ELECTRON SPIN RESONANCE INVESTIGATIONS OF METHYL HYDROGEN PEROXIDE COMPOUND II OF CATALASE

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1. Introduction

Catalase reacts with methyl hydrogen peroxide to form a red compound designated as Compound II of catalase. The structure of this compound is still of unclear origin. Chance [1] discusses an enzyme-substrate complex with trivalent heme iron. George [2] assumes that the heme iron exists in a quadrivalent state in Compound II of the peroxidases. Measurements of the magnetic susceptibility of Compound II made by Theorell and Ehrenberg [3] failed to reveal the valency of heme iron. Therefore electron spin resonance was used to investigate Compound II of catalase, because this method is known to give typical spectra of ferrihemoproteins [4, 5].

2. Materials and methods

Bovine liver catalase was prepared according to the method of Schnuchel [6] with slight modification [5]. The Kat-f-value determined according to Bergmeyer [7] ranged from 50,000 to 70,000. For the electron spin resonance measurements, 0.6 ml of a 1.25×10^{-4} M catalase solution were mixed with 40 μl of a 0.4 M aqueous methyl hydrogen peroxide solution and subsequently frozen in liquid nitrogen. The electron spin resonance measurements were made at 77°K and at 20°K.

3. Results

Hemoprotein complexes with trivalent heme iron are reported to exist in 2 electronic configurations in the basic state [8]. One of them with 5 unpaired electrons is called high spin and the other one, with 1 unpaired electron, low spin. Both have the same chemical structure with minor differences in the bond lengths between the iron and its ligands.

In the electron spin resonance spectrum the high-spin form is represented by strong absorption with *g*-factor 6 and a small absorption with *g*-factor 2. The low spin form exhibits 3 absorption bands around *g*-factor 2. Consistent with this, the electron spin resonance spectrum of Compound II of catalase (fig. 1) recorded at 77°K proved to be a typical mixed complex of the high and low spin form, because the spectrum exhibits absorptions typical of both electronic configurations. This type of spectrum is observed with various hemoproteins [4, 5]. On the contrary, the low spin portion can no longer be reliably detected at 20°K. This temperature behaviour suggests that the high spin form of Compound II is the preferred one. The temperature dependence of the mixed complex is in conformity with the optical spectrum at room temperature which mainly shows low spin character [1].

The electron spin resonance spectrum of Compound II also shows a radical signal of lower intensity. The radical concentration of this signal does not correspond to an oxidation equivalent and should be attributed to a side reaction of peroxide with the catalase protein. The absorption at $g = 4.2$ corresponds to nonheme rhombic iron [9].

4. Discussion

As it is revealed by the typical electron spin reso-

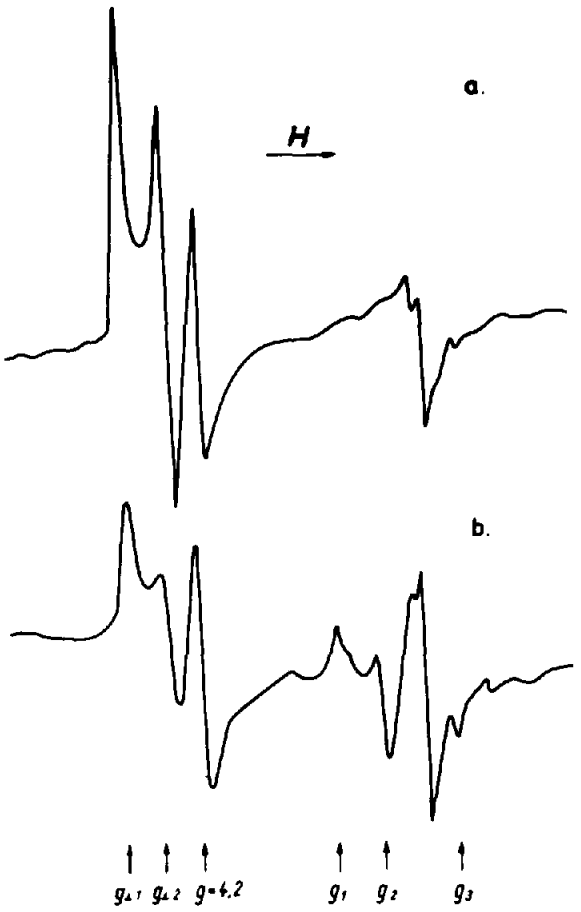

Fig. 1. Electron spin resonance spectra of Compound II of catalase: $g\perp_1 = 6.5$; $g\perp_2 = 5.2$; $g_1 = 2.52$; $g_2 = 2.22$; $g_3 = 1.84$. Temperature: a) 20°K; b) 77°K.

nance spectrum of Compound II of catalase, heme iron is in a trivalent state. This finding is in conformity with the conceptions maintained by Chance who concluded from the optical behaviour of Compound II that this complex must contain ferric iron [1]. But our results are in contradiction to the views expressed by George [2] who assumes quadrivalent heme iron for the Compound II of the peroxidases, on the basis of his redox investigations of Compound II of peroxidase. George has extended this finding to all Compound II of other heme enzymes, but this assumption is refuted by the phenomenon that the electron spin resonance spectra of d^4-complexes differ from d^5-complexes [10] and that no stable quadrivalent iron complexes are known as yet. The formation of Compound II is conceivable as an isomerization of Compound I, an enzyme-substrate complex of catalase [1] in which both oxidation equivalents are maintained. The assumption of one oxidation equivalent for Compound II might be compatible only with the presence of a quadrivalent heme iron or of a radical equivalent, but either has been precluded by electron spin resonance measurement. Hence, according to our studies, the reaction of catalase proceeds without single-step intermediate stages. Compound I or Compound II must rather be reconverted into catalase by a 2-equivalent reduction.

References

[1] B.Chance, J. Biol. Chem. 179 (1949) 1331.
[2] P.George, Biochem. J. 54 (1953) 267; 55 (1953) 220.
[3] H.Theorell and A.Ehrenberg, Arch. Biochem. Biophys. 41 (1952) 442.
[4] H.Rein and O.Ristau, Biochim. Biophys. Acta 94 (1965) 516.
[5] H.Rein, O.Ristau, F.Hackenberger and F.Jung, Biochim. Biophys. Acta 167 (1968) 538.
[6] G.Schnuchel, Z. Physiol. Chem. 298 (1954) 241.
[7] H.W.Bergmeyer, Biochem. Z. 327 (1955) 255.
[8] J.S.Griffith, in: Molecular Biophysics, eds. B.Pullman and M.Weissbluth (Academic Press, New York, London, 1965) p. 191.
[9] H.Beinert and R.H.Sands, Biochem. Biophys. Res. Commun. 3 (1960) 41.
[10] K.Ono, S.Koide, H.Sekiyama and H.Abe, Phys. Rev. 96 (1954) 38.