INVESTIGATION OF TEMPERATURE DEPENDENCE OF D₂O SOLUTIONS BY 400 MHZ NMR

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Abstract: The rate of water proton relaxation of protein solutions were studied in the presence and absence of the paramagnetic ions [gadolinium (III), manganese (II), chromium (III), iron (III), nickel (II), copper (II), and cobalt (II)] in the previous studies. However, these studies were carried out rather at low frequencies. Therefore, studying of temperature dependence of relaxation rates for absence and presence of 2 % albumin in pure D₂O by 400 MHz will be a novelty.

In this study, T₁ and T₂ relaxation ratios of D₂O and 0.1 H₂O/0.9D₂O solutions were investigated with respect to temperature for pure and for constant albumin concentration(2%). The experiments were carried out by using Bruker Avance 400 MHZ NMR. Inversion Recovery (180-τ-90) pulse step were used for T₁, whereas Carr-Purcell-Meiboom-Gill pulse step were used for T₂. The experiments were performed for temperature range of 20°C-40°C by using automatic temperature control unit. 1/T₁ and 1/T₂ decrease linearly with increasing temperature for pure D₂O solutions. However, for 0.1H₂O/0.9D₂O solutions, the relaxation rates of T₁ increase with increasing temperature while T₂ decreases with increasing temperature. The decrease in both relaxation rates of the D₂O solution with respect to the increased temperature suggests that relaxation is due to spin relaxation interaction. Increasing of relaxation rates with the increasing temperature, in the presence of albumin demonstrates the validity of the dipolar mechanism

Keywords: NMR, T₁, T₂, relaxation, albumin, manganese (Mn)

1. Introduction

Human serum albumin (HSA) is the most abundant serum protein in plasma and has a high ligand binding capacity that can bind a wide variety of compounds. HSA contributes to various physiological functions such as homeostasis, metabolism, protection, and also the passage and binding of endo-exogenous substrates [1, 2, 3]. Spin-lattice (T₁) and spin-spin (T₂) relaxation times of albumin solutions were studied in detail by Nuclear Magnetic Resonance Dispersion (NMRD) and Nuclear Magnetic Resonance (NMR) techniques [4-21]. Several methods have been applied to explain the mechanisms of the reaction. One of these methods is based on the derivation of albumin's rotational correlation (interest) time from the Stokes-Einstein association [8, 13, 16, 17]. The other is based on
the time of interest obtained from $T_1/T_2$ ratios [21, 22]. The interest times in microseconds in the first studies are derived from nanoseconds in subsequent studies. The difference is explained by the high protein concentration (such as 10% or 15%) used in the initial studies.

The development of contrast agents to bind a ligand to HSA has a central value in view of displaying vessels with abnormal vascular permeability [23, 24]. For this reason, studies on albumin solutions containing paramagnetic centers are still valuable. The albumin solutions containing manganese were investigated by many people [20, 25–29]. However, the effect of the manganese found in albumin D$_2$O solutions on $T_1$ and $T_2$ relaxation has not yet been investigated at 400 MHz. In this study, $T_1$ and $T_2$ of D$_2$O solutions containing various ion concentrations were investigated in NMR. The same ion concentrations were then examined in terms of the effects of $T_1$ and $T_2$ in the presence of 0.02 g of albumin. In addition, measurements were made by varying protein concentrations for specific ion concentrations.

2. Materials and Methods

Preparation of Stock solutions - A stock solution was prepared by adding 0.003 g of MnCl$_2$ in 20 ml of D$_2$O. The prepared stock solution was thoroughly shaken and the mouth part was completely closed with parafilm to prevent air ingress. 20 μl of this solution contains 1 μg Mn (II).

Temperature measurements were carry out for the following solutions : - The temperature-dependent change of ion-containing D$_2$O solution was investigated for sample prepared by taking 40 μl of the stock solution and 960 μl of D$_2$O. The temperature dependence of this solution was also investigated by adding 0.02 g of albumin.

NMR Measurements - NMR $T_1$ and $T_2$ relaxation times measurements of the prepared samples were made at 20$^{\circ}$ C, 25$^{\circ}$ C, 30$^{\circ}$ C, 35$^{\circ}$ C and 40$^{\circ}$ C. Temperatures were changed with the help of automatic temperature control system. Relaxation time measurements were performed with the Bruker Avance 400 MHz NMR spectrometer. $T_1$ and $T_2$ relaxation times were performed by using Inversion Recovery and Carr-Purcell-Meiboom-Gill pulse steps, respectively. Pulse repetition time was taken as $5T_1$ for $T_1$ measurements. Inversion Recovery and Spin Echo delay times were changed in accordance with relaxation recovery and decay processes.

3. Results and Discussion

In this study, we presented measured $T_1$ and $T_2$ relaxation times of HSA solutions at temperatures 20–45 $^{\circ}$ C. $1/T_1$ and $1/T_2$ relaxation ratios were investigated with respect to temperature for pure and for constant albumin concentration (2%). The value of the measurements were summarized in Table 1. and 2. We plotted $T_1$ and $T_2$ relaxation rates versus $T$ in order to understand the influence of temperature. Graphs are shown in Figure 1.
Table 1. The Spin-lattice relaxation rates \( (1/T_1) \) of albumin and albumin free solutions as a function of temperature \( (T) \)

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>0.02g alb+D(_2)O (1/T_1(\text{s}^{-1}))</th>
<th>pure D(_2)O (1/T_1(\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>1.592</td>
<td>1.197</td>
</tr>
<tr>
<td>298</td>
<td>1.6</td>
<td>1.075</td>
</tr>
<tr>
<td>303</td>
<td>1.63</td>
<td>0.946</td>
</tr>
<tr>
<td>308</td>
<td>1.65</td>
<td>0.846</td>
</tr>
<tr>
<td>313</td>
<td>1.66</td>
<td>0.767</td>
</tr>
</tbody>
</table>

Table 2. The spin-spin relaxation rates \( (1/T_2) \) of albumin and albumin free solutions as a function of temperature \( (T) \)

<table>
<thead>
<tr>
<th>Temperature(K)</th>
<th>0.02 g alb+ D(_2)O (1/T_2(\text{s}^{-1}))</th>
<th>pure D(_2)O (1/T_2(\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>26.52</td>
<td>46.14</td>
</tr>
<tr>
<td>298</td>
<td>23.64</td>
<td>41.89</td>
</tr>
<tr>
<td>303</td>
<td>20.32</td>
<td>38.87</td>
</tr>
<tr>
<td>308</td>
<td>18.11</td>
<td>33.3</td>
</tr>
<tr>
<td>313</td>
<td>15.87</td>
<td>29.1</td>
</tr>
</tbody>
</table>
Figure 1. Temperature dependence of the spin–lattice ($R_1$) (A) and spin–spin relaxation rate ($R_2$) (B), for pure and protein-contained D$_2$O solutions.

It can be seen that the change of $1/T_1$ and $1/T_2$ relaxation rates with temperature ($T$) is linear, but in the presence of 0.02 g albumin, the $1/T_1$ ratio of D$_2$O increases linearly with increasing temperature while decreases linearly in the absence of albumin. In addition, in the presence and
absence of 0.02 g albumin, the \(\frac{1}{T_2}\) relaxation rate of \(\text{D}_2\text{O}\) decreases linearly with increasing temperature (Fig.1).

Decreasing of the \(\frac{1}{T_1}\) and \(\frac{1}{T_2}\) values with temperature in \(\text{D}_2\text{O}\) solutions suggests that the dipole-dipole interaction mechanism is predominant [19-21]. In addition, the increase of \(\frac{1}{T_1}\) with temperature in the \(\text{D}_2\text{O}\) solutions shows that the mechanism of spin-rotation interaction is predominant. The relaxation rates in all solutions has been found that high correlation for each fit and vary linearly with temperature [22].

4. Conclusions

The \(\frac{1}{T_1}\) and \(\frac{1}{T_2}\) vary linearly with temperature and has a high influence to alter relaxation of solution studied. The least-squares fitting of \(\frac{1}{T_1}\) and \(\frac{1}{T_2}\) versus \(T\) gives a linear relationship, and the data suggest that the relaxation mechanism of HSA is caused by a fast chemical exchange of water molecules between protein-bound water and free water. Decreasing and increasing of the relaxation rates (\(\frac{1}{T_1}\) and \(\frac{1}{T_2}\)) values with increasing temperature in \(\text{D}_2\text{O}\) solutions suggests that the dipole-dipole interaction and spin-rotation interaction mechanism is predominant, respectively.

References