CHAPTER - IV

AQUEOUS SOLUTIONS OF PROTEINS AND AMINO ACIDS

INTRODUCTION:

Proton magnetic relaxation times of water protons in biological systems have been widely studied (1-3) with the intention of getting a better understanding of the state of water in living systems and elucidating the molecular mechanisms underlying relaxation. Such studies are also becoming increasingly important due the development of magnetic resonance imaging, since proton relaxation times being one of the parameters for image contrast (4,5).

In biology the most frequently studied parameters are spin-lattice and spin-spin relaxation times (T_1 and T_2) and their dependence on NMR frequency. The frequency dependence of the longitudinal relaxation rate of tissues shows low field dispersion (6,7). Such low field dispersion is also seen in solutions of macromolecules (1,8) which originates from a scalar coupling between ^1H and ^{17}O 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 and protein solutions were chosen because of their relevance to biology and their well-established relaxation mechanisms in their crystalline state (9-11).

In a recent paper of Gruker et al (12) 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 governed by a proton exchange between the protons of water and NH_3^+ group of the amino acids. In a recent paper, Hill et al (13) have reported transverse proton relaxation dispersion as a function of Carr-Purcell-Meiboom-Gill (CPMG) pulse spacing in aqueous solutions of native bovine serum albumin (BSA). This 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 chain of the protein. However, for amino acids digest of BSA the fit of the data is not as 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 glycine, L-proline, tyrosine, tryptophan (amino acids) and bovine serum albumin (protein) to understand the nature of molecular interactions in these solutions. The effects of temperature, the influence of body pH and isoelectric pH in these solutions were also studied at particular solute concentration and the results are reported in this chapter.

EXPERIMENTAL SET UP

Protein and amino acid solutions were prepared by dissolving AR/BDH samples in double distilled water. Aqueous solutions of amino acids glycine, L-proline were prepared in the concentration range of 1% to 9% by weight. Aqueous solutions of tyrosine and tryptophan were prepared in the concentration range of 0.5% to 3% due to the non availability of the chemical in large quantities. Aqueous solutions of bovine serum albumin (BSA) were prepared in the concentration range of 0.5% to 4%. All the chemicals were used as such without further purification. In the case of aqueous solutions tyrosine and tryptophan, 0.5 ml of dilute HCl was added to get a clear solution. A preliminary experiment has shown that the addition of small amount of HCl does not effect the relaxation time significantly (14). 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 aqueous solutions of amino acids were degassed using standard technique (15). The spin-lattice and spin-spin relaxation times (T_1 and T_2) were measured at a frequency of 20 MHz using the 'Inversion Recovery' and 'Carr-Purcell-Meiboom-Gill' programs. 5-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. 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.

RESULTS AND DISCUSSION

AQUEOUS SOLUTIONS OF GLYCINE AND L-PROLINE

The variation of relaxation times (T_1 and T_2) for aqueous solutions of glycine and L-proline with different solute concentrations is shown in figure 4.1-4.3. 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 (T_1 & T_2) for glycine is large as compared to L-proline at any given concentration. The relaxation times (T_1 & T_2), viscosity, density for several solute concentrations are shown in tables 4.1,4.2. From the table, it can be seen that generally viscosity is found to be higher for glycine as compared to L-proline for all the concentration studied. To test whether the decay of magnetization in the solution is mono exponential or multi exponential, the variation of magnetisation M with the time interval t for some aqueous solutions of glycine and L-proline are shown in figure 4.4,4.5. From the figure, it can be seen that the decay of magnetisation is mono exponential in these solutions and hence we have a unique T_1 or T_2 value.

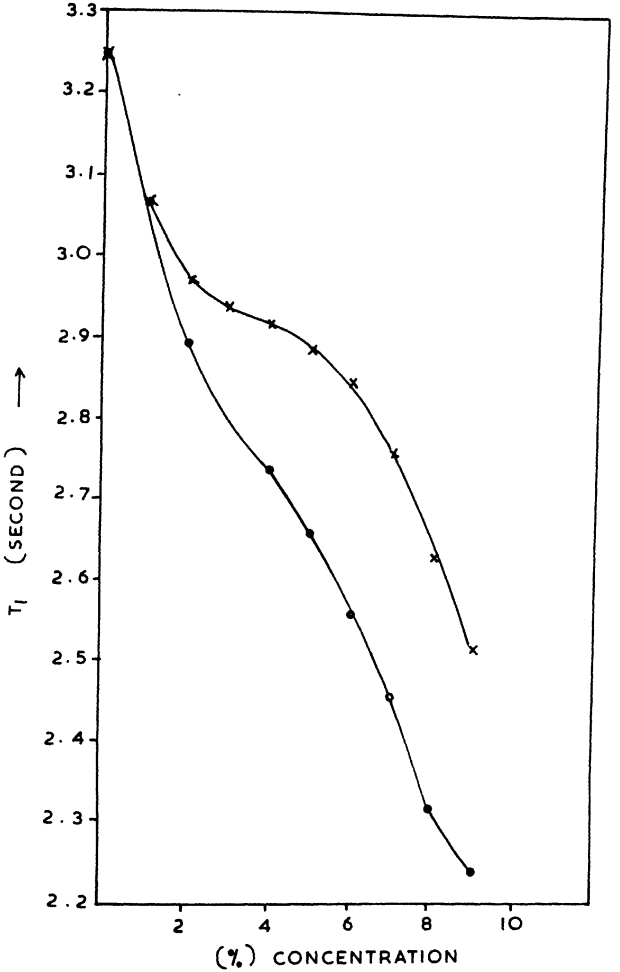

FIG. 4.1 RELAXATION TIME (T_1) VS (%) CONCENTRATION OF GLYCINE (•) AND L-PROLINE (X)

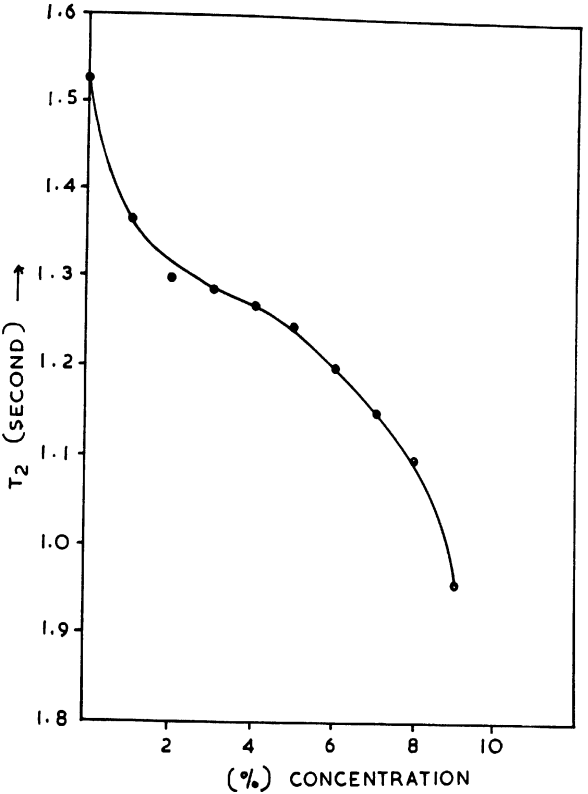

FIG. 4.2 RELAXATION TIME (T_2) VS (%) CONCENTRATION OF L · PROLINE

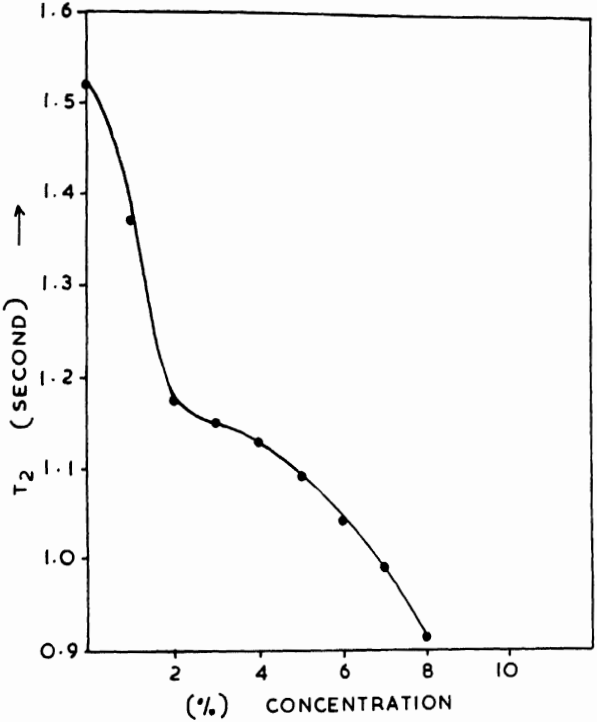

FIG. 4.3 RELAXATIME (T_2) VS (%) CONCENTRATION OF GLYCINE

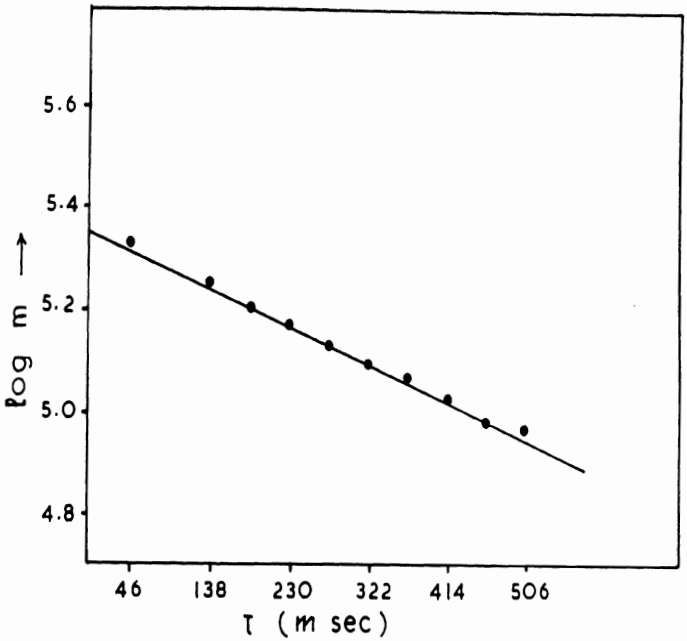

FIG.4.4 log m VERSUS T OF 3(%) GLYCINE SOLUTION

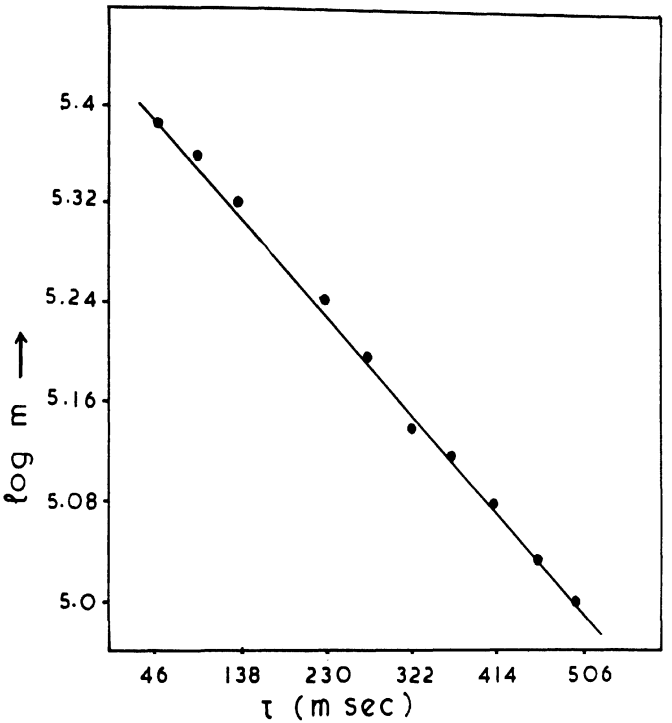

FIG. 4.5 log m VERSUS τ OF L.PROLINE SOLUTION

The general change observed in the values of T_1 and T_2 with increase of solute concentration may be understood by the water structure making and breaking properties of the solute (16). From the figure 4.1, it can be seen that the relaxation times T_1 decreases with increase of solute concentration. It has been already established that glycine and L-proline are weak water structure breaker by Ultrasonic methods (17,18).

Our earlier studies in aqueous solutions of fructose and rare-earth chlorides established that water structure breakers increases the values of relaxation time T_1 whereas structure makers have a tendency to decrease the relaxation times (16,19). Proton magnetic relaxation studies in several alcohols reveals that relaxation times T_1 and T_2 decreases with increase of hydrogen bond energy (20). Since L-proline and glycine are known water structure breakers, one would expect that T_1 and T_2 should increase with increase of solute concentration. But the present experimental observation contradicts the expected result. The presently observed decrease in the relaxation times T_1 and T_2 may be attributed due to the strong hydrogen bond formation between glycine /L-proline molecule with water molecules. Such strong hydrogen formation results in a decrease in the values of relaxation times T_1 and T_2. The present observation indicates that the 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 (Blandamer) (21). According to the cluster model,water is supposed to consist of hydrogen bonded clusters and unbonded water molecules. The molecules in the interior of the clusters are quadruply bonded (ice like) and unbonded 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 break down of clusters is a cooperative process. When one hydrogen bond breaks in the cluster the whole cluster breaks down and increases the closed packed structure water.

On the basis of the model, glycine and L-proline molecules break 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 T_1 for both glycine and L-proline. Figure 4.1 shows the relaxation times T_1 for aqueous solutions of glycine and L-proline for different solute concentration. From the figure it can be seen that the decrease in the value of relaxation time T_1 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 for L-proline it is 1.85 (22,23) for a given concentration at same 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 T_1 for glycine as compared to L-proline at the same concentration studied (19). This difference in hydration may be due to fact that glycine is hydrophilic while L-proline is hydrophobic in nature. The variation of spin-spin relaxation time T_2 for aqueous solutions of glycine and L-proline with solute concentration are shown in figures 4.2,4.3. The explanation proposed for the variation of T_1 in aqueous solutions of glycine and L-proline appears to be valid for this case also.

AQUEOUS SOLUTIONS OF TYROSINE AND TRYPTOPHAN

The variations of spin-lattice and spin-spin relaxation times (T_1 & T_2) with solute concentration in the range from 0.5 % to 3 % for tyrosine and tryptophan are shown in figure 4.6-4.8. The observed values of relaxation times T_1, T_2, viscosity and density for several solute concentration are shown in table 4.3,4.4. The decay of magnetisation is found to be mono exponential for all the solutions.

In aqueous solution of tyrosine, the relaxation time T_1 decreases with increase of solute concentration and shows a

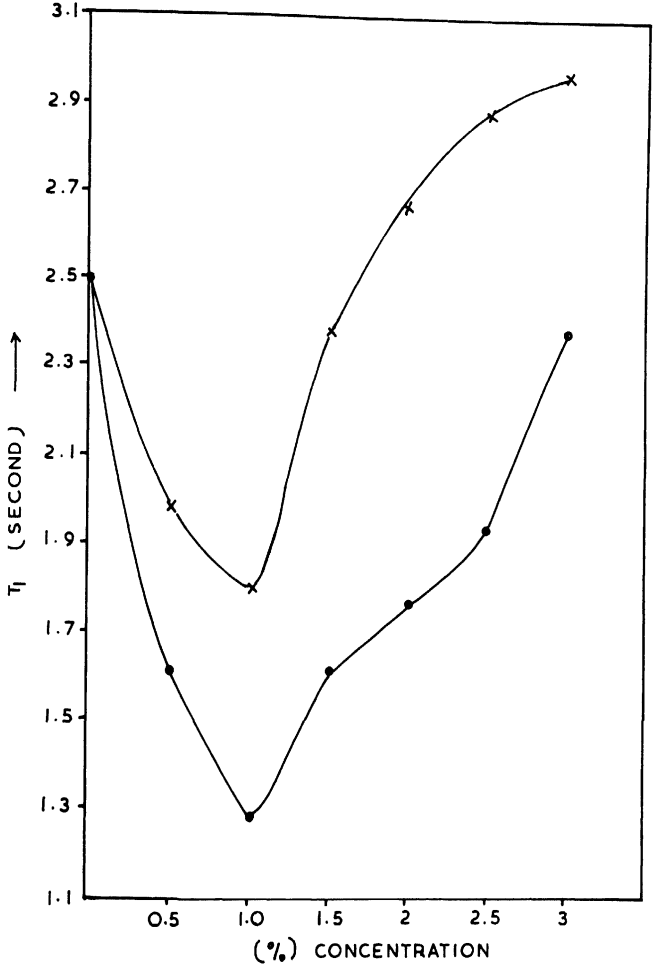

FIG.4.6 RELAXATION TIME (T_1) VS (%) CONCENTRATION
TRYPTOPHAN (X) AND TYROSINE (•)

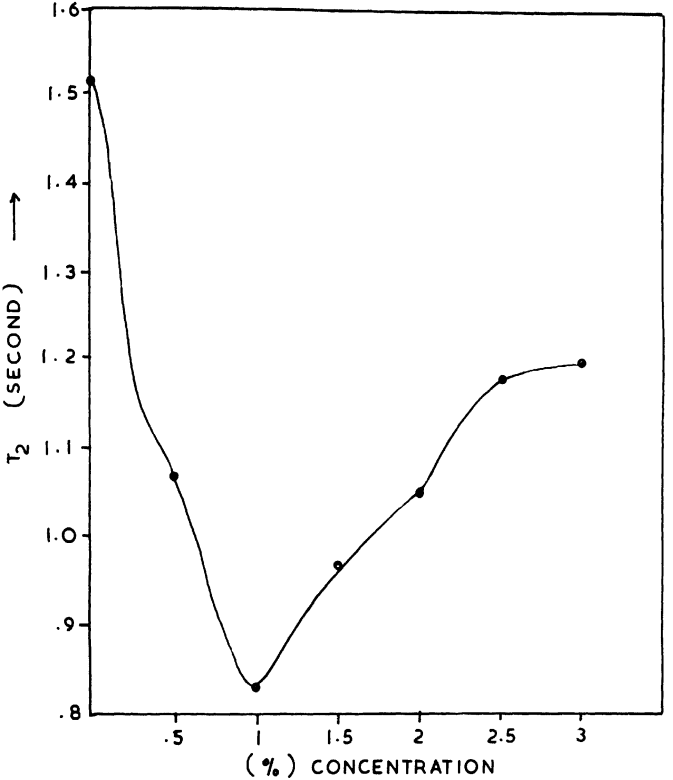

FIG.4.7 RELAXATION TIME (T_2) VS (%) CONCENTRATION OF TYROSINE

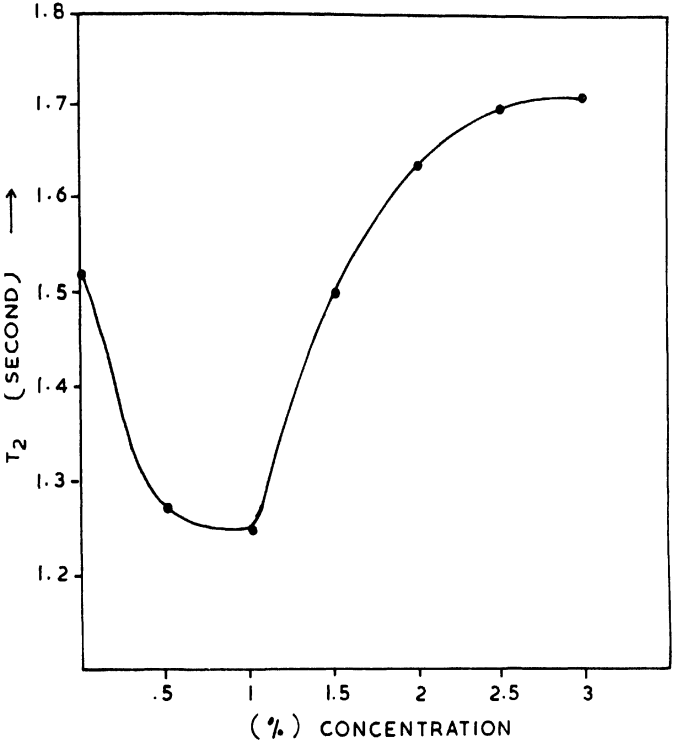

FIG. 4.8 RELAXATION TIME (T_2) VS (%) CONCENTRATION OF TRYPTOPHAN

minimum at 1% solute concentration and then increases with further increase of solute concentration. Similar variation is also seen in aqueous solutions of tryptophan. The initial decrease in the values of relaxation times T_1 and T_2 with increase of solute concentration of these amino acids may be due to a combination of water structure breaking effect of the solute and hydrogen bond formation between the solute and solvent molecules. The initial decrease of T_1 indicates that the hydrogen bond formation dominates over the structure breaking effect.

The decrease in the value of T_1 upto 1% concentration of tyrosine may be explained as follows . The tyrosine molecules initially breaks the water structure and forms hydration shells with the solute molecule and the hydration increases upto 1% solute concentration which results a decrease in the values of relaxation time T_1 and shows a minimum at 1% solute concentration. This may also be due to the formation of fairly strong hydrogen bonds in these solutions as it is known that T_1 decreases with increase of hydrogen bond energy (Rajalakshmi) (20). Further increase of solute concentration beyond 1% the relaxation time T_1 increases gradually. The observed increase may be explained as follows. When the solute concentration reached 1% the water structure is completely broken and the hydration becomes maximum. Further increase of solute concentration above 1% the formation of hydration decreases and there is

a possibility of micelle formation (24). The micelles are held by strong hydrogen bonds in a closed manner and their formation may result in a increase of the size of the micelles as it is known that the formation of strong hydrogen bonds results in an increase of inter proton-proton distance (Lippincott and Srinivasa Rao) (25). This miscelles have to be accommodated in the water structure and this may result in weakening of inter molecular forces. This may probably is the reason for the observed increase in the value of relaxation time T_1. Further studies are necessary to establish unequivocally the formation of micelles in the above solutions. Similar explanation also holds good for the variation of relaxation time T_2 with solute concentration. Figures 4.7,4.8 show the variation of relaxation times T_1 and T_2 with solute concentration for aqueous solutions of tryptophan. From the figure, it can be seen that the relaxation time decreases with increase of solute concentration and shows a minimum at 1% solute concentration. The relaxation times increases with further increase of concentration. The explanation proposed for aqueous solutions of tyrosine appears to be valid for this case also.

pH VARIATION STUDY

The spin-lattice and spin-spin relaxation times T_1 and T_2 were measured in aqueous solutions of amino acids (L-lysine, L-serine, L-proline , glycine and L-aspartic) at a

concentration of 1% for two pH values namely body pH (7.4) and iso-electric pH. The values of relaxation times, viscosity and density are listed in Tables 4.5, 4.6. The results indicate that the relaxation times do not show any significant differences between body pH and iso-electric pH. This observation is similar to the results obtained by Gruker et al (12).

STUDY OF AQUEOUS SOLUTIONS OF PROTEIN

The investigation of the proton spin-lattice relaxation as a function of the temperature, concentration, provides valuable insight into molecular dynamics in solutions (26-30). Hennel et al (31) first reported that the spin-lattice relaxation time (T_1) in a denatured protein is shorter than in native one. Proton denaturation causes some changes of NMR line parameters in high resolution NMR spectrum (Bradbury et al) (32). The denaturing processes have also been investigated by Raman spectroscopy and Ultrasonic methods (33,34). The relaxation studies are important for understanding the state of water in protein solutions and these may be useful in the application of the NMR technique to cancer diagnosis and NMR imaging of living system. Hence the present study has been undertaken in aqueous solutions of Bovine Serum Albumin (BSA) at different temperatures in the range (15 C - 75 C). These studies may throw more light on the nature of molecular interactions between

the solvent and solute molecules. PMR relaxation study may also provide some information on denaturing of proteins.

High purity protein (BSA) powder with different percentage concentrations was dissolved in the phosphate buffer of pH 7.4. The temperature variation study was performed in 5% BSA powder dissolved in double distilled water. The relaxation times were measured using Bruker PC 120 NMR processes analyser at a temperature of 37 C. 180- - 90 pulse sequences was used for the measurement of T_1. The error of the T_1 values was less than 1 percent. The decay of magnetisation was checked and the decay is found to be mono-exponential for all the solutions.

RESULTS AND DISCUSSION

The variation of relaxation times (T_1) versus concentrations of BSA are shown in figure 4.9. From the figure, it can be seen that the relaxation time (T_1) is found to decrease with increase of BSA concentration. It has been already found that (T_1) decreases with increase of hydrogen bond energy (20). It may be interpreted in the present observation that the observed decrease may be due to the formation of strong hydrogen bonds in these solutions. It has been observed in protein solutions that the spin polarization of the water proteins is transported first by rapid material exchange from the bulk solvent to the hydration

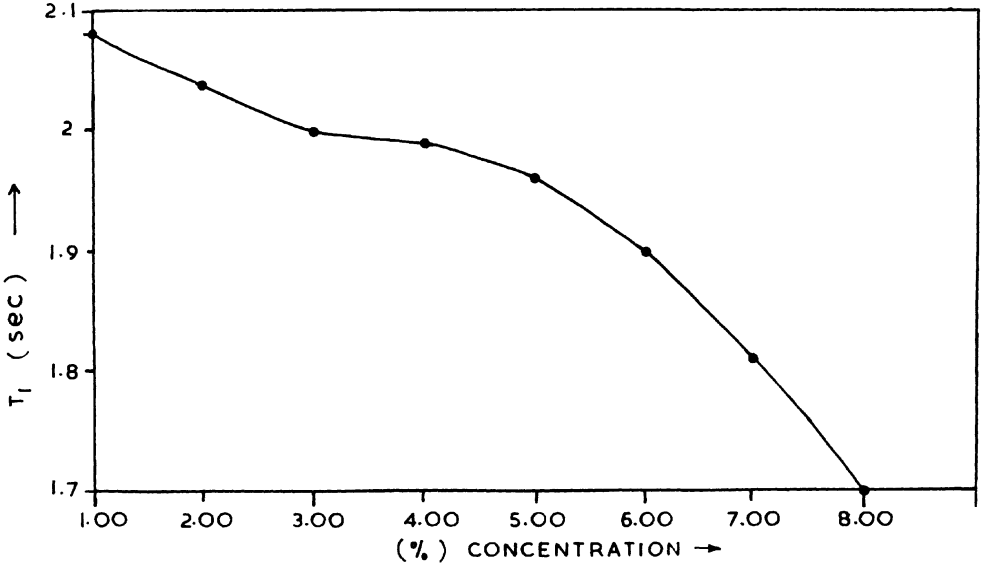

FIG. 4·9 RELAXATION TIME (T_1) VS (%) CONCENTRATION OF BSA

shell and there to the relaxation centers by dipolar interaction and spin diffusion (35). It is possible that hydration shells may also form in the present study and the formation of hydration shells requires the presence of strong hydrogen bonds. Hence the present decrease observed in the value of T_1 with increase of solute concentration may be interpreted as due to the formation of hydration shells and the spin-lattice relaxation time (T_1) may be due to rapid exchange of protons between the hydration shells and the bulk solvent.

TEMPERATURE VARIATION STUDY

The temperature variation studies were carried out in 5 percent aqueous solutions of BSA in the temperature range of 15 C to 75 C. The variation of relaxation time with temperature is shown in figures 4.10, 4.11 for BSA and pure water respectively. From the graph, it can be seen that the relaxation time (T_1) for aqueous solution of BSA increases with increase of temperature upto 70 C, and then decreases with further increase in temperature. In the case of pure water the relaxation time (T_1) increases gradually with increase of temperature.

According to the previous investigations in protein solutions at low concentration (28), the relaxation time of the solution fulfill the following equation

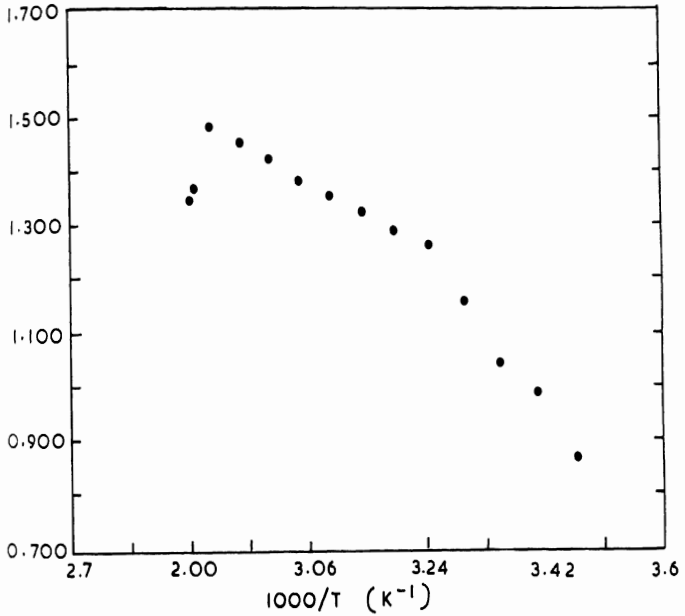

FIG. 4.10 RELAXATION TIME (T_1) VS 1000/T FOR BSA

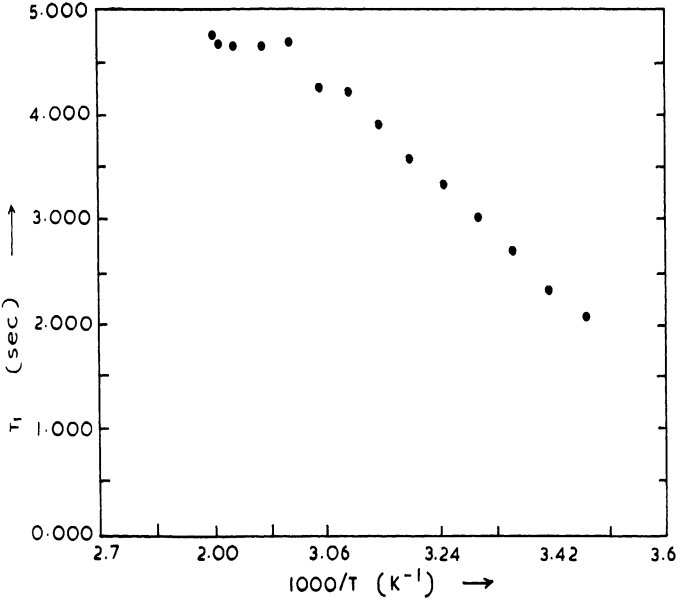

FIG. 4.11 RELAXATION TIME VS 1000 / T FOR WATER

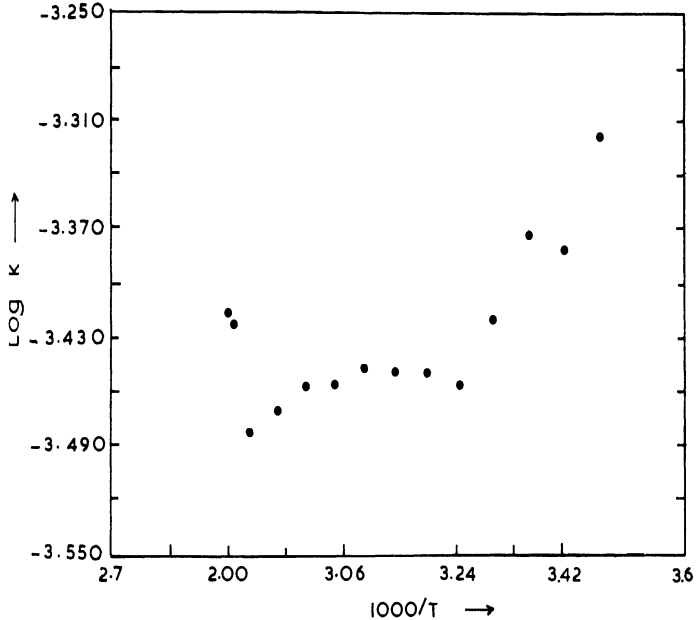

FIG. 4-12 LOG K VS 1000/T

$$\frac{1}{T_1} = \frac{1}{T_{1W}} + KC \qquad \text{----- (4.1)}$$

where T_{1W} is the relaxation time for pure water (solvent), C is the concentration of protein and the factor K is proportional to the relaxation rate of the protein. The calculated values of log K are plotted against 1000/T (figure 4.12). It has been already established (28) in aqueous solution of proteins that in the low temperature region (-5 to 45 C) the protein will be in the native state. Thermal denaturation occurs in the temperature range 45 C to 75 C and complete denaturation occurs when the temperature exceeds 75 C. From figure 4.12, it can be seen that the graph can be divided into three regions namely native region corresponding to low temperature range, thermal denaturation region ranging from 40 C to 70 C and complete denaturation region above 70 C. The calculated activation energy for the first region is found to be 10.54 KJ/mole and for the second region the activation energy is found to be 3.21 KJ/mole and these agree fairly with earlier studies in other protein solutions.

In conclusion it may be mentioned that the present NMR study in aqueous solutions of amino acids 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. This results in a decrease of spin-lattice relaxation with increase of solute

concentration. The present NMR study in aqueous solution of BSA established that partial denaturation of BSA molecules occur in the temperature range of 40 C - 70 C.

TABLE.4.1

PMR RELAXATION TIMES, VISCOSITY AND DENSITY OF AQUEOUS SOLUTIONS OF GLYCINE

(%)Con.	T1 (Sec)	T2 (Sec)	$\eta \times 10^3$ NSm^{-2}	ρ g/cm^3
1	3.067	1.369	0.8080	0.8847
2	2.894	1.255	0.8240	0.8898
3	2.766	1.170	0.8285	0.8915
4	2.739	1.147	0.8715	0.8956
5	2.661	1.132	0.9257	0.8982
6	2.557	1.086	0.9410	0.9011
7	2.456	1.042	0.9055	0.9028
8	2.324	0.992	0.9468	0.9073
9	2.237	0.907	0.9786	0.9102

T_1 - Spin-lattice relaxation time

T_2 - Spin-spin relaxation time

η - Viscosity

ρ - Density

TABLE.4.2

PMR RELAXATION TIMES, VISCOSITY AND DENSITY OF AQUEOUS SOLUTIONS OF L.PROLINE

(%)Con.	T1 (Sec)	T2 (Sec)	$\eta \times 10^3$ NSm^{-2}	ρ g/cm^3
1	3.069	1.369	0.8080	0.8850
2	2.968	1.301	0.8250	0.8900
3	2.940	1.290	0.8312	0.8917
4	2.919	1.271	0.8816	0.8925
5	2.887	1.251	0.9360	0.8990
6	2.847	1.200	0.9560	0.9016
7	2.760	1.150	0.9570	0.9112
8	2.627	1.001	0.9512	0.9100
9	2.515	0.961	0.9701	0.9130

T_1 - Spin-lattice relaxation time

T_2 - Spin-spin relaxation time

η - Viscosity

ρ - Density

TABLE.4.3

PMR REELAXATION TIMES, VISCOSITY AND DENSITY OF AQUEOUS SOLUTIONS OF TYROSINE

(%)Con.	T1 (Sec)	T2 (Sec)	η x 10^3 NS m^{-2}	ρ g/cm^3
0.5	1.612	1.068	0.7884	1.0035
1.0	1.285	0.831	0.8020	1.0035
1.5	1.623	0.971	0.8173	1.0085
2.0	1.766	1.051	0.8114	1.0116
2.5	1.936	1.182	0.8537	1.0123
3.0	2.394	1.197	0.8648	1.0167

T_1 - Spin-lattice relaxation time

T_2 - Spin-spin relaxation time

η - Viscosity

ρ - Density

TABLE.4.4

PMR RELAXATION TIMES, VISCOSITY AND DENSITY OF AQUEOUS SOLUTIONS OF TRYPTOPAN

(%)Con.	T1 (Sec)	T2 (Sec)	$\eta \times 10^3$ NSm^{-2}	ρ g/cm^3
0.5	1.982	1.273	0.8120	1.995
1.0	1.798	1.251	0.8207	1.037
1.5	2.392	1.501	0.8125	1.068
2.0	2.673	1.635	0.8182	1.012
2.5	2.892	1.701	0.8656	1.099
3.0	2.967	1.701	0.8954	1.108

T_1 - Spin-lattice relaxation time

T_2 - Spin-spin relaxation time

η - Viscosity

ρ - Density

TABLE 4.5

Relaxation Times, Viscosity , Density Data of Aqueous Solutions in Amino Aci (1%) at 38 C of Body pH

Compound	T1 (sec)	T2(sec)	$\eta \times 10^3$ NS/m^2	ρ g/cm^3
L-Lysine	3.612±0.030(9)	1.886±0.040(9)	0.6667	0.993
L-Serine	3.626±0.012(6)	2.161±0.009(6)	0.6527	0.977
L-Proline	3.591±0.007(6)	2.261±0.001(6)	0.6725	0.989
Glycine	3.552±0.010(6)	2.171±0.005(6)	0.6511	1.002
L-Aspartic	3.452±0.012(5)	1.821±0.010(5)	0.6850	1.004

TABLE 4.6

Relaxation Times, Viscosity , Density Data of Aqueous Solutions in Amino Acids (1%) at 38 C of Isoelectric pH

Compound	ipH	T1 (sec)	T2(sec)	$\eta \times 10^3$ NS/m^2	ρ g/cm^3
L-Lysine	9.7	3.504±0.010(5)	2.248±0.006(5)	0.6667	0.999
L-Serine	5.7	3.609±0.015(5)	1.562±0.012(5)	0.6650	0.998
L-Proline	6.4	3.573±0.016(5)	1.722±0.060(5)	0.6467	0.997
Glycine	6.1	3.482±0.012(5)	1.499±0.012(5)	0.6290	0.999
L-Aspartic	3.0	3.486±0.005(5)	2.248±0.060(5)	0.6670	0.999

Mean ± S.D (N); N = Number of measurements

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