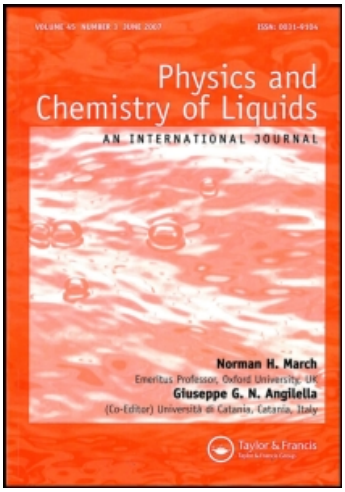

Physics and Chemistry of Liquids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646857

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To cite this Article Arulmozhi, V. and Rao, A. Srinivasa(1994) 'NMR Relaxation Studies in Aqueous Solutions of Amino Acids', Physics and Chemistry of Liquids, 27: 4, 219 — 224
To link to this Article: DOI: 10.1080/00319109408029530
URL: http://dx.doi.org/10.1080/00319109408029530

Phys. Chem. Liq., 1994, Vol. 27, pp. 219–224
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NMR RELAXATION STUDIES IN AQUEOUS SOLUTIONS OF AMINO ACIDS

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(Received 20 September 1993)

The proton magnetic resonance (PMR) spin-lattice and spin–spin relaxation times ($T1$ and $T2$) were measured in aqueous solutions of glycine and L-proline as a function of solute concentrations and at a temperature of 32 °C. The relaxation times were measured using Bruker PC 120 NMR process analyser. The relaxation times were found to decrease with increase of solute concentrations. The results are interpreted on the basis of flickering cluster model and hydrogen bond formation between solute and solvent molecules.

KEY WORDS: Nuclear Magnetic Resonance, Hydrogen bonding, Glycine, L–Proline.

INTRODUCTION

Proton magnetic relaxation times of water protons in biological systems have been widely studied (1–5) with the intention of getting a better understanding of the state of water in living systems and elucidating the molecular mechanisms underlying rela-xation (6–8). Such studies are also becoming increasingly important due to the development of magnetic resonance imaging, since proton relaxation times being one of the parameters for image contrast (9, 10).

In biology, the most frequently studied parameters are spin-lattice and spin–spin relaxation times ($T1$ & $T2$), and their dependence on NMR frequency. The frequency dependence of the longitudinal relaxation rate of tissues shows low field dispersion (11, 12). Such low field dispersion is also seen in solutions of macromolecules (1, 13) which originates from a scalar coupling between 1H and 17O within the water molecules. Such slow processes are conveniently studied using transverse relaxation rate measurements. In order to understand both relaxation mechanisms and the nature of molecular interactions in biological systems, it is essential to study relatively simple systems. Amino acids were chosen because of their relevance to biology and their well established relaxation mechanisms in their crystalline state (14–16).

In a recent paper, Grucker *et al.* (17) have reported proton relaxation rates of some aqueous solutions of amino acids. The results reveal that longitudinal relaxation rate is only slightly different from that of pure water and their transverse relaxation rate is

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governed by a proton exchange between the protons of water and NH3 group of the amino acids. Hills *et al.* (18) have reported transverse proton relaxation dispersion as a function of Carr-Purcell-Meiboom-Gill pulse spacing in aqueous solutions of native bovine serum albumin. The dispersion is only seen at high frequency (> 100 MHz) corresponding to the fast exchange of water with the NH and OH protons of the amino acids side chains of the protein. However, for amino acids digest of BSA the fit of the data is not good as for the native protein, suggesting that the observed dispersion curves is an envelope of many dispersions arising from various types of exchangeable protons. The present study has been undertaken in aqueous solutions of amino acids such as glycine and L-proline with a view to understand the nature of molecular interactions in these solutions. The influence of body pH and isoelectric pH in some aqueous solutions of amino acids were also studied at particular solute concentration and the results are reported in this note.

EXPERIMENTAL DETAILS

Amino acids were purchased from the Sigma Chemical Co and were used without further purification. Aqueous Solutions of glycine and L-proline were made in distilled, deionized water in the concentration range of 1% to 9% (w/v). The pH of the solutions was adjusted to the body pH (7.4) and isoelectric pH by the addition of either NaOH or HCl. The samples were degassed using the standard technique (19). The relaxation times ($T1$ and $T2$) were measured using Bruker PC 120 NMR process analyser at a frequency of 20 MHz using 'Inversion Recovery' and 'Carr-Purcell-Meiboom-Gill' programs. Five fold accumulation was used for the reduction of error, which was usually less than 2%. The error of the 3-parameter fitting of relaxation curve was less than 1%. The measurements were carried out at a temperature of 32 °C by circulating water from a thermostatically controlled water bath provided by Concord Instruments (P) Ltd, Bangalore. The viscosity and density of these solutions were determined by using Ostwald's viscometer and specific gravity bottle of capacity of 10 ml at the same temperature. The accuracy in the measurement of viscosity and density is of the order of + 1%.

RESULTS AND DISCUSSION

The variation of spin-lattice and spin–spin relaxation times ($T1$ and $T2$) for aqueous solutions of glycine and L-proline with different solute concentrations is shown in Figures 1–2. In aqueous solutions of glycine and L-proline, the relaxation time decreases with increase of solute concentration. The decrease in the values of relaxation times ($T1$ and $T2$) for glycine is large as compared to L-proline at any given concentration. The viscosity is found to be slightly higher for glycine as compared to L-proline for all the concentrations studied. The decay of magnetization in these solutions is found to be mono exponential.

The general change observed in the values of $T1$ and $T2$ with the increases of solute concentrations may be understood by the water structure making and breaking

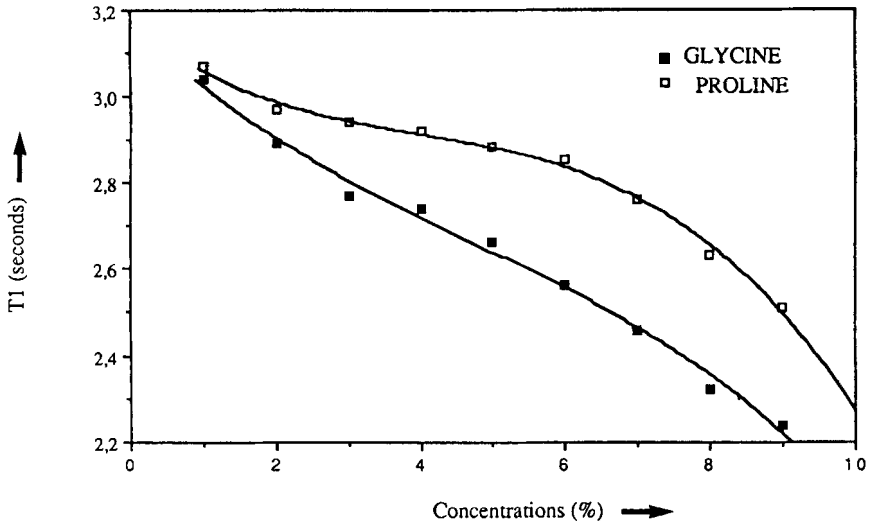

Figure 1 Spin-lattice relaxation time (T1) as a function of increasing Glycine and Proline concentration.

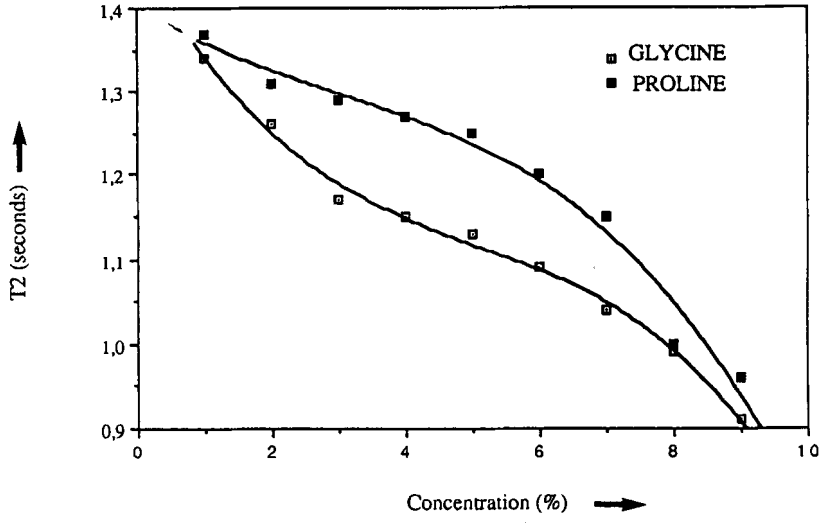

Figure 2 Spin–spin relaxation time (T2) as a function of increasing concentration.

properties of the solute (20, 21). From the figures, it is apparent that the relaxation time $T1$ decreases with the increase of solute concentration. It has been already established that glycine and L-proline are weak water structure breaker by ultrasonic studies (22, 23).

Earlier studies in aqueous solutions of fructose, rare-earth chlorides and nitrates have established that water structure breakers increase the values of relaxation time

$T1$, whereas structure makers have a tendency to decrease the relaxation times (21,24,25). Proton magnetic relaxation studies in several alcohols reveal that relaxation times $T1$ and $T2$ decrease with the increase of hydrogen bond energy (4,26). Since glycine and L-proline are known water structure breakers, one would expect that $T1$ and $T2$ should increase with the increase of solute concentration. But the present experimental observation contradicts the expected result. The presently observed decrease in the relaxation times $T1$ and $T2$ may be attributed due to the strong hydrogen bond formation between glycine/L-proline molecule with water molecules. Such hydrogen bond formation in these solutions more than compensates the structure breaking ability of the solute molecules.

The above observation may further be qualitatively explained by resorting to the flickering cluster model of water proposed by Blandamer (27). According to this cluster model, water is supposed to consist of hydrogen bonded clusters and unbounded water molecules. The molecules in the interior of the clusters are quadruply bonded (ice like) and unbounded water molecules are supposed to occupy the space in between the clusters. The clusters are sometimes referred as 'Open Structure' water and dense monomeric fluid is referred to as 'Closed Structure' water. The mixture is dynamic mixture and the breaking down of clusters is a co-operative process. When one hydrogen bond breaks in the cluster the whole cluster breaks down and increases the closed packed structure. On the basis of the model, glycine and L-proline breaks the open packed structure of water and form hydrogen bonds with the solute molecules and resulting in the formation of hydration shells. This hydration results a decrease in the values of relaxation times $T1$ and $T2$ for both glycine and L-proline. It is also interesting to note that the decrease in the value of relaxation times is quite large for glycine as compared to L-proline for any solute concentrations. This may be understood by noting the hydration numbers of the solute. The hydration number of glycine is calculated to be 2.73 while that for L-proline is 1.85 (28) for a given concentration at an identical temperature. The increased bound water content in aqueous solution of glycine decreases the freedom of movement of water molecules, which may be the cause for the observed decrease in the value of relaxation time $T1$ for glycine as compared to L-proline at the same concentration studied. This difference in hydration may be due to the fact that glycine is hydrophilic while L-proline is hydrophobic in nature.

The results of the relaxation times in aqueous solutions of simple amino acids such as L-proline, L-lysine, L-serine, glycine, valine and L-aspartic at a fixed concentration of 1% for two pH values namely body pH, isoelectric pH, viscosity and density reveals that the relaxation times do not show any significant difference between body pH and iso-electric pH. Hence, it is not possible to draw any meaningful conclusions on the basis of relaxation mechanism.

Furthermore, the dependence of interpulse delay of the Carr-Purcell-Meiboom-Gill pulse sequence on transverse relaxation rates (R2) of hearts, liver, spleen and brain tissue samples disected from normal rat tissue samples and some aqueous solutions of amino acids were studied by Gruker *et al.* (29). The transverse relaxation rates increases as the interpulse delay is increased. The results reveal that in aqueous solutions of amino acids at low pH value of all amino acids increased with increase of interpulse delay. The increase is large for glycine and lysine as compared to cysteine

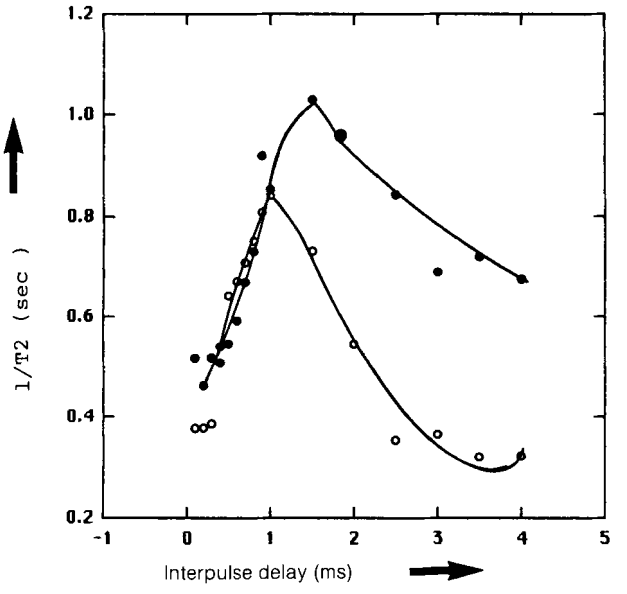

Figure 3 Transverse relaxation rate dependence on the echo delay in a CPMG sequence.

and aspartate. This can be explained on the basis of exchange process. The exchange process may be due to proton exchange between H O and $-NH_3^+$. On the otherhand, in the present study on aqueous solutions of glycine and L-proline (Figure 3) at the same frequency, temperature and at low pH values do not follow the trend reported by Gruker *et al.* (29). Further studies is in progress to understand the importance of interpulse delay on exchange process.

In conclusion, it may be mentioned that the present NMR study in aqueous solutions of glycine and L-proline shows that the structure breaking ability of the amino acids is more than compensated by hydrogen bond formation in these solutions for the low solute concentration.

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