ELECTRON PARAMAGNETIC RESONANCE SPECTRA OF MYELOPEROXIDASE IN POLYMORPHONUCLEAR LEUKOCYTES

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1. Introduction

Myeloperoxidase (EC 1.11.1.7) is found in remarkably high concentrations in leukocytes [1] and is thought to have a role in the bacteriacidal capabilities of phagocytosing cells by catalysing the halogenation, proteolysis and decarboxylation of bacterial cell walls in the presence of halide and hydrogen peroxide [2,3]. The electron paramagnetic resonance (EPR) spectrum of myeloperoxidase, first reported [4], is that of high-spin ferric heme with rhombic distortion, each of the two subunits [5] apparently giving identical signals. If the heme moiety has axial symmetry, the x and y components of the g-tensor, in the plane of the heme, will be equal ($g \approx 6$) while the third component will be $g \approx 2$. With rhombic distortion, g_x and g_y deviate from g 6, but g_z remains near g 2. Examination at low temperatures reveals that there are multiple rhombic environments for the heme suggesting several conformations of the enzyme. In itself, this is not unusual, since numerous reports of this phenomenon have been made with other heme proteins, such as catalase [6–9], horseradish peroxidase [10], cytochrome c peroxidase [11], hemoglobin [7,12–14] and denatured myoglobin [15,16]. However, none of these changes have been correlated with a physiological function.

Recent reports of EPR studies on human granulocytes [17] and guinea pig polymorphonuclear leukocytes (PMNL) [18] have suggested a correlation of the appearance of an axial signal at g 6.0 with the physiological process of phagocytosis. Both reports have attributed the axial signal to myeloperoxidase, although this signal cannot be induced in the isolated enzyme when physiological parameters such as pH, H_2O_2 or halide are varied [18].

In this report, we show multiple rhombic conformations for the heme environment of purified myeloperoxidase and PMNL. We agree that axial symmetry cannot be induced in purified myeloperoxidase without denaturation. However, we do not find any significant changes in the EPR spectra of PMNL during phagocytosis. Since an intense axial signal is associated with both serum and whole blood, we attribute the occurrence of this signal in PMNL suspensions to contamination by either free or erythrocyte-bound methemoglobin.

2. Materials and methods

Purified canine myeloperoxidase was obtained through the courtesy of Dr J. E. Harrison, Papanicolaou Cancer Res. Inst., Miami, FL. Bovine liver catalase and polystyrene latex particles were purchased from Sigma Chemical (St Louis, MO) and DOW Chemical (Midland, MI), respectively. Both myristic acid and guinea pig serum were purchased from Grand Island Biological (Grand Island, NY). Guinea pig PNML were isolated as described [19] and suspended in Krebs Ringer phosphate solution (KRPS) at pH 7.4. Quantities of PMNL were determined with a hemocytometer, and differential cell counts performed on Wright stained smears showed homogeneities of greater than 98% PMNL. Leukocytes from other species were obtained from whole blood

after erythrocyte sedimentation [20] and after repetitive hypotonic lysis of the erythrocytes with 0.2% (w/v) NaCl.

Bacillus cereus, Bacillus mycoides and *Escherichia coli* were grown at 37°C in 1% (w/v) trypticase soy broth, whereas *Photobacterium phosphoreum* was grown under similar conditions but in McElroy's medium [21]. Bacteria were heat-killed at 90°C for 15 min and resuspended in KRPS containing 10% guinea pig serum. Phagocytosis was induced in PMNL suspensions by adding aliquots of either bacteria, polystyrene latex particles, or a myristic acid sonicate. Phagocytosing cells were incubated in open test tubes at 37°C and were shaken continuously to avoid anaerobiosis. Immediately after incubation, PMNL were centrifuged at 3000 × g for 1 min and transferred to cold (4°C) EPR tubes. EPR tubes containing approx. 1.0×10^8 PMNL were subsequently immersed and stored in liquid nitrogen. Aliquots of stimulant were in each case sufficient to increase PMNL respiration as determined by closed system polarography [21].

EPR spectra were taken on a Varian E-4 EPR spectrometer described [22].

3. Results and discussion

Low field EPR signals from dog myeloperoxidase, beef liver catalase, guinea pig PMNL and guinea pig serum are shown in fig.1. The myeloperoxidase (fig.1A) shows a major rhombic component with g_x 6.77, g_y 5.02 and a minor rhombic component with g_x 6.33, g_y 5.52. The signal near g 4.2 is associated with ferric iron in a non-heme environment. Similar pairs of rhombic signals are seen in the catalase (fig.1B) and resting guinea pig PMNL (fig.1C) spectra. As will be discussed later, the particular values of g_x and g_y are both enzyme and species dependent. The third g-tensor component (g_z) present near g 2 is not shown; it is not sensitive to rhombic distortion, and is much weaker and is located in a region where other signals are predominant [7].

Guinea pig serum (fig.1D) shows a strong axial signal at $g \sim 6.0$ which can be attributed to methemoglobin [7], but this is clearly absent from the resting guinea pig PMNL (fig.1C). The axial signal becomes noticeable if the PMNL are stimulated with polystyrene latex particles in 10% guinea pig serum

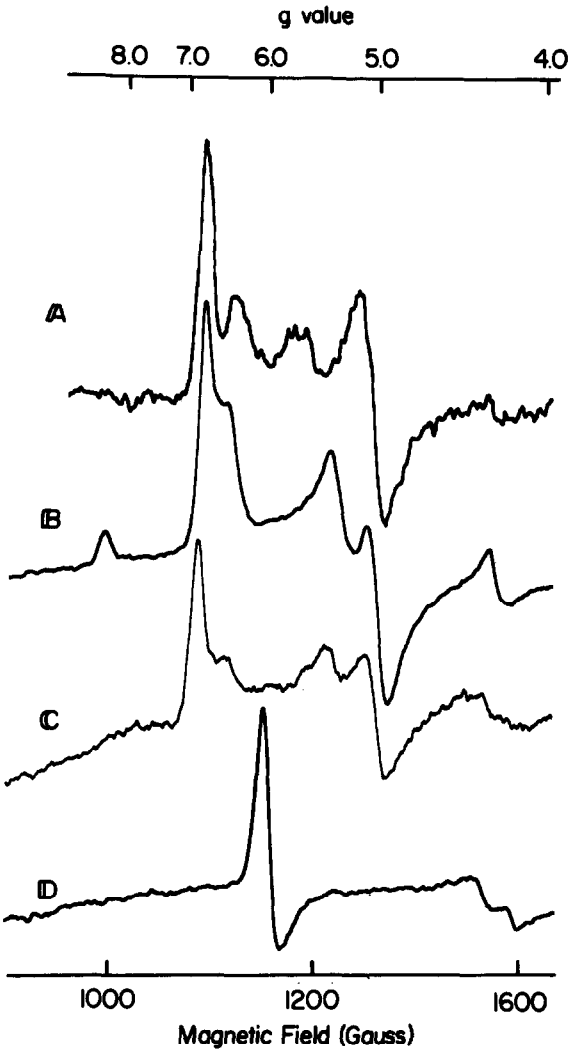

Fig.1. Low field EPR spectra of (A) purified dog myeloperoxidase, (B) purified beef liver catalase, (C) guinea pig polymorphonuclear leukocytes, and (D) serum from guinea pig. Measurements were made at 6–10°K.

(fig.2) or with a variety of bacteria in 10% guinea pig serum (fig.3). However the intensity of the axial signal did not significantly change in the following 60 min. The axial signal was not seen if the PMNL were treated with latex particles alone, although respiration was markedly stimulated by all these additions (data not shown). It thus seems that the axial $g \sim 6.0$ signal seen in small amounts in fig.2 and 3, and reported [17,18] is an artefact probably arising

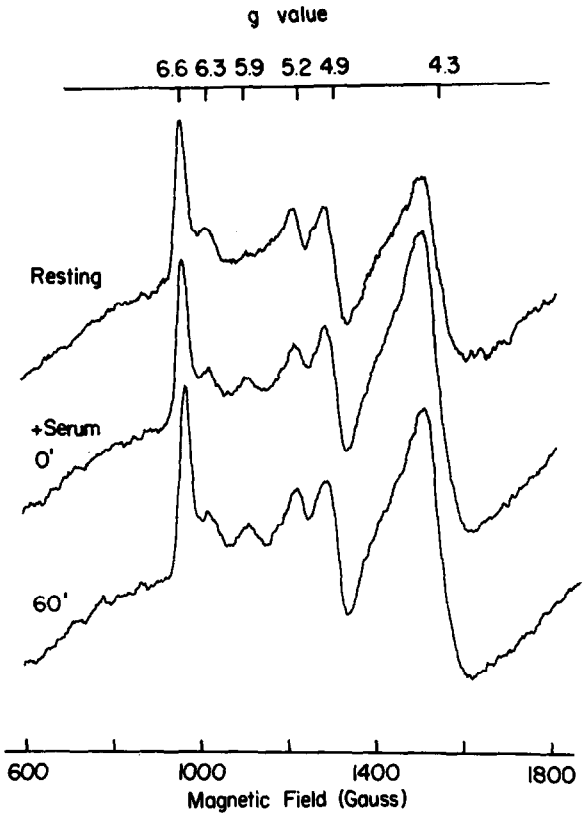

Fig.2. Low field EPR spectra of resting and phagocytosing guinea pig PMNL. Phagocytosing cells were incubated with polystyrene latex particles plus 10% guinea pig serum for the indicated time at 37°C.

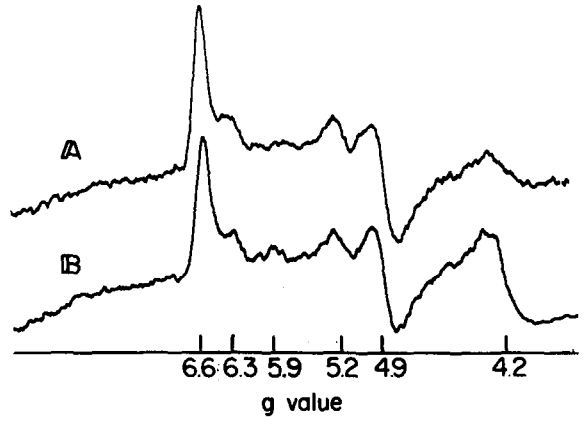

Fig.3. Low field EPR spectra of (A) resting and (B) phagocytosing guinea pig PMNL. Phagocytosing cells were stimulated with opsinized *B. mycoides* for 60 min at 37°C. Similar results were seen with *B. cereus*, *E. coli* and *P. phosphoreum*.

Table 1
Effect of erythrocyte contamination on EPR spectra of polymorphonuclear leukocytes

Cellular ratio (erythrocytes/PMNL)	Signal intensities ratio (g 6.0/g 6.7)
0	0
0.2	0.23
1.0	1.9
5.0	16
whole blood	all g 6.0

from methemoglobin contamination in the guinea pig serum. Indeed weak axial signals in preparations of resting PMNL could be eliminated or enhanced by changing the amounts of erythrocyte contamination (table 1). An axial signal at $g \sim 6.0$ can be generated at low pH in PMNL but this is associated with protein denaturation. Even in this case the axial signal is not as narrow as the signal seen in serum (fig.1D) which we attribute to methemoglobin.

Leukocytes from a variety of mammalian sources possess high concentrations of myeloperoxidase [1,23] and exhibit rhombic signals associated with high-spin ferric hemes (table 2). Guinea pig, pig and horse leukocytes show two rhombic signals, while only one was sufficiently abundant to be measured in dog, sheep, cow and human leukocytes. Similar signals are also seen in alveolar macrophages (table 2). The physiological basis of the multiple signals remains to be determined. It is interesting that chicken leukocytes, where the circulating phagocytic cells are heterophils, possess little or no rhombic signal ([17], unpublished observations) and apparently lack myeloperoxidase [24]. Whether the axial $g \sim 6.0$ ferric high-spin heme signal seen in chicken heterophils is associated with the phagocytic process remains to be seen. The lack of myeloperoxidase-like activity [25] in chicken heterophils does not seem to be disadvantageous to the chicken, because this species is not unusually susceptible to infectious diseases.

Table 2
EPR signals from leukocytes and hemeproteins (g values)

Source	Rhombic		Axial	Ref.
	g_x	g_y	g	
Leukocytes				
Guinea pig	6.59	4.88	5.92	This work
	6.31	5.19		
Pig	6.59	5.04	–	This work
	6.37	5.25		
Horse	6.80	5.00	–	This work
	6.50	5.30		
Dog	6.77	5.00	5.92	This work
Sheep	6.68	5.21	–	This work
Cow and Calf	6.78	5.00	–	This work
Man	6.90	5.07	–	[17]
Chicken	–	–	6.0	[17], this work
Myeloperoxidase				
Dog uterus	6.77	5.02		
	6.33	5.52	–	This work
	6.3	5.3	–	[4]
Alveolar Macrophages				
Rhesus monkey	6.08	5.86		This work
Dog	6.77	5.00		This work
	6.41	5.32		

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