HEME COMPLEXES OF RABBIT HEMOPEXIN, HUMAN HEMOPEXIN AND HUMAN SERUM ALBUMIN: ELECTRON SPIN RESONANCE AND MÖSSBAUER SPECTROSCOPIC STUDIES

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SUMMARY

The electron spin resonance (ESR) spectra of human and rabbit ferriheme-hemopexin complexes at 30°K show an ESR absorption characterized by $g_x = 1.60$, $g_y = 2.25$ and $g_z = 2.86$, characteristic of low-spin ferrihemeproteins. The low-spin nature of the heme-iron in heme-hemopexin is corroborated by the observation of nuclear hyperfine splitting in Mössbauer spectra at 4.2°K. The similarity of the ESR spectra of ferriheme-hemopexin with those of low-spin cytochromes which coordinate heme *via* two histidine residues points to a similar coordination mechanism in hemopexin. In contrast, the ESR spectra of the 1:1 and 2:1 complexes of heme with human serum albumin display signals at $g = 6.0$ and $g = 2.0$, characteristic of high-spin ferrihemeproteins.

Two porphyrin-binding proteins, hemopexin and albumin, have been found in mammalian serum (1). Hemopexin, a β-glycoprotein which binds one mole of heme[2] with a higher affinity than albumin (2-4), transports circulating heme to the hepatocytes (5). Absorption spectra of both oxidized and reduced heme-hemopexin (1,6), unlike those of albumin (1,4), resemble those of low-spin heme proteins. This observation in conjunction with recent magnetic circular dichroism (MCD) (7) and chemical modification studies (8) indicate that the heme iron in heme-hemopexin is coordinated *via* two histidine residues. The MCD data show that the reduced

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[2] Abbreviations used are: heme, iron-protoporphyrin IX; ESR, electron spin resonance; MCD, magnetic circular dichroism.

heme-hemopexin complex is low-spin but do not distinguish the spin state of the oxidized complex. In contrast, neither the residues involved in heme coordination nor the spin state of methemalbumin are definitely known (1,4).

Low temperature electron spin resonance (ESR) and Mössbauer spectroscopy are useful in establishing the spin state of the iron in oxidized heme proteins. In this communication, we report on the application of these techniques to rabbit and human heme-hemopexin as well as human methemalbumin.

MATERIALS AND METHODS

The apo-hemopexins of rabbit and human were isolated, tested for purity (6) and their concentrations determined (9) as previously described. Human heme-hemopexin was also donated by Dr. G. Schwick of the Behringwerke, Marburg, Germany. Human albumin was prepared by

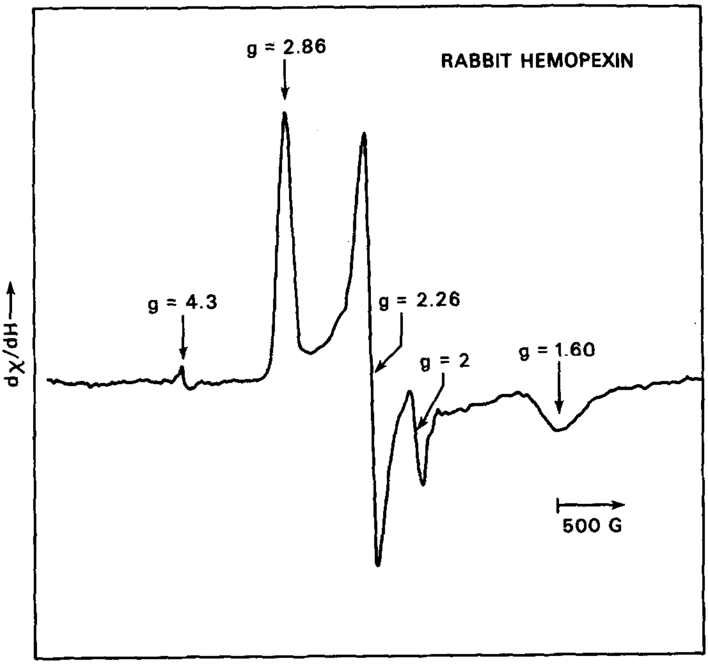

Figure 1: ESR spectrum of rabbit heme-hemopexin at 30°K. The spectrometer conditions were: frequency, 9.263 GHz, power to cavity, 10mW; modulation, 10G. Protein concentration was 7.5 mg/ml.

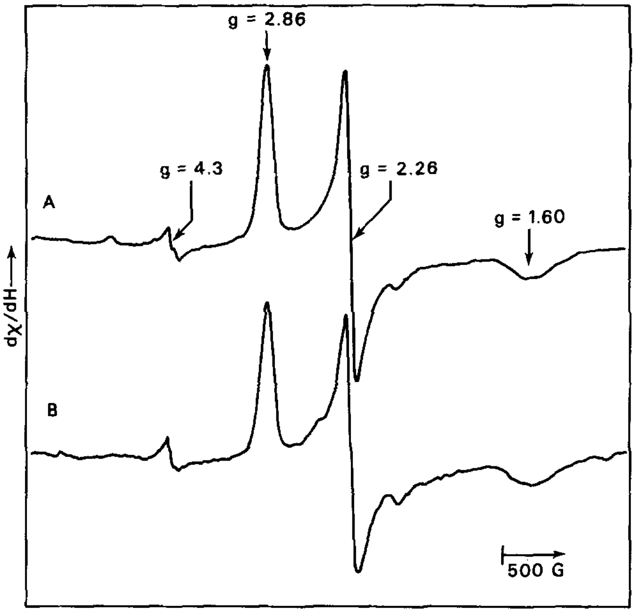

Figure 2: ESR spectra of human heme-hemopexin at 30°K. Trace A was obtained from a sample prepared as described in Materials and Methods, and Trace B from a preparation from the Behringwerke, Marburg, Germany. The spectrometer conditions were the same as in Figure 1. The protein concentration was 13 mg/ml.

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zone electrophoresis employing Pevikon (10), and heme (Eastman Organic Chemicals) was added in a 1:1 or 2:1 molar ratio. Fully oxidized (Fe III) heme-hemopexin was obtained by mixing an equimolar amount of heme with protein and was characterized by optical absorption spectra (6,11). The hemeproteins were exhaustively dialyzed at 4° against 0.02 M sodium phosphate, pH 6.3 or 7.4, and 0.02 M sodium borate, pH 9.3, with 0.05 M EDTA. Protein concentrations employed for ESR studies ranged from 7.5 to 13.0 mg/ml. For Mössbauer spectroscopy, ^{57}Fe-heme (a gift of Dr. W.S. Caughey) was added in a 1:1 molar ratio to 85 mg of rabbit apo-hemopexin. The sample was lyophilized after dialysis against 0.02 M sodium phosphate buffer, pH 7.4, 0.05 M EDTA, and then against triple-distilled water.

X-band ESR spectra were recorded at 30°K using a modified JEOL ME-IX spectrometer (12) and the Mössbauer spectra were obtained (13) under controlled temperature conditions at 4°K and 200°K.

RESULTS AND DISCUSSION

The ESR spectra taken at 30°K of both rabbit (Fig. 1) and human (Fig. 2) heme-hemopexin show principal absorptions at g-values of 1.60, 2.26, and 2.86, characteristic of a low spin ($S = 1/2$) state of ferric iron. These values are similar to those observed for cytochrome b_5 at pH 11.5 ($g = 1.68$, 2.28 and 2.82) (14) and for cytochrome b_2 at pH 12.1 ($g = 1.75$, 2.24 and 2.70) (15). The two different human heme-hemopexin preparations were identical (Fig. 2), and the minor signals at $g = 4.3$ and $g = 2.0$ (Figs. 1 and 2) are probably due to small amounts of free heme or heme bound to denatured protein. The ESR spectra for both human and rabbit heme-hemopexin like the absorption spectrum of rabbit deuteroheme-hemopexin (11) were unaffected by pH over the range pH 6.3 to 9.3 (not shown).

Rabbit heme-hemopexin treated at 20°C with a five molar excess of fluoride, a ligand which forms a high spin complex with accessible heme, did not display an altered ESR spectrum at 30°K (Fig. 3A). However, when this sample was heated to 85°C for one minute and re-examined at 30°K, its ESR spectrum (Fig. 3B) showed that most of the heme was converted to the high-spin form with g values of 6 and 2. This heat treatment produced denatured forms of heme-hemopexin on polyacrylamide gels (not shown). Replacing a ligand of the heme iron in native ferriheme-hemopexin with fluoride is difficult as is forming its cyanide complex. Carbon monoxide, however, readily complexes with ferroheme-hemopexin (1).

Mössbauer spectra of ^{57}Fe-enriched rabbit ferroheme-hemopexin at 4.2°K and 200°K are depicted in Figure 4. The spectrum taken at 200°K shows only a single quadrupole pair of Mössbauer absorption lines with a quadrupole splitting of 2.25 mm/sec and an isomer shift of -0.20 mm/sec

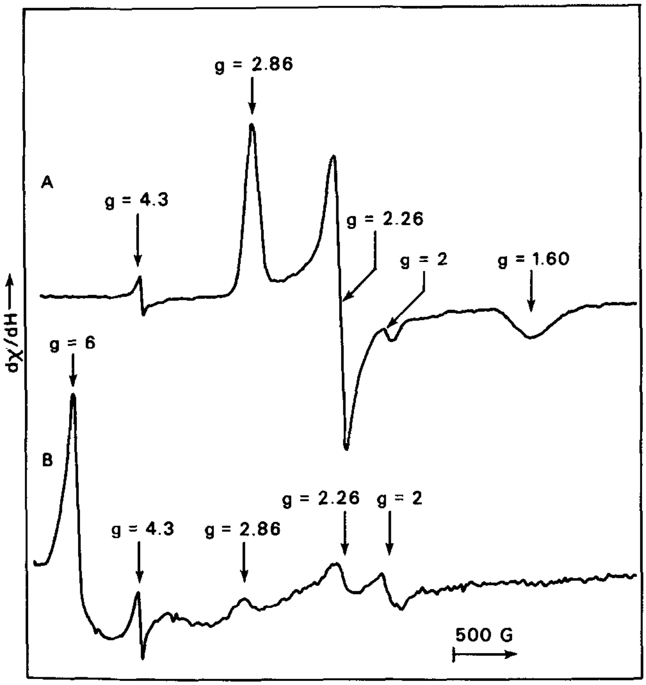

Figure 3: Effect of fluoride on the ESR spectra of rabbit heme-hemopexin. The ESR spectrometer conditions were the same as in Figure 1. Trace A was obtained at 30°K after exposure of the protein to a five molar excess of sodium fluoride at room temperature and Trace B after heating the sample to 85°C for one minute and re-examining it at 30°K.

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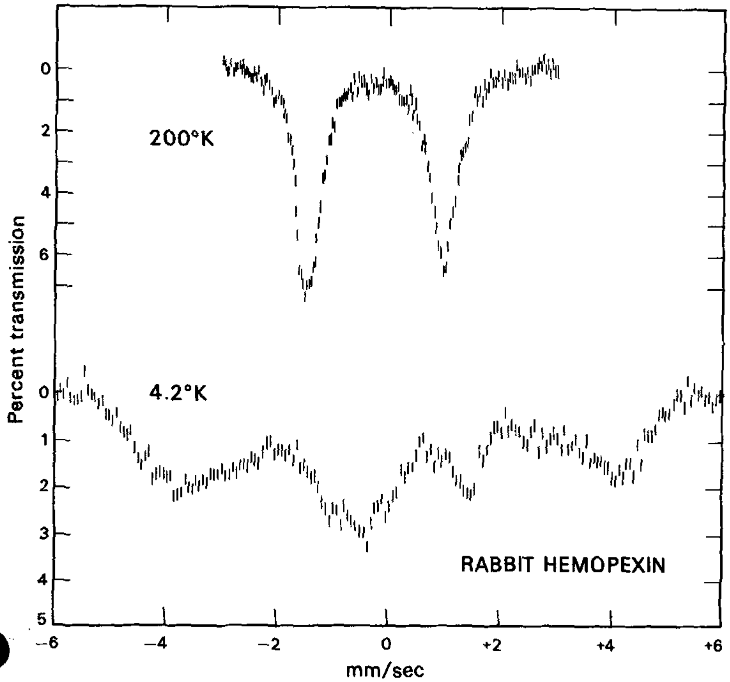
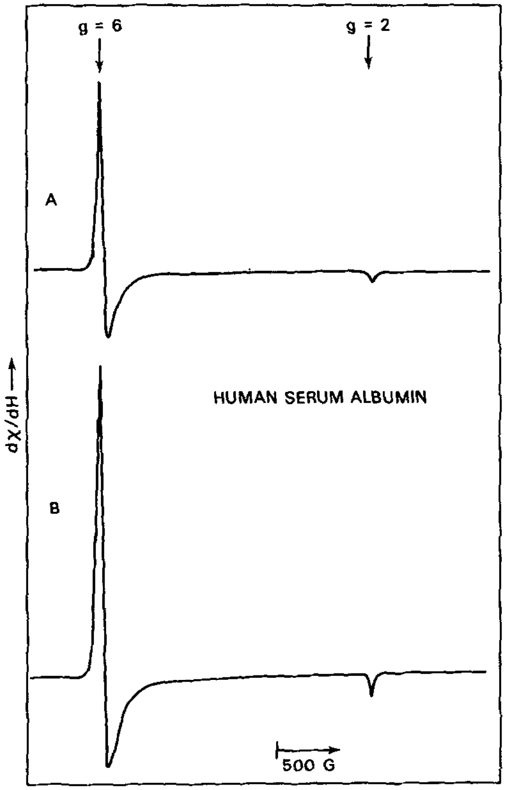
relative to a ^{57}Fe:Pt source or +0.27 mm/sec referred to Fe metal. These values of quadrupole splitting and isomer shift are within the range expected for low-spin Fe (III) compounds (13,16); nevertheless, this spectrum does not allow a definite assignment of either spin or oxidation state. At 4.2°K, the Mössbauer absorption pattern shows additional features indicative of a nuclear hyperfine interaction at the ^{57}Fe position; this is quite similar to the nuclear hyperfine effect shown in other low-spin ferrihemeproteins (16).

In contrast, the ESR spectra of human serum albumin at 30°K with a 1:1 (Fig. 5A) and 2:1 (Fig. 5B) ratio of heme to protein show prominent g-values at $g_x = g_y = 6$ and $g_z = 2$. The observed ESR signals,


200°K
4.2°K
RABBIT HEMOPEXIN
Percent transmission
0
2
4
6
0
1
2
3
4
5
−6
−4
−2
0
+2
+4
+6
mm/sec

4


g = 6
g = 2
A
B
HUMAN SERUM ALBUMIN
dχ/dH
500 G

5

which were proportional to the amount of heme bound to the protein, are typical of high spin (S = 5/2) ferric hemeproteins and do not indicate any rhombic distortion of the heme. No low-spin Fe (III) signals were seen between 12°K and 100°K.

In conclusion, the ESR and Mössbauer spectra of ferriheme-hemopexin reported here demonstrate the low-spin nature of this complex. Since ferroheme-hemopexin is also low-spin (7), the spin state of this hemeprotein is independent of oxidation state. Further, the resemblance of the ESR spectra of ferriheme-hemopexin to those of cytochrome b_5 (14), known to have dihistidyl heme coordination (17), provides additional evidence that the heme in heme-hemopexin is similarly coordinated.

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Figure 4: Mössbauer spectra of rabbit ^{57}Fe-heme-hemopexin. The spectra were obtained on 85 mg of lyophilized material at 200°K (upper trace) and at 4.2°K (lower trace).

Figure 5: ESR spectra of human methemalbumin at 30°K. Shown are: (A) 1:1 molar ratio of heme to protein and (B) 2:1 ratio. ESR spectrometer conditions were the same as in Figure 1. The protein concentration was 8 mg/ml.

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