Pure & Appl. Chem. Vol.54, No.3, pp.585-594, 1982.
Printed in Great Britain.

0033-4545/82/030585-10$03.00/0
Pergamon Press Ltd

PROTON RELAXATION STUDIES OF DYNAMICS OF PROTEINS IN THE SOLID STATE

E. R. Andrew, D. N. Bone, D. J. Bryant, E. M. Cashell*, R. Gaspar Jr.† and Q. A. Meng¶

Department of Physics, University of Nottingham, University Park, Nottingham, UK

Abstract - Dynamical information concerning proteins and other macromolecules in the solid state may be obtained from investigations of proton magnetic relaxation. Measurements have been made of T_1 for protons at 18, 30 and 60 MHz on polycrystalline α-chymotrypsin, insulin, lysozyme and ribonuclease A in the temperature range 10 to 300 K. Analysis identifies the predominant source of relaxation between 70 and 250 K as methyl group reorientation in amino acid sidechains. Activation parameters characterizing a distribution of methyl rotors are deduced. Deuteration gives information concerning the relaxation contribution of water and other exchangeable protons. Investigations were extended to solid polymers devoid of methyl groups such as polyglycine and poly-L-proline to give information concerning relaxation contributions from main chain motions and ring puckering. The measurement of dipolar relaxation enables slower motions to be investigated. Measurements of T_{1D} in solid α-chymotrypsin and solid lysozyme exhibit behaviour similar to T_1 but displaced about 100 K lower in temperature, and enable other dynamical effects to be observed at higher temperatures.

INTRODUCTION

In the functional performance of proteins in living systems their structure, conformation and dynamical behaviour play a central role. X-ray diffraction has been conspicuously successful in determining the detailed three-dimensional structure adopted by protein molecules in the crystalline state and has more recently given information concerning their dynamical behaviour (Ref. 1,2). Dynamical information is also gained from fluorescence polarization spectroscopy (Ref. 3), fluorescence quenching (Ref. 4), thermodynamics and statistical mechanics (Ref. 5), ab initio dynamics (Ref. 6,7) and from high-resolution NMR spectroscopy of proteins in solution (Ref. 8-10). Moreover a recent comparison of high-resolution ^{13}C NMR spectra of lysozyme in polycrystalline form with that of lysozyme in solution (Ref. 11) is consistent with the view that the molecular conformation is closely similar in solid and liquid states.

It is therefore of interest to investigate the dynamical properties of protein molecules in the solid state by proton NMR relaxation studies. Such studies complement those obtained in solution and relate to the motions of the molecules in the environment in which their X-ray structures were determined. Moreover in the solid state a much wider range of temperature is accessible without molecular degradation and this gives the opportunity to characterize the molecular motions with activation parameters.

Four proteins have been investigated in polycrystalline form, namely α-chymotrypsin, insulin, lysozyme and ribonuclease A (Ref. 12,13). These protein molecules are composed of 241, 51, 129 and 124 amino acid residues respectively; lysozyme and ribonuclease consist of single polypeptide chains, insulin of two chains (21 and 30 residues) and α-chymotrypsin of three chains (13, 131 and 97 residues). Since methyl groups are found to play an important role in the proton spin-lattice relaxation of these four solid proteins, work has also been carried out on solid polyglycine and poly-L-proline, both of which contain no methyl groups, and this enables the contribution of other molecular motions to be assessed.

Large biomolecules have many degrees of freedom and the many modes of molecular motion are characterized by a wide range of frequencies each with its own temperature-dependence. Measurements of the nuclear spin-lattice relaxation time T_1 are particularly responsive to dynamic behaviour in the spectral range from 10^5 to 10^{10} Hz and yield information in this range. We have recently extended investigations to the measurement of the proton dipolar relaxation time T_{1D} in solid proteins. This parameter is responsive to much slower motions and gives information in the spectral range 1 to 10^5 Hz, enabling a wide range of dynamical behaviour to be covered overall.

*Present Address: Cork Regional Technical College, Bishopstown, Cork, Ireland.
†Present Address: Department of Biophysics, Medical University of Debrecen, Hungary-4012.
¶Present Address: Institute of Physics, Chinese Academy of Sciences, Peking, China.

MATERIALS

The α-chymotrypsin was supplied by Sigma from bovine pancreas, 3x crystallized and lyophilised, dialysed salt free (type II).

A high purity sample of solid bovine insulin was kindly provided by the Research Department of the Boots Company, Nottingham. Assay by disc polyacrylamide gel electrophoresis showed < 0.1% each of pro-insulin, arginine insulin, intermediate, mono- and di-desamidoinsulin; pro-insulin by radioimmunoassay 3.6 ± 0.6 ppm, high molecular weight impurity 0.33%; zinc 0.3%. The sample had been crystallized and dried in vacuo. It was stored at -20°C to minimize formation of high-molecular weight impurity. A second sample of bovine pancreatic insulin, obtained from Sigma, product no. 15500, gave results within experimental error the same as the Boots sample.

The lysozyme was obtained from BDH, described as crystalline from egg white, 3x crystallized, approximately 25000 units per mg. A sample of 'deuterated' lysozyme was also prepared by three recrystallizations from D_2O, replacing all exchangeable protons with deuterons.

The ribonuclease A was supplied by Sigma from bovine pancreas, 5x crystallized (type 1-A), protease-free and essentially salt-free.

The poly-L-proline was supplied in powder form by Sigma who confirmed that the material was in poly-L-proline II form. 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 NMR spectra in D_2O, kindly obtained by Dr. H. Booth, 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).

In each case specimens of about 1 g were pumped for 24 hours at room temperature and sealed off.

EXPERIMENTAL PROCEDURE

Measurements of the proton NMR spin-lattice relaxation time T_1 were made at frequencies $\omega_o/2\pi$ of 18, 30 and 60 MHz using a Bruker B-KR 322s variable-frequency pulsed NMR spectrometer in conjunction with an AEI RS2 electromagnet and Datalab signal averaging facilities, over a range of temperature from 10 K to 300 K, and in the case of polyglycine to 400 K. A 90^o-τ-90^o or sat-τ-90^o pulse sequence was used. Recoveries of nuclear magnetization were exponential within experimental error, and could therefore be characterized by a single relaxation time T_1. The accuracy of T_1 values was typically 5 to 10%.

Measurements of the proton dipolar relaxation time T_{1D} were measured using the three-pulse sequency of Jeneer and Brockaert (Ref. 14) namely $90^o_{0^o}$-t_1-$45^o_{90^o}$-t_2-$45^o_{90^o}$. The maximum signal following the third (observation) pulse was found to follow a strictly exponential decay within experimental error, whose time constant is T_{1D}; the accuracy of measured values was about 5%. Measurements at 18 and 60 MHz were the same within experimental error.

RESULTS AND DISCUSSION: SOLID PROTEINS

Work was first carried out between 120 and 300 K and the results for solid α-chymotrypsin and solid lysozyme are shown in Figs. 1 and 2 respectively. The temperature variation of the proton spin-lattice relaxation time T_1 exhibits minima characteristic of nuclear relaxation effected by thermally activated molecular motions. However unlike the behaviour found earlier for solid monomeric amino acids (Refs. 15-17), the data in Figs. 1 and 2 cannot be analyzed in a consistent manner at all three frequencies on the assumption that the molecular motions are characterized by a single correlation time τ_c. The curves are broad and shallow, and T_1 is not independent of ω_o at high temperatures nor proportional to ω_o^2 at low temperatures as predicted by the dipolar relaxation theory of Kubo and Tomita (Ref. 18) for motion characterized by a single correlation time. These features are typical of molecular motions characterized by a distribution of correlation times τ_c and for large molecules such as proteins such a conclusion is not unexpected. It should be noted however that measurements made at a single NMR frequency over a restricted range of temperature can often be fitted to the Kubo-Tomita theory using a single thermally-activated correlation time, and this underlines the importance of making measurements at several measuring frequencies.

We have therefore analyzed the data in terms of the Kubo-Tomita relaxation equation extended in the manner suggested by Connor (Ref. 19) to cover a distribution of correlation times:

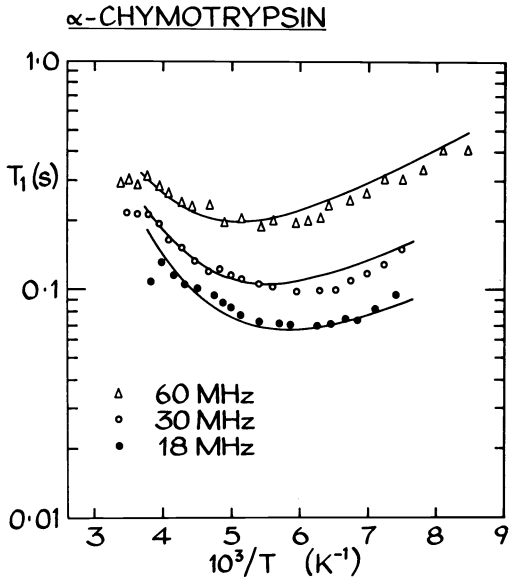

Fig. 1. The temperature dependence of T_1 in solid α-chymotrypsin between 120 and 300 K. The full lines are theoretical curves calculated in the manner described in the text.

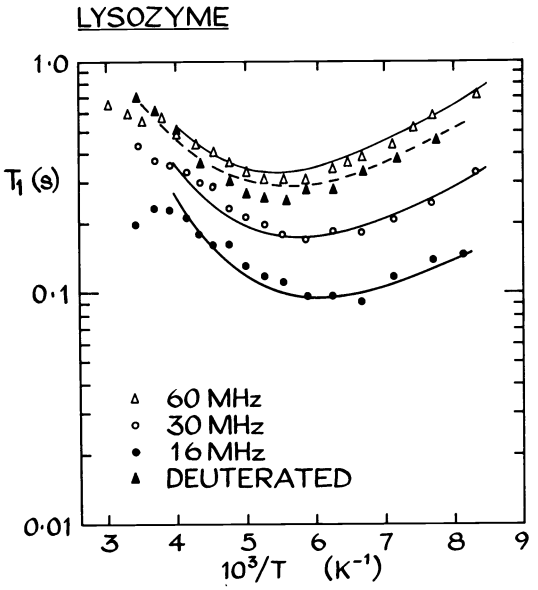

Fig. 2. The temperature dependence of T_1 in solid lysozyme between 120 and 300 K. The full lines are theoretical curves calculated in the manner described in the text.

$$T_1^{-1} = C \int_{-\infty}^{\infty} F(S)[\tau_c(1 + \omega_o^2\tau_c^2)^{-1} + 4\tau_c(1 + 4\omega_o^2\tau_c^2)^{-1}]dS \qquad (1)$$

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

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

We suppose that the median correlation time $\tau_{c\,m}$ follows a simple activation law:

$$\tau_{c\,m} = \tau_{o\,m} \exp(E_A/RT). \tag{3}$$

The full lines in Figs. 1 and 2 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:

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

in which the distribution parameter β is temperature-dependent (Ref. 20):

$$\beta^2 = \beta_o^2 + (\beta_Q/RT)^2. \tag{5}$$

The same parameters C, $\tau_{o\,m}$, E_A, β_o, β_Q were used for the three frequencies; the RMS deviation of the points was 7.5%. Several other distribution functions F(S) were considered and in particular the Fuoss-Kirkwood distribution (Ref. 21) and the Gaussian distribution with temperature-independent parameter β; for these two distributions a closely similar quality of fit was obtained over this temperature range.

In order to discriminate between the three distributions F(S) which fitted the data satisfactorily down to 120 K, experiments were extended to 10 K since the behaviour forecast by the three distributions diverges strongly from each other at lower temperatures. Results for solid lysozyme are shown in Fig. 3. Over this much wider range of relaxation times only the lognormal distribution with temperature-dependent β gave a tolerable fit at all three frequencies, with parameters given in Table 1, and shown as full lines in Fig. 3. It will be noticed that the experimental points fall systematically below the theoretical curves below 50 K and also above 250 K. The additional sources of relaxation responsible are discussed later. In the computer fitting procedure experimental points outside the range 50 to 250 K have therefore not been included.

Measurements of T_1 for solid insulin are shown in Fig. 4 together with computer-fitted theoretical curves (Ref. 13). Qualitatively similar results have been obtained for solid α-chymotrypsin and solid ribonuclease A (Ref. 12). The average RMS deviation of the experimental points from the theoretical lines for the four proteins is 17%, which is very satisfactory considering that the values of T_1 cover two decades.

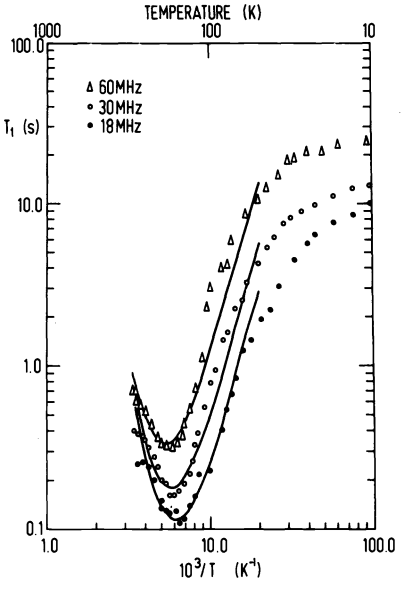

Fig. 3. The temperature dependence of T_1 in solid lysozyme between 10 and 300 K. The full lines are theoretical curves calculated in the manner described in the text.

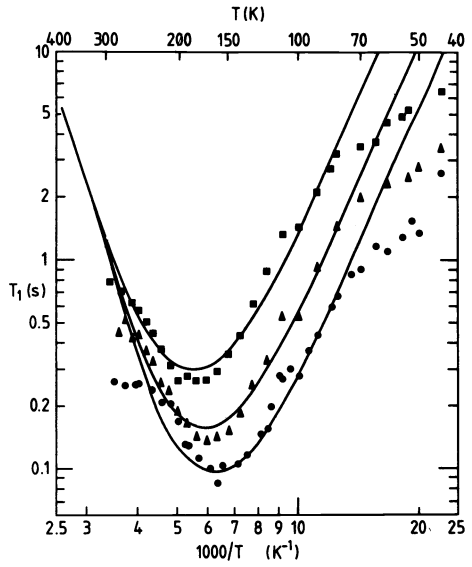

Fig. 4. The temperature dependence of T_1 in solid insulin between 40 and 300 K. Squares 60 MHz, triangles 30 MHz, circles 18 MHz. The full lines are theoretical curves calculated in the manner described in the text.

TABLE 1. Relaxation parameters for solid proteins

Protein	C (10^9 s^{-2})	E_A (kJ/mol)	log $\tau_{o\,m}$ (s)	β_o	β_Q (kJ/mol)
α-Chymotrypsin	3.07	10.1	-11.9	0	6.4
Insulin	1.97	13.5	-12.9	0.5	5.1
Lysozyme	1.81	12.6	-12.6	0	5.6
Ribonuclease A	2.30	16.2	-13.6	0	8.0
Accuracy	±0.2	± 1.5	± 0.6	±0.5	±0.6

The best values of C, $\tau_{o\,m}$, E_A, β_o and β_Q are given for all four proteins in Table 1. The quoted estimates of accuracy were obtained by holding one parameter constant at a series of fixed values in the vicinity of the optimum value and the other parameters were then varied to minimize the RMS deviation. In this manner it was possible to explore the minimum in the 5-parameter space.

We notice from Table 1 that the constant part β_Q of the distribution parameter β is negligibly small, so that β is effectively given by the temperature-dependent part (β_Q/RT). Thus it appears that the distribution of correlation times arises essentially from a distribution of activation energies; the range $E_A \pm \beta_Q$ encompasses some 84% of the distribution, since the error function I(1) = 0.843.

We now consider the source of this spin-lattice relaxation. Previous work on amino acids (Ref. 15-17), peptides (Ref. 22) and homopolypeptides (Ref. 23) in the solid state has shown that an important source of proton spin-lattice relaxation arises from the hindered rotation of methyl groups in the sidechains of the residues alanine, isoleucine, leucine, methionine, threonine and valine. Several pieces of evidence support the view that this is the case in the four solid proteins also. First we notice that the minimum value of T_1 at 60 MHz in all four occurs at about 180 K as in most of the related materials (Ref. 15-17,22, 23). Secondly the distributions of activation energies $E_A \pm \beta_Q$ given in Table 1,for example 13.5 ± 5.1 kJ/mol for solid insulin, 12.6 ± 5.6 kJ/mol for solid lysozyme, are similar to the range 14.5 ± 8 kJ/mol found for the methyl groups in the solid amino acids (Ref. 16,17). While we should not expect the hindrances to methyl reorientation to be the same in the protein as in the pure polycrystalline amino acid, nevertheless it is satisfactory that they are not very different.

Next we consider the strength of the relaxation process, given by the relaxation constant C. If the reorientation of the methyl groups provided the only source of relaxation in the solid protein, the whole assembly of protons being maintained at a common spin temperature by rapid spin diffusion, the relaxation constant would be given by

$$C' = \frac{3}{n} C_m \ , \qquad (6)$$

where C_m is the relaxation constant for isolated methyl groups, and n is the number of protons which the three methyl protons must, on average, relax. The relaxation constant C_m for isolated methyl groups is 8×10^9 s^{-2} (Ref. 17). In Table 2 the values of n, C' and C are given for the four solid proteins. From the ratios C'/C we see that methyl group reorientation can account for 62 to 89% of the proton spin-lattice relaxation. This leaves 11 to 38% to be accounted for by other sidechain motions, segmental motion, reptation and whole-body motions.

TABLE 2. Relaxation constants

Protein	No. of Protons	No. of Methyl Groups	n	C' (10^9 s^{-2})	C (10^9 s^{-2})	$\frac{C'}{C}$
α-Chymotrypsin	1752	149	11.8	2.03	3.07	0.66
Insulin	377	28	13.5	1.78	1.97	0.89
Lysozyme	956	61	15.7	1.53	1.81	0.84
Ribonuclease A	907	54	16.8	1.43	2.30	0.62

Data in columns 2 and 3 compiled from 'Atlas of Protein Sequence and Structure 1967-1968' by M. O. Dayhoff and R. V. Eck, National Biomedical Research Foundation, Silver Spring, USA (1968).

It is to be seen from Fig. 2 that above 250 K the experimental values of T_1 for solid lysozyme fall below the extrapolated theoretical curves; on the other hand for the sample of 'deuterated' lysozyme they do not. This suggests that the additional proton relaxation above 250 K in ordinary lysozyme arises from motion of water molecules and side groups containing exchangeable protons. Below 250 K the values of T_1 for the 'deuterated' sample are about 10% lower than for normal lysozyme, suggesting that about 10% of the protons in the sample were exchanged.

Below 70 K the experimental values of T_1 fall systematically below the theoretical curves for all four solid proteins, as illustrated for solid lysozyme in Fig. 3 and for solid insulin in Fig. 4. Here the correlation times for methyl group reorientation by excitation over hindering barriers become too long for efficient relaxation and other motional processes evidently become more important. One of these is the motion of groups with weaker dipolar interactions, such as methylene and imino groups, brought about by sidechain and backbone motions. We make further reference to these when discussing solid polyglycine in the next section. A second class of relaxation processes, particularly important at low temperatures, are non-classical processes such as phonon-assisted methyl group tunnelling and phonon-scattered Raman processes (Ref. 24,25).

RESULTS AND DISCUSSION: HOMOPOLYPEPTIDES

The measured values of T_1 for the higher molecular weight sample of solid poly-L-proline for the three measuring frequencies are displayed as a function of temperature in Fig. 5. Measurements with the lower molecular weight sample gave values within experimental error the same as for the higher molecular weight sample.

The T_1 data for solid poly-L-proline between 50 and 300 K have been computer-fitted to the extended Kubo-Tomita relaxation equation (1) in conjunction with Equations (2) to (5) using the same procedures as for the solid proteins. The best values of the relaxation parameters are given in Table 3. The RMS deviation of the measured points from the theoretical curves is 15%.

We note that the relaxation mechanism is quite strong for a polymer without methyl groups or sidechains. The relaxation constant C has the value 0.96×10^9 s^{-2}, about half of the value for the solid proteins (Table 1) and about one-third of the values for solid polyalanine, polyleucine and polyvaline. The strength of the mechanism suggests a source of

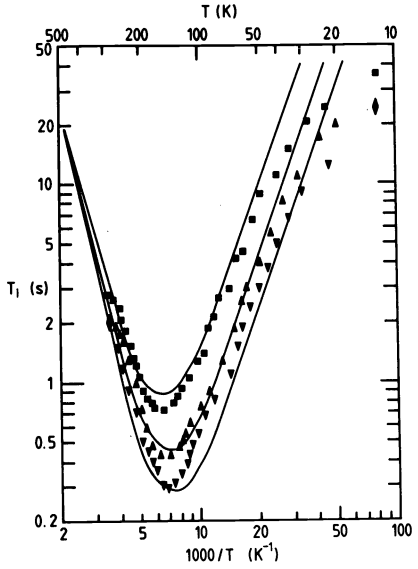

Fig. 5. The temperature dependence of T_1 in solid poly-L-proline between 10 and 300 K. Squares 60 MHz, upright triangles 30 MHz, inverted triangles 18 MHz. The full lines are theoretical curves calculated in the manner described in the text.

TABLE 3. Relaxation parameters for solid homopolypeptides

Material	C (10^9 s^{-2})	E_A (kJ/mol)	log $\tau_{o\,m}$ (s)	β_o	β_Q (kJ/mol)
Poly-L-proline	0.96 ± 0.1	11.7 ± 1.5	-13.2 ± 1	2.2 ± 0.5	6.0 ± 1
Polyglycine	0.10 ± 0.03	7 ± 1.5	-11 ± 1	0 ± 0.5	7 ± 2

relaxation within each monomeric unit of the chain, a conclusion which is consistent with the experimental 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 for which there is evidence from ^{13}C relaxation of proline and proline peptides in solution (Ref. 26) and from broad-line proton studies in poly-L-proline (Ref. 27). Analysis of the contribution of ring puckering motions to the proton spin-lattice relaxation of poly-L-proline yields a relaxation constant C close to the measured value (Ref. 28), and provides good support for this identification of the molecular motions responsible for the observed proton relaxation behaviour in this polymer.

In α-chymotrypsin there are nine proline residues, in insulin one, in lysozyme two and in ribonuclease A four. Ring puckering motions, causing reorientation of proton pairs on the ring, can therefore be expected to contribute to proton relaxation in proteins.

The behaviour of T_1 for solid polyglycine (Ref. 28) 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. Equation (1) has been computer-fitted to the data between 70 and 300 K and gives a reasonable fit (13% RMS deviation), yielding the parameters listed in Table 3.

The relaxation constant C for solid polyglycine is about ten times smaller than that for poly-L-proline, and about 25 times smaller than those for solid proteins (Table 1) and methyl-containing homopolypeptides (Ref. 23), 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 . These facts may 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 which modulate the dipolar interactions of the methylene

and imino groups. Furthermore the rather large mean pre-exponential factor $\tau_{o\,m}$ (Table 3), about forty times the value of h/kT at 250 K, suggests that the relaxation is generated by motions which require a considerable degree of co-operation of the elements of the chain and characterized by a substantial entropy of activation.

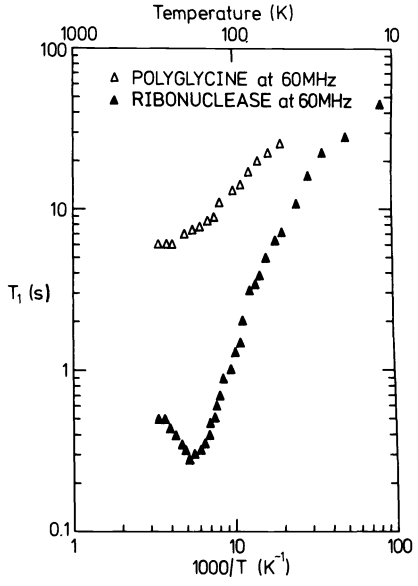

Fig. 6. Comparison of the variation with temperature of T_1 for solid polyglycine and solid ribonuclease between 10 and 300 K.

The constrast of relaxation behaviour between the solid proteins, typified by ribonuclease, and solid polyglycine is illustrated by the relaxation times T_1 at 60 MHz in Fig. 6. Between 50 and 300 K the methyl contributions dominate the relaxation process for solid ribonuclease and at the temperature of the minimum of T_1 its value is 25 times shorter than that for polyglycine. On the other hand below 50 K the main chain motions are most important for both polymers and their relaxation times are more nearly equal.

RESULTS AND DISCUSSION: DIPOLAR RELAXATION

Experimental values of the proton dipolar relaxation time T_{1D} for solid α-chymotrypsin between 40 and 300 K are shown in Fig. 7. Results for solid lysozyme and 'deuterated' lysozyme are qualitatively similar to those of α-chymotrypsin. All display a minimum in T_{1D} at about 90 K. Such minima reflect the optimum efficiency of relaxation of the dipolar reservoir by some dynamical process; this efficiency is greatest when the correlation time τ_c characterizing this process satisfies $\omega_L\tau_c \sim 1$, where $\omega_L = \gamma H_L$ and H_L is the effective local dipolar field.

While a rigorous theoretical description of the dependence of T_{1D} on τ_c has not yet been given which spans both the weak collision regime ($\omega_L\tau_c < 1$) and the strong collision regime ($\omega_L\tau_c > 1$), nevertheless an expression which reasonably bridges both regimes is the following (Ref. 29-31):

$$T_{1D}^{-1} = C\tau_c(1 + \omega_L^2\tau_c^2)^{-1} \quad , \tag{7}$$

where C is the relaxation constant encountered in Equation (1). For molecular motion characterized by a distribution of correlation times, we may generalize Equation (7) to

$$T_{1D}^{-1} = C \int_{-\infty}^{\infty} F(S)\tau_c(1 + \omega_L^2\tau_c^2)^{-1}\, dS \tag{8}$$

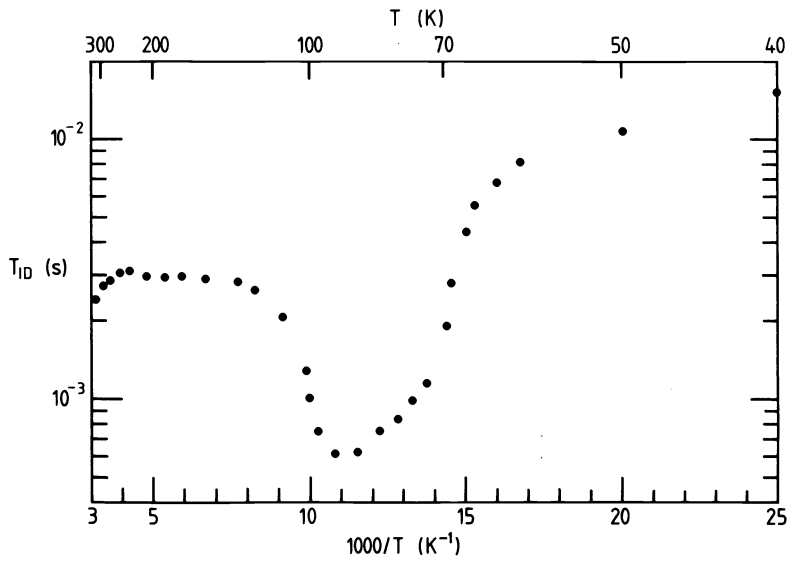

Fig. 7. The temperature dependence of the dipolar relaxation time T_{1D} for solid α-chymotrypsin between 40 and 300 K.

where S is defined in (2).

From Equation (7) we see that for a single correlation time τ_c the minimum value of T_{1D} should occur when $\omega_L\tau_c = 1$. For a distribution of correlation times symmetrical about τ_{cm}, such as the Gaussian distribution of Equation (4), we see from (8) that the minimum value of T_{1D} should still occur close to

$$\omega_L\tau_{cm} = 1. \qquad (9)$$

Taking Equation (9) in conjunction with (3) and the data in Table 1 we predict that the minimum in T_{1D} should occur close to 90 K for solid α-chymotrypsin and solid lysozyme as was found experimentally. We may therefore confidently conclude that the T_{1D} minimum is associated with the same dynamic processes as the T_1 minima, predominantly methyl group reorientation. However, whereas the median correlation time τ_{cm} was of order 10^{-9} s at 190 K, the temperature of the T_1 minimum for 60 MHz, the processes are much slower at the T_{1D} minimum at 90 K, with τ_{cm} of the order 10^{-5} s.

A comparison of Equations (1) and (8) shows that the minimum value of T_{1D} should be very much shorter than the minimum value of T_1 for the same molecular motion and indeed this was found to be the case experimentally. In Fig. 8 measured values of T_{1D} are shown together with those of T_1 at the three measuring frequencies; at their respective minima T_1 is more than two orders of magnitude longer than T_{1D}.

Above 130 K the rise in T_{1D} for α-chymotrypsin (Fig. 7) is arrested and levels off with the constant value 3 ms, falling again above 260 K; a similar behaviour was found for 'deuterated' lysozyme. Other motions are evidently contributing to the dipolar relaxation here. Since these motions have a substantially lower relaxation efficiency than the methyl group reorientations responsible for the minimum at 90 K, and occur at a higher temperature, they may tentatively be associated with more hindered backbone and sidechain motions.

With normal lysozyme a more continuous decrease in T_{1D} was found above 180 K (Fig 8). This suggests a significant dipolar relaxation contribution in this region from slow motions associated with water molecules and other groups with exchangeable protons. A small, but reproducible, discontinuity in T_{1D} was noted at 273 K (Fig. 8) and may be associated with water in the structure.

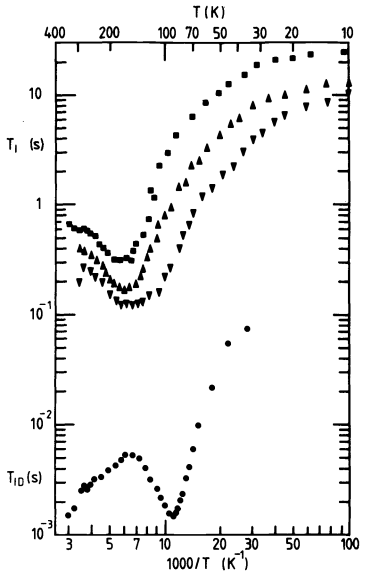

Fig. 8. Comparison of the variation with temperature of T_1 and T_{1D} for solid lysozyme between 10 and 300 K. Squares T_1 at 60 MHz, upright triangles T_1 at 30 MHz, inverted triangles T_1 at 18 MHz, circles T_{1D}.

REFERENCES

1. H. Frauenfelder, G. A. Petsko and D. Tsernoglou, Nature 280, 558-563 (1979).
2. P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips and M. J. E. Sternberg, Nature 280, 563-568 (1979).
3. I. Munro, I. Pecht and L. Stryer, Proc. Natl. Acad. Sci. US 76, 56-60 (1979).
4. J. R. Lakowicz and G. Weber, Biochemistry 12, 4171-4179 (1973).
5. A. Cooper, Proc. Natl. Acad. Sci. US 73, 2740-2741 (1976).
6. J. A. McCammon, B. R. Gelin, M. Karplus and P. G. Wolyner, Nature 262, 325-326 (1976).
7. J. A. McCammon, B. R. Gelin and M. Karplus, Nature 267, 585-590 (1977).
8. I. D. Campbell, C. M. Dobson and R. J. P. Williams, Proc. Roy. Soc. B 189, 503-509 (1975).
9. D. J. Nelson, S. J. Opella and O. Jardetzky, Biochemistry 15, 5552-5560 (1976).
10. K. Wuthrich, NMR in Biological Research: Peptides and Proteins, Elsevier/North-Holland, Amsterdam (1976).
11. O. Jardetzky and N. G. Wade-Jardetzky, FEBS Letters 110, 133-135 (1980).
12. E. R. Andrew, D. J. Bryant and E. M. Cashell, Chem. Phys. Lett. 69, 551-554 (1980).
13. E. R. Andrew, D. J. Bryant, E. M. Cashell and Q. A. Meng, FEBS Letters 126, 208-210 (1981).
14. J. Jeneer and P. Broekaert, Phys. Rev. 157, 232-240 (1967).
15. E. R. Andrew, W. S. Hinshaw, M. G. Hutchins and R. O. I. Sjöblom, Mol. Phys. 31, 1479-1488 (1976).
16. E. R. Andrew, W. S. Hinshaw, M. G. Hutchins, R. O. I. Sjöblom and P. C. Canepa, Mol. Phys. 32, 795-806 (1976).
17. E. R. Andrew, W. S. Hinshaw, M. G. Hutchins and R. O. I. Sjöblom, Mol. Phys. 34, 1695-1706 (1977).
18. R. Kubo and K. Tomita, J. Phys. Soc. Japan 9, 888-919 (1954).
19. T. M. Connor, Trans. Faraday Soc. 60, 1574-1591 (1964).
20. A. S. Nowick and B. S. Berry, IBM J. 297-311 (1961).
21. R. M. Fuoss and J. G. Kirkwood, J. Am. Chem. Soc. 63, 385-394 (1941).
22. E. R. Andrew, T. J. Green and M. J. R. Hoch, J. Mag. Res. 29, 331-339 (1978).
23. E. R. Andrew, R. Gaspar and W. Vennart, Biopolymers 17, 1913-1925 (1978).
24. S. Clough, B. Alefeld and J. B. Suck, Phys. Rev. Letters 41, 124-128 (1978).
25. S. Clough and A. Heidemann, J. Phys. C 12, 761-770 (1979).
26. R. E. London, J. Am. Chem. Soc. 100, 2678-2685 (1978).
27. H. Stefanou and A. E. Woodward, J. Macromol. Sci. Phys B 10, 641-646 (1974).
28. E. R. Andrew, D. J. Bryant, E. M. Cashell, R. Gaspar Jr. and Q. A. Meng, Polymer 22, 715-717 (1981).
29. R. Van Steenwinkel, Z. Naturf. 249, 1526-1531 (1969).
30. O. Lauer, D. Stehlik and K. H. Hausser, J. Mag. Res. 6, 524-532 (1972).
31. D. Wolf, Spin Temperature and Nuclear Spin Relaxation in Matter, O.U.P. (1979)