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Deuterium Magnetic Relaxation Process during the Polymerization of Hemoglobin S

Y. Cabrales, **M. Lores**, and **Y. Machado**

Biophysics and Medical Physics Center, University of Oriente, Santiago de Cuba, Cuba

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Abstract. The deuterium and the proton spin–spin relaxation times are investigated at 4 MHz in partially deuterated samples of hemoglobin A and S at 36 °C during spontaneous deoxygenation. Deuterium relaxation shows a sigmoidal behavior describing the three characteristic phases of hemoglobin S (HbS) polymerization. The coincidence between the behaviors of deuterium and proton relaxation supports the dominant effect of agglutination on macromolecular mobility. Evidences of an increase of the internal electric field gradient and the appearance of a modified charge distribution inside the HbS solution, as a result of the polymerization process under spontaneous deoxygenation, were not found.

1 Introduction

As stated in previous papers [1, 2], the hemoglobin S (HbS) polymerization [3, 4] is the basic molecular process in sickle cell disease (SCD) [5]. On the other hand, the pathophysiology of this disease is related to the change in the shape and the physical properties of the red blood cell (RBC) [6]. For that reason a connection must exist between the macromolecular agglutination and the erythrocyte membrane: a modified HbS–membrane interaction. The interaction between deoxygenated HbS (deoxyHbS) and RBC membrane includes an electrostatic interaction [7], which could be changed if modified charge distributions appear inside the Hb solution as a result of polymerization. In that case the internal electric field gradients of the Hb solution could be modified.

Physical parameters, such as proton magnetic relaxation times [1], macromolecular mobility and microviscosity [2] inside the Hb solution, have been characterized showing significant changes and giving us new information about HbS polymerization under spontaneous deoxygenation conditions. Nevertheless, no evidence of modified charge distributions has been found.

The sensitivity of the deuteron (d) magnetic relaxation to changes of the electric field gradients around the nucleus [8], in contrast to the proton (p) mag-

netic relaxation [1], could give us new information about the change of the internal electric field gradients and the appearance of modified charge distributions inside the HbS solution under spontaneous deoxygenation conditions.

A rapid exchange is established between the free water molecules inside the solvent (F) and those bound to the Hb (B) in a deuterated Hb solution under physiological pH, concentration and temperature. Then, both the proton [9–13] and the deuteron [14–17] spin–spin relaxation measured (T_2) can be expressed as

$$\left(\frac{1}{T_2}\right)^{\mathrm{p}} = \left(\frac{P_{\mathrm{b}}}{T_{2\mathrm{b}}}\right)^{\mathrm{p}} + \left(\frac{P_{\mathrm{f}}}{T_{2\mathrm{f}}}\right)^{\mathrm{p}}, \quad \left(\frac{1}{T_2}\right)^{\mathrm{d}} = \left(\frac{P_{\mathrm{b}}}{T_{2\mathrm{b}}}\right)^{\mathrm{d}} + \left(\frac{P_{\mathrm{f}}}{T_{2\mathrm{f}}}\right)^{\mathrm{d}},$$

where P_{b} and P_{f} represent the bound and free water populations, respectively. As it was demonstrated in a previous paper [1], the quotient corresponding to the water bound to the Hb dominates the relaxation

$$\left(\frac{1}{T_2}\right)^{\mathrm{p}} = \left(\frac{P_{\mathrm{b}}}{T_{2\mathrm{b}}}\right)^{\mathrm{p}}, \quad \left(\frac{1}{T_2}\right)^{\mathrm{d}} = \left(\frac{P_{\mathrm{b}}}{T_{2\mathrm{b}}}\right)^{\mathrm{d}}.$$

Starting from the evaluation of the change in the internal electric field gradients, in this paper we analyze the appearance of modified charge distributions inside the HbS solution as a result of polymerization under spontaneous deoxygenation conditions. With this objective, we compare the temporal behavior of the proton and deuteron spin–spin relaxation times determined simultaneously in partially deuterated Hb samples.

2 Materials and Methods

Hb samples preparation. RBC samples were obtained from fresh venous whole blood donated by voluntary individuals and patients [1, 18]. To obtain the deuterated Hb solution, the RBC samples were incubated in a deuterated phosphate saline buffer (DPSB) solution for 2 h, washed three times with DPSB, lysed by freezing and centrifuged (2000 rpm, 10 min) to remove the stroma [1]. The nondeuterated hemoglobin A (HbA) and HbS solutions were obtained by conventional procedures [1, 2]. After that, twenty samples of 350 μl were prepared by mixing 80% and 20% of deuterated and nondeuterated Hb solutions, respectively, to be used in the nuclear magnetic resonance experiments.

Proton magnetic relaxation method. T_2 was determined on a Giromag 01 relaxometer (4 MHz) with the Hahn pulse sequence, at 36 °C, and with an error of less than 5%. The measurements were performed during 8 h of spontaneous deoxygenation.

Statistical analysis. To compare the values of the physical parameters analyzed, Student's test ($\alpha = 0.05$) was employed. Before that, the normal distribution of the data was demonstrated and Fisher's test ($\alpha = 0.05$) was used for the variances analysis.

3 Results

The typical proton and deuteron T_2 temporal behaviors in samples of partially deuterated HbA and HbS are shown in Fig. 1. A detailed description and explanation of the proton T_2 behavior have been published in ref. 1. In HbA, a constant temporal behavior of deuteron T_2 takes place. However, in HbS, a three-region sigmoidal temporal behavior is observed: regions I and III, where the effectiveness of relaxation is practically constant; and region II, in which T_2 decreases on average by 2.68 ± 0.35 times. The beginning of this decrease is defined by the delay time (t_d) [1, 2, 18].

A comparison made by taking into account different parameters characterizing the spin–spin magnetic relaxation along the HbS polymerization process, such as initial and final T_2 values (T_{2in}, T_{2fin}) and t_d, is presented in Table 1.

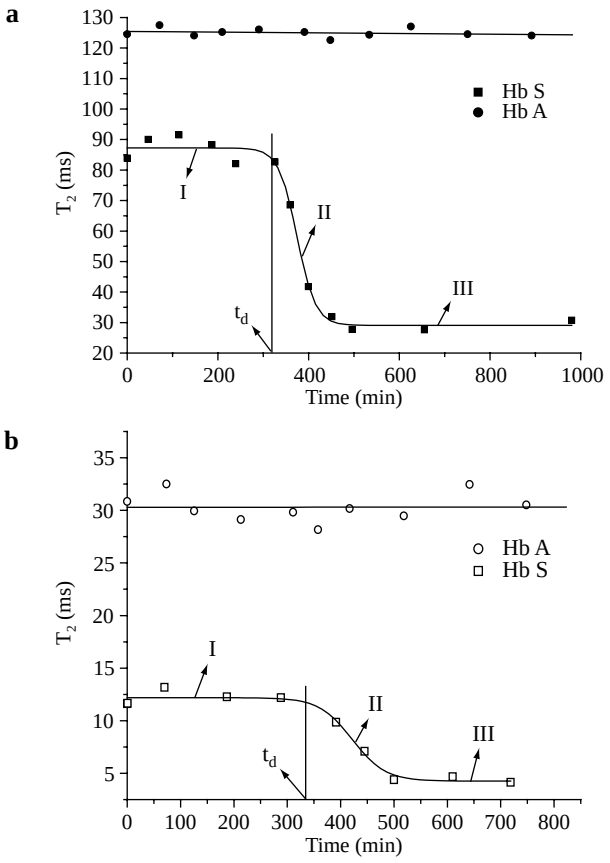

Fig. 1. Typical temporal behavior of T_2 in a mixture of HbS (80% deuterated, 20% nondeuterated) at 36 °C under spontaneous deoxygenation. **a** Proton T_2, **b** deuteron T_2. The continuous lines represent the sigmoidal and linear fittings of experimental data obtained for HbS and HbA samples, respectively. Regions I, II and III coincide with the three characteristic phases of the HbS polymerization process, and t_d with the irreversible beginning of macromolecular agglutination.

Table 1. Physical parameters obtained from the proton and the deuteron temporal behavior during HbS polymerization.

Parameter	Mean value ± standard error for:	
	Proton (^{1}H)	Deuterium (^{1}H)
T_{2in} (ms)	104±24	16±4
T_{2fin} (ms)	49±20	9±2
T_{2in}/T_{2fin}	2.86±0.42	2.68±0.35
t_d (min)	335±25	371±28

4 Discussion

The proton and deuteron T_2 values obtained for HbA and HbS samples agree with the theoretical considerations found in the literature [1, 14, 19].

In ref. 1, it was demonstrated that the proton T_2 depends on the rotational correlation time of the water bound to the protein (τ_C) and on the Larmor frequency (w) used in the experiment

$$\left(\frac{1}{T_{2b}}\right)^{p} = C_{p\text{-}p}\left(3\tau_C + \frac{5\tau_C}{1+w^2\tau_C^2} + \frac{4\tau_C}{1+4w^2\tau_C^2}\right), \tag{1}$$

where $C_{p\text{-}p}$ is the mean quadratic value of the proton dipolar interaction energy.

In contrast, the deuteron spin–spin magnetic relaxation includes magnetic (M) and electric (E) contributions

$$\left(\frac{1}{T_{2b}}\right)^{d} = \left(\frac{1}{T_{2b}}\right)^{dM} + \left(\frac{1}{T_{2b}}\right)^{dE}. \tag{2}$$

The magnetic contribution can be determined by Eq. (1), considering the intramolecular dipolar interaction of the deuteron inside the water bound to Hb. In a deuterated Hb solution, we can find three kinds of bound water depending on its deuterium content: nondeuterated (ppO), partially deuterated (pdO) and completely deuterated (ddO) water. The value of C for p–d interaction ($C_{p\text{-}d}$) will dominate the deuterium dipolar interaction, taking into consideration the proton and the deuteron magnetogyric ratio values (γ_p and γ_d) [19]. On the other hand, the electric component of the deuteron magnetic relaxation depends on the deuterium electric quadrupole moment (Q), the mobility of the water molecule (τ_C) and the electric field gradient (q) around the molecule [14]

$$\left(\frac{1}{T_{2b}}\right)^{dE} = C_Q\left(\frac{2\pi e^2 qQ}{h}\right)^2 \tau_C, \tag{3}$$

where the quadrupole coupling constant $2\pi e^2qQ$ also includes the fundamental constants e and h. The constant C_Q contains information about the deuteron spin quantum number and the molecular asymmetry.

By taking into consideration the above explanation, the dipolar contribution to magnetic relaxation in the case of the deuteron will be smaller than that in the case of the proton giving place to smaller proton T_2 values. Nevertheless, the deuteron T_2 values are less than the proton T_2 values during the whole experiment in HbS and HbA samples (Fig. 1). This result supports the dominant contribution of the electric component to deuterium magnetic relaxation (Eqs. (2) and (3)) and the presence of internal electric field gradients inside the Hb solution.

The sigmoidal behavior of deuteron T_2 for HbS samples shown in Fig. 1 can be explained considering the development of the polymerization process and the constant behavior of T_2 for HbA samples supports this conclusion. Regions I, II, and III coincide with the three characteristic phases of macromolecular agglutination [1, 2]: nucleation, irreversible polymerization and the formation of structural microdomains. During polymerization the HbS rotational correlation time (τ_R) increases twice on average [2], causing τ_C to increase [1] and T_2 to decrease (Eqs. (1)–(3)).

The deuteron relaxation is potentially determined by the bound water mobility (τ_C) and electric field gradient changes (Eqs. (1) and (3)), while the proton relaxation is affected only by τ_C variations (Eq. (1)); therefore, the drop in the deuteron value (Fig. 1b) should be greater than that of proton T_2 (Fig. 1a), considering the possibility of an increase of the internal electric field gradient as a result of the HbS polymerization. However, Table 1 shows no statistical difference between the proton and deuteron values of the physical parameters representing the irreversible beginning (t_d) and the magnitude (T_{2in}/T_{2fin}) of the HbS polymerization process. In other words, deuteron and proton T_2 describe the polymerization process of HbS under spontaneous deoxygenation conditions in the same way due to the presence of a common factor that determines their behavior: the increase of τ_R and τ_C.

The results obtained show no evidence of an increase of the internal electric field gradients inside the HbS solution as a result of polymerization. For that reason we cannot find experimental evidences related to the appearance of a modified charge distribution inside the HbS solution during the polymerization process under spontaneous deoxygenation.

5 Conclusions

The deuteron spin–spin magnetic relaxation time shows a three-region sigmoidal behavior during the polymerization of HbS under spontaneous deoxygenation conditions, thus describing the well-known three phases of the macromolecular agglutination: nucleation, irreversible polymerization and the formation of structural microdomains. That behavior is related to a decrease of Hb and water mobility as a result of macromolecular agglutination. Experimental evidences of an increase of the internal electric field gradient and the appearance of a modi-

fied charge distribution inside the HbS solution as a result of the polymerization process under spontaneous deoxygenation were not found.

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Authors' address: Yunior Cabrales, Biophysics and Medical Physics Center, University of Oriente, Santiago de Cuba, Patricio Lumumba 90 500, Cuba
E-mail: yunior@cbm.uo.edu.cu