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Investigation of the Hydration of Bovine Serum Albumin by an NMR Titration Method

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In this study, NMR titration method was used to determine the dynamics of hydration water. Solutions were made by dehydration procedures and relaxation measurements were carried out on a Vnmr (Varian NMR) spectrometer operating at 300 MHz for proton and relaxation rates ($1/T_1$) were plotted *versus* M_s/M_w. It was seen that the NMR titration data was separated into 2 line segments. The intercepts of the first line segment with lower mass of solute/mass of water (M_s/M_w) ratio and the second line segment with higher M_s/M_w are 2.15 and 2.57, respectively. The correlation times for free and structured water were obtained as 5.56×10^{-12} s and 1.5×10^{-11} s, respectively. The $1/T_1$ and $1/T_2$ relaxation rates increase as the protein concentration increases. This implies that the protein is reducing the motion of water molecules. The correlation times (τ_c) were calculated by using the experimental data and relaxation theory related. These results suggest that the relaxation times of water layers surrounding a protein could be determined by T_1 measured *versus* M_p/M_w. Present results were correlated well with the results from other measurements techniques. The data also suggest that the relaxation mechanism of bovine serum albumin (or serum proteins) can be explained in terms of fast chemical exchange of protons between bulk water and water bound to proteins.

Key Words: Bovine serum albumin, Correlation, Relaxation times.

INTRODUCTION

The interactions of water with globular proteins has been investigated by several authors using two different approaches and presenting some methods[1-3]. The first approach is based on the analysis of NMR T_1 and T_2 relaxation mechanisms in protein solutions. These studies inferred the presence of different water types in solutions. For example, water was classified as free and bound water molecules on protein in 1 work[1,4], while it was classified as free, translationaly hindered and rotationally bound[5] or bulk and bound water in others[6,7]. The relaxation rate, $1/T_1$, is dependent on correlation time, τ_c, which characterizes the molecular motions. Therefore; determination of τ_c gives useful information about molecular mobility

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and intramolecular motions. All the studies in the first approach are based on fast chemical exchange of water molecules among layers. NMR titration method was used to determine the dynamics of hydration water. Titration data was separated into different line segments and each segment was attributed to the different compartment of water in solution. T_1, T_2 and τ_c of each department were determined[1]. Water molecules in a hydration shell around a macromolecule are normally less mobile than water molecules in the bulk liquid. Numerous experimental and theoretical studies have demonstrated that the properties of protein hydration water are different from those of bulk water[8].

EXPERIMENTAL

In this study, the protein used was grade BSA from sigma Company. Solutions were made by dehydration procedures and relaxation measurements were carried out by NMR device.

Dehydration procedure: Samples, which was 0.15 g of BSA, was weighed in the NMR tubes and 0.85 mL of water was added to each samples. Following the initial NMR measurements, the samples were dehydrated over a period of 1 h by placing under the air flow at room temperature and then the samples were removed, reweighed and measured T_1 and T_2 relaxation times by the NMR device. This process was periodically repeated until the measurements of spin-lattice (T_1) relaxation times become unreliable. The difference between the initial mass at each measurements was attributed entirely to water, which allowed calculation of the mass of solute/mass of water ratio (M_s/M_w) during each relaxation time determination.

NMR measurements: NMR relaxation times ($1/T_1$ and $1/T_2$) were measured in BSA solutions with increasing concentration for three identical sample. The measurements were carried out on a Vnmr (Varian NMR) spectrometer operating at 300 MHz for proton. The spin-lattice (T_1) relaxation times were determined with a inversion recovery (IR) pulse sequence using different time interval of τ, being varied from 0.2 s to 15 s. Spin-spin relaxation times measurements were measured with a Carr-Purcell-Meiboom-Gill pulse sequence (CPMG), using 7 echo. Echo delays were changed from 16 to 1000 ms. Pulse repetition time was chosen as 7 s. All of the measurements were carried out at room temperature, which was 23 ± 1 ºC.

RESULTS AND DISCUSSION

Results show that the values the relaxation rate ($1/T_1$) increase as the concentration of bovine serum albumin is increased. Relaxation times are also influenced by the interactions of water molecules with macromolecular structures. Fig. 1 shows M_s/M_w dependence of spin-lattice relaxation rate, $1/T_1$, of protons in bovine serum albumin solutions. The data can clearly be separated into two line segments. These were shown on Fig. 2. The intercepts of the first line segment with lower M_s/M_w and the second line segment with higher M_s/M_w are 2.15 and 2.57, respectively. It is assumed that the dehydration starts from outer compartment (or hydration layer) to

inner one and each segment corresponds to one compartment in the NMR titration method. Under this explanation, the first and the second line should correspond to the free and structured water, respectively.

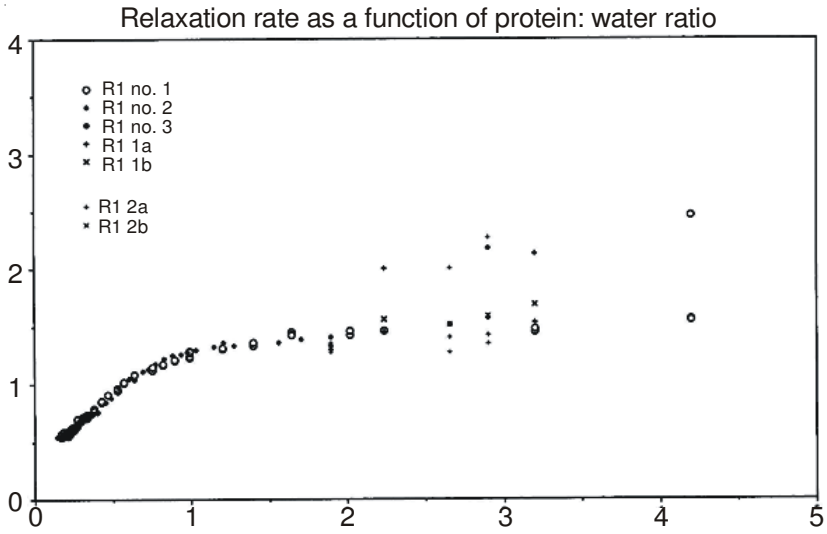

Fig. 1. A dehydration from dilute solution study of the spin-lattice relaxation rates, $1/T_1$, *versus* concentration, M_s/M_w, for three identical concentrations of BSA

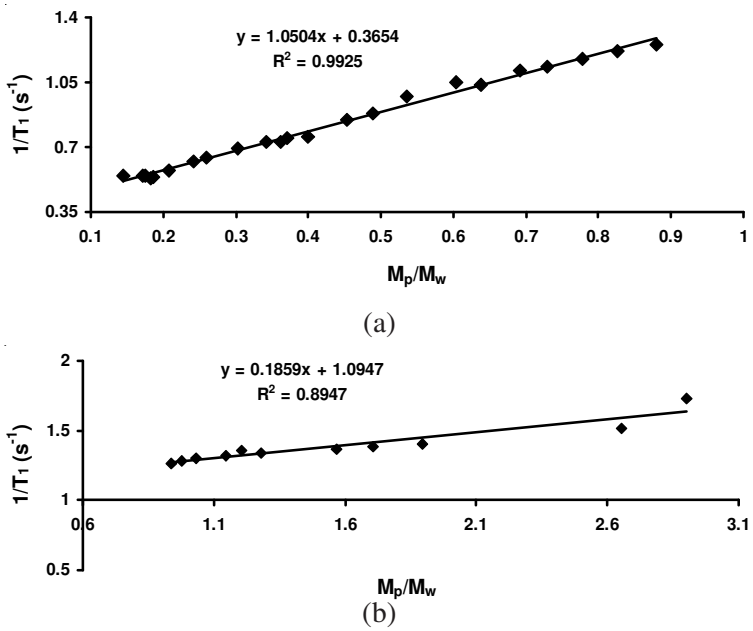

Fig. 2. Proton NMR spin-lattice relaxation rates for free (a) and hydration (b) layers as functions of M_p/M_w

It can be described mathematically by two linear section with one point of singularity between them which is consistent with FPD model interpretation[9].

A theoretical expression for T_1 relaxation time in water protein solutions can be written as:

$$\frac{1}{T_1} = k\left[\frac{\tau_c}{1+\omega^2\tau_c^2} + \frac{4\tau_c}{1+4\omega^2\tau_c^2}\right] \quad (1)$$

If, we take the separation of the two protons to be 1.52 Å, as is typical water, then $k = 1.4 \times 10^{10}$ s^{-2}. If we take the separation of two protons to be 1.79 Å which is typical structured water, then $k = 6.2 \times 10^9$ s^{-2}.

Assuming $T_1 = 2.57$ s for relaxation of free water and $T_1 = 2.15$ s for relaxation of structured water and using $\omega = 300$ MHz in eqn. 1, the correlation time of free and structured water can be calculated as 5.56×10^{-12} s and 1.5×10^{-11} s, respectively.

The work mentioned in the introduction shows that some water molecules have different behaviour from free water molecules. This water have been described as a compartment[5,6,10-12] and τ_c correlation times of the first approach can be summarized as follows: The correlation times for free and bound water have been found 10^{-11} s and 10^{-5} s[6], The correlation times for free, translationally hindered and rotationally bulk water molecules have been found as $\tau_F \approx 10^{-11}$ s, $\tau_{HT} \approx 10^{-9}$ s and $\tau_{HR} \approx 10^{-8}$ s, respectively[5]. The correlation times for superbound, polar bound, structured and bulk water have been found 10^{-6} s, 10^{-9} s, 10^{-11} s and 10^{-12} s, respectively[11]. Present results correlate well with results from other measurements techniques described above.

There are mainly two states for water in protein solutions. These are free water molecules and water molecules which form a hydration layer around the macromolecules in the sample (the bound fraction). Several different experiments have demonstrated that the exchange of water between two states is rapid and relaxation rate in a protein solution can be analyzed in terms of chemical exchange which is in the form of eqn. 2[8,13-15].

If a proton exchanges rapidly between two states, bulk or free water and water associated with the protein, then relaxation rate,

$$\frac{1}{T_1} = \frac{P_b}{T_{1b}} + \frac{(1-P_b)}{T_{1f}} \quad (2)$$

where P_b is the fraction of time that a proton spends associated with the protein. T_{1b} and T_{1f} are the spin-lattice relaxation times of the bound and free water fractions, respectively[16].

Therefore, protein fraction, P_b, proportional to contain protein, eqn. 2 becomes:

$$\frac{1}{T_1} = \frac{1}{T_{1w}} + RC \quad (3)$$

The proton T_1 relaxivitie(R_{T1}) is $R_{T1} = \dfrac{\Delta\left(1/T_1\right)}{C} = \dfrac{1/T_1 - 1/T_{1w}}{C}$

where C is concentration of protein(M_p/M_w)[17].

The correlation coefficients between $1/T_1$ with M_s/M_w for the first and second layers are 0.99 and 0.89, respectively. The strong correlation demonstrated that, at 300 MHz, $1/T_1$ measurements are linearly proportional to the concentration of protein in solution for each layer. This can be described as:

$$\frac{1}{T_1} = 0.3654 + 1.0504\frac{M_s}{M_w} \quad (4)$$

where the first term on the right hand approximates the relaxation rate of free water[13].

It is experimentally well known that the so-called two state model can be used to describe proton magnetic relaxation in biological samples[14,18].

Conclusion

In this model, water molecules can exchange between two states and form a hydration layer around the macromolecules in the sample (bound fraction and the remainder of the water molecules which are similar ordinary liquid water). Relaxation rates show characteristic variations with a inversion recovery (IR) pulse spacing which can be interpreted on the basis of chemical exchange between solute and solvent molecules. The similarity of eqn. 4 to the eqn. 3 implies that the relaxation mechanism in bovine serum albumin solution can be analyzed in terms of fast chemical exchange of water molecules between hydration layer and bulk phase. The experimental results show the importance of water interactions with bovine serum albumin molecules and this can be lead to a better understanding of the effect of hydration water in bovine serum albumin solutions.

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