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THE INTERACTION OF MYELOPEROXIDASE WITH LIGANDS AS STUDIED BY EPR

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Summary

1. The reaction of myeloperoxidase with fluoride, chloride and azide has been studied by EPR.

2. Fluoride decreases the rhombicity of the high-spin heme signal of myeloperoxidase and the nuclear spin of the fluoride atom induces a splitting in $g_{//}$ of 35 G. This observation demonstrates that fluoride binds as an axial ligand to the heme iron of the enzyme.

3. Addition of chloride to the fluoride-treated enzyme increases the rhombicity of the high-spin heme signal and brings about a disappearance of the splitting at $g_{//}$. The addition of azide to the fluoride-treated enzyme changes the spin state of the heme iron from a high- to a low-spin state (g_x = 2.68, g_y = 2.22 and g_z = 1.80).

4. Upon addition of chloride or fluoride to low-spin azido-myeloperoxidase this compound is converted into the high-spin chlorido- or fluorido-myeloperoxidase. These observations demonstrate that these ligands compete for a binding site at or close to the heme iron of myeloperoxidase.

Introduction

Human granulocytes contain large amounts of myeloperoxidase [1], a ferric hemoprotein which is involved in the antimicrobial activity of these white blood cells [2]. The bactericidal action of myeloperoxidase is probably due to its property to catalyze the oxidation of Cl^- by H_2O_2 to HOCl [3—5]. The latter compound reacts with bacterial cell walls and amino acids, resulting in cell death [5—7]. The effect of chloride on the absorbance spectrum of the enzyme has been studied in some detail [4,8]. These studies have indicated that

the Cl^-, which is in fact a substrate of myeloperoxidase, combines with the prosthetic group of the enzyme. Whether the halide is bound directly to the heme iron of the enzyme is not known. For chloroperoxidase [9] from the fungus *Caldariomyces fumago*, which like myeloperoxidase is able to catalyze chlorination reactions, it has been suggested that the Cl^- does not bind as an axial ligand to the heme iron. In the present paper new high- and low-spin EPR signals of various myeloperoxidase compounds are reported and it is shown that chloride, fluoride and azide compete for a common binding site on myeloperoxidase at or near the heme. The results have partly been reported in a preliminary form [10].

Materials and Methods

Myeloperoxidase was prepared from human leucocytes as described previously [11] except that the final gel filtration step was omitted. The purity index (A_{430nm}/A_{280nm}) of the preparations used was 0.72—0.81. The concentration was calculated with an absorption coefficient of 89 $mM^{-1} \cdot cm^{-1}$ at 430 nm [12,13].

Chemicals were analar grade, mainly obtained from British Drug Houses. EPR spectra were obtained with a Varian E-9 EPR spectrometer. Temperature, magnetic field and microwave frequency were measured as described previously [14].

Results

Fig. 1A shows the EPR spectrum of myeloperoxidase with resonance lines near $g = 6$ and $g = 2$ characteristic for high-spin hemoproteins. A major high-spin heme signal is present with $g_x = 6.82$ and $g_y = 5.05$. In addition to this signal a minor signal is discernible with a slightly lower rhombicity ($g_x = 6.6$, $g_y = 5.3$). In the presence of fluoride (Fig. 1B) the rhombicity of the high-spin heme signals decreases and a single high-spin species is formed with $g_x = 6.43$ and $g_y = 5.48$ which exhibits a clear splitting of 35 G at $g_z = 1.99$. The well-resolved hyperfine splitting in g_z (Fig. 2B) caused by the nuclear spin of the fluoride atom, demonstrates that fluoride coordinates directly as an axial ligand to the heme iron atom of myeloperoxidase.

Fig. 3 illustrates that addition of chloride to the fluoride-treated myeloperoxidase increases the rhombicity of the heme signal and a spectrum similar to that observed before addition of fluoride is obtained with $g_x = 6.82$ and $g_y = 5.05$. Similarly, the addition of excess fluoride (0.69 M) to chloride-incubated (0.2 M) myeloperoxidase again changes the EPR spectrum to that of myeloperoxidase fluoride. These observations demonstrate that both chloride and fluoride compete for a binding site on myeloperoxidase and that the affinity of the two ligands is of the same order of magnitude.

Since azide binds as an axial ligand to the heme iron in several hemoproteins [15], the competition between this ligand and chloride and fluoride was also studied. Fig. 4 demonstrates that azide converts the high-spin heme signal of myeloperoxidase to that of a low-spin compound with $g_x = 2.68$, $g_y = 2.22$ and $g_z = 1.80$. It is interesting to note that the minor species with g values 6.6 and

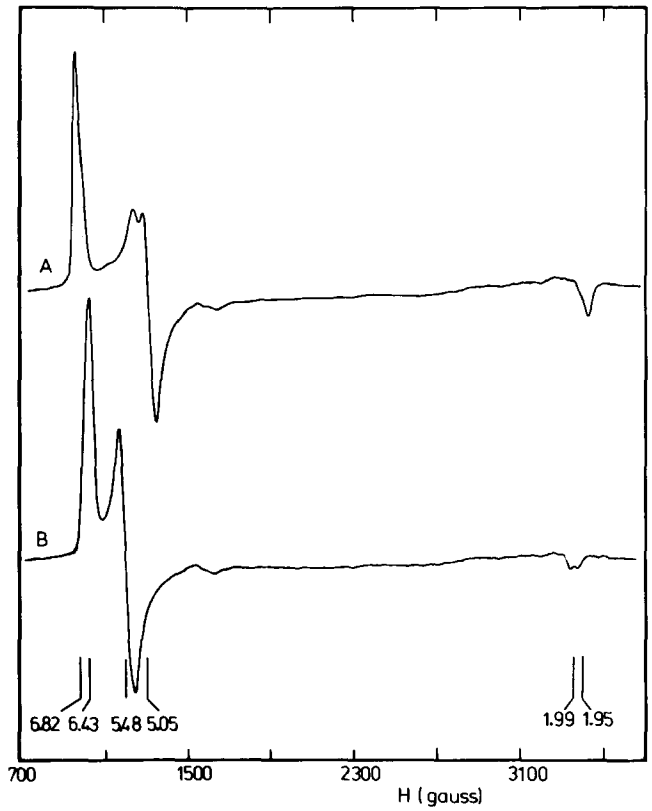

Fig. 1. Effect of fluoride on the EPR spectrum of myeloperoxidase. (A) 75 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8). (B) After addition of 100 mM KF. Incubation for 10 min at room temperature. Conditions of EPR spectroscopy: frequency, 9.308 GHz; microwave power, 36 mW; modulation amplitude, 16 G; temperature, 9.5 K. The gain in (A) was 1.3-fold higher than that in (B).

5.3 appears to be less reactive towards this ligand since it disappears only at a high (1—5 mM) azide concentration. The addition of chloride to azido-myeloperoxidase readily converts the low-spin compound into the high-spin species with $g_x = 6.82$ and $g_y = 5.05$. Similar experiments carried out with fluoride

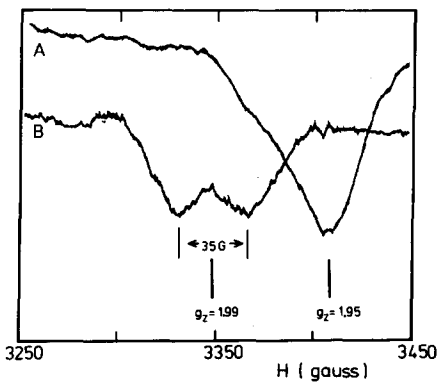

Fig. 2. Effect of fluoride on the g_z line of myeloperoxidase. Conditions as in Fig. 1A and B.

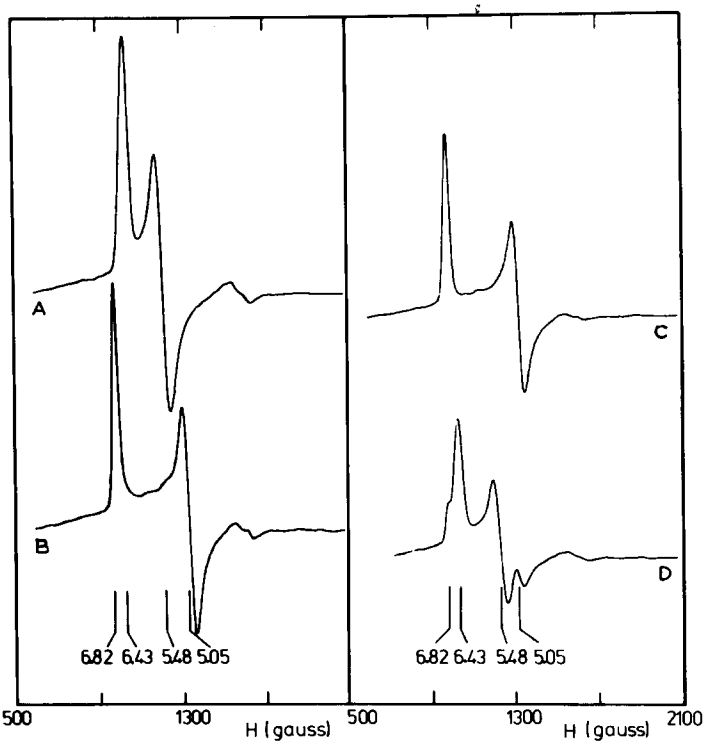

Fig. 3. Competition between chloride and fluoride for myeloperoxidase. (A) 75 μM myeloperoxidase in 0.1 M KF. Incubation for 2 min at room temperature. (B) After addition of 0.83 M NaCl to (A). Incubation at room temperature for 5 min. (C) 75 μM myeloperoxidase in 0.1 M potassium phosphate (pH 6.8) and 0.2 M NaCl. Incubation for 2 min at room temperature. (D) After addition of 0.69 M KF to (C). Incubation for 5 min at room temperature. Conditions of EPR spectroscopy as in Fig. 1, except for the temperature, which was 15 K.

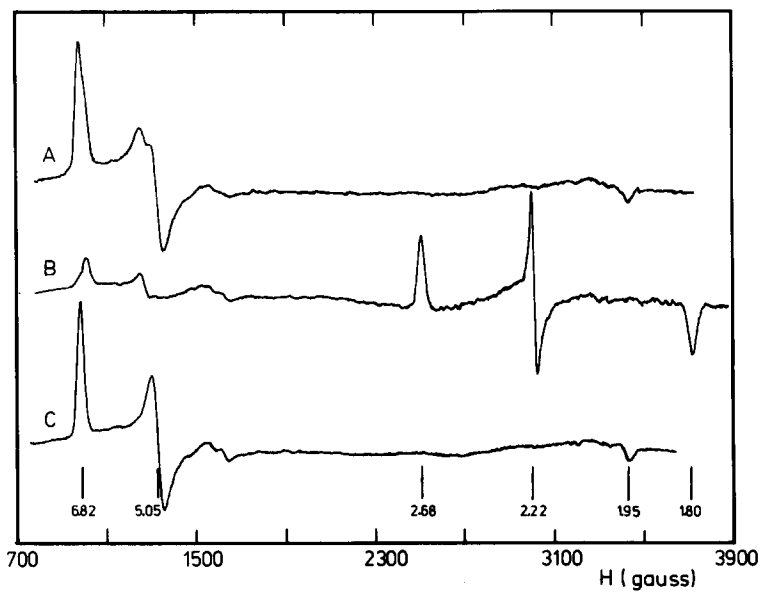

Fig. 4. Competition between azide and chloride for myeloperoxidase. (A) 75 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8). (B) After addition of 0.5 mM sodium azide to (A). Incubation for 5 min at room temperaure. (C) After addition of 0.83 M NaCl to (B). Incubation for 5 min at room temperature. Conditions of EPR spectroscopy: frequency, 9.309 GHz; microwave power, 4 mW; modulation amplitude, 10 G; temperature, 14 K.

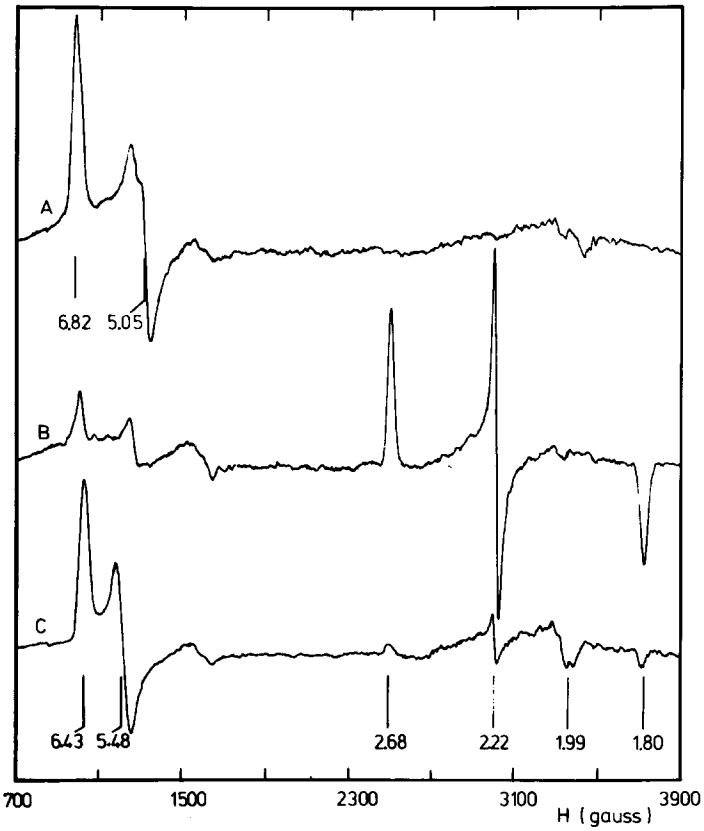

Fig. 5. Competition between azide and fluoride for myeloperoxidase. (A) 75 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8). (B) After addition of 0.5 mM sodium azide to (A). Incubation for 10 min at room temperature. (C) After addition of 0.1 M KF to (B). Incubation for 10 min at room temperature. Conditions of EPR spectroscopy as in Fig. 4, except for the microwave power which was 2 mW. The gain in (C) was half that in (A) and (B).

instead of chloride (Fig. 5) demonstrate that these ligands bind at a site which may be at or very close to the heme iron of myeloperoxidase.

Discussion

The results presented in this paper show that azide and the halide anions, chloride and fluoride, form reversible complexes with myeloperoxidase and compete for a binding site on the enzyme. For fluoride and azide there is little doubt that the binding site is the sixth heme ligand position. Therefore it is likely that also chloride coordinates as an axial heme ligand. Similarly, in intestine peroxidase [16] cyanide and chloride appear to react at the same enzyme site. It is, of course, also possible that there is a different binding site for chloride which has a strong anticooperative interaction with the other ligand binding site. In chloroperoxidase, an enzyme which also catalyzes chlorination reactions, NMR [9] and Mössbauer studies [17] have clearly shown that chloride does not coordinate to the iron atom.

The EPR spectra of isolated myeloperoxidase show a superposition of two high-spin heme signals with a small difference in rhombic distortion of the heme group. The relative concentration of the two species appears to depend on the experimental conditions like pH and buffer medium used as has been observed previously for other high-spin hemoproteins [18,19]. Interconversion between the high-spin species is possible since in the presence of chloride the minor signal disappears and the major signal with $g_x = 6.82$ and $g_y = 5.05$ intensifies. This shows that the EPR spectrum of isolated myeloperoxidase is very similar to that of the enzyme-chloride compound.

In some myeloperoxidase preparations a low-spin heme species [11] is present ($g_x = 2.56$, $g_y = 2.31$ and $g_z = 1.81$), whereas in others (Figs. 1—5) it is absent. If present, this species is converted into the high-spin state upon addition of cloride or fluoride (not shown). This may suggest that, like in other peroxidases [20,21], an equilibrium exists between a high- and low-spin state of myeloperoxidase although it is not known which factors determine the equilibrium position between the high- and low-spin structure.

There is very little information available about the active site of myeloperoxidase. Even the structure of the heme prosthetic group has not been elucidated completely [22] although it is distinctly different from that in other peroxidases. From the g values and ligand-field parameters of low-spin hemoproteins it is possible to get some information about the nature of the ligands of the heme iron [15]. These ligand-field parameters can be calculated easily with the method introduced by Taylor [23]. Although it has been suggested previously [24] that the myeloperoxidase contains a chlorin type of heme, a recent study [22] indicates that the structure of the heme is a porphyrin with a formyl substituent as in heme *a*. Hence for comparison of the parameters in the literature, the axis system as used by Blumberg and Peisach [15] for normal hemes, was used in the calculations. For azido-myeloperoxidase, parameters are obtained which lie near the boundary of type 0 and type H in the socalled truth diagrams [15]. Similarly, the low-spin form of myeloperoxidase at high pH, which is presumably the hydroxide derivative, has parameters which lie close to those of the hydroxide forms of the horseradish and Japanese radish peroxidase. Furthermore, the cyanide derivative of myeloperoxidase ($g_x = 2.83$, $g_y = 2.25$ and $g_z = 1.64$) has parameters which correspond to those of horse erythrocyte catalase cyanide [15]. Thus, the iron-ligand structure in myeloperoxidase is probably not very different from that in catalase and other peroxidases.

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