

## Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spin-Lattice Relaxation Time Measurements of Amino Acids

(progressive saturation)

IAN M. ARMITAGE, H. HUBER, H. PEARSON, AND JOHN D. ROBERTS

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif. 91109

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**ABSTRACT** The  $^{13}\text{C}$  spin-lattice relaxation times,  $T_1$ 's, of several amino acids have been measured as a function of pD and concentration. A strong dependence of the carboxyl carbon  $T_1$  was observed for both pD and concentration and is believed to be due to intermolecular associations. For the carboxyl carbon, spin rotation is proposed as the predominant relaxation mechanism, whereas the other carbons are relaxed mainly by the dipole-dipole mechanism, and their relaxation times are relatively independent of changes in concentration and pD.

There has been considerable recent interest in  $^{13}\text{C}$  spin-lattice relaxation times as a means of structural analysis for peptides (1-3). However, it would seem that fundamental to the understanding of  $^{13}\text{C}$   $T_1$ 's in peptides and proteins is a knowledge of the behavior of the relaxation times of individual amino acids as a function of pH. We report here such studies for  $\text{D}_2\text{O}$  solutions of glycine, DL-alanine, and  $\gamma$ -aminobutyric acid.

Relaxation times were measured using the progressive saturation method (4) with our pulsed Fourier transform (PFT) modified DFS-60 "Brukarian" spectrometer operating at 15.09 MHz. Nuclear Overhauser enhancements (NOE) were measured using a gating technique wherein, after a delay time of about five times  $T_1$ , the proton decoupling frequency was electronically switched far off resonance and then switched back on resonance for an acquisition time short compared to  $T_1$  (5).

Table 1 contains the  $^{13}\text{C}$   $T_1$ 's determined at the natural-abundance level for the carbons of amino acids at 2 M in deuterium oxide at or near the isoelectric point, and the change in the carboxyl  $T_1$  at several pD values.

The glycine carboxyl carbon  $T_1$  is very sensitive to concentration and was found to change from 6.8 sec for a 2 M solution to 47.0 sec for a 0.1 M solution at a pD of 7.3. In contrast, the methylene carbon  $T_1$  of 3.0 sec, measured with a natural-abundance sample, was found to be relatively independent of concentration and pD. To obtain more detailed data on the carboxyl carbon, a pD study was carried out on a 1 M glycine- $\text{D}_2\text{O}$  solution with the carboxyl carbon enriched to 90.8%  $^{13}\text{C}$ . The results are shown in Table 2. The remarkable feature is the nearly symmetrical decrease in  $T_1$  from the pD, where the zwitterionic form predominates, to minima at about  $\text{pK}_1$  and  $\text{pK}_2$ ; this was also observed for 0.3 M solutions.

A close look at the  $T_1$  values for C2 measured on 90%- $^{13}\text{C}_2$ -enriched glycine showed no change with pD. In addition,

the NOE for the methylene carbon was 2.8, and this was also invariant with pD. On the other hand, the NOE for the enriched carboxyl carbon (1 M at pD 6.5) was 1.3. It is thus clear that, while the methylene carbon is almost totally relaxed by the dipolar mechanism, the carboxyl carbon is only about 15% relaxed by this process. Scalar relaxation does not seem possible in this case, and contributions to the  $^{13}\text{C}$  relaxation behavior from chemical-shift anisotropy have been shown to be negligible, except for the few special cases (6). To rule out the possibility that deuterium exchange on and off the carboxyl group causes a dipolar relaxation, the  $T_1$  of a 1 M solution in  $\text{H}_2\text{O}$  was measured and found to be identical to that determined for the corresponding  $\text{D}_2\text{O}$  solution. Because the magnetic moment of protons is about six times greater than deuterons, one can immediately rule out any effect from this quarter.

We conclude that spin rotation is the important relaxation mechanism, and, indeed, this seems reasonable because there

TABLE 1. pD Dependence of  $^{13}\text{C}$  spin-lattice relaxation times ( $T_1$ ) for 2 M  $\text{D}_2\text{O}$  solutions of amino acids

pD	$T_1$ , sec			
	<i>Glycine</i>			
	CO	CH <sub>2</sub>		
3.0	15.7			
3.6	13.6			
7.3	6.8	3.0		
10.0	14.0			
<i>DL-Alanine</i>				
	CO	CH	CH <sub>3</sub>	
0.8	19.0			
3.0	12.0			
6.2	5.1	3.1	1.8	
7.4	8.7			
<i><math>\gamma</math>-Aminobutyric acid</i>				
	CO	C- $\alpha$	C- $\beta$	C- $\gamma$
0.0	15.0			
1.5	22.9			
3.3	10.2			
6.1	5.9	1.7	1.2	2.1
7.6	8.6			
9.7	15.0			
11.0	12.6			

Abbreviations:  $T_1$ , spin-lattice relaxation time; NOE, nuclear Overhauser enhancement; PFT, pulsed Fourier transform.

TABLE 2. Variation in  $T_1$  with pD for the carboxyl carbon of glycine, 1 M in  $D_2O$ 

pD	$T_1$ , sec
0.8	29.0
2.3	6.8
3.0	5.0
3.5	4.8
7.1	11.7
9.4	5.7
9.9	5.0
10.4	4.0
13.3	44.6

should only be a very low barrier to rotation about the C—CO<sub>2</sub> bond. A preliminary temperature-dependence study to confirm this relaxation mechanism showed that as temperature was increased,  $T_1$  also increased. However, amino-acid molecules are not really isotropic rotors so that the spin-rotation dependence of  $T_1$  with temperature may be more complex than predicted from equations for isotropic rotors.

The striking concentration- and pD-dependence of  $T_1$  suggests an important contribution from intermolecular association. We have been able to simulate the observed concentration- and pD-dependence of  $T_1$  by assigning separate relaxation rates to the possible moieties (anion, zwitterion, etc.)

and equilibrium constants for their mutual associations. Obviously, if these equilibria are responsible for the observed concentration- and pD-dependence of  $T_1$ , they will also complicate the temperature-dependence of  $T_1$ .

The proposed relaxation time of about 13 sec for spin rotation of the 1 M glycine appears to be the smallest so far measured for <sup>13</sup>C.

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