Molecular Recognition of R- and T-States of Human Adult Hemoglobin by a Paramagnetic Gd(III) Complex by Means of the Measurement of Solvent Water Proton Relaxation Rate

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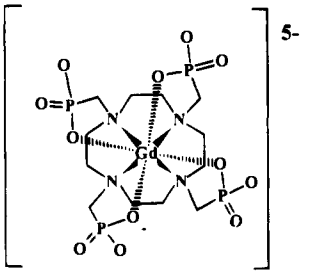
In recent years, Ln(III) complexes have been under intense scrutiny because of their potential applications as contrast agents for magnetic resonance imaging (MRI).[1-5] For these applications, Gd(III) is often the metal of choice because of its high effective magnetic moment (seven unpaired electrons) and its relatively long electronic relaxation time. The high coordination number (8−10) displayed by the Gd(III) ion allows it to be chelated by ligands of high denticity (to limit the toxicity of the lanthanide ion) while maintaining one or more water molecules directly coordinated to the paramagnetic center.[1,3] This directly coordinated water is usually in fast exchange (on the NMR time scale) with bulk water, thus allowing an overall relaxation enhancement of solvent water protons.[4-6] In MRI, an enhanced contrast is then determined by the increase of the water proton relaxation rate in the tissue, which depends on the concentration and the relaxivity of the paramagnetic complex.[1,2,7] Large relaxation enhancements are observed when a Gd complex is characterized by a long reorientational correlation time.[6] This property has been exploited in MRI applications both by covalently linking Gd complexes to macromolecular systems (such as albumin,[8] polylysine,[9] and dextran[10]) and by the inducement of noncovalent interactions between slowly tumbling substrates (such as micelles[11] and albumin[12]) and suitable functionalities on the surface of the ligand.

In addition to the search for increased relaxivities, further development of Gd complexes in biomedical investigations[13] lies chiefly in improving their specificity for discriminating given pathologies and in turn in improving their sensitivity to the biochemical environment.[14] In this respect, the binding of the [Gd(1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetrakis-(methylenephosphonate))]$^{5-}$ complex[15] (Gd-DOTP; see Chart 1) to human adult hemoglobin[16,17] (human Hb) has been investigated by measuring the water proton relaxation enhancement.[6]

Chart 1



Gd-DOTP

Human Hb is an allosteric protein in equilibrium between two conformations, the T- and the R-states, displaying low and high oxygen affinity, respectively. The quaternary T- and R-conformations are related by a rotation and translation of the $\alpha_1\beta_1$ dimer relative to the $\alpha_2\beta_2$ dimer upon ligand binding.[16-18] In red blood cells, 2,3-diphosphoglycerate (DPG) acts as a natural allosteric effector of human Hb by stabilizing the T-form. The negatively charged groups of DPG form salt bridges with the positively charged *N*-terminal nitrogen of Val1 and with side chains of His2, Lys82, and His143 of opposite β chains of human Hb.[16-18] Thus, we may expect that a negatively charged metal complex, such as Gd-DOTP, sets up an electrostatic interaction with human Hb in a way similar to that reported for DPG.[16]

The Gd-DOTP complex displays high thermodynamic stability, kinetic inertia, and stereochemical rigidity as the result of an octacoordinate bonding geometry made up of four nitrogen and four oxygen atoms[15] (see Chart 1). The coordination sphere of the lanthanide ion is completed by one water molecule which rapidly exchanges with the bulk solvent water.[5,15]

The ability of Gd complexes to enhance the water proton longitudinal relaxation rate R_1^{obsd} is well understood on the basis of the sum of three contributions (eq 1): $R_{1,p}^{is}$ is the contribution from the exchange of water molecules in the inner coordination sphere of the paramagnetic metal ion to the bulk water; $R_{1,p}^{os}$ is the contribution from water molecules freely diffusing in the proximity of the coordination sphere of the paramagnetic metal ion; and R_1^{dia} represents the solvent relaxation rate in the presence of an analogous diamagnetic compound.[6] The inner sphere contribution, $R_{1,p}^{is}$, is described by eq 2, where N is the molar concentration of the metal complex, q is the number of water molecules directly coordinated to the Gd center, τ_M is their mean residence lifetime, and T_{1M} is their longitudinal relaxation time. T_{1M} depends upon the strength of the dipolar interaction between the electronic and nuclear magnetic moments (D) and on its modulation rate ($f(\tau_c)$) (eq 3). τ_c is the correlation time, primarily determined by the shortest of the three values τ_R (the molecular reorientational correlation time),

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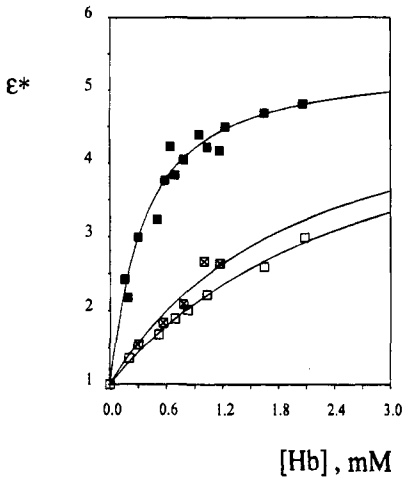

Figure 1. Dependence of the water proton relaxation enhancement of a 0.10 mM Gd-DOTP solution on the human deoxy-Hb (■), HbO_2 (□) and met-Hb (box with a "×" inside) concentrations, at pH = 7.2 and 25 °C. Data have been analyzed according to the proton relaxation enhancement (PRE)[6] procedure (see eq 6). The asymptotic value (ε^b = 5.7) of ε^* at [Hb] > $10K_D$ was independent of the human Hb derivative considered. Analysis of the data yields K_D values for Gd-DOTP binding to deoxy-Hb (3.0×10^{-4} M), HbO_2 (2.6×10^{-3} M), and met-Hb (1.9×10^{-3} M).

τ_S (the electronic relaxation time), and τ_M respectively, according to eq 4.[6]

$$R_1^{obsd} = R_{1,p}^{is} + R_{1,p}^{os} + R_1^{dia} \quad (1)$$

$$R_{1,p}^{is} = \frac{Nq}{55.56}\frac{1}{T_{1M} + \tau_M} \quad (2)$$

$$\frac{1}{T_{1M}} = D f(\tau_c) \quad (3)$$

$$\frac{1}{\tau_c} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{\tau_S} \quad (4)$$

At 25 °C, the relaxivity[7] of Gd-DOTP is 4.75 mM^{-1} s^{-1} and the inner-sphere contribution is determined primarily by the molecular reorientational time τ_R.[5] A relaxation enhancement is expected to occur upon the interaction of a low-molecular-weight paramagnetic complex with a slowly tumbling protein, as the longer reorientational time associated with this adduct is still the shortest among the three values in eq 4, and determines the dominant contribution to τ_c.[11] In fact, upon addition of human Hb to a solution of Gd-DOTP at pH = 7.2 and 25 °C (0.15 M PBS buffer), an increase of the solvent proton relaxation rate at 20 MHz is observed. On the other hand, no enhancement was detected in the presence of horse heart myoglobin and cytochrome *c*. These observations suggest that Gd-DOTP interacts with the Hb tetramer. The dependence of the water proton relaxation enhancement of a 0.10 mM Gd-DOTP solution on human Hb concentration (Figure 1) has been analyzed according to the proton relaxation enhancement (PRE)[19] procedure. The observed relaxation enhancement ($\varepsilon^* = R_{1,p}^*/R_{1,p}$) is the result of the higher relaxivity of the human Hb:Gd-DOTP adduct, $R_{1,p}^*$ and $R_{1,p}$ being the paramagnetic contributions to the observed R_1^{obsd} rates in solutions of the paramagnetic complex in the presence and in the absence of the macromolecule, respectively. The enhancement factor ε^* is then related to the molar fraction of the human Hb:Gd-DOTP adduct (α) (eq 5):

$$\varepsilon^* = \alpha(\varepsilon_b - 1) + 1 \quad (5)$$

where ε_b is the enhancement factor of the adduct. The molar fraction α can be expressed in terms of the dissociation equilibrium constant, K_D, by eq 6:

$$\alpha = \frac{\varepsilon^* - 1}{\varepsilon_b - 1} = \frac{[Hb]}{K_D + [Hb]} \quad (6)$$

Analysis of data given in Figure 1 yields K_D values for Gd-DOTP binding to deoxy-Hb (3.0×10^{-4} M), HbO_2 (2.6×10^{-3} M), and met-Hb (1.9×10^{-3} M) at pH = 7.2 and 25 °C. In analogy with the behavior earlier reported for DPG, these results indicate that the affinity of Gd-DOTP for deoxy-Hb (T-state) is higher by about 1 order of magnitude than that for HbO_2 and met-Hb (R-state).[16,18] The observed relaxation enhancements are readily quenched out as inositol hexaphosphate is added to the solutions containing Gd-DOTP and human Hb at pH = 7.2 and 25 °C. Inositol hexaphosphate is a well-known allosteric effector which strongly binds at the same cleft as does DPG in human Hb.[16] In accord with the principle of linked functions,[16,20] the affinity of O_2 for human Hb in the absence of Gd-DOTP (P_{50} = 7 mmHg; pH = 7.2, 20 °C) is higher by about 1 order of magnitude than that measured in the presence of the allosteric effector ($P_{50} = 6 \times 10^1$ mmHg; pH = 7.2, 20 °C) (0.15 M PBS buffer).

Present results indicate that the peculiar stereochemical and NMR properties of suitable lanthanide complexes may provide a useful tool for assessing changes in the protein structure. Furthermore, as the protein structure is dependent upon the biochemical environment, the observed relaxation behavior may be taken as a probe of the functional state of the investigated system. As far as MRI applications are concerned, the inclusion in red blood cells of small amounts of a paramagnetic complex whose relaxivity is dependent on T- or R-states of Hb may provide useful insights for novel functional imaging[21] experiments which report on the oxygen delivery and consumption. In the context of these applications, it would be necessary to consider also the effects on T_2 and T_2*.

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Supporting Information Available: Text describing materials and methods (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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