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INTERNAL DYNAMICS IN SOLID POLY-L-ASPARAGINE

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A proton magnetic relaxation study on poly-L-asparagine in the solid state has been carried out in the temperature range 10 K to 400 K at three different frequencies, 60, 30 and 18 MHz.

The temperature dependence curve of spin-lattice relaxation times exhibits a single minimum, typical of dipolar relaxation. The depth of the minimum predicts the presence of some extra NH_3^+ groups in the poly-peptide. Activation parameters characterizing the molecular dynamics have been determined.

Key words: Poly-L-Asparagine, Poly peptide, Molecular dynamics.

INTRODUCTION

Nuclear Magnetic Resonance (NMR) is a very useful technique to get a direct and detailed insight into processes at molecular and atomic levels. The measurement of proton relaxation as a function of frequency and temperature gives a valuable insight into the molecular dynamics of polymers, both natural and synthetic [1.2].

Investigations into the internal dynamics of proteins and polypeptides are very useful in understanding the molecular details of protein functions. Efforts are being made to correlate different types of motions to different functions of proteins. Homopoly-peptides are very good model compounds for proteins.

Homopoly-peptides studied uptil now include poly-L-alanine, poly-leucine, poly-valine [3,4]. Poly-glycine and polyproline [5]. The first three homopoly-peptides have methyl groups in their side chains; therefore, their study demonstrated the strong proton relaxation generated by methyl groups reorientation. The dominant sources of relaxation of poly-proline and poly-glycine are proline ring puckering and main chain motions respectively.

Poly-L-asparagine was chosen for this study to assess the role of other motions in aliphatic side chains which are often dominated by strong relaxation due to methyl bearing side chains in proteins.

EXPERIMENTAL

The polycrystalline poly-L-asparagine was obtained from Sigma Chemical Company (p.8137) Lot Number 52-F-5066 with average degree of polymerisation 90 (mol. wt. 10,000). The sample was used without further purification. It was pumped for more than 48 hr. to remove absorbed moisture and oxygen and was sealed in glass tube under vacuum. A sample of deuterated poly-L-asparagine was also prepared by three recrystallizations from D_2O, replacing all exchangeable protons with deuterons.

Measurements were made at 60,30 and 18 MHz using a Bruker B-KR 322S variable-frequency pulsed NMR spectrometer in conjunction with an $AEIRS_2$. Electromagnet and Datalab DL 922/4000 B signal Average. The spin relaxation times (T_1) were mostly measured using $180^o - t - 90^o$ pulse sequence, while long T_1 values (at very low temperature) were measured by saturations-t-90^o sequence. The recoveries of nuclear magnetization in all cases were found to be exponential allowing their characterization by a single spin lattice relaxation time. The accuracy in T_1 values varied with frequency and temperature of the measurement but was typically 5 to 10%.

The temperature of the sample was controlled by one of the two systems, both based upon the gas flow system. One was Bruker B. ST 100/700 which employed nitrogen gas flow and was used to obtain temperatures above 100K. The other was Oxford system which was used for temperatures below 120K and employed helium gas flow.

RESULTS AND DISCUSSION

The proton spin lattice relaxation times in Poly-L-asparagine observed over the full range of temperature at all three frequencies are shown in Fig. 1. The relaxation curve displays a single minimum at about 180K which is typical of dipolar relaxation generated by molecular motions which are characterized by thermally activated motion with a correlation time τ_c. The width of the minimum and the non-proportionality of T_1s to ω_o^2 at low temperature indicate a distribution of correlation times at each temperature.

The data have therefore been analysed in terms of Kubo-tomita relaxation equation [6] extended in the manner suggested by Connor [7] to cover a distribution of correlation times

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

where C is the relaxation constant, F(s) is a normalized logarithmic distribution function of the correlation times τ_c, with

$$S = \mathrm{Ln}\,(\tau_c / \tau cm) \qquad \ldots (2)$$

where the median correlation time τ_{cm} follows a simple activation law :

$$\tau_{cm} = \tau_{cm} \exp (E_A/RT) \qquad \ldots (3)$$

where τ_{cm} is the pre-exponential factor, E_A the activation energy, R the gas constant and T the absolute temperature. The full lines in Fig. 1 are theoretical curves least squares fitted to Equations 1,2 and 3 using the Nottingham University ICL 1906A computer, for a Gaussian or log-normal distribution :

$$F(s) = (\beta\pi^{1/2})^{-1} \exp(-S2/\beta^2)$$

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

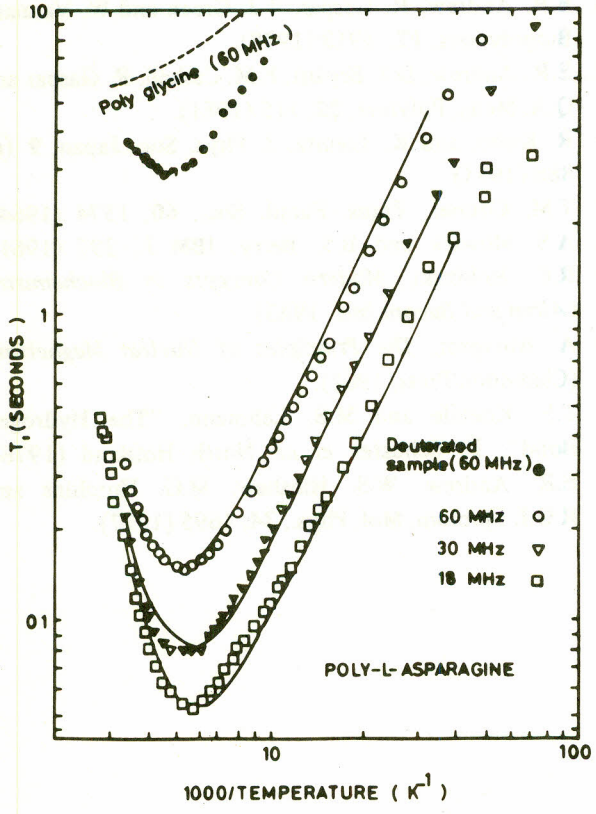

Fig. 1. Proton spin lattice relaxation times in poly-L-asparagine at three different frequencies with best fit theoretical curves, as compared to T_1S in the deuterated sample of poly-L-asparagine (only at 60 MHz) T_1S in poly-glycine at 60 MHz from Andrew *et al* [5] are also shown for comparison.

$$\beta^2 = \beta_o^2 + (\beta_\epsilon/RT)^2$$

The same parameters C, τom, E_A, β_o and β_E were used for the three frequencies. The RMS deviation of the points from the theoretical curves was 9.8 %. The relxation parameters of the best fit curves are :

C	=	4.5×10^9 (S^{-2})
τ_{om}	=	9.2×10^{-13} Sec.
E_A	=	10.4 KJ/mole
β_o	=	1.2×10^{-9}
β_E	=	6.7 KJ/mole.

It may be noted that the points below 20K have not been included in the analysis as they fall systematically below the theoretical curves due to some anomalous relaxation.

In order to identify the molecular motions responsible for the observed minimum in the proton relaxation curve, a deuterated sample was also prepared. The T_1S in the deuterated poly-L-asparagine (at 60 MHz only) are also shown in the figure. Very long relaxation times were observed in the deuterated sample as compared to those in the undeuterated one. This shows quite clearly that the observed minimum in the relaxation curve of poly-L-asparagine is due to reorientation of a group which consists of exchangeable protons. The only group in poly-asparagine side chain which consists of protons exchangeable in aqueous solution is the NH_2 group, though the NH group protons in the peptide backbone are also exchangeable.

Comparison of the data with the relaxation curve of poly-glycine (the homopoly-peptide without any side chain) shows that the backbone contribution to the observed relaxation in poly-L-asparagine is quite negligible, at least at or near the temperature where the minimum is observed. We will thus have to associate the observed minimum in the relaxation curve with the reorientation of NH_2 group of the asparagine side chain.

The amino group of asparagine side chain is a planar group and its twofold reorientation about an axis normal to the proton – proton vector generates little relaxation since the intra group proton-proton contribution to the dipolar Hamiltonion of the system is unchanged by this transposition. However the two-fold reorientation does modulate the dipolar interaction between the protons in the-NH_2 group and other protons in their environment, and between the protons and nitrogen nucleus giving a weaker relaxation mechanism.

Reorientation of the terminal $-NH_3^+$ group of the main chain cannot have a measurable contribution to the relaxation as this would require 542 protons to be relaxed

per $-NH_3^+$ group (degree of polymerization of the poly-L-asparagine sample used was 90).

We may suspect that the terminal $-NH_2$ groups of the side chains in poly-L-asparagine are protonated to $-NH_3^+$ groups, like those found in the lysine side chains [9] and give the extra relaxation observed.

Assuming the terminal-NH_2 groups of all the side chains of poly-L-asparagine to be in the protonated form and considering $-NH_3^+$ group reorientation to be the cause of observed relaxation minimum, the theoretical value of relaxation constant 'C' may be calculated as

$$C = \frac{3}{n} Co$$

Where n is the number of protons to be relaxed by each $-NH_3^+$ group and Co is the intrinsic relaxation constant of $-NH_3^+$ group given by [10].

$$Co = \frac{9}{20} \times \frac{\gamma^4 \hbar^2}{b^6}$$

γ is the gyromagnetic ratio of protons, $\hbar$ the *Planek constant* and b the interprotons distance. The calculated value of 2π C thus obtained taking b = $1.68A^{O11}$ and n = 7 comes out to be 4.9×10^9 S^{-2} which is very close to the best fitted relaxation constant 4.5×10^9 S^{-2}.

The low value of mean activation energy (10.4KJ/mole) in poly-L-asparagine as compared to the high activation energies (27.8 – 51.7KJ/mole) for $-NH_3^+$ group reorientations in simple amino acids in Zwitterion form [12], shows that the $-NH_3^+$ groups are very weakly hydrogen bonded in the polymer.

A large distribution in activation energies (E_A = 10.4 ± 6.7 KJ/mole) suggests that the hindering barriers are to a large extent either inter-molecular or at least inter-chain and thus might depend upon crystal packing, hydrogen bonding, secondary and tertiary structures of the polypeptide and electrostatic interactions.

The value of τ_{om} (9.2×10^{-13}), the mean pre-exponential factor ($\frac{\hbar}{KT}$ at 180K $\sim$ 10^{-13}) suggests that the reorientation takes place without much cooperative interaction of the neighbouring groups.

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