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THE MOLECULAR MECHANISM OF TEMPERATURE ENHANCEMENT OF PROTON MAGNETIC RELAXATION RATES IN METHAEMOPROTEIN SOLUTIONS

III The effect of sixth ligand in high-spin derivatives*

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The distinctions of the solvent-proton longitudinal magnetic relaxation (PMR) mechanisms between high-spin ferric aquo and fluoro complexes of some haemoproteins are discussed here. It becomes apparent that the "transition" from the exchange-limited PMR to the fast-exchange PMR mechanism upon addition of fluoride to some of the aquocomplexes is due to a more intense solvent-dynamics in the vicinity of the paramagnetic haem in the fluoromet derivatives. This can be rationalized by a conformational change induced by the fluoride ion, an effect not observed by X-ray analysis thus far. A possible mechanism of this change is indicated here.

1. Introduction

A considerable amount of experimental data has already been accumulated on the structures, allosteric mechanisms and conformational alterations of various met (=ferric) haemoproteins in solution using the solvent-proton (mainly longitudinal) magnetic relaxation (PMR) rates. In order to fully understand the actual relaxation mechanisms as well as to elucidate the structural parameters, both temperature and frequency dependence studies have been carried out. The latter may yield a more complete insight into the physical nature of relaxation phenomena [1]. Such measurements have only recently been carried out with methaemoproteins [2–5], while the experimentally simpler temperature dependence studies of these metalloproteins were begun as early as 1966 [6].

The temperature dependence of the paramagnetically induced PMR rates are usually interpreted by the Luz–Meiboom model of solvent-nuclei relaxation in solutions of paramagnetic ions [7]:

$$\left(\frac{1}{T_1}\right)_{\mathrm{f,pmg}} = \frac{nN}{N_p}\,\frac{1}{\tau_M + T_{1M}}\,. \qquad (1)$$

Here $(1/T_1)_{\mathrm{f,pmg}}$ stands for the paramagnetically induced PMR rates due to protons fluctuating between the haem-pocket and bulk solvent; n for the number of relaxing protons within the "sphere of closest approach" to the methaem-iron [8] which may or may not be identical with the first-coordination sphere of inorganic ions; N for the molarity of paramagnetic haems; N_p for the concentration of the relaxant nuclei in the solution; τ_M is the time the protons spend within the sphere of closest approach and T_{1M} is the relaxation time of a proton while at such a site near the metal ion.

An examination of eq. (1) shows that it is the ratio of τ_M and T_{1M} that determines the dominant PMR mechanism and that the reversal of the inequality $T_{1M} \ll \tau_M$ brings about the "transition" from the exchange-limited to the fast-exchange PMR (cf. fig. 6 in ref. [8]). It has previously been estimated that the fast-exchange condition pertains to the ratio $T_{1M}/\tau_M \geq 10$ [8].

The presently available data on the PMR mechanisms in the aqueous solutions of high-spin methaemoprotein derivatives obtained within the *same* temperature range between 0 and some 50°C show that the replacement of the water molecule at the sixth-coordination site in the aquomet complexes by the fluor-

* Previous papers in this series are refs. [9] and [21].

ide ion affects the PMR rates in two ways: (i) it either simply increases the PMR rates in aquomet derivatives exhibiting the fast-exchange relaxation [4,9,10] (group I in table 1), or (ii) it converts the exchange-limited PMR mechanism ($\tau_M \gg T_{1M}$, eq. (1)) to a fast-exchange PMR ($\tau_M \ll T_{1M}$, group II in table 1) [5,9,11].

The aim of this paper is to establish the nature of the fluoride effect upon the relaxation mechanism in methaemoprotein solutions. We demonstrate here that this is of steric origine. In the last section we discuss a possible mechanism of this effect.

2. Proton magnetic relaxation in the solutions of fluoromet derivatives

It can be seen from table 1 that in those cases when the fast-exchange dominates the PMR in both aquomet and fluoromet forms (group I), so that T_{1M} can be calculated for both from eq. (1) because τ_M may be neglected [4,9,12,13], the values of T_{1M} do not differ by more than a factor of two. For the solutions of aquomet derivatives, where the proton-fluctuation governs the PMR mechanism (group II, table 1), only an *upper* limit of T_{1M} can be calculated. Even these limits are not more than about six times larger than the corresponding T_{1M} in fluoromet solutions (for human adult haemoglobin). As mentioned above, in order to attain the fast-exchange PMR from the exchange-limited PMR, the inequality $T_{1M} \ll \tau_M$ must be reversed. An increase in T_{1M} and/or a diminution of τ_M may provide such an effect. And T_{1M} can, in turn, change due to an alteration of the correlation time, τ_C, for the interacting spins or/and their distance, r.

First we shall consider horseradish peroxidase, a representative of group I, because for the solutions of this enzyme all the experimental data needed for further discussion are known. It has been shown that in this case the observed increase of the paramagnetically induced PMR rates upon binding of fluoride is mainly due to an increase of the electron-spin relaxation time (i.e., of the correlation time, τ_C), and not to a smaller radius-vector joining the nuclear and electron spins in interaction [12]. In other words, the position(s) of the proton(s) within the sphere of closest approach with regard to haem-iron are not very different in the ferric aquo and fluoro horseradish peroxidase complexes, because in the case of the aquo-complex the water molecule at the sixth coordination site (recently proved to exist, ref. [14]) is ineffective for the PMR mechanism, a suggestion propounded earlier for haemoglobin and myoglobin [8] and later verified for other cases as well [4,9,10]. Although at present we lack data about the actual correlation times for both the aquomet and fluoromet derivatives of Chironomus haemoglobin, leghaemoglobin, Petromyzon marinus (fraction II), Lampetra fluviatilis

Table 1
The molar (per haem) paramagnetically induced solvent-proton longitudinal magnetic relaxation rates, R_{pmg}, in solutions of aquomet and fluoromet haemoprotein derivatives. The values of T_{1M} are calculated from eq. (1) with the assumptions described in the text. The data are taken at 30°C, except for pigeon haemoglobin at 50°C

Protein	Sixth ligand				
	H_2O		F^-		
	R_{pmg} $(Ms)^{-1}$	T_{1M} (μs)	R_{pmg} $(Ms)^{-1}$	T_{1M} (μs)	Reference
Group I					
Chironomus haemoglobin	780 [a]	23			9
Leghaemoglobin	880	20	1300	14	4
Horseradish peroxidase	500	36	1200	15	12
Petromyzon marinus (fraction II)	800	23			13
Lampetra fluviatilis	1120	16	1120	16	b)
Pigeon	1250	14			21
Group II					
Petromyzon marinus	900	< 20			13
Seal myoglobin	500	< 36	1700	11	11
Human haemoglobin A	250	< 70	1500	12	5
Human haemoglobin A + imositol hexaphosphate	660	< 27	1650	11	5

[a] Measured in a solution containing 35% ethane diol and expressed here per 111 moles of exchangeable solvent-protons.
[b] Unpublished data from this laboratory.

and pigeon haemoglobin (other fast-exchange PMR cases of group I), it is plausible to assume that conclusions concerning horseradish peroxidase [12] may also be applied to these haemoglobins. All the T_{1M}'s in table 1 are calculated from eq. (1) assuming that two protons (from a water molecule) relax equidistant from the methaem-iron both in aquo and fluoro derivatives. Although this is probably not strictly true, the values of T_{1M} calculated in such a way may be used for the sake of comparison, as the uncertainties involved in n (eq. (1)) could influence T_{1M} certainly much less than an order of magnitude. The important point is that when the substitution of ligands (F^- for H_2O) is *not* accompanied by a change of the dominant fast-exchange relaxation mechanism, the value of T_{1M} *shortens*, i.e., it changes towards the values favourable for the exchange-limited PMR and yet the mechanism of relaxation does not alter.

On the other hand, the values of T_{1M} in solutions of fluoromet derivatives of haemoproteins exhibiting exchange-limited PMR in their aquomet forms (group II) are almost identical to such values in fluoro derivatives of group I. Hence, it is plausible to assume that T_{1M} values in the aquomet forms (group II) which cannot be measured are also similar to the analogous values for the aquo derivatives of group I. This is corroborated by the calculable upper limits of the values of T_{1M} for aquomet complexes of the group II.

We have shown here that the relaxation times of the solvent-protons when "bound" near to the methaem-iron, T_{1M}, are very similar in aquomet and fluoromet solutions of haemoproteins belonging to either of the two groups. Therefore, it may be only the rate of proton-interchange, τ_M^{-1}, which determines which type of PMR mechanism will dominate in the same temperature range between 0 and 50°C. The differences between these rates in the solutions of various haemoproteins may only be due to specific accessibilities of the immediate neighbourhood of the haem. This conclusion is further supported by the observed increase of the rate of proton-interchange, τ_M^{-1}, upon binding of inositol hexaphosphate to human aquomet [15] and nitric oxide haemoglobin [16] as well as by similar effects observed in a solvent perturbation study of human high-spin methaemoglobin derivatives [17].

3. On the structural effects of fluoride inside the methaem-pocket

The question of the factors which determine the magnitude of τ_M^{-1} cannot be answered with certainty at present. Austin et al. have recently shown the complexity of the energetics of penetration of a ligand into the haem-crevice [18]. Here the propionyls of the haem and the hydration sheath at the mouthpiece of the haem-pocket as well as the distal histidine (E7) probably play an essential part. The importance of the latter seems to be supported by the comparison of the PMR behaviour in the solutions of the mammalian haemoglobins (exchange-limited PMR) and those lacking the distal histidine [9] or having an extremely open haem-crevice (fast-exchange PMR) [4]. The effect of fluoride, which may "convert" the exchange-limited PMR into the fast-exchange mechanism, may be rationalized by a rearrangement of the protein environment of the haem. Although an alteration which would allow solvent-protons to fluctuate more freely than in the aquomet complex and to fulfil the fast-exchange condition, $\tau_M \ll T_{1M}$, (while at the same time T_{1M} may even diminish) was not observed in the *crystalline* state [19], it is likely that in *solutions* of fluoromethaemoglobin and fluorometmyoglobin the conformation of the distal histidyl is altered in such a way by an interaction with the fluoride ion. An exception to this appears to have been found by Fabry and Eisenstadt [20] in both aquomet and fluoromet forms of monomeric haemoglobin from the radular muscle of marine gastropod Aplysia showing very small paramagnetically induced PMR rates in both forms. It is suggestive of a conformation of the haem-environment which hinders the fluctuation of solvent-nuclei in the vicinity of the iron. However, irrespective of the degree of contribution of any particular mechanism, the higher accessibility of the haem (on the PMR time scale) in the fluoromet complex of human haemoglobin as distinct from the aquomet complex has recently been directly demonstrated [21].

The present discussion demonstrates that the data on solvent dynamics in the immediate neighbourhood of the paramagnetic haem in the solutions of ferric high-spin methaemoprotein derivatives obtained by the PMR technique are more informative than the structural parameters which can be calculated using the well-known Solomon's equation [22].

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