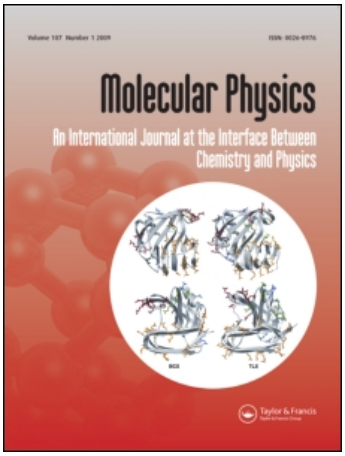

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Proton magnetic relaxation and molecular motion in polycrystalline amino acids

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Proton magnetic relaxation and molecular motion in polycrystalline amino acids

I. Aspartic acid, cystine, glycine, histidine, serine, tryptophan and tyrosine

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A proton magnetic resonance and relaxation study has been carried out on seven polycrystalline amino acids from 130 to 500 K at 60·2 MHz. These amino acids contain no methyl groups and all exhibit a single relaxation minimum. The measurements are well accounted for by the Kubo–Tomita theory of relaxation assuming a single correlation time which follows a simple activation law. In all cases the source of relaxation is provided by reorientation of the $-NH_3$ groups in the zwitterion form of the molecules ; other protons are relaxed by spin-exchange with those of the $-NH_3$ group. The measured relaxation constants are consistent with inter-proton distances in the $-NH_3$ groups recently measured by neutron diffraction.

1. INTRODUCTION

Nuclear magnetic resonance and relaxation provide a well-established method for investigating the dynamics of molecules in crystals [1, 3, 25]. In this investigation N.M.R. is used to study molecular motion in the amino acids commonly encountered in proteins, not only on account of the fundamental importance of these materials, but also as a contribution towards understanding the dynamical behaviour of proteins and polypeptides generally. The information gained from N.M.R. complements that obtained by diffraction methods, and the current intensity of solid-state diffraction investigations, especially by neutron diffraction, into the molecular components of biopolymers, makes a proton N.M.R. investigation of these materials particularly opportune.

A feature of this study is the importance of nuclear relaxation generated in each amino acid by reorientation of the triangular rotor group $-NH_3$, confirming the appropriateness of the zwitterion description $^+H_3N–CHR–COO^-$ for most of the amino acids. In a number of the amino acids the side group R provides an additional source of relaxation, especially when mobile methyl groups $-CH_3$ are present. These highly mobile molecular rotors contribute to efficient relaxation at lower temperatures, whereas the hydrogen bonding of the $-NH_3$ groups causes them to be effective at higher temperatures.

The amino acids occurring in proteins fall into two categories. First, there are the classical 20, or 21 if one counts cysteine and cystine separately. The

second category includes at least 16 amino acids presumed to be derived from one or other of the 20 by enzyme-directed reactions of already assembled polypeptide chains [29]; these amino acids have a limited range of occurrence. In addition there are at least a dozen more amino acids for which there is some evidence of occurrence in proteins. Our work has concentrated on members of the classical 20. Although norleucine is not now considered an authentic protein amino acid we have included it in our investigations for comparison with the isomeric amino acids leucine and isoleucine. A few have been examined previously by other workers to whom reference will be made in later sections. Glycine, the simplest member of the series, has been the subject of a number of earlier N.M.R. investigations. It has nevertheless been included for completeness, and especially so that comparisons may be made at the same N.M.R. frequency for all the compounds. Some of our work has been published in preliminary form [4, 5]. Quite recently our attention has been drawn to a related study by Zaripov [31], which includes about half the compounds covered here.

The D, L, and DL forms of each amino acid have not been separately studied. No difference in N.M.R. behaviour is to be expected between D and L forms, and such small differences as have been reported in the past are perhaps to be attributed to small differences in the physical and chemical state of the specimens and to experimental error in the measurements. Our choice between L and DL forms has been made on the basis of availability of pure material and of the form for which the greatest structural information from diffraction measurements was known at the time.

This first paper is concerned with seven polycrystalline amino acids which display only one proton relaxation mechanism, attributed to reorientation of the $-NH_3$ group. The second paper is concerned with a further group of seven polycrystalline amino acids which display two distinct relaxation mechanisms, the second attributed to methyl groups in the side chain R. The third paper is concerned with amino acids whose side chain R includes an amino group, and with some special cases. In this third paper some overall comparisons for the whole series are made.

2. Experimental details

The amino acids were obtained in polycrystalline form from BDH Chemicals Limited, Poole, Dorset, of chromatographically homogeneous purity grade. Some of the amino acids were recrystallized from distilled water, but this was not found to change the measured relaxation times within experimental error. Trouble was taken to remove water from the specimens. They were placed in the experimental phial in a dessicator with silica gel under vacuum for three weeks, after which they were pumped for several hours at temperatures up to 160°C and then sealed off. Each specimen contained about 1 g of material.

Measurements of proton spin-lattice relaxation time T_1 were carried out between 130 and 500 K using a Bruker B–KR 322s variable-frequency pulsed N.M.R. spectrometer in conjunction with an AEI RS2 electromagnet. Measurements were made at 60·2 MHz on all the amino acids, and on some also at 38·8 and 20·0 MHz. The radiofrequencies were accurate to one part in 10^8; the magnetic field had a uniformity over the specimen and stability of a few parts in

10^6. The temperature of the specimen was controlled by a Bruker B–ST 100/700 temperature-control unit, the accuracy of which was measured by a copper–constantan thermocouple to within 2 K. The accuracy of measurements of T_1 varied with specimen and temperature, but were typically ± 5 per cent. Proton N.M.R. spectra were also recorded over a wider temperature range either by Fourier transformation of the free induction decay, or using a marginal oscillator CW spectrometer with a cryostat capable of measurements down to 4 K. Values of the second moment of the proton spectra in plateau regions were typically ± 5 per cent by either method.

Starting from room temperature measurements were made with decreasing temperature and then with increasing temperature. Hysteresis effects were not observed. When all low temperature measurements had been completed, measurements were taken above room temperature with increasing temperature only since near the melting-point chemical changes are possible, while after melting and freezing again random polycrystallinity may not be preserved.

3. Results and analysis

Using a 90°–τ–90°, 180°–τ–90°, or sat–τ–90° double pulse sequence with varying time intervals τ between pulses, the recovery of nuclear magnetization was found to be exponential within experimental error for all the seven amino

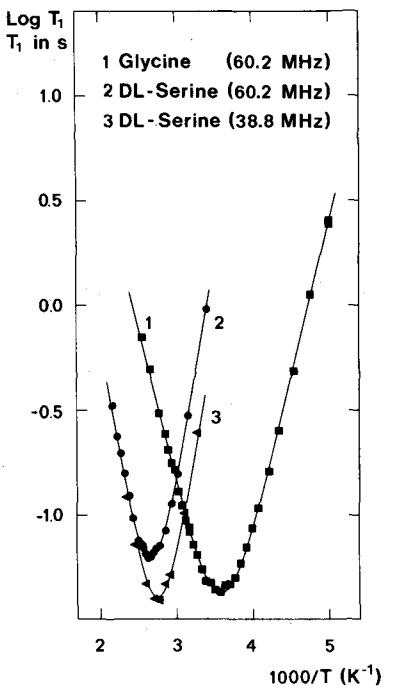


Figure 1. The variation of proton spin-lattice relaxation time T_1 with inverse temperature T^{-1} for polycrystalline amino acids. Curve 1 : glycine (60·2 MHz) ; curve 2 : DL-serine (60·2 MHz) ; curve 3 : DL-serine (38·8 MHz). The full lines are theoretical curves calculated in the manner described in the text.

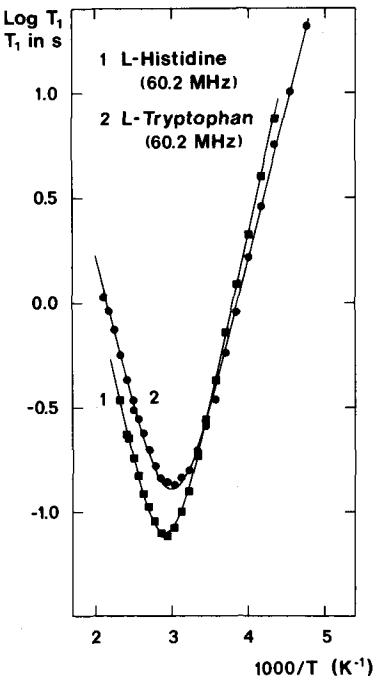


Figure 2. The variation of proton spin-lattice relaxation time T_1 with inverse temperature T^{-1} for polycrystalline amino acids at 60·2 MHz. Curve 1 : L-histidine ; curve 2 : L-tryptophan. The full lines are theoretical curves calculated in the manner described in the text.

acids discussed in this paper, at each temperature of measurement. A unique spin-lattice relaxation time T_1 could therefore be measured at each temperature for each compound. The results are shown in figures 1, 2 and 3. All the measurements shown were made at 60·2 MHz except for DL-serine for which measurements were also made at 38·8 MHz. In each of these figures the full line is a calculated theoretical line obtained in a manner to be described presently.

In all cases the variation of T_1 with temperature T exhibits a single minimum, a feature characteristic of nuclear relaxation generated by the random reorientation of molecules or molecular groups within the solid. The results have therefore been analysed using the well-known relaxation expression of Kubo and Tomita [14] :

$$T_1^{-1} = C[\tau_c(1+\omega^2\tau_c^2)^{-1} + 4\tau_c(1+4\omega^2\tau_c^2)^{-1}], \quad (1)$$

where $\omega/2\pi$ is the N.M.R. frequency of measurement, and τ_c is the correlation time of the random molecular motion. The analysis assumed that the motion could be characterized by a single correlation time at each temperature and that this followed a simple activation law :

$$\tau_c = \tau_0 \exp E/kT. \quad (2)$$

A computer programme minimized the r.m.s. percentage difference between observed and calculated values of T_1, and the best values of C, τ_0 and E are listed in table 1. The excellent agreement between the calculated theoretical

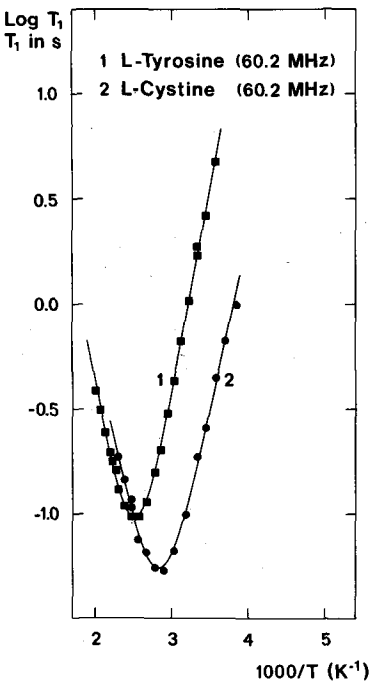


Figure 3. The variation of proton spin-lattice relaxation time T_1 with inverse temperature T^{-1} for polycrystalline amino acids at 60·2 MHz. Curve 1 : L-tyrosine ; curve 2 : L-cystine. The full lines are theoretical curves calculated in the manner described in the text. Note that these two amino acids have almost identical activation parameters (table 1).

curves and the experimental data over the whole temperature range shows that the assumption of a single correlation time which follows a simple activation law is remarkably good. In the case of serine the same parameters have been used to fit the data at both measuring frequencies (figure 1). It follows from equation (1) that at high temperatures, where $\omega^2\tau_c^2 \ll 1$, the relaxation time is proportional to τ_c^{-1} and is independent of frequency, as found, while for low temperatures, where $\omega^2\tau_c^2 \gg 1$, T_1 is proportional to $\omega^2\tau_c$, also in agreement with experiment ; furthermore, the value of the relaxation time at the minimum, $T_{1\,\mathrm{min}}$, is proportional to frequency in agreement with (1). The r.m.s. percentage differences between observed and calculated values of T_1 after minimization are shown in table 1 and are all of order 5 per cent, which is as good as can be expected in view of the accuracy of measurement of T_1. The accuracy of the values of C and E in table 1 are typically 5 per cent, and of τ_0 typically 50 per cent.

For glycine our value of activation energy E of 28 ± 1 kJ/mole agrees well with other measured values : 28 kJ/mole [19], 27 kJ/mole [31]. For L-tyrosine our value of 37 ± 2 kJ/mole appears to be significantly higher than the value of 30 kJ/mole of McElroy *et al.* [19]. However the latter value is said to be based on the slope of a plot of T_1 against T^{-1} on the low temperature side of the minimum, whereas our value is obtained by fitting the data over the whole range

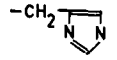
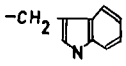
Amino acid	Side chain	Number of protons n	R.M.S. (obs-calc) per cent	T_1 min at 60 MHz / ms	Temp. at $T_{1\ min}$ / K	Relaxation constant C / $10^8\ s^{-2}$	Activation energy E / kJ/mole	Time factor τ_0 / 10^{-14} s
α-Glycine	$-H$	5	3·9	42	279	62·9	28·6	0·7
DL-Serine	$-CH_2OH$	7	4·1	62	376	42·6	40·0	0·45
L-Aspartic acid	$-CH_2COOH$	7	7·9	75	397	35·6	29·8	19
L-Tyrosine		11	5·5	96	399	27·5	37·0	2·3
L-Cystine	$-CH_2SSCH_2-$	6	6·1	55	354	48·1	31·5	3·6
L-Histidine		9	2·9	79	344	33·6	31·4	2·8
L-Tryptophan		12	5·6	129	333	20·6	27·8	7

Table 1. Relaxation parameters for the molecular motion of the NH_3 group.

of temperature. For DL-serine our value of 40 ± 2 kJ/mole is rather higher than the value of 35 kJ/mole obtained by Zaripov [31], though details are not given of the derivation of this value from the data. For the other four amino acids discussed in this paper there appear to be no other values for comparison.

4. Discussion

There is strong evidence from X-ray and neutron-diffraction studies that these seven amino acids are in the zwitterion form in the crystalline state. The sources of this evidence are listed in table 2. All the molecules therefore include the $-NH_3$ group whose reorientation is a potential source of proton spin-lattice relaxation.

	References
L-Aspartic acid	[9]
L-Cystine	[8, 20]
α-Glycine	[2, 12, 18]
L-Histidine	[15–17]
DL-Serine	[11, 23]
L-Tryptophan	[27]
L-Tyrosine	[10]

Table 2. X-ray and neutron diffraction references.

Continuous-wave N.M.R. studies of the proton spectra of α-glycine have exhibited spectral narrowing above 150 K [13, 21, 22, 30]. The reduced second moment in the plateau region above 200 K was found to be consistent with the assumption that the motional narrowing was caused by rapid reorientation of the $-NH_3$ group.

For aspartic acid we have found that the second moment decreases from a low temperature plateau value of 25 ± 1 G^2 to a high temperature plateau value of 12 ± 1 G^2, the narrowing taking place between 200 and 350 K†. We have computed the rigid-lattice second moment [24, 28], and obtained the value 25·4 G^2 in good agreement with the low temperature experimental value. We have also computed the second moment for three-fold reorientation of the $-NH_3$ groups and obtained the value 12·4 G^2 in good agreement with the high temperature value.

In serine also there is evidence of proton spectral narrowing due to $-NH_3$ group reorientation. We have found that the second moment decreases from a plateau value of 29 ± 2 G^2 below 150 K to a plateau value of 12 ± 2 G^2 above 300 K. The calculated rigid-lattice second moment is 26·7 G^2 and that for three-fold reorientation of the $-NH_3$ groups is 14·2 G^2.

Rapid random reorientation of the $-NH_3$ groups in these solid amino acids will modulate the dipolar interaction between the protons in the groups and provide an efficient mechanism for their relaxation. This motion will also modulate the dipolar interaction between protons of the $-NH_3$ groups and protons elsewhere in the same molecule or in neighbouring molecules. However, remembering that the contribution to the relaxation rate of these other

† $G = 10^{-4}$ T.

protons is proportional to r^{-6}, where r is the inter-proton separation, this provides a comparatively inefficient mechanism for their relaxation. A more potent source of relaxation for these extra-NH_3 group protons is through the the spin-exchange mechanism [7]. Since the transverse relaxation time T_2 is some three orders of magnitude less than the shortest measured spin-lattice relaxation time $T_{1\,\mathrm{min}}$, this mechanism will maintain a constant spin temperature throughout each crystal during the relaxation process. This is supported by the observation that the recovery of magnetization was found to be exponential in all these materials. The motion of the three protons in each $-NH_3$ rotor has therefore also to relax the $(n-3)$ other protons in each molecule, where n is the number of protons in the molecule. Disregarding the small direct contributions to the relaxation of these other $(n-3)$ protons, it follows that

$$\frac{1}{T_1}=\frac{3}{n}\left(\frac{1}{T_1}\right)_{NH_3}, \tag{3}$$

where $(1/T_1)_{NH_3}$ is the proton relaxation rate that would be observed if the $-NH_3$ group motions had only to relax their own three protons.

The value of $(1/T_1)_{NH_3}$ for any particular ω and τ_c depends, as equation (1) indicates, on the relaxation constant C, which in turn depends on the inter-proton distance in the $-NH_3$ group. If the geometry of the $-NH_3$ groups were identical in all the amino acids, it therefore follows from equations (1) and (3) that the observed values of $T_{1\,\mathrm{min}}$ should be proportional to n, and the measured values of C would be proportional to n^{-1}. This hypothesis is tested in figure 4. Remembering that the assumption of identical geometry for the $-NH_3$ groups in all seven amino acids is only approximately true, the proportionality of both $T_{1\,\mathrm{min}}$ and C^{-1} to n is borne out quite well, and gives good support to the view that the protons in all these amino acids are relaxed by reorientation of $-NH_3$ groups.

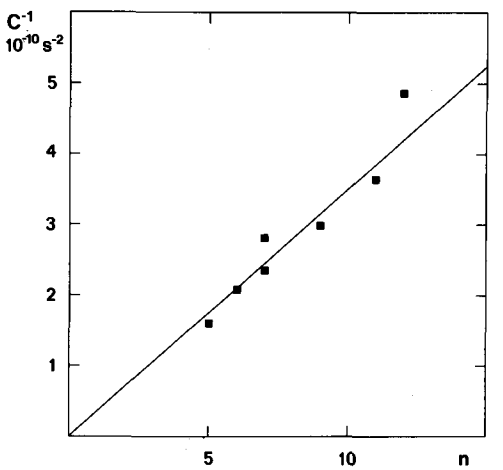


Figure 4. The variation of the inverse relaxation constant C^{-1} with n, the number of protons in each amino acid molecule.

From the straight line in figure 4, one reads off the value of C of $1{\cdot}0 \times 10^{10}$ s^2 for $n=3$, the value appropriate to a solid in which the $-NH_3$ group protons are the only protons present. Assuming the three protons are at the vertices of an equilateral triangle of side b, we expect [1]

$$C=\frac{9}{20}\frac{\gamma^4\hbar^2}{b^6}. \tag{4}$$

Insertion of the value $1{\cdot}0 \times 10^{10}$ s^{-2} for C in (4) yields a value of b of 1·717 Å†. From figure 4 the uncertainty in C for $n=3$ is of order 10 per cent, yielding a corresponding uncertainty in b of order 0·03 Å. Accurate positions for the protons have been obtained by neutron diffraction for four of the seven amino acids, namely α-glycine, L-histidine, DL-serine and L-tryosine. The inter-proton distances in the $-NH_3$ groups for these four amino acids, corrected for thermal motion, are given in table 3. The approximation of an equilateral triangle configuration is seen to be correct within ±0·03 Å. The inter-proton distances range from 1·654 to 1·717 Å with an overall mean of 1·689 Å. Thus within their combined uncertainties the value of b is in good agreement with the directly measured inter-proton distances in the $-NH_3$ groups, and provides strong support for the proposed relaxation mechanism.

Amino acid	H^1–H^2	H^1–H^3	H^2–H^3	Mean	References
α-Glycine	1·711	1·685	1·661	1·686	[12]
L-Histidine	1·667	1·712	1·694	1·691	[15]
DL-Serine	1·696	1·686	1·717	1·700	[11]
L-Tyrosine	1·675	1·654	1·704	1·678	[10]

Table 3. Inter-proton distances in $-NH_3$ groups from neutron-diffraction studies (in Å).

While the agreement is good we nevertheless mention some factors which could have affected the measured and calculated values. First we have neglected the small direct contributions to C from the dipolar interactions between protons in the NH_3 groups and their remoter neighbours in the crystal, which depend on r^{-6}. On the other hand, we have taken no account of librational motion of the NH_3 group or of the whole amino acid molecule which would reduce C [26]. Any distribution of correlation times τ_c would reduce the measured value of C; however, the excellent agreement between the experimental points in figures 1, 2 and 3 and the curves calculated on the assumption of a single correlation time at each temperature, suggests that this assumption is a very good one for these materials. In principle T_1 is anisotropic [6], and the recovery of magnetization is integrated over all the microcrystals in the polycrystalline specimen. The absence of significant non-exponential recovery of magnetization suggests that substantial anisotropy is not present, and that any systematic errors in C from this cause are small. The good agreement obtained using equation (4) without taking account of these corrections, some of them difficult to estimate exactly, suggests that they are indeed small, or tend to cancel.

† Å = 10^{-10} m.

The activation energies for $-NH_3$ group reorientation (table 1) range from 28·6 to 40·0 kJ/mol. This suggests that the barriers are to a large extent intermolecular in origin, dependent on details of the packing, hydrogen bonding and electrostatic interactions. Further discussion is deferred to paper III so that a comparison may be made for all the amino acids studied.

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