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Assessment of Contribution of Curie-Spin Mechanism in Proton Relaxation During Aggregation Process of Hemoglobin S

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Abstract
Previous works showed a significant increase in the rotational correlation time of the water bound to the hemoglobin S during the aggregation process under sickle cell disease. In this case, the contribution of "Curie-Spin" relaxation mechanism to proton relaxation may be expected. The Curie-Spin relaxation mechanism has been well described theoretically but only a few experimental evidences have been presented. Based on the reported correlation times, the contribution of the Curie-spin relaxation mechanism to proton relaxation times (T_1 and T_2) has been estimated in comparison with the contribution of the dipole–dipole relaxation mechanism at the extreme stages of the aggregation process of the hemoglobin S. This contribution is about 25% and 50% in the spin–spin relaxation rates at the magnetic field of 1.5 T during the latent and ending stages of the aggregation process, respectively. At lower magnetic fields, this mechanism gives an insignificant contribution. The contribution to the spin–lattice relaxation is negligible even at 1.5 T. In particular, this relaxation mechanism should be taken into account when interpreting experiments related to MRI.

1 Introduction

The potentials of the magnetic resonance methods in the research of the biomedical systems have been well-known. Nuclear magnetic relaxation plays an important role in the investigations of molecular mobility in those objects [1]. Many diseases are associated with the existence of molecular aggregates. The polymerization (aggregation) of the hemoglobin S (HbS) is the underlying molecular process in sickle cell

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disease (SCD) that is distributed worldwide [2]. The aggregation process of HbS starts at low oxygenation pressure inside the red blood cell. After some time, called "delay time", irreversible molecular aggregates of the HbS are formed. In connection with these modifications, the red blood cells produce some disorders in the microcirculation and the hemodynamics conducing to the painful vaso-occlusive crisis [2–9].

Many techniques have been used to monitor the polymerization processes and the proton magnetic relaxation method has reached noticeable successes in studying them [4–9]. An adequate understanding of the mechanisms of the relaxation processes, associated with molecular agglutination, is very important for the development of new diagnostic and therapeutic strategies. The HbS aggregation process includes three characteristic stages: (1) induction state (delitescence), (2) polymerization (nucleation, aggregation) and (3) ending stage (termination) [3–6]. It has been found that this behavior is accompanied by the increase in the rotational correlation time, τ_R, of the water molecules bound to the HbS during the aggregation process [3–9]. The rotational correlation time τ_R was experimentally estimated by ^{1}H relaxation at 4 MHz [6] and confirmed by electron paramagnetic resonance (EPR) [8]. More recently the rotational correlation time measurements were also assessed using nuclear magnetic relaxation dispersion (NMRD) in the range of resonance frequencies between 10 kHz and 10 MHz [9]. Besides, T_1 and T_2 were measured under the same experimental condition at the resonance frequencies $\nu_0 = 20$ MHz and 60 MHz [9]. As it was indicated in the works [6, 8, 9], although the dipole–dipole interaction was the most important mechanism of proton relaxation in the HbS presence, the further analysis was needed in order to assess the possible contributions of other relaxation mechanisms to the measured relaxation parameters. Thus, different relaxation mechanisms in the presence of the HbS polymerization were discussed in ref. [6] and it was concluded that the dipole–dipole interaction was the most important at low magnetic fields. However, other possible magnetic relaxation mechanisms can be present at the ending stage and produce variations of the T_1/T_2 ratio. We believe that it is necessary to consider the possible influence of the Curie-Spin relaxation mechanism on the proton relaxation in the presence of HbS aggregates that may be manifested in the case of slow molecular motion.

The hemoglobin S is a complex iron-containing protein possessing paramagnetic properties in the deoxygenated state. It should be noted that the correlation time, τ_c, for the dipole–dipole interaction in case of uncorrelated molecule and "spin" dynamics is determined by the expression:

$$\tau_c^{-1} = \tau_R^{-1} + T_{1e}^{-1},$$

where T_{1e} is the electron spin relaxation time. If the electron spin relaxation is fast, i.e. $\tau_R \gg T_{1e}$, the correlation time becomes $\tau_c \approx T_{1e}$. Thus, molecules are almost motionless on the time scale of electronic relaxation, and the magnetic moments of nuclei in that molecules experience the average value of the magnetic moment of a paramagnet, which is proportional to the difference in population δn of the states of unpaired electrons when oriented along and against the static magnetic field, and δn obeys the Curie law. Hence, the name of this relaxation mechanism is Curie-Spin

(CS) relaxation. This mechanism is the dipolar interaction of nuclear magnetic moments with the average, in time, electronic magnetic moment (spin) modulated by random molecular rotation but not by the electronic spin relaxation. Therefore, in this case, the correlation time will be τ_R, and the mechanism of the CS relaxation can contribute to the total relaxation rate. The resulting spin-lattice relaxation time, T_1, and spin-spin relaxation time, T_2, can be expressed via the contributions of the "classical" dipole-dipole relaxation (T_{1S}) and Curie-Spin relaxation ($T_{1\chi}$):

$$T_{1,2}^{-1} = T_{1,2S}^{-1} + T_{1,2\chi}^{-1}.$$

The Curie-Spin relaxation was well described for a long time [10, 11], however, just a few authors have reported experimental evidence (see, for example, [12]). In this work, we present assessments of the CS relaxation contribution to the T_1 and T_2 of water protons in the extreme stages of the aggregation process when the molecular rotation in the HbS aggregates becomes slow. This assessment is done relative to the dipole-dipole interaction. The estimations are based on the data of experimental determination of the rotational correlation times reported in the works [6, 8, 9].

2 Results and Discussion

Keeping constant the experimental conditions, the changes of rotational correlation time during the aggregation process were determined by three independent methods: NMR-relaxation at a constant frequency, EPR and NMRD [6, 8, 9]. The good agreement of data for these three methods is observed. The reported rotational correlation times in the induction and ending stages of HbS aggregation are $\left(\tau_R\right)_{\text{induction}} \approx 50$ ns and $\left(\tau_R\right)_{\text{ending}} \approx 100$ ns, respectively. The authors of the papers [10, 11] demonstrated the existence of the CS relaxation mechanism when the $\tau_R \gg T_{1e}$. Below we use the results of Vega and Fiat [11] who estimated the relation between contributions of the CS relaxation and well-known dipole–dipole mechanism, which was elegantly described by Solomon [13] (see also, for example, the monographs [14, 15]). Typical values of electron relaxation times T_{1e} for relevant paramagnetic metal ions in solutions are of the order of 10^{-12} s [11]. For example, in the works [16–18] the information is reported on electron relaxation times in the Fe-complexes: for Fe^{+3} $T_{1e} \approx 4.6 \cdot 10^{-12}$ s [16]; for Fe^{+2} $T_{1e} \approx 4.7 \cdot 10^{-12}$ s [17] and $T_{1e} < 4.7 \cdot 10^{-12}$ s [18]. From these values of the electron relaxation times and our data on the rotational correlation time, one can conclude that $\tau_R \gg T_{1e}$ at least in four orders of magnitude: $\left(\tau_R\right)_{\text{ending}} \approx 100\ \text{ns} \geq 10^4\ T_{1e}$. This means, that the presence of the CS relaxation is possible, and, definitely, its contribution is expected to be greater in high magnetic fields B_0 (or at high resonance frequency $\omega_0 = \gamma B_0$, γ is the nuclear gyromagnetic ratio) because the average value of the magnetic moment of a paramagnet is proportional to B_0.

Let the relaxation rates of the CS contribution are represented as $R_{1\chi} = 1/T_{1\chi}$, $R_{2\chi} = 1/T_{2\chi}$ and for the dipole–dipole contribution by $R_{1s} = 1/T_{1S}$, $R_{2s} = 1/T_{2S}$. According to ref. [11] one can evaluate the relative contribution of these two relaxation mechanisms by the following expressions:

$$\begin{aligned}\frac{R_{1\chi}}{R_{1s}} &= \frac{6}{7}\Delta\frac{\tau_2}{T_{1e}}, \text{if } \omega_0\tau_2 \ll 1\\ \frac{R_{1\chi}}{R_{1s}} &= \frac{6}{7}\frac{1}{\omega_0^2\tau_2^2}\Delta\frac{\tau_2}{T_{1e}}, \text{if } \omega_0\tau_2 \gg 1\end{aligned} \tag{1}$$

$$\begin{aligned}\frac{R_{2\chi}}{R_{2s}} &= \Delta\frac{\tau_2}{T_{1e}}, \text{if } \omega_0\tau_2 \ll 1\\ \frac{R_{2\chi}}{R_{2s}} &= \frac{4}{7}\Delta\frac{\tau_2}{T_{1e}}, \text{if } \omega_0\tau_2 \gg 1\end{aligned} \tag{2}$$

$$\begin{aligned}\Delta &= \frac{21}{20}\frac{g^2\mu_\beta^2}{(3kT)^2}S(S+1)B_0^2\\ &\text{and}\\ \tau_2 &= \frac{1}{6}\tau_R\end{aligned} \tag{3}$$

In Eq. (3) g is the Lande factor, μ_β is the Bohr magneton, S is the total electron spin of a paramagnetic component, k is the Boltzmann constant, T is the absolute temperature. It is obvious from Eqs. (1–3) that except the case of R_1, when $\omega_0\tau_2 \gg 1$, the relative contribution of the CS relaxation is proportional to the square of the magnetic field B_0 [11, 12].

First, the estimation of the value $\Delta_{Fe^{+2}}$ in Eq. (3) has been done assuming that $B_0 = 1.5$ T ($\nu_0 = 60$ MHz, the highest frequency in our experiments) and 308 K (close to the temperature in our experiments): $\Delta_{Fe^{+2}} \approx 0.25\cdots10^{-4}$. For $B_0 = 0.1$ T ($\nu_0 = 4.4$ MHz) the value $\Delta_{Fe^{+2}}$ is ca. 200 times smaller. Then, based on Eqs. (1–3) it is possible to evaluate the relative contribution of the CS relaxation mechanism. The results of this assessment for the induction and ending stages at three different frequencies are presented in Table 1 where the relatively significant contribution of CS relaxation is indicated in bold. Obviously, for the ^{1}H resonance at 4.4 MHz the values of $\omega_0\tau_R$ in the induction and ending stages are close to unity. The evaluations of the CS contributions for both conditions in Eqs. (1) and (2) gave very small

Table 1 The relative contribution of the Curie-Spin relaxation mechanism at different magnetic field strength

ν_0, MHz	$(\omega_0\tau_R)_{\text{induction}}$	$(\omega_0\tau_R)_{\text{ending}}$	$\left(\frac{R_{1\chi}}{R_{1s}}\right)_{\text{induction}}$	$\left(\frac{R_{2\chi}}{R_{2s}}\right)_{\text{induction}}$	$\left(\frac{R_{1\chi}}{R_{1s}}\right)_{\text{ending}}$	$\left(\frac{R_{2\chi}}{R_{2s}}\right)_{\text{ending}}$
4.4	1.4	2.8	$<10^{-6}$	$3{\cdot}10^{-3}$	$<10^{-5}$	$6{\cdot}10^{-3}$
20	6.26	12.6	$<10^{-5}$	<0.03	$<10^{-5}$	0.05
60	18.8	37.7	$<10^{-5}$	0.25*	10^{-5}	0.5*

*Curie-Spin relaxation mechanism may contribute up to 25% at induction stage and 50% at ending stage of HbS aggregation

values in all cases (Table 1), i.e., the CS mechanism at the low magnetic field, when $\omega_0\tau_R \approx 1$ ($\nu_0 = 4.4$ MHz), is non-effective that corresponds to the conclusions formulated in the work [6]. It is worth noting that the relative contribution of the CS mechanism to the spin–spin relaxation becomes more remarkable, if the aggregation process is present, especially with the increase of the magnetic field, i.e., when $\omega_0\tau_R \gg 1$ (in our experiments at 60 MHz). In this case, the relative contribution of the SC mechanism in the total relaxation rate can be up to 50% at the ending stage. The presence of the CS contribution allows us to explain the fact that, without taking it into account, the estimation of τ_R from the data at 60 MHz is somewhat higher [9] than at lower resonance frequencies (certainly, the molecular motion does not depend on the resonance frequency). Indeed, the Curie-Spin contribution to the spin–lattice relaxation rate R_1 is negligible even at 1.5 T. Therefore, the T_1/T_2 ratio may change not only because of the increase in τ_R (and, as a result, the manifestation of R_1 dispersion) but also due to the Curie-Spin relaxation contribution in R_2.

3 Conclusion

The presence of the Curie-Spin relaxation mechanism in the aggregation process of the hemoglobin S has been evaluated. The Curie-Spin contribution to the total spin-spin relaxation rate R_2 is about 25% and 50% at the magnetic field of 1.5 T (60 MHz for ^{1}H resonance) during the induction and ending stages of the aggregation process, respectively. At a lower magnetic field, this mechanism gives an insignificant contribution. The Curie-Spin contribution to the spin–lattice relaxation rate R_1 is negligible in the range of magnetic fields investigated. Therefore, the T_1/T_2 ratio may change not only because of the R_1 dispersion but also due to the Curie Spin relaxation contribution. The dependence of the T_1/T_2 ratio on the magnetic field magnitude during the aggregation processes will be discussed in an upcoming work for what higher field experiments on the NMR-relaxation in the presence of the HbS agglutination process need to be done. Definitely, at $B_0 > 1.5$T, the CS contribution to the spin-spin relaxation will increase even before the ending stage and its contribution might appear to the spin–lattice relaxation. In other words: if $T_1/T_2 \approx 1$, it means that the aggregation process in the blood sample has not started or is reversible, and if this ratio increases, then based on the dipole-dipole model it was previously assumed that the aggregation becomes significant (τ_R increases). It is shown here that for studies in high magnetic fields, it is first necessary to evaluate the contribution of Curie-Spin relaxation, otherwise, the conclusions may be erroneous. Since the CS and dipole-dipole relaxation have different dependences on the magnetic field and molecular mobility, it is advisable to raise the question of choosing the optimal magnetic field for the relaxation method of the sickle cell disease diagnostics.

In high field magnetic resonance imaging experiments (modern trend), the contribution of the Curie-Spin mechanism may also be expected. Thus, to obtain higher contrast in the molecular magnetic resonance imaging (mMRI) high-molecular structures conjugated with certain paramagnetic ions (for example, Pr^{+3}, Sm^{3+}, Fe^{2+}, Fe^{3+}) having short electron relaxation times are used. The mMRI experiments

are done in very high field machines and in these cases, the CS contribution should also be considerable.

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