ON THE SIXTH COORDINATION POSITION OF BEEF LIVER CATALASE

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1. Introduction

The identity of the ligand occupying the sixth coordination position of the catalase heme iron has been a matter of discussion in the past. There is a general belief (for a review see [1]) that this ligand is a water molecule [2,3], although this was never directly proved. In this paper we present nmr evidence for the presence of a fast exchanging water molecule in the first coordination sphere of ferric catalase. Nmr spectroscopy has the potential of counting the hydration number of a paramagnetic metal ion bound to a biological macromolecule [4]. If the various parameters which are responsible for the proton relaxation mechanisms can be precisely evaluated, it is possible to decide whether OH^- or H_2O groups are coordinated to the metal ion [5]. This technique was previously applied in the determination of the hydration number of various high spin ferric hemoproteins [6–8]. In this work we have examined the longitudinal proton relaxation rate in solutions of beef liver catalase. It was concluded that the iron in catalase is coordinated by a water molecule.

2. Materials and methods

Beef liver catalase was obtained from Boehringer Mannhein GmbH. 3 ml of the enzyme suspension (1 g/50 ml) were centrifuged and the enzyme was dissolved in 1 ml of 10% NaCl.

At the concentrations required for the nmr experiment, below pH 6.0 and above pH 9.0 the enzyme was partly precipitated. Heme concentrations were determined from measurements of the Soret band intensity at 405 nm using a molar absorptivity of 1.485×10^5 cm^{-1} M^{-1} per heme [9]. Longitudinal relaxation rates at 100 MHz were measured using a spin echo attachment to a Varian HA-100 nmr spectrometer [10], by the 180°–90° null method.

3. Results

Fig.1 presents the pH dependence of the net longitudinal relaxation rate ($1/T_{1p}$) measured at 100 MHz, of water protons in aqueous solutions of beef liver catalase. The catalase–cyanide complex has a low-spin ferric iron. Since the longitudinal electronic relaxation time of the low spin ferric ion is extremely short, the T_1^{-1} value in solutions of

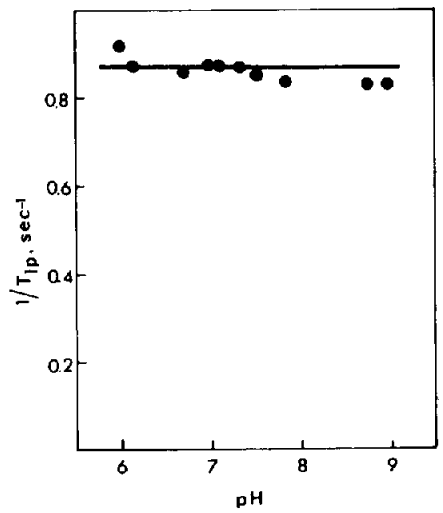

Fig.1. The pH dependence of the net longitudinal relaxation rate at 100 MHz, 30°, for 3.15×10^{-4} M heme of beef liver catalase, in 0.1 M phosphate buffer.

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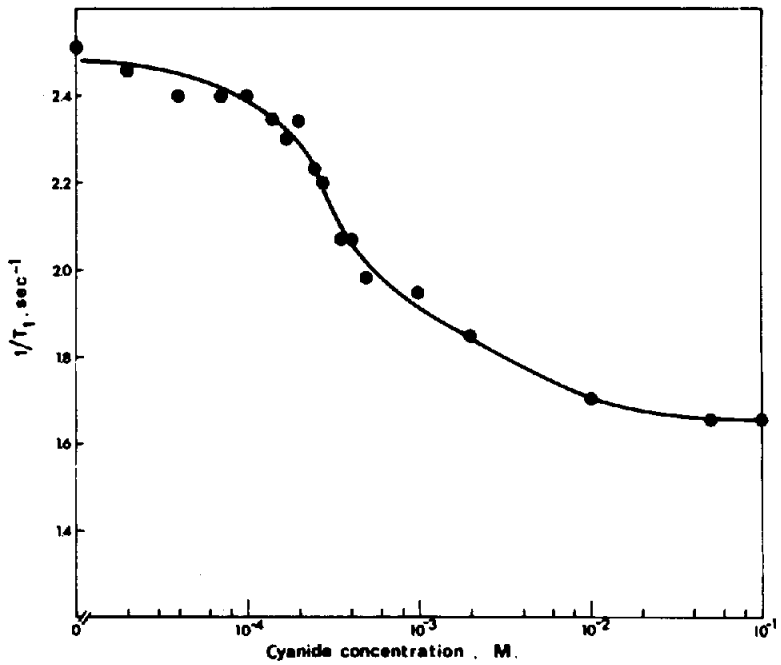

Fig. 2. The relaxation plotted against cyanide concentration. The effect was studied at 100 MHz, 30°, in 0.1 M phosphate buffer pH 7.0.

CN–catalase may be considered as being very close to the diamagnetic contribution of the catalase protein to the relaxation. Hence, the difference between the relaxation rates of water in catalase and its cyanide complex can be taken as the paramagnetic contribution of the high spin ferric ion to the relaxation.

Fig. 2 presents the titration of the relaxation effect by CN^-. The paramagnetic contribution to the longitudinal relaxation rate $1/T_{1p}$ of a ligand nucleus is a weighted average for the coordinated and uncoordinated ligand and depends on the residence time, τ_M, in the coordination sphere:

$$\frac{1}{T_{1p}} = \frac{N \cdot q}{55.6} \cdot \frac{1}{\tau_M + T_{1M}} \qquad (1)$$

In our case, N is the concentration of the heme, q its hydration number; T_{1M} the longitudinal relaxation time of water protons in the first coordination sphere. In a series of experiments it was established that T_{1p} is temperature and frequency dependent (A. Lanir and A. Schejter, unpublished results). The dependencies indicate that the condition of fast exchange holds for the present system. T_{1M} can be affected both by dipolar interaction and by scalar coupling to the electron magnetic moment of the metal ion. However, for water molecules bound to high spin Fe^{3+} in hemoproteins it was found that the scalar coupling term is much smaller than the dipolar term [7]. Thus:

$$\frac{1}{T_{1M}} = \frac{K}{r^6} \left(\frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{7\tau_c}{1 + \omega_s^2 \tau_c^2} \right) \qquad (2)$$

In equation (2), r is the Fe^{3+}-proton separation, ω_I and ω_s are the precession frequencies of the proton spin moment and the high spin Fe^{3+} electron spin moment, respectively. τ_c is the correlation time. In our system τ_c is determined by the electron spin correlation time of the high spin Fe^{3+} which, for catalase was determined from the frequency dependence of the relaxation rate below 25 MHz to be $(7 \pm 1) \times 10^{-11}$ sec. K is the product of physical constants determined by the electron spin of Fe^{3+} ($s = 5/2$) and the magnetogyric ratio of the proton.

The numerical value of K is 2.87×10^{-31}. Taking $r = 2.84$ Å [7] and $\tau_s = 7 \times 10^{-9}$ sec the number of water molecules bound per heme iron in catalase is calculated to be 1.3 ± 0.2. Considering the small second sphere contribution to the relaxation it is clear that the heme iron in catalase is coordinated to a water molecule. This water molecule could be displaced by inhibitors like formate or acetate, both of which do not change the spin state of catalase, and thus the displacement of the water molecule can be directly demonstrated.

4. Discussion

Catalases and peroxidases are similar in a number of properties. Both enzymes are high spin ferric heme proteins, and both can be activated by hydrogen and alkyl peroxides. They differ, however, in the reactivity of their higher oxidation states towards hydrogen donors: while the activated state of catalase is extremely reactive with two electron donors (H_2O_2, alcohols), and poorly reactive with one electron donor (phenols, aromatic amines), the converse is true for the peroxidases [11]. The similarity in the spectral and magnetic properties of the catalases and peroxidases has been usually taken as indication of analogous structural features in the environment of their prosthetic groups. The results reported in this paper for beef liver catalase, and those given elsewhere for horseradish peroxidase [8], indicate a major structural difference between the two enzymes. In catalase, one of the iron ligands is a water molecule; in peroxidase, there is no iron-coordinated water: either it is penta-coordinated, or the sixth ligand is a protein residue [8].

The immediate implication of these findings is that the mechansims of ligand reactions with the two enzymes must be different. Thus, for example, the reaction of peroxidase with ionic ligands such as azide and cyanide probably proceed via an S_{N_1} mechanism, as in the case of cytochrome c [12], while this is not all necessary with catalase. Furthermore, the absence of water bound to the peroxidase iron suggested that oxidation by H_2O_2 could not be preceded by direct binding of the oxidant to the iron [8]; this argument is obviously not valid in the case of catalase.

A major argument against the presence of iron bound water in the two enzymes was that complex formation was accompanied by proton binding, and it was suggested that the proton became bound to a protein residue acting as sixth ligand in the native enzyme [13]. This argument may still be valid for peroxidase, but it is no longer tenable for catalase.

Finally, if the easy accessibility of the catalase iron to the solvent persists in the higher oxidation state of the enzyme, it is quite possible that the two electron donors bind the catalase iron directly. This may explain the very high rate of the reaction of activated catalase with H_2O_2, and the comparably low rate of the corresponding reaction of peroxidase.

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