MÖSSBAUER EFFECT IN PEROXIDASE-HYDROGEN PEROXIDE COMPOUNDS

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Received October 31, 1967

In the previous communication, we demonstrated Mössbauer effect in Japanese-radish peroxidase a (JRP-a) and some of its derivatives such as hydroxide, azide and cyanide compounds, all of which exhibited resonance spectra attributed to low-spin ferric iron (Maeda, Higashimura, and Morita (1967)). Such results were valuable for the elucidation of the electronic configurations of the heme-iron in the enzyme and its derivatives, complementary to information derived from magnetic susceptometry and electron spin resonance spectroscopy on this enzyme. Although the electronic configurations of the intermediate compounds of peroxidase in the course of peroxidatic and oxidatic reactions are indispensable to elucidate the mechanism of the reactions, less information has been obtained. Theorell and Ehrenberg (1952) demonstrated the magnetic susceptibilities of Compounds I and II, which were proposed to have intermediate spin states. Morita and Mason (1965) studied on the compounds by means of electron spin resonance spectroscopy, but they failed to detect any absorption attributed to the heme-iron atom of the compounds, as reported early by Chance and Fergusson (1954) and most recently by Yonetani, Schleyer, and Ehrenberg (1966). So, in spite of valuable information by optical spectroscopy and redox-titration experiments, the electronic configurations of these compounds are still uncertain. In this communication we present some experimental results on Mössbauer effect in JRP-a-hydrogen peroxide Compounds I, II, and III enriched with ^{57}Fe and discuss briefly on the electronic configurations of these compounds.

MATERIAL AND METHOD JRP-a enriched to 68 % with ^{57}Fe was synthesized by combining apo-JRP-a with protohematin enriched with ^{57}Fe and purified further by chromatography on a DEAE-Sephadex A-25 column and a Sephadex G-25 column, as described in the previous paper (Maeda, Higashimura, and Morita (1967)). The concentrated solution of JRP-a, 2.2 mM, was poured into a Lucite absorber cell, 5 mm thick and 15 mm in diameter, and mixed very rapidly with a slight excess of hydrogen peroxide in molar ratio. The cell was then rapidly dipped into liquid nitrogen to freeze the mixture. At this moment, the mixture was dark green in color, and at least 80 % of the enzyme was in the form of Compound I, the remaining part being in the form of Compound II. For the formation of Compound II, the mixture was allowed to stand for five minutes and then frozen, when more than 90 % of the enzyme was in the form of red Compound II. Compound III was formed by mixing JRP-a solution with 50 times excess amounts of hydrogen peroxide in molar ratio and the mixture was frozen. The experiments were performed at two sample temperatures, i.e., 77°K (liquid nitrogen) and 195°K (dry ice-acetone), by means of a spectrometer of the conventional linear velocity type, equipped with a source of 30 mCi of ^{57}Co diffused into metallic Pd and with a multichannel analyzer operating in multiscaler mode. The velocity scale was calibrated absolutely from an independent Mössbauer run using a thin metallic iron absorber, taking the center of symmetry of this spectrum as the velocity zero. The velocity was determined to an accuracy of $\pm$ 0.03 mm/sec.

RESULTS AND DISCUSSION Mössbauer spectra of Compound II at two sample temperatures are shown in Fig. 1, and the Mössbauer parameters are summarized in Table I together with those of Compounds I and III. As seen from Fig. 1, the spectrum of Compound II exhibited two symmetric lines, with a quadrupole splitting of 1.46 mm/sec and an isomer shift of 0.11 mm/sec at 77°K. It should be noticed that the latter parameter was about 0.2 mm/sec more negative than those of the usual low-spin ferric compounds. This implies that the s-electron density at the nucleus in Compound II was larger and hence the

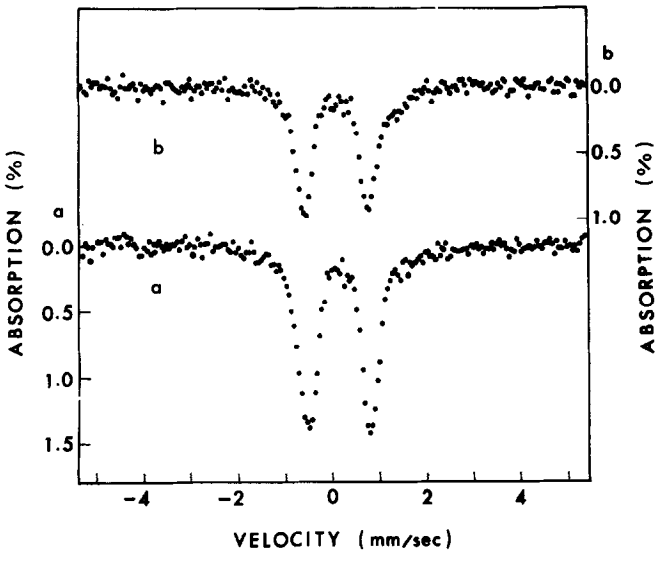

Fig. 1. Mössbauer absorption spectrum of JRP-a-hydrogen peroxide Compound II at (a) 77°K and (b) 195°K.

shielding effect of d-electrons was less than in the low-spin ferric compounds. In this connection, Theorell and Ehrenberg (1952) demonstrated in their magnetic susceptometric experiments that Compound II had two unpaired electrons, and Chance, Theorell and Ehrenberg (1952) proposed the structure of Compound II to be in a ferryl form, in which the iron was oxidized to Fe^{4+}. The present Mössbauer effect strongly supported this proposal, because in such ferryl form the iron has less d-electrons giving the shielding effect on s-electron density than in the case of low-spin ferric iron.

The most striking result in the present experiment is that Compound I gave the same Mössbauer absorption spectrum as that of Compound II, as shown in Fig. 2 and Table I. This suggests that the electronic configuration of the heme-iron should be the same in both compounds. As stated by Theorell and Ehrenberg, Compound I is thought to have three unpaired electrons, so that the configuration might be Fe^{4+}:Ö·, if Compound I would be in a ferryl form as

Table I. Isomer Shift and Quadrupole Splitting for Japanese-radish Peroxidase a-Hydrogen Peroxide Compounds

Material	Temperature (°K)	Quadrupole splitting (mm/sec)	Isomer shift* (mm/sec)
Compound I	195	1.38	0.04
	77	1.33	0.10
Compound II	195	1.44	0.07
	77	1.46	0.11
Compound III	195	2.33	0.24
	77	2.37	0.29

* Isomer shifts include the second-order Doppler shift due to the temperature difference between source and absorbers. The temperature of source was 293°K.

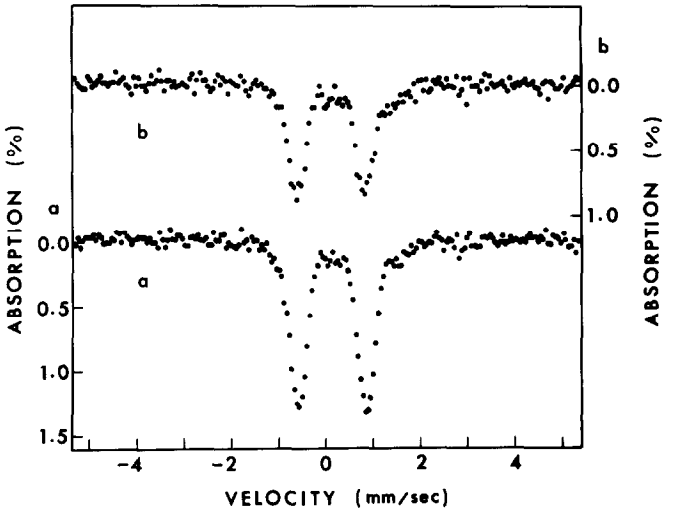

Fig. 2. Mössbauer absorption spectrum of JRP-a-hydrogen peroxide Compound I at (a) 77°K and (b) 195°K.

well as in Compound II, or one unpaired electron might be carried on porphyrin ring orbitals.

Fig. 3 shows the spectrum of Compound III, which exhibits two widely splitted lines with a quadrupole splitting of 2.37 mm/sec and an isomer shift of 0.29 mm/sec at 77°K. The spectrum is very similar to that of oxyhemoglobin reported by Lang and Marshall (1966), so that Compound III will be in the oxy-

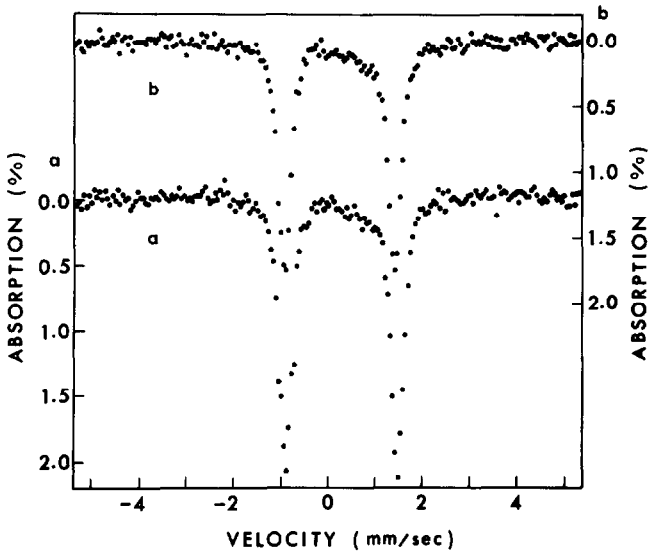

Fig. 3. Mössbauer absorption spectrum of JRP-a-hydrogen peroxide Compound III at (a) 77°K and (b) 195°K.

ferrous form, as reported by George (1953) and by Mason (1957). In this form the oxygen molecule may be linked to the iron atom by π-bonding, and it situates parallel to the heme-plane. In this case, the compound should be diamagnetic. However, as the magnetic susceptibility of Compound III has not been determined, there still remains a possibility that Compound III might have an oxyferric form, in which there would exist two unpaired electrons, one being carried on the oxygen atom, because the Mössbauer parameters resembled very closely to those of low-spin ferric compounds.

ACKNOWLEDGMENT The authors are much indebted to Dr. T. Higashimura for valuable discussions.

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