polymer communications

Proton magnetic relaxation and dynamics of solid poly-L-proline and polyglycine

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Introduction

The investigation of proton relaxation as a function of frequency and temperature provides valuable insight into the molecular dynamics of polymers both natural and synthetic[1]. In particular the study of proton relaxation in solid homopolypeptides provides basic information for understanding not only the dynamical behaviour of related synthetic polymers but also for understanding the more complex dynamics of solid proteins[2]. Previous work on solid polyalanine, polyleucine and polyvaline[3] has demonstrated the strong proton relaxation generated by methyl group reorientation in the polymer sidechains. So dominant in fact is methyl group relaxation that the contribution of other polymer motions to proton relaxation can be difficult to assess. We have therefore undertaken a proton relaxation investigation of two solid homopolypeptides, namely poly-L-proline and polyglycine, which contain no methyl groups and have no sidechains, in order to investigate the contributions to proton relaxation from polymer main chain motions.

Experimental

Poly-L-proline was supplied in powder form by Sigma, who confirmed that the material was in poly-L-proline II form; in this form the polymer is a left-handed helix with all peptide bonds in the *trans* configuration[4,5]. Two samples were used: one, P-3886, had an average molecular weight of 40000, and the other, P-2254, had an average molecular weight of 9000; these molecular weights correspond to polymer chains of about 400 and 90 monomeric units respectively. 250 MHz high resolution proton n.m.r. spectra in D_2O confirmed the absence of methyl groups in both samples. Polyglycine was supplied in powder form by Sigma (P-0254), average molecular weight 6000 (about 100 monomeric units). Specimens of about 1 g were pumped for 24 h at room temperature and sealed off.

Measurements of the proton n.m.r. spin-lattice relaxation were made at 18, 30 and 60 MHz using a Bruker B-KR 332s variable-frequency pulsed n.m.r. spectrometer in conjunction with an AEI RS2 electromagnet and Datalab signal averaging facilities, over a range of temperature from 10 to 300K, and in the case of polyglycine continued to 400K. A 90 –τ–90 pulse sequence was used, the signals being recorded from the free induction decay about 10 μs after the exciting pulse. Recovery of the nuclear magnetization was exponential within experimental error, and could therefore be characterized by a single spin-lattice relaxation time T_1. The accuracy of T_1 values varied with frequency and temperature of measurement but was typically 5 to 10%.

Results

Experimental values of T_1 for the higher molecular weight sample of solid poly-L-proline are shown as a function of temperature for the three measuring frequencies in *Figure 1*. Measurements of T_1 for the lower molecular weight sample of solid poly-L-proline, made at

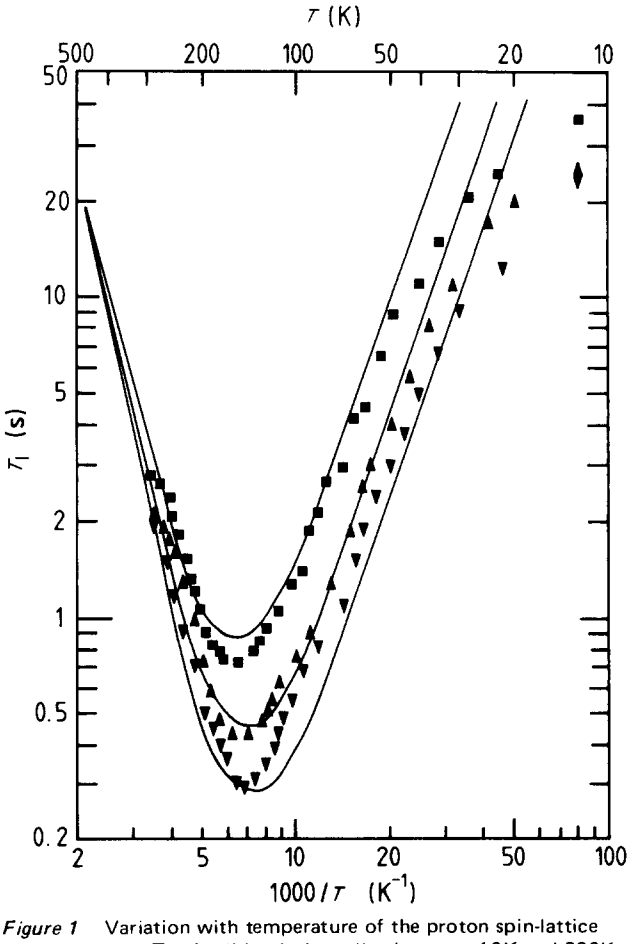

Figure 1 Variation with temperature of the proton spin-lattice relaxation time T_1 of solid poly-L-proline between 10K and 300K. (■), 60 MHz; (▲), 30 MHz; (▼), 18 MHz

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30 MHz only, gave values within experimental error the same as for the higher molecular weight sample.

Experimental values of T_1 for solid polyglycine are shown in *Figure 2*.

Analysis and discussion

The temperature-dependence of T_1 shown in *Figure 1* for poly-L-proline for all three measuring frequencies $\omega_o/2\pi$ display minima which are typical of dipolar relaxation generated by molecular motions which are characterized by thermally activated correlation times τ_c. Moreover the frequency-dependence is indicative of a distribution of correlation times at each temperature, since on the low temperature side of the minima T_1 is not proportional to ω_o^2 and on the high temperature side T_1 is not independent of ω_o as required by the simple Kubo–Tomita theory[6] of nuclear dipolar relaxation based on a single correlation time.

The data have therefore been fitted by computer to an extended Kubo–Tomita relaxation equation[2,7] which includes a normalized logarithmic distribution $F(S)$ of correlation times:

$$T_1^{-1} = C \int_{-\infty}^{\infty} F(S)[\tau_c(1+\omega_o^2\tau_c^2)^{-1} + 4\tau_c(1+4\omega_o^2\tau_c^2)^{-1}]\mathrm{d}S, \quad (1)$$

where

$$S = \ln(\tau_c/\tau_{cm}) \quad (2)$$

and C is the relaxation constant. The median correlation time τ_{cm} is assumed to follow a simple activation law

$$\tau_{cm} = \tau_{om} \exp(E_A/RT) \quad (3)$$

The full lines in *Figure 1* are theoretical curves least-squares fitted by computer to the data for a Gaussian or log-normal distribution:

$$F(S) = (\beta\pi^{1/2})^{-1} \exp(-S^2/\beta^2) \quad (4)$$

in which the distribution parameter β is temperature dependent[8],

$$\beta^2 = \beta_o^2 + (\beta_Q/RT)^2 \quad (5)$$

Below 40K the experimental points fall systematically below the theoretical curves and for this reason experimental values have been included in the fitting procedure down to 50K.

The same parameters $C, \tau_{om}, E_A, \beta_o, \beta_Q$ have been used to fit the data at all three measuring frequencies. The *RMS* deviation of the points from the theoretical curves is 15% which is satisfactory since the values of T_1 cover two decades. The quoted estimates of accuracy of C and E_A were obtained by holding them constant at a series of fixed values in the vicinity of the optimum value and the other parameters were varied to minimize the *RMS* deviation. The overall quality of fit may be regarded as a measure of the success in the choice of distribution function.

In order to identify the molecular motions responsible for the proton relaxation in solid poly-L-proline we first

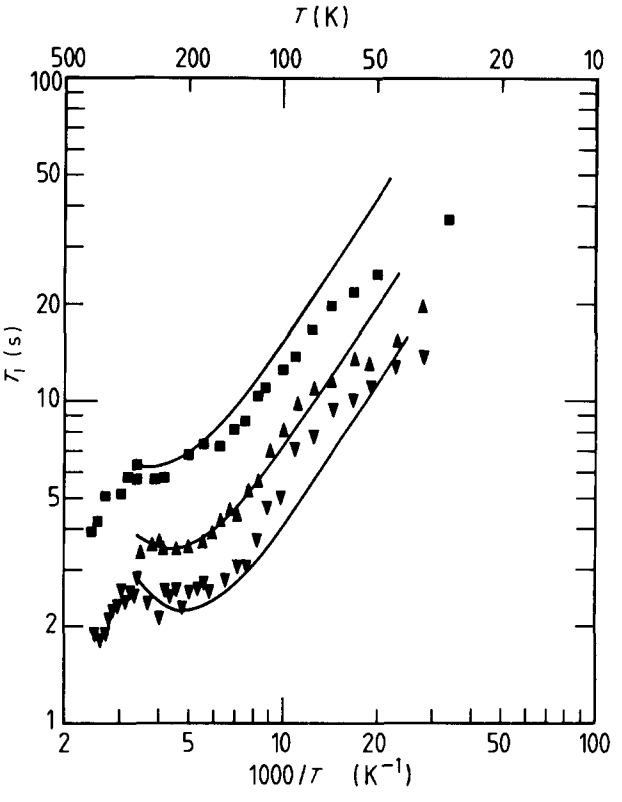

Figure 2 Variation with temperature of the proton spin-lattice relaxation time T_1 of solid polyglycine between 30K and 400K. (■), 60 MHz; (▲), 30 MHz; (▲), 18 MHz

Table 1 Relaxation parameters

	Poly-L-proline	Polyglycine
Relaxation constant C (10^9 s^{-2})	0.96 ± 0.1	0.10 ± 0.03
Mean activation energy E_A (kJ mol^{-1})	11.7 ± 1.5	7 ± 1.5
Pre-exponential factor $\log_{10}\tau_{om}$ (s)	−13.2 ± 1	−11 ± 1
Distribution parameter β_o	2.2 ± 0.5	0 ± 0.5
Distribution parameter β_Q (kJ mol^{-1})	6.0 ± 1	7 ± 2

note that the relaxation mechanism is surprisingly strong for a polymer devoid of methyl groups or side-chains. The relaxation constant C has the value 0.96×10^9 s^{-1} (*Table 1*) which is about one-third of the values found for polyalanine, polyleucine and polyvaline[3] and about a half of the values found for solid proteins[2,9]. Such a strong mechanism points to a source of relaxation within each monomeric unit of the chain; this conclusion is moreover consistent with our finding that T_1 is the same for poly-L-proline samples of different chain length.

The most likely source of relaxation is conformational motion of the proline ring. The proline ring is not a rigid structure and there is evidence of ring puckering motions from ^{13}C relaxation of proline and proline peptides in solution[10]. Such motions have also been postulated to account for line narrowing in broad-line proton n.m.r. of solid poly-L-proline[11]. Although the conformational changes may be somewhat complicated, the simplest motion envisages the C_γ, which lies on one side of the plane of the other four atoms of the ring, moving to its mirror position on the other side of the ring[10,12]. This motion shifts the proton–proton vector of the $C_\gamma H_2$ group between direc-

tions 2γ apart, where γ is the dihedral angle between the plane defined by the C_β, C_γ, C_δ carbon atoms and the plane of the rest of the proline ring.

The effect of reorientation of proton pairs in solids has been considered by Andrew and Brookeman[13]. Assuming equal residence times in the two conformations, the relaxation constant C_o for an ensemble of reorienting pairs is calculated to be

$$C_o = \tfrac{1}{2} C_m \sin^2 2\gamma, \qquad (6)$$

where C_m is the relaxation constant for reorienting methyl groups with the same proton–proton separation. From the geometry of the proline molecule[12,14] and from ^{13}C relaxation studies[10] the angle 2γ is estimated to be about 65°. Taken with the value of C_m of 8×10^9 s^{-2}, based on both experiment and theory[15], we find from equation 6 a value for C_o of 3.3×10^9 s^{-2}. Each reorienting $C_\gamma H_2$ proton has on the average to assist in the relaxation of all seven protons in the monomeric unit. Consequently the expected contribution to the theoretical relaxation constant C is $\frac{2}{7} C_o$, namely 0.94×10^9 s^{-2}. To this, smaller contributions from the concomitant motions of the $C_\beta H_2$ and $C_\delta H_2$ groups must be added. However, the residence times of the two conformations may not be equal[10], which has the effect[16] of reducing C. Moreover there are other ring puckering modes. Nevertheless it is clear that a ring puckering mechanism generates a relaxation mechanism with a relaxation constant of the right order of magnitude to account for the measured value of 0.96×10^9 s^{-2} (*Table 1*), and lends good support to this identification of the molecular motions responsible for the observed proton relaxation behaviour of this polymer.

The behaviour of T_1 for solid polyglycine shown in *Figure 2* contrasts significantly with that for poly-L-proline. The values of T_1 are typically an order of magnitude longer and vary less rapidly with temperature. From 30K to 300K, T_1 falls to an ill-defined minimum, and then above room temperature falls again. Equation 1 has been computer-fitted to the data between 70K and 300K and gives a reasonable fit (13% *RMS* deviation), yielding the parameters listed in *Table 1*.

The relaxation constant C for polyglycine is about 10 times smaller than that for poly-L-proline, and about 25 times smaller than those found for solid proteins[2,9] and methyl-containing homopolypeptides[3], indicating a much weaker relaxation mechanism in solid polyglycine. A wide distribution of correlation times is indicated by the spread of activation energies $E_A \pm \beta_Q = 7 \pm 7$ kJ mol^{-1}. These facts suggest that unlike poly-L-proline, there is not a source of relaxation in each monomeric unit, and that the relaxation reflects the motions of segments of the chain. Moreover the rather large pre-exponential factor τ_{om} (*Table 1*), about 40 times the value of h/kT at 250K, suggests that the relaxation is generated by motions which require a considerable degree of cooperation of the elements of the chain, characterized by a substantial entropy of activation[17]. At temperatures below 50K the values of T_1 are comparable with those found in solid proteins, thus supporting the view that segmental and other main chain motions make an important contribution to relaxation in solid proteins at low temperatures[2,9].

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