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PROTON MAGNETIC RELAXATION AND INTRA-MOLECULAR DYNAMICS IN SOLID POLY-L-GLUTAMIC ACID

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Proton spin-lattice relaxation times (T_1s) have been measured in solid polycrystalline poly-*L*-glutamic acid at three different frequencies 60, 30 and 18 MHz in the temperature range 10K to 400K. Two well resolved and one partially resolved minima have been observed in the relaxation curves (T_1-versus temperature) of poly-*L*-glutamic acid. The comparison of the relaxation behaviour of poly-*L*-glutamic-acid with those of other homopolypeptides suggests considerable amount of motion in the side chains even in the absence of free rotors such as $-CH_3$ and $-NH_3^+$ groups.

The data have been analysed by computer using the Kubo-Tomita relaxation theory modified to include log-normal distribution of correlation times. Parameters characterizing the molecular dynamics have been determined.

Key words: Poly-glutamic acid, Polypeptide, Molecular dynamics.

INTRODUCTION

Using pulsed NMR techniques Andrew *et al.* have performed a series of proton relaxation studies to understand the dynamics of complex protein molecules by stepwise investigations of simple amino acids [1-3], dipeptides, tripeptides and some homopolypeptides [4-6] and a few proteins [7-10]. These experiments have shown that the main cause of proton relaxation in proteins is methyl group reorientation in the side chains of the amino acids residues, though the contribution of other motions such as $-NH_3^+$ group reorientations, main chain motions, ring puckerings and segmental motions was also estimated. Strong relaxation due to reorientation of $-NH_3$ groups in the protonated side chains of poly-*L*-asparagine has also been observed [11].

Poly-*L*-glutamic acid side chain ($-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O^-$) has no free rotors like $-NH_3^+$ or $-CH_3$ groups and therefore is an important polypeptides for the assessment of role of aliphatic side chains, which is often dominated by the strong relaxation due to $-CH_3$ or $-NH_3^+$ bearing side chains.

EXPERIMENTAL

The polycrystalline poly-*L*-glutamic acid (type II B) Na salt was obtained from Sigma Chem. Co. (P-4761) Lot. No. 110f-5038, with average degree of polymerization 140 (mol. wt. 21000). The sample was used without further purification. A deuterated sample was also prepared.

Measurements of spin-lattice relaxation times were made at 60, 30 and 18 MHz mostly using 180-τ-90 pulse sequence and also saturation-τ-90 pulse sequence for very long relaxation times at very low temperatures. The accuracy in T_1 values is 5 to 10 percent.

The details of the sample preparations and the experimental techniques can be found elsewhere [11, 12].

RESULTS AND DISCUSSION

The temperature dependence of the observed spin lattice relaxation times in poly-*L*-glutamic acid at three resonant frequencies is shown in Fig. 1. Two well resolved minima and a partially resolved one observed in the relaxation curve, show that atleast two or probably three separate mechanisms are responsible for the observed relaxation in the homopolypeptides. The frequency dependence of T_1s through out the temperature range and the widths of the minima indicate a distribution of correlation times. The asymmetry of the relaxation curves when drawn on usual log-linear graphs suggests the temperature dependence of the width parameter, (Note that the reciprocal temperature scale in Fig. 1 is also logarithmic in order to show the data conveniently).

The data were therefore fitted to the theoretical curves obtained by adding the relaxation rates due to three independent processes, each described by the well known Kubo-Tomita Equation [13] modified to include a normalized logarithmic distribution of correlation times [14]. The width parameter β is considered to be temperature dependent and distributions in $\ln \tau_o$ and E_A are assumed to be un-

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correlated.

$$\beta = [\beta_o^2 + [\frac{\beta_E}{RT}]^2]^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Theoretical values of T_1 were generated numerically by a computer using the following equation [12].

$$\frac{1}{T_1} = \frac{C\,\tau_c^+}{\beta\,\pi_c^{1/2}} \int_{-\infty}^{+\infty} \left[\frac{\exp(S-(S^2/\beta^2))}{1+\omega_o^2\,\tau_c^{*2}\exp(2S)} + \frac{4\exp(S-(S^2/\beta^2))}{1+4\,\omega_o^2\,\tau_c^{*2}\exp(2S)} \right] ds \quad \ldots\ldots\ldots (2)$$

where the Gaussian or log-normal distribution function is given by

$$F(s) = \frac{1}{\beta\,\pi^{1/2}} \exp(-\frac{S^2}{\beta^2}) \quad \ldots\ldots\ldots\ldots\ldots (3)$$

where C is the relaxation constant, τ_c^* the median correlation time given by the simple activation law

$$\ln \tau_c^* = \ln \tau_o^* + \frac{E_A^*}{RT} \quad \ldots\ldots\ldots\ldots\ldots (4)$$

where τ_o^* and E_A^* are the most probable values τ_o and E_A respectively.

The same parameters C, τ_o^*, E_A^*, β_o and β_E were used for all the three frequencies. The RMS deviation of the points from the theoretical curves was 8.8%. The relaxation parameters of the best fit curves are shown in Table 1, and the best fit curves obtained are shown in Fig. 1 as solid lines. Points below 30K which lower down due to some additional source of relaxation were not included in the fitting.

Comparison of these relaxation curves with those of poly-glycine [6] shows that contribution of the main chain motions to the observed relaxation is negligible. In order to confirm this T_1 were also measured in deuterated samples of poly-*L*-glutamic acid only at 60 MHz which are shown in Fig. 1. The T_1 values in the deuterated sample are 5/6 of those in the undeuterated one which shows that the total number of protons to be relaxed via some relaxation source has decreased from six to five per monomeric unit by the deuteration. The amide protons in the backbone of the polypeptide are the only exchangeable protons and the results suggests that these amide protons do not contribute to the obseved relaxation and only add to the proton load to be relaxed. This confirms that all the observed minima in the relaxation curve are due to side chain mobilities.

There are no free rotors such as $-NH_3^+$ or $-CH_3$ groups in the glutamic acid residue, which could be responsible for the observed minima. In a glutamic acid side chain (CH_2-CH_2-$\overset{O}{\overset{\|}{C}}$-$O^-$) reorientation of $C_\gamma H_2$ about C_β-C_γ bond and $C_\beta H_2$ about C_α-C_β bond are allowed and can cause the proton-proton dipolar hamiltonian to be modulated, thus providing a source of relaxation in the sample.

The mobility of the side chains is generally restricted by collisions with themselves and with the back-bone components. As the distance between the residues is relatively large to facilitate any collision, the side chain components near the back bone are more heavily hindered and thus we expect higher reorientation rates for side chain components which are more remote from the backbone [15]. The reori-

Table 1. Best fit relaxation parameters for Gaussian temperatue dependent distribution in poly-*L*-glutamic acid.

Relaxation parameters	(RMS deviation 8.8%) High temperature 1	Low temperature 2	Lowest temperature 3
$C(S^{-2})$	4.0×10^8	4.5×10^8	4.6×10^8
β_o	2.4	1.2	6.3
β_E	2.2×10^{-2}	1.6	2.2
τ_o^* (S)	5×10^{-12}	9.9×10^{-13}	5.4×10^{-13}
E_A	23.1	9.6	5.5

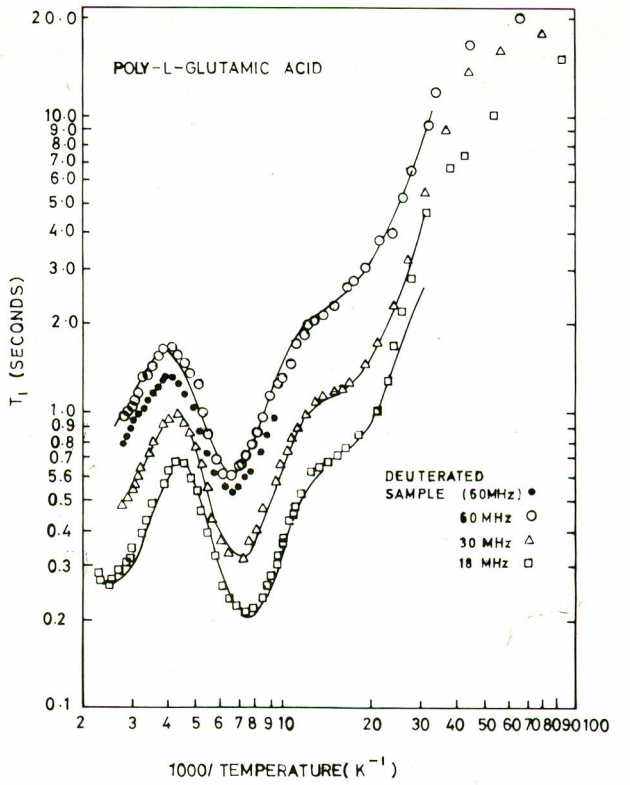

Fig. 1. Proton spin lattice relaxation times in poly-L-glutamic acid at three resonant frequencies with the best fit theoretical curves, as compared to T_1S in deuterated sample of poly-L-glutamic acid at 60 MHz only.

entation of $C_\gamma H_2$ might therefore be at a higher rate as compared to the $C_\beta H_2$ group reorientation. The high temperature minimum may therefore be attributed to the reorientation of $C_\beta H_2$ group about C_α-C_β bond which may require concerted and correlated motion of the chain and the low temperatue minima to $C_\gamma H_2$ group reorientation about C_β-C_γ bond. We may also suspect that the charge at the terminal carbo-oxyl group may have some damping interactions with the surrounding charge lattice and the kinetic unit may vary according to charge state [15].

In order to get some more information about the molecular processes causing the observed minima in the relaxation curve of poly-*L*-glutamic acid, a sample of poly-*L*-aspartic acid (side chain -CH_2-$\overset{O}{\overset{\|}{C}}$-O) was also studied only at 60 MHz in a relatively narrow temperature range (400K-110K). Spin lattice relaxation times as measured in poly-*L*-aspartic acid are shown in Fig. 2 with the T_1 curve of poly-*L*-glutamic acid at 60 MHz (only shown in the narrow temperature range).

It is evident from Fig. 2 that the high temperature minimum which we assumed to be due to $C_\beta H_2$ group reorientation, is also present in poly-*L*-aspartic acid but the lower temperature minimum which we assumed to be due to $C_\gamma H_2$ group reorientation is essentially absent in it. As there is no $C_\gamma H_2$ group present in poly-*L*-aspartic acid these results go in the favour of our assumptions.

The third partially resolved lowest temperature minimum is still difficult to associate to any particular relaxation mechanism. Nearly the same values of relaxation constants obtained from data fitting Table 1 for all the three minima observed suggest that this third minimum might also be due to similar CH_2 group reorientations with slightly different hindering barriers.

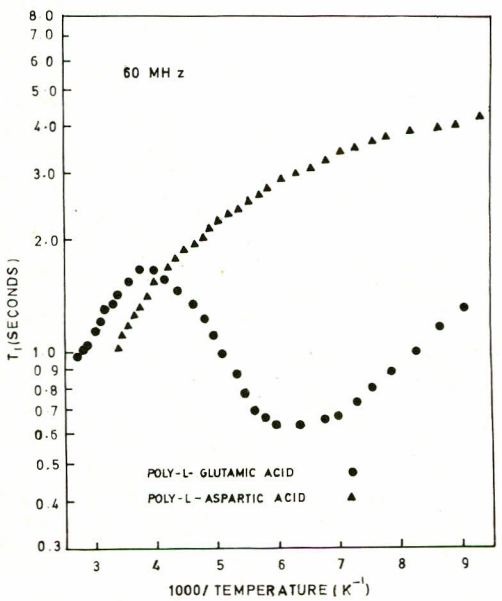

Fig. 2. A comparison of T_1S in poly-L-glutamic acid and poly-L-aspartic acid in a relatively narrow temperature range at 60 MHz.

For a more quantitative analysis we consider a pair of identical nuclei of spin 1/2 reorienting rapidly with correlation time τ_c between two fixed directions, subtending an angle 2α, spending equal times on average in the two directions, the intrinsic relaxation constant is given by

$$C_o = \frac{9}{40} \gamma_p^4 h^2 b^{-6} \sin^2 2\alpha \quad \ldots\ldots\ldots\ldots\ldots (5)$$

where γ_p is the gyromagnetic ratio of a proton, h the plancks constant/2π, b the inter proton distance.

It is of interest to compare this result with the value of C_m for relaxation by reorienting groups of three nuceli is in -CH_3 groups, where the intrinsic relaxation constant is given as

$$C_m = \frac{9}{20} \gamma^4 h^2 b^{-6} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Assuming the same inter proton distances in the methyl and the methylene groups we get

$$\frac{C_o}{C_m} = \frac{1}{2} \sin^2 2\alpha \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Assuming that only one -CH- group reorientation between two fixed directions with equal residence times is causing the relaxation at the temperature of each minimum, we can obtain C_o from the best fitted parameters C for all the three relaxation processes of our experimental results.

$$C_o = \frac{6}{2} C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

The factor 6/2 is due to the assumption that only two protons are relaxing the total of 6 protons per monomeric unit in poly-*L*-glutamic acid at the temperture of each minimum. Comparing equations 7 and 8 we get 2α as 33°, 35.4°, for the high and the prominent low temperature minima respectively. A larger value of 2α for reorientation of $C_\gamma H_2$ group as compared to $C_\beta H_2$ group is also as should be expected.

Other relaxation parameters obtained from the fitting of the data also seem to be reasonable. A very high activation energy (23kJ/mole) and high value of τ_o^* (5×10^{-12} s) (as compared to h/kT which ~1.4×10^{-13} at 340K) for the highest temperature relaxation process (see Table 1) suggests larger hindrances of the surrounding components to the sunmolecular mobilities causing the minimum and a correlated motion of the neighbouring components. This is more

likely to happen for reorientation of groups closer to the backbone. A small distribution inactivation energies (β_E = 2.2 x 10^{-2} kJ/mole) indicate that the hindering barriers are very similar.

The values of τ_o of the two low tempeature minima are roughly the ordre of h/kT at their temperatures of minima, which suggests that the reorientation takes place without much cooperation of other neighbouring components. A very large distribution of correlation times, obtained by fitting (β_o = 6.3) for the lowest temperature minimum suggests that the reorienting submolecular groups causing the dominant relaxation in this region are situated in a variety of surroundings and this is more probable to happen in the case of more remote side chain components particularly if the sample has not a homogenously crystalline environment.

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