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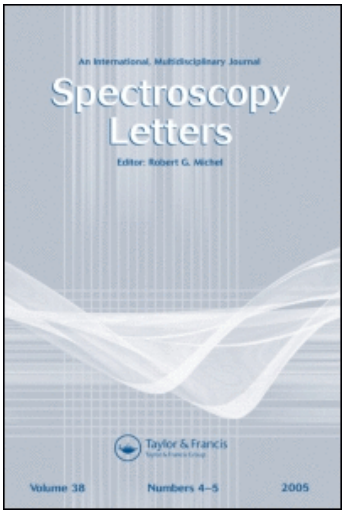

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NMR Water Proton T1 Mechanism in Blood Diluted by its Own Plasma

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NMR WATER PROTON T1 MECHANISM IN BLOOD DILUTED BY ITS OWN PLASMA

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ABSTRACT

The water proton T1 in human blood diluted by its own plasma was measured with a FT-NMR spectrometer operating at 60MHz for protons. A linear relationship (with a correlation of 0.99) was found between the 1/T1 and hemoglobin content(Hb) in the blood.The exchange of water between the extracellular plasma and the intracellular Hb in blood is known to satisfy the fast chemical exchange conditions , and the decay of magnetization in blood is reported to have a single exponential. Therefore, the obtained relationship

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should represent fast chemical exchange between the intracellular Hb and the extracellular plasma.

INTRODUCTION

Relaxation of water protons in hydrated protein solutions is dominated by chemical exchange of protons between free and bound water.[1-2] If exchange between this phases is sufficiently rapid,all protons exhibit similar relaxation behavior as a result of the mixing, the overall relaxation observed is then a weighed average of relaxation rates in each local proton environment.

On the other hand , published data on T1 of whole blood reveal that the exchange of water between the extracellular plasma and the intracellular Hemoglobin is sufficiently rapid,[3,4] and the total magnetization of water protons in blood follows a single exponantial decay.[5,6] Therefore,the T1 mechanism in blood is attributed to the extracellular and the intracellular phases with fast exchange between them.This attribution implies that the 1/T1 in blood fulfils the chemical exchange formula valid in the fast exchange limit. To the best of the author's knowledge , no direct formula has been obtained from healthy blood data so far.Therefore,an experimental formula representing fast chemical exchange in blood should be interesting for confirming the previous data. Since the relaxation rate of a protein solution that is

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caused by fast chemical exchange is proportional to its protein content,[1,2] changing the Hb concentration of blood should provide an experimental derivation of such a formula.

In order to obtain the formula desired , the T1 measurements in blood samples containing increasing amounts of Hb have been undertaken in this study.

THEORY

The presence of proton - exchanging phases in a system can cause a relaxation mechanism called chemical exchange. In the case of two exchanging phases the relaxation time has been evaluated in the fast, intermediate and slow exchange limits. If the subscripts a and b refer to the exchanging phases , T1a and T1b denote the relaxation times in phase a and in phase b, respectively,and T1 denotes the relaxation time in whole system,then for the slow exchange case(T1 << lifetime in each phase) , the total magnetic signal contains two separate components, decaying with time constants T1a and T1b, respectively. For the case of fast chemical exchange (T1 >> lifetime in each phase) , whole system relaxes with a single characteristic time T1 given by,[7,8]

$$1/T1 = Pa/T1a + Pb/T1b \qquad (1)$$

where Pa and Pb denote fraction of nuclei in each phase,respectively, (Pa + Pb = 1).

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Most analyses of NMR relaxation data from hydrated protein solutions make the assumption of two phases known as "bound" and "free" with fast chemical exchange between them. Then Eq.1 can be rewritten as [1,2]

$$1/T1 = 1/T1f + Pb(1/T1b - 1/T1f) \quad (2)$$

In this case T1f and T1b denote the relaxation times in the free and bound phases, respectively, and Pb stands for fraction of nuclei in bound phase. Since the fraction of bound water is proportional to protein content C, Eq.2 can be rearranged as

$$1/T1 = 1/T1f + kC \quad (3)$$

where k is the relaxation rate enhancement per unit protein concentration.

EXPERIMENTAL

Plasma for this experiment was obtained from heparinized blood of a healthy volunteer. Blood samples with different Hb content were obtained by making serial dilutions of the blood with its own plasma. The Hb of the samples was measured by DHB-3 hemoglobinometer (Atago Co. Ltd.). All measurements were made immediately after the donation of blood. T1 measurements were carried out on a JEOL FX-60Q FT NMR spectrometer operating at 60MHz for protons. The probe temperature was kept at (20 $\pm$ 0.5)C by means of a JNM-VT-

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automatic temperature controller unit. The inversion recovery pulse sequence was used with pulse spacing, τ, being varied from 0.4s to 2.2s, and the infinite τ was 12s.[9-11] Pulse repetition time was set at 15s. The pulse separations are large enough to ensure that any effects from hydrogen atoms in the protein have decayed away,[10-11] leaving only the water-dominated signal. The magnetization decay curve was found to be a single exponential,[5-6,9-11] and experimental errors for the T1 and the Hb measurements were estimated to be about($\pm$ 0.03)s and ($\pm$ 0.1) g/dl, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the dependence of the 1/T1 on the Hb content for the blood diluted by its own plasma. The least squares fit of the 1/T1 versus the Hb gives a linear relationship (with a correlation of 0.99) as follows:

$$1/T1b = 1/T1p + kHb \qquad (4)$$

where k is the slope of the straightline which measures the relaxation rate change per unit Hb content, and 1/T1b and 1/T1p are the relaxation rates of blood and plasma, respectively. The intercept of the straightline on 1/T1-axis corresponds to the 1/T1p of the plasma used. Eq.4 has the same form as the fast chemical exchange

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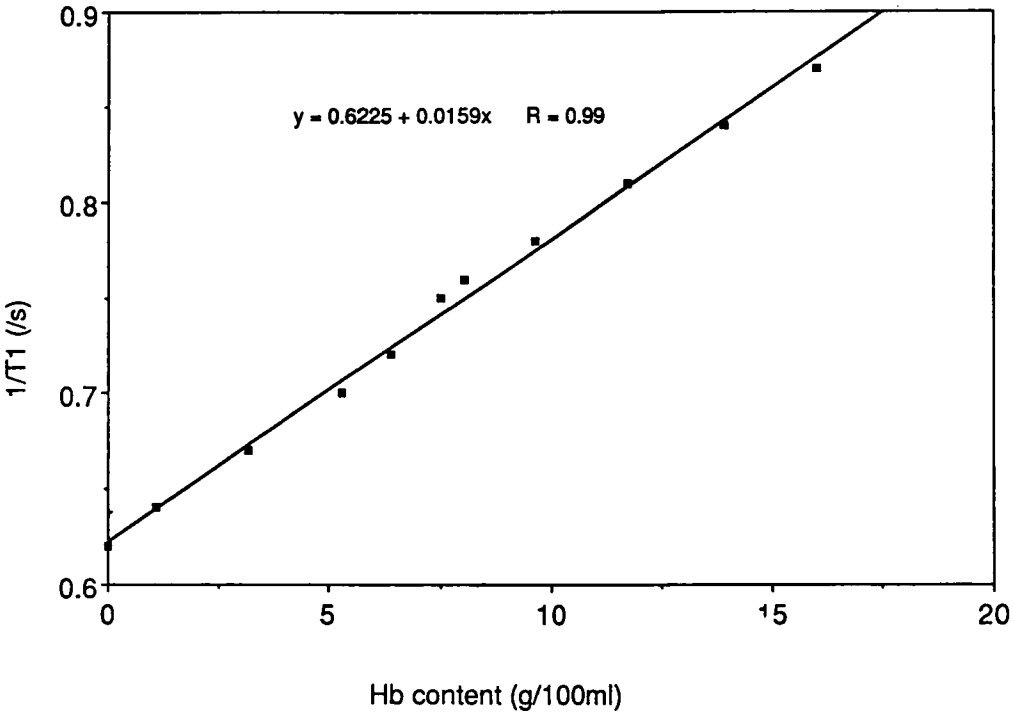

Figure 1 : Spin-lattice relaxation rate of blood diluted by its own plasma versus its hemoglobin content.

formula given in Eq.3, and indicates that the contributions of the Hb and plasma to the relaxation rate in whole blood are averaged by exchange. This is consistent with the single exponential behavior of total magnetization of each of the plasma and cytoplasm(intracellular phase),indicating that the exchange of water between bulk water and water bound to proteins is very rapid.[9,11] In fact, the contributions from the bulk and

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bound water to the relaxation rate of each of plasma and packed cells,too,are effectively averaged by exchange.[12-15] This makes possible applying the theory of chemical exchange to the exchange of water molecules through the red cell membrane.

It is known that blood consists of a fluid portion (plasma) and cellular portion(mainly erythrocytes). The erythrocytes contain a rich aqueous Hb solutions within a thin membrane capsule. The plasma contains about 91% water, 7% various proteins and assorted electrolytes. Blood cells are suspended in plasma. There are basically two chemical environments for water in blood: bulk water and water bound to protein molecules (to Hb mainly). Bond is not strong,however,so that there is a continual fast-exchange of water molecules between the bulk phase and the hydration sphere . In addition,the erythrocyte membrane is highly permeable to water and water is continually exchanging between the intracellular Hb and the extracellular plasma in a time about 8ms.[3,4] The relaxation time in whole blood is about 1s.[5,11] Then the life time of bound water is much shorter than the relaxation time in whole blood , and water exchange between the intracellular Hb and extracellular plasma satisfies the fast chemical exchange conditions. Furthermore,the total magnetization in whole blood has a single exponential decay attributed to fast chemical

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exchange in blood.[5-6,9,11] Therefore, the relaxation mechanism of water protons in whole blood should be analyzed in terms of fast chemical exchange, and Eq.4 should represent fast chemical exchange of water protons between the intracellular Hb and extracellular plasma.

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