$$

with $\tau^*_{\max} = (4\sigma k_F)^{-1}$, where k_F is the rate constant for the forward growth reaction. The explicit expression for the function φ^* is available elsewhere [11]. For the limiting case of "infinite" chains we obtain (within the transition range)

$$\tau^* = \frac{1}{\sigma k_F}\theta(1-\theta) = 4\theta(1-\theta)\tau^*_{\max} \qquad (5)$$

This τ^* is obviously a symmetrical function of θ with a maximum at $\theta = 0.5$. For finite chain lengths τ^* will be smaller and the shape of the curve (when plotted versus θ) becomes asymmetrical.

3. Experimental

3.1. Material

The PHPG was first synthetized and described by Lupu-Lotan et al. [9]. It is soluble in water and in methanol, but has no ionizing side groups. The helix-coil transition is induced by temperature, pressure and variation of solvent composition (e.g. methanol/water).

The samples used in this work were purchased from Miles-Yeda Ltd., where they were produced with poly-(γ-benzyl-L-glutamate) as starting material.

The criterion of purity reported is "no absorption due to benzyl group at 257 nm proving that de-estrification was practically complete". The product was used without further purification.

The molecular weight was determined through the diffusion and sedimentation constants (extrapolated to zero concentration) using the Svedberg relation. A weight average molecular weight $M_w = 25\,000$ was obtained, resulting in a mean degree of polymerization of $N = 135$.

3.2. Optical and calorimetric measurements

Rotation angle measurements were carried out with a Perkin-Elmer 141 polarimeter at 546 nm wavelength and a temperature interval between 273 K and 343 K.

Calorimetric studies were performed by means of a differential adiabatic calorimeter, developed by Grubert and Ackermann [15]. With this technique the energy absorbed in the solution upon slowly raising the temperature is compared with that absorbed in the solvent. The differential signal measures the heat capacity of the investigated solute. In the transition range it reflects an apparent contribution δC_p due to the helix-coil conversion. Accordingly the corresponding enthalpy change when increasing the temperature from T_1 to T_2 is given by

$$\Delta H = \int_{T_1}^{T_2} \delta C_p \, \mathrm{d}T \tag{6}$$

For our system and the applied concentration the total calorimetric effect amounted to about 2 J at a measuring accuracy of ± 10%.

3.3. Ultrasonic measurements

The ultrasonic resonator technique and the evaluation of data used in this investigation have been described before [16,17]. This was developed on the basis of a method originally introduced by Eggers [18]. Our 5 ml cell could be applied at frequencies between 0.5 and 36 MHz. A non-relaxing NaCl-solution was used as a reference. To at least four significant figures its density was kept equal to the one of the solution under investigation. With our equipment and evaluation method we could determine both the sound absorption and velocity dispersion with a relatively high precision as has already been demonstrated in another study published elsewhere [19]. One spectrum consists of 25 frequency points in the average, depending on the position of the relaxation frequency in the accessible frequency range.

In order to avoid sound wave scattering at small air bubbles we heated all solutions to 328 K for one hour thereby increasing the bubble size and removing them by shaking. Possible artefacts originating from the particular cell construction were shown to be negligible by measuring one of the solutions (38.8 g PHPG per kg standard solvent, i.e. 20% (v/v) methanol in water) in two different cells with different lengths (10.6 mm and 8.01 mm) at the same temperature.

The ultrasonic absorption and dispersion caused by a chemical relaxation process with a single relaxation time τ is described according to

$$\frac{\alpha}{\omega} = g\,\frac{\omega\tau}{1+(\omega\tau)^2}, \quad \frac{k}{\omega} = g\,\frac{1}{1+(\omega\tau)^2} + \frac{1}{v_\infty} = \frac{1}{v}, \tag{7a, b}$$

α is the excess absorption coefficient of the sound amplitude, k the wave number, v the sound velocity with v_∞ being its limit at the high frequency side of the dispersion curve. The amplitude factor, g, as well as τ are determined by the underlying chemical reaction [14].

Our experiments on the helix-coil transition of PHPG did not show any noticeable deviation from the frequency course given by the eqs. (7). This is gathered from the fact that the amplitude factor g can be calculated independently (see below) and agrees satisfactorily with the one experimentally obtained. Such behaviour implies a practically single relaxation process with $\tau = \tau^*$ [14]. The corresponding amplitude factor has been deduced before [7,8,20] to be

$$g = \frac{\rho v_\infty}{2\mathrm{RT}} \left(\Delta V - \Delta H \frac{\alpha_p^\infty}{\rho c_p^\infty} \right)^2 \frac{\partial \theta}{\partial \ln s}\,. \tag{8}$$

Here α_p^∞ is the expansion coefficient and c_p^∞ the specific heat capacity, both taken at constant pressure but without the contribution due to the transition. These quantities are practically determined by the solvent alone. ΔV and ΔH are the reaction volume and enthalpy of the elementary growth reaction, respectively. The term $\partial\theta/\partial \ln s$ can be calculated from eq. (1).

Numerical values of α_p^∞ and c_p^∞ were taken from the "International Critical Tables" and linearly interpolated or extrapolated, if there were no data at the desired temperatures. The density ρ of the solution was measured for all temperatures and concentrations.

4. Results

4.1. Thermodynamic and optical evaluations

The reaction volume was determined by measuring the partial specific volume of PHPG in the standard solvent (concentration: 9.3 g l^{-1}) as a function of the degree of transition by varying the temperature (see below for the relation between θ and T) (fig. 1). We obtained a ΔV of 5.5 $\mathrm{cm}^3\ \mathrm{mol}^{-1}$, whereas Okita et al. [21] found a ΔV of 8.0 $\mathrm{cm}^3\ \mathrm{mol}^{-1}$ by varying the solvent composition. These reaction volumes ate amazingly large in comparison with those obtained for α-

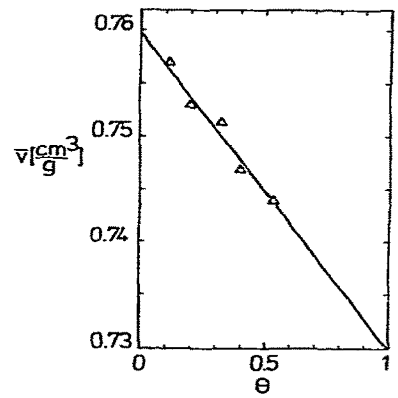

Fig. 1. Partial specific volume of PHPG in the standard solvent as a function of the degree of transition obtained by varying the temperature. Concentration was 9.3 g l^{-1}.

helix-coil transitions of ionic polypeptides like poly(L-glutamic acid), for which a ΔV of 1 to 2 cm^3 mol^{-1} has been reported [7,22].

The remaining thermodynamic quantities contributing to the size of g were determined independently from differential scanning calorimetry and optical rotation.

Since optical rotation angles α can be linearly related to the percentage of helix structure [23] we have set

$$\theta = (\hat{\alpha} - \hat{\alpha}_c)/(\hat{\alpha}_h - \hat{\alpha}_c) , \qquad (9)$$

where $\hat{\alpha}_c$ and $\hat{\alpha}_h$ are the rotation angles associated with the pure coil and helix states, respectively. On this basis the theoretical expression for $\theta(s, \sigma, \beta', \beta'', N)$ was fitted to the experimental $\hat{\alpha}$ versus T data with

$$s = \exp\{-\Delta H/RT + \Delta S/R\} , \qquad (10)$$

employing a non-linear processing method described in the literature [24] (also used in the evaluation of the calorimetric data, see below). For our method it is necessary to measure as wide a portion of the transition curve as possible in order to obtain sufficiently accurate values of the final $\hat{\alpha}$-levels corresponding to the two pure conformational states. That such levels actually do not depend on temperature was checked by varying the solvent composition so that the pure helical and coil states, respectively, are assumed in the accessible temperature range [9]. We note that in addition to the experimental quantities T and $\hat{\alpha}$ the fitting process involves the adjustable parameters $\hat{\alpha}_c$, $\hat{\alpha}_h$, ΔH, ΔS, σ, β', β'', N. For N we have always inserted the above mentioned experimentally obtained mean value of 135. The end effect parameters can be set equal to each other, so that $\beta' = \beta'' = \beta$, because for α-helix-coil transitions there is no apparent difference in the helix formation and disruption at the two chain ends [11]. This has been confirmed by an earlier analysis of pertinent data including PHPG [25]. Although the four remaining thermodynamic parameters may have been taken from the literature or equally well from our independent calorimetric results, we could determine them together with $\hat{\alpha}_c$, $\hat{\alpha}_h$ surprisingly well without prior knowledge from the above fitting procedure. It was necessary to determine the transition curve for each concentration of polymer which was used in the kinetic experiments, because the location of these curves and their shape — and therefore the thermodynamic parameters — depend considerably on the concentration. This phenomenon has been observed before in similar systems [26] but is not really understood at the present time.

Fig. 2a shows the transition curves for three different concentrations of PHPG in the standard solvent as obtained from the optical rotation studies. The results of the fitting procedure are collected in table 1. The trend of ΔH, ΔS and simultaneously $T_{\theta=0.5}$ with concentration is obvious and shown in fig. 3. A (merely empirical) rough extrapolation to infinite dilution as suggested by Ackermann and Rüterjans [26] is listed in the last column of table 1. In the calorimetric experiments the excess heat capacity due to the helix-coil transition, δC_p, is measured directly. Independent of any model the expression for δC_p yields

$$\delta C_p = (\partial H/\partial\theta)_{T,P}(\partial\theta/\partial T)_P = \Delta H(\partial\theta/\partial T)_P \qquad (11)$$

Because of eq. (1) it follows in principle that

$$\delta C_p = f'_T(s, \sigma, \beta, N) \cdot \Delta H = \varphi'_T(\theta, \sigma, \beta, N) \cdot \Delta H \qquad (12)$$

where f'_T is the derivative of f in eq. (1) with respect to T(φ'_T follows by expressing s in terms of θ and the other parameters). The relation (12) degenerates for $N \gg N_0$ into [27]

$$\delta C_p = (\partial\theta/\partial \ln s)_{T,P}(\partial \ln s/\partial T)_P \cdot \Delta H$$
$$= \frac{s+1}{\sqrt{\sigma s}}[\theta(1-\theta)]^{3/2} \cdot \frac{(\Delta H)^2}{RT^2} . \qquad (13)$$

This predicts that δC_p passes through a maximum

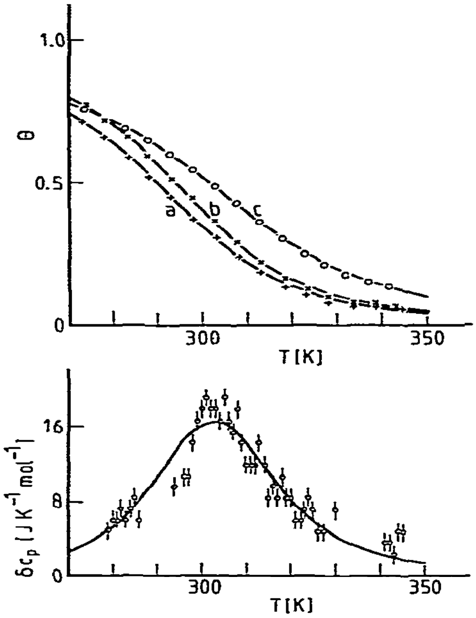

Fig. 2. (a) The degree of transition as a function of temperature obtained from optical rotation at 546 nm wavelength. Concentrations of PHPG in the standard solvent: a = 0.10 mol/kg solv.; b = 0.16 mol/kg solv.; c = 0.21 mol/kg solv. The solid curves were fitted with eqs. (1) and (9). The thermodynamic fitting parameters are collected in table 1. (b) The excess heat capacity as a function of temperature. Concentration was 0.21 mol/kg stand. solv. The solid curve was fitted with eq. (13). The thermodynamic fitting parameters were $\Delta H = -730$ J mol^{-1}, $\Delta S = -2.42$ J mol^{-1} K^{-1}, $\sigma = 1.1 \times 10^{-4}$.

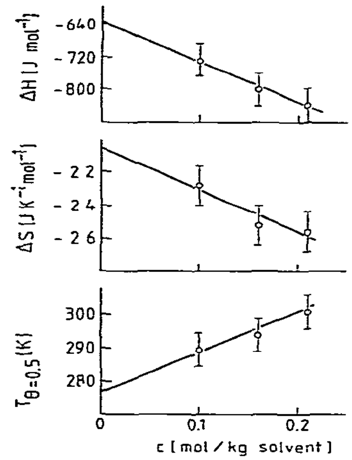

Fig. 3. Transition enthalpy and entropy as well as temperature of the transition midpoint as a function of concentration as obtained from optical rotation measurements.

within the transition region and is expected to be practically symmetrical for infinitely long chains when plotted versus θ. Such a behaviour is generally confirmed in practice.

Our calorimetric results are shown in fig. 2b. The dots represent the excess heat capacity at given temperatures; they were read from the originally analog heat absorption curve. The relatively strong scattering of points is due to the small effect of 2.16 J excess heat (at the already rather high concentration) spread over the accessible temperatures range. For the theoretical fitting of the $\delta C_p(T)$-curve the quality of the data justified the application of eq. (13) without consideration of finite chain length. A fitting procedure analogous to the one for the processing of optical ro-

Table 1
Thermodynamic parameters of the α-helix-coil transition of PHPG in 20% methanol as obtained from optical rotation, see also figs. 2 and 3.

	Concentration [mol/kg solvent]			
	0.1	0.16	0.21	0
ΔH [J mol^{-1}]	−730 ± 70	−800 ± 80	−840 ± 80	−640 ± 100
ΔS [J K^{-1} mol^{-1}]	−2.3 ± 0.2	−2.5 ± 0.3	−2.55 ± 0.3	−2.0 ± 0.4
$\sigma \times 10^4$	0.5	0.8	1.6	
$\beta \times 10^4$	3.2	57	2.8	
$T_{\theta=0.5}$ [K]	289.5	294	301	277 ± 5

Table 2
Synopsis of thermodynamic parameters for the α-helix-coil transition of PHPG.

	ΔH [J mol^{-1}]	ΔS [J K^{-1} mol^{-1}]	$\sigma \times 10^4$	$\beta \times 10^4$
Lotan et al. [9] (c)	−629			
Lotan et al. [45] (c)	−712 ± 126	−1.89 ± 0.21		
Okita et al. [21] (a)	−704 ± 21			
(c)	−419 ± 21		3.2 ± 0.6	
Winklmair [25] (c)	−712 ± 377	−1.84 ± 2.68	2.0 ± 0.3	30 ± 20
This work (b)	−800 ± 80	−2.5 ± 0.4	1 ± 0.5	3 ± 2

These refer to the following solvents: (a) 10% methanol, (b) 20% methanol, (c) 30% methanol.

tation data yielded $\Delta H = -730$ J mol^{-1}, $\Delta S = -2.42$ J K^{-1} mol^{-1}, $\sigma = 1.1 \times 10^{-4}$. The thermodynamic parameters as well as the location of the δC_p-maximum are apparently well consistent with the independent results obtained from optical rotation. The most probable thermodynamic parameters according to the present work are listed in table 2, where they are compared with relevant results reported in the literature.

4.2. Ultrasonic and kinetic evaluations

The helix-coil transition of PHPG is rather broad with respect to the change of temperature (cf. fig. 2). Its location on the temperature scale is influenced by the methanol/water composition of the solvent [9]. In order to shift the transition and its midpoint temperature into a convenient temperature range, a methanol content of 20 vol % in water was here employed.

Within the transition region we observed in all PHPG solutions with this standard solvent composition two ultrasonic relaxation processes, one between 0.5 and 2 MHz and the other from 20 MHz upwards. The frequency of either one of them was found to fall fully within the accessible frequency range. Since the pure solvent showed one single relaxation in the frequency range above 20 MHz (a rough estimate for the relaxation frequency is 70 MHz), we attribute this fast process to the water/methanol interaction. This is supported by the literature according to which water/alcohol mixtures exhibit relaxation frequencies between 50 and 100 MHz [28,29]. On the other hand the slower process is attributed to the helix-coil transition of the polypeptide. In fact, only the water absorption was observed for a solution with pure water as the solvent, where the polypeptide exists in a practically complete coil state.

In fig. 4 typical excess absorption and dispersion curves are depicted. The data were numerically fitted to eqs. (7) with $\tau = \tau^*$. The amplitude g was first fixed to the values obtained from the independently determined thermodynamic parameters according to eq. (8). This leaves only τ^* as an adjustable quantity. Treating then the same data with variable τ^* and g resulted in the same τ^*- and g-values within experimental error. These values both exhibit a maximum in the transition range clearly indicating that the relaxation is caused by the conformational change. The collected relaxation times are shown in fig. 5 as a function of the degree of transition. Two different concentrations of the polypeptide in the same solvent mixture were used. For a quantitative theoretical fit according to eq. (4) a best set of

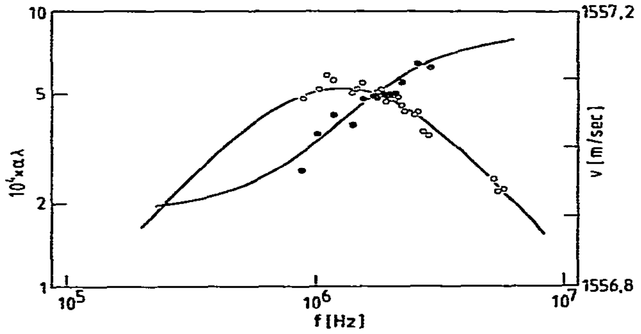

Fig. 4. A selected set of ultrasonic absorption and dispersion curves for 29.8 g PHPG/kg stand. solv. Fitting parameters of eqs. (7) were $\tau = 0.2154 \times 10^{-6}$ s and $g = 3.64 \times 10^{-4}$ s m^{-1}.

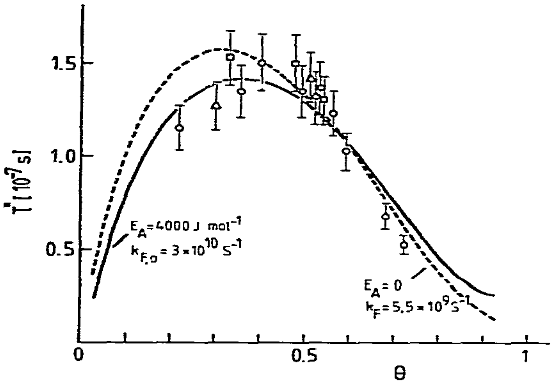

Fig. 5. Mean relaxation time τ^* as a function of degree of transition. Concentrations were ○ and □ = 38.8 g/kg stand. solv.; △ = 19.9 g/kg stand. solv. The broken and solid lines were fitted with eqs. (4) and (14). The fitting parameters were $\Delta H = -876$ J mol^{-1}, $\Delta S = -2.69$ J mol^{-1} K^{-1}, $\sigma = 1.35 \times 10^{-4}$, $\beta = 2.16 \times 10^{-4}$, plus those indicated in the figure.

parameters obtained from the thermodynamic experiments was chosen as indicated in the legend of fig. 5. Since the investigated interval of θ corresponds to a fairly broad temperature range, the dependence of $\tau^*_{\max} = (4\sigma k_F)^{-1}$ on the temperature may not be negligible. Assuming an Arrhenius expression for the rate constant k_F we used

$$\tau^*_{\max} = \exp(E_A/RT)/4\sigma k_F^{\infty} \ , \qquad (14)$$

where σ and k_F^{∞} are assumed to be independent of T. With the two adjustable parameters k_F^{∞} and E_A we have calculated the curves in fig. 5. All other parameters were taken from the independent experiments as given in table 2. The best fit yielded $E_A = 4$ kJ mol^{-1}. This rather small value is in good agreement with the recent work of Tsuji et al. [43] on the α-helix-coil transition of poly(α-L-glutamic acid) induced by electric field jumps at varying pH. Since this polypeptide exhibits no appreciable thermotropic transition in aqueous solution, temperature could easily be used for the determination of activation parameters leading to $E_A = 3.5 \pm 1.6$ kJ mol^{-1}. As indicated in the legend to fig. (5) k_F^{∞} was found to be 3×10^{10} s^{-1}. Thus the actual rate constant at $T = 298$ K is $k_F = 5.6 \times 10^9$ s^{-1}.

In fig. 6 a plot of $\tau^*/\tau^*_{\max}$ versus θ is presented. This clearly shows the theoretically predicted features

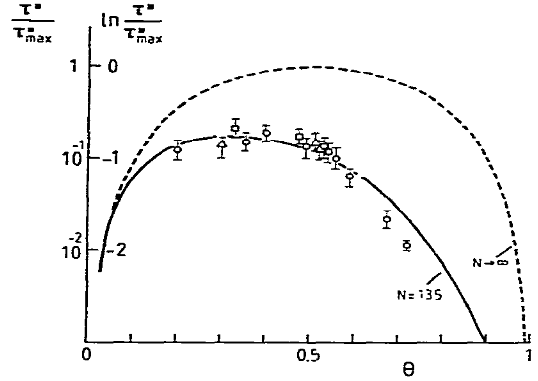

Fig. 6. $\log(\tau^*/\tau^*_{\max})$ as a function of θ. The solid line corresponds to the solid line in fig. 5. The broken line was obtained for the same parameters and $E_A = 0$, but with eq. (5) (case of infinite chain length). Cf. ref. [11].

[11], namely the maximum of τ^*, its shift towards lower θ for our finite N and the simultaneous decrease of the height of the maximum in comparison with the case of $N \to \infty$. In order to have an independent check for the rate constant k_F at 298 K, we performed temperature jump experiments with the same solutions as were used in the ultrasonic apparatus. Light scattering signals were registered with a measurable amplitude but a relaxation time too fast to be detected in the accessible time range. Since the minimum time constant observable was about 10^{-6} s, this only provides a lower limit for the rate constant, namely $k_F \gg 3 \times 10^8$ s^{-1}.

5. Discussion

5.1. Elementary reaction steps and relaxation time

For slight perturbations within the transition region of highly cooperative α-helix-coil conversions the relaxation process is determined by the growth reaction of already existing helix structures. The elementary growth step

$$\text{cch} \underset{k_D}{\overset{k_F}{\rightleftharpoons}} \text{chh} \qquad (15)$$

may be split up as

$$\mathrm{cch} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ch'h} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{chh}\,. \tag{16}$$

The first step represents the diffusion of the peptide segment considered from a random position in the coil form to its proper steric position in the helical state but without a hydrogen bridge (this could be called an encounter "complex"), whereas the second step stands for the formation of a corresponding hydrogen bond. The actual hydrogen bonding requires no activation energy implying that $k_2 \gtrsim 10^{11}\ \mathrm{s}^{-1}$ [30]. Rate limiting for the overall process is the restrained diffusion of the first step which involves the rotation of individual segments about their $\mathrm{C}^\alpha - \mathrm{C}$ and $\mathrm{C}^\alpha - \mathrm{N}$ bonds. Reasonable values of the torsion frequencies about these bond ($\approx 10^{13}\ \mathrm{s}^{-1}$) and their activation energies ($\approx 15\ \mathrm{kJ\ mol}^{-1}$) lead to a time constant of approximately $10^{-10} - 10^{-11}$ s. Taking into account the random walk in the confined space of a peptide segment likewise results in a time constant of this order of magnitude. Thus one can estimate

$$k_{-1} \approx 10^{10}\ \mathrm{s}^{-1} \ll k_2\,, \tag{17}$$

and accordingly

$$k_{\mathrm{F}} = k_1 \lesssim 10^{10}\ \mathrm{s}^{-1}\,, \tag{18}$$

which is to be considered an upper boundary value of k_{F} for the following reasons. Solvent molecules may compete for hydrogen bonding to the NH and CO groups of the peptide linkages. If these competitional bonding reactions are assumed to be fast in comparison with the bond rotations mentioned above, a simple calculation yields for the effective value of the forward rate constant

$$k_{\mathrm{F}} = k_{\mathrm{F}}^0 \, \frac{1}{1 + K_{\mathrm{c}} a}\,, \tag{19}$$

where k_{F}^0 applies to the case of no competition, K_{c} is the equilibrium constant for the competitive hydrogen bonding to the coil form and a is the thermodynamic activity of the solvent. In addition to this retarding solvation effect we note [31] that in polyvinylic chains rotation about $\mathrm{C}^\alpha - \mathrm{C}$ and $\mathrm{C}^\alpha - \mathrm{N}$ bonds may apparently be slower than estimated above, as is indicated by ultrasonic absorption measured in such a system.

According to the theory we always have $\tau^* \leq \tau^*_{\max} = (4\sigma k_{\mathrm{F}})^{-1}$. With the assumption of an upper limit of k_{F} and $\sigma \approx 10^{-4}$, which is well confirmed for a number of nonionic polyamino acids, one therefore must expect that $\tau^* \lesssim 10^{-6}$ s. Such a fast relaxation process was indeed observed by means of earlier temperature jump techniques [32,33], although it could not definitely be resolved because of an inadequate lower time limit. However, the predicted relaxation time falls into a time range, which is quite accessible by ultrasonic methods [34]. Hence these methods were first suggested as an appropriate experimental approach.

5.2. Earlier ultrasonic work

Unfortunately the earlier efforts seriously suffered from interfering effects due to the ionic nature of the polyamino acids which were investigated for their α-helix-coil transition. For poly(L-glutamic acid), poly (L-ornithine) and poly(L-lysine) protolytic reactions and counterion binding could be shown to produce contributions to the ultrasonic absorption greatly exceeding those of the conformational conversion proving thereby that most of the ultrasonic results had been misinterpreted [5,35]. It has been pointed out, however, that those ultrasonic investigations on poly(L-glutamic acid) which were carried out around pH = 5.1 really reflected the α-helix-coil transition [5].

Obviously protolytic contributions to the ultrasonic absorption do not exist in the present system. The observed effect must be due to the helix-coil transition, as clearly suggested by the observed maximum values of amplitude and relaxation time within the transition region. In addition the measured amplitudes agree with the calculated factor g determined on the basis of independently obtained parameters. Within experimental accuracy a single relaxation time τ^* is observed, whose asymmetrical course with the degree of conversion θ is correctly predicted by the theory. Quantitatively τ^* versus θ was fitted with adjustable $k_{\mathrm{F}} = k_{\mathrm{F}}^{\infty} \exp(-E_{\mathrm{A}}/RT)$. As pointed out above E_{A} was found to be about 4 kJ mol^{-1} which is smaller than the expected 12–16 kJ mol^{-1} for valence bond rotation and solvent viscosity, respectively. This discrepancy is likely due to the disregarded temperature dependence of σ. A different approach to the interpretation of ultrasonic absorption due to the α-helix-coil transition was undertaken by Cerf [36]. He introduced an additional reaction volume associated with the fluctuation of the number of uninterrupted helix sequences (whereas the simultaneously occurring "normal" reaction volume is defined as due to conformational changes). This theoretical treatment which leads to a shift of maximum absorption to θ-values between 0.5 and 1 for relatively short chains with $N \gtrsim N_0$ is not confirmed by our experiments.

5.3. Other previous relevant work

A number of investigations on the α-helix-coil transition has been carried out by the method of dielectric relaxation, so far for technical reasons with nonaqueous solutions only. Two electric field induced processes may be seen with this technique: (i) rotation of the dipolar helix fragments and (ii) growth of helices due to a displacement of the helix-coil equilibrium. The chemical effect is in principle observable if it is much faster than the rotational one [37].

In case of α-helix-coil transitions with an estimated $\tau^* \lesssim 10^{-6}$ s the corresponding dielectric dispersion was predicted to occur in the 100 kHz region, at considerably higher frequency than the molecular rotation. For poly(γ-benzyl-L-glutamate) in mixture of dichloroethane and dichloroacetic acid these two dielectric relaxations were indeed observed, yielding the predicted τ^* (θ)-curve and a k_F of about 10^{10} s^{-1} [38]. There are great experimental difficulties, however, owing to the highly aggressive solvent which required a platinum/teflon cell. Standard equipment proved to be inadequate. This may be the reason why Marchal [4] could not reproduce the reported results. The suspected solvent effect is certainly disqualified by the fact that the phenomenon in question passes through a maximum at the midpoint of the helix-coil transition. Further clear evidence for the observability of α-helix-coil transitions by means of dielectric relaxation has been produced by two more laboratories. For poly(β-benzyl-L-apartate) in m-cresol Wada et al. [39] measured a dispersion effect around 3 MHz which they interpreted as being due to the conformational transition because it passed through a maximum around the midpoint of transition as expected.

In addition, we note that Omura et al. [40] reported dielectric relaxation of poly(ε-carbobenzoxy-L-lysine) in m-cresol and obtained relaxation times τ which decrease monotonously with increasing θ, or go through a slight maximum, respectively. This relaxation may not be interpreted on the basis of pure rotational relaxation, because that would imply a steady increase of τ with θ (since the dipolar helix fragments become longer with increasing θ). Instead, we propose that the observed behaviour is to some extent determined by the helix-coil transition kinetics. The latter leads to a pronounced decrease of τ versus θ when the chain length parameter N falls below N_0 which is clearly the case here.

Ultrasonic and dielectric methods are ordinarily associated with very small perturbations of the transition equilibrium. Jump techniques involve much greater effects which may lead to less justified application of theoretical results to experimental data. On the other hand, these techniques provide rather direct insight into the kinetics. Only in recent years has it been possible to extend the time scale accessible for jump methods to times sufficiently short for the observation of α-helix-coil transition. This was achieved by appropriate cable discharge devices for the generation of electric field as well as temperature jumps together with improved signal detection. Most significant relevant findings were reported for poly(L-glutamic acid) by Sano et al. [41] using a temperature jump and by Cummings and Eyring [42] with a field jump. Both techniques were equipped with polarimetric registration, which responds more directly to structural changes than any other available detection method. These studies confirmed the predicted τ (θ)-behaviour yielding good agreement regarding $\tau^*_{\max}$ (see table 3). At least these results appear to represent comparatively clear evidence. In addition, field jump experiments on poly(L-glutamic acid) have been carried out in which proton reactions coupled to the helix-coil transition were monitored. Tsuji et al. [43] conducted field jump experiments following changes of electrical conductivity. They interpreted their effects in terms of proton dissociation due to conformational changes of the polymer. This yielded relaxation times well consistent with those obtained on the basis of optical rotation signals. The fact that this is true in spite of the substantial difference in the concentrations would also support the intramolecular nature of the underlying conversion. On the other hand, Cummings and Eyring [44] trying to reproduce these results by monitoring the absorption of a proton sensitive indicator obtained a quite different τ (pH)-dependence. Both approaches were later challenged by Madsen and Slutsky [35] who doubted the unambiguity of experiments with proton coupled reactions for the interpretation of structural transitions on theoretical grounds.

Table 3
Synopsis of kinetic parameters for various polypeptides as obtained from the literature.

Polymer [a)]	Solvent [b)]	Method [c)]	T[°C]	$\tau^*_{\max}$[s]	σ	k_F[s^{-1}]	Ref.
PDGA	H_2O	US	30	1.1×10^{-6}	2.5×10^{-3}	9×10^{7}	Inoue [46]
PLGA	H_2O	US	37	1×10^{-6}	2.5×10^{-3}	1×10^{8}	Barksdale + Stuehr [47]
PLGA	H_2O	TJ	22	3×10^{-6}	2.5×10^{-3}	3×10^{7}	Sano et al. [41]
PLGA	H_2O	FJ	24	1.4×10^{-6}	2.5×10^{-3}	7×10^{7}	Cummings + Eyring [42]
PHPG	H_2O/CH_3OH	US	25	3.3×10^{-7}	1.35×10^{-4}	5.6×10^{9}	this paper
PBG	DCA/DCE	diel.	25	5×10^{-7}	0.4×10^{-4}	1.3×10^{10}	Schwarz + Seelig [38]
PBA	m-cresol	diel.	50	5×10^{-8}	1.6×10^{-4}	3×10^{10}	Wada et al. [39]

a) PDGA = poly(D-glutamic acid), PLGA = poly(L-glutamic acid), PHPG = poly-N^5-(3-hydroxypropyl)-L-glutamine, PBG = poly-(γ-benzyl-L-glutamate), PBA = poly-(γ-benzyl-L-aspartate).
b) DCA = dichloroacetic acid, DCE = dichloroethane.
c) US = ultrasonic absorption, TJ = temperature jump, FJ = E-field jump, diel. = dielectric relaxation.

The apparently most reliable experimental data for the kinetics of α-helix-coil transitions are compiled in table 3. They suggest that the forward rate constants k_F for nonionic polymers are scattered around a value of 10^{10} s^{-1} which corresponds to the upper limit. With regard to ionic polymers, only measurements of poly(L-glumatic acid) can be taken into account. In this case k_F is found to be two orders of magnitude smaller (using a best value for σ of about 2.5×10^{-3} as discussed by Zana [31] which, however, implicates greater uncertainty than for nonionic polymers). Such a considerably reduced forward rate constant of the ionic polymer probably stems from the solvent competition effect discussed above. At any rate the now available experimental results can be quite well understood on the basis of the previously developed theory.

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