Elimination of Radiation Damping Effects from the NMR Relaxation Curves of H$_2$O/D$_2$O Mixtures Containing Protein and Ions

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In this work the T$_1$ and T$_2$ curves of H$_2$O/D$_2$O mixtures were obtained for several fractions of H$_2$O and also for different amounts of albumin and manganese. The experiments were carried out with a 400 MHz proton NMR spectrometer. The T$_1$ and the T$_2$ curves were determined by the inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) sequences, respectively. The relaxation times in residual water and in a mixture with 0.05 ml H$_2$O were found to be reliable either in the presence or in the absence of albumin. The mixtures containing 0.1 ml or higher H$_2$O suffer from radiation damping (RD). RD was removed gradually by addition of albumin or manganese. RD in the protein solution with 0.1 ml H$_2$O was removed by adding 1 µg of manganese to one ml of solution. The data suggest that the relaxation times in H$_2$O/D$_2$O mixtures are measurable by conventional methods upon the addition of appropriate amounts of manganese and albumin.

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I. INTRODUCTION

Radiation damping (RD) is caused by a strong interaction between a high proton magnetization of the solvent and a free induction decay (FID) current induced in RF coils [1–4]. The effects of RD on the NMR signal intensities, relaxation times, and line shapes have already been reported in several works [5–12]. RD was found to interfere with the NMR relaxation times. Therefore, RD must be eliminated when relaxation measurements are performed at higher fields. RD can be suppressed by using several techniques, such as pulsed bipolar field gradients, electronic negative feedback, and Q-switching during acquisition [13–19]. Since relaxation times are decreased by paramagnetic ions or proteins, RD in H$_2$O solutions should also be reduced by the addition of such materials to the solutions. The conventional inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) sequences may then become convenient for reliable measurements of the relaxations. Such a work should be useful for high-field relaxation studies of protein solutions by conventional methods.

The aim of this work is to demonstrate the elimination of RD in H$_2$O/D$_2$O mixtures by the addition of albumin and manganese. For this purpose, the T$_1$ and T$_2$ curves of the
H₂O/D₂O mixtures were obtained for several fractions of H₂O. They were also found in the presence of albumin and manganese.

II. EXPERIMENTAL

II-1. Preparation of samples

A set of pure H₂O/D₂O mixtures were prepared by adding a certain volume of H₂O to that of D₂O so that the total volume is 1ml. The volumes of H₂O were residual water, 0.05 ml, 0.10 ml, 0.15 ml, 0.20 ml, and 0.30 ml in 1 ml of H₂O/D₂O. These samples were used to demonstrate the effect of RD in pure H₂O/D₂O mixtures. A set of 6 samples was also prepared by adding each of 0.05 g, 0.10 g, 0.15 g, 0.20 g, 0.25 g, and 0.30 g albumin to 1 ml of a mixture (0.10 ml H₂O and 0.90 ml D₂O). The samples in this set were used to demonstrate the elimination of the RD by the addition of albumin. In addition, a mixture of water (0.10 ml H₂O/0.9 ml D₂O) containing manganese was also prepared in the absence and in the presence of 0.02 g albumin. Prior to NMR measurements, all samples were deoxygenated with a flowing nitrogen stream for one hour to remove oxygen by a method similar to that given in a previous work [20].

II-2. Measurements of the relaxation times

The experiments were carried out with a BRUKER AVANCE-400 MHZ proton NMR spectrometer operating at 400.132 MHz. The IR and CPMG sequences were used for measuring the relaxation times. The pulse repetition time of each experiment was set to a value above 5T₁. The T₁ and T₂ of each sample were determined first by pioneer experiments. The successive FID signals were acquired versus one hundred delay times, while the successive echo decays of the same samples were obtained by using fifty echo times. The delay times in the IR experiments, and also the echo times in the CPMG experiments were altered by equal steps. The FT signals of the FID or echo decays were plotted versus the delay or echo times for obtaining the T₁ and T₂ curves. All measurements were carried out at 25 °C by using an automatic temperature controller unit.

III. RESULTS

The representative T₁ and T₂ curves of some H₂O/D₂O mixtures are shown in Fig. 1, while those of a mixture (0.10 ml H₂O/0.90 ml D₂O) containing increasing amounts of protein are shown in Fig. 2. The T₁ and T₂ curves of D₂O and 0.05 ml H₂O/0.95 ml D₂O fit the relevant single relaxation formulas very well, but the relaxations of 0.10 ml H₂O/0.90 ml D₂O and other mixtures with higher H₂O or more suffer from RD. RD in the mixture of 0.10 ml H₂O/0.90 ml D₂O is gradually diminished by the gradual addition of protein. The perfect removal of RD was obtained at high protein concentrations. Fig. 3 shows that 1µ g of manganese is a minimum concentration to remove RD from the solution of 0.10 ml
H$_2$O/0.90 ml D$_2$O and a solution with albumin.

FIG. 1: T$_1$ (left hand side) and T$_2$ (right hand side) relaxation curves of pure H$_2$O/D$_2$O mixtures. Up to down: the water content of the samples were 0.05 ml H$_2$O, 0.10ml H$_2$O, and 0.20 ml H$_2$O per 1 ml of solution.

The relaxations obtained for pure mixtures are given in Table I, whereas the relaxation rates in protein solutions are given in Table II. These tables show the quantitative values of the relaxations obtained from the IR or CPMG curves.
FIG. 2: $T_1$ (left hand side) and $T_2$ (right hand side) relaxation curves of a mixture of 0.10 ml H$_2$O and 0.90 ml D$_2$O mixtures obtained for increasing concentration of albumin. Up to down: the albumin contents of the samples were 0.05 g, 0.10 g, and 0.20 g per 1 ml of solution.

IV. DISCUSSION

Solvent $T_1$ and $T_2$ relaxations of albumin solutions obey a single relaxation behavior [21, 22]. In the absence of RD, the $T_1$ data of such solutions fits $M_z = M_0[1 - 2\exp(-t/T_1)]$, while the $T_2$ decay obeys the relation $M_{xy} = M_0[\exp(-t/T_2)]$. In the presence of RD, such samples display a multiple relaxation behavior, since RD acts as a pseudo relaxation mechanism [1, 2]. As a result, the relaxation curves of the samples with RD do not fit the plotted data when single relaxation formulas are used. The good
FIG. 3: $T_1$ (left hand side) and $T_2$ (right hand side) relaxation curves of the mixture (0.10 ml H$_2$O and 0.90 ml D$_2$O) containing 1 µg of manganese in the absence (top) and presence of 0.02 g albumin (bottom).

<table>
<thead>
<tr>
<th>Fraction of Water</th>
<th>Pure $T_1$ (s)</th>
<th>Pure $T_2$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 ml</td>
<td>15.37</td>
<td>2.915</td>
</tr>
<tr>
<td>0.05 ml</td>
<td>12.66</td>
<td>6.148</td>
</tr>
<tr>
<td>0.10 ml</td>
<td>RD</td>
<td>RD</td>
</tr>
<tr>
<td>0.15 ml</td>
<td>RD</td>
<td>RD</td>
</tr>
<tr>
<td>0.20 ml</td>
<td>RD</td>
<td>RD</td>
</tr>
<tr>
<td>0.30 ml</td>
<td>RD</td>
<td>RD</td>
</tr>
</tbody>
</table>
TABLE II: The observed $1/T_1$ and $1/T_2$ relaxation rates in a mixture (0.10 ml H$_2$O/0.90 ml D$_2$O) versus increasing amounts of added albumin. RD denotes the presence of radiation damping.

<table>
<thead>
<tr>
<th>Albumin Concentration (g/dl)</th>
<th>$1/T_1$ (1/s)</th>
<th>$1/T_2$ (1/ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.21</td>
<td>2.044</td>
</tr>
<tr>
<td>0.10</td>
<td>0.36</td>
<td>3.46</td>
</tr>
<tr>
<td>0.15</td>
<td>0.53</td>
<td>5.46</td>
</tr>
<tr>
<td>0.20</td>
<td>0.59</td>
<td>7.30</td>
</tr>
<tr>
<td>0.25</td>
<td>0.75</td>
<td>10.53</td>
</tr>
<tr>
<td>0.30</td>
<td>0.87</td>
<td>11.76</td>
</tr>
</tbody>
</table>

and bad fits confirming this explanation are clearly seen in Fig. 1. The transition from the good fit to the bad one is clearly related to increasing the signal intensity, which is caused by increasing the water.

The relaxation rates in protein solutions and aqueous solutions of paramagnetic ions increase significantly with increasing amounts of proteins and paramagnetic ions. Then one can calculate much smaller intensities of magnetizations in such solutions. The gradually diminished RD in Fig. 2 is consistent with gradually increased amounts of albumin leading to higher relaxation rates. On the other hand, $1/T_1$ and $1/T_2$ are known to be linearly proportional to protein concentration [21, 22]. Such a linear dependence must be obtained if the present relaxation values are reliable. The dependencies of $1/T_1$ and $1/T_2$ on protein concentration are given Fig. 4. The linearities presented in this figure imply that the obtained relaxation values are quite reliable.

![FIG. 4: The relaxation rates in 0.10 ml H$_2$O / 0.90 ml D$_2$O mixture versus increasing albumin content.](image)

Despite this, protein aggregation is known to have an effect on the $1/T_2$ in the presence of a high amount of bovine serum albumin (BSA) [24]. Therefore, H$_2$O/D$_2$O
mixtures containing a high amount of albumin may not be suitable for relaxation studies. However, manganese is a quite effective relaxer. The presence of 1 µg of manganese in solution of 0.1 ml H₂O/0.90 ml D₂O removes the RD, even in the presence of 0.02 g albumin. This makes it possible to study protein-paramagnetic ion complexes at high magnetic fields.

The present study indicates that RD in protein solutions is effectively diminished by the addition of a suitable concentration of albumin and manganese to a H₂O/D₂O solution. However, the suitable concentration of albumin or manganese is different for each fraction of H₂O. It should also be different for different types of proteins and paramagnetic ions. Therefore, the present study provides only a clue for high field studies of protein-paramagnetic ion complexes by the conventional sequences. Suitable concentrations in H₂O/D₂O mixtures need to be determined for each type of protein and ion separately.

V. CONCLUSION

In conclusion, RD in H₂O/D₂O mixtures is diminished by the addition of albumin or manganese. The data suggest that the relaxation times in H₂O/D₂O mixtures are measurable by conventional methods upon the addition of appropriate amounts of manganese and albumin.

References