Vol. 86, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1979 Pages 319-324

SPIN-STATE EXCHANGE IN FUSARIUM LIPOXYGENASE ON BINDING OF LINOLEIC ACID

Yuzuru Matsuda*, Teruhiko Beppu and Kei Arima

Department of Agricultural Chemistry, The University of Tokyo,

Bunkyo-ku, Tokyo(Japan)

Received November 1, 1978

SUMMARY

The electron paramagnetic resonance(EPR) signals of Fusarium lipoxygenase were measured at liquid nitrogen temperature in the presence or absence of substrate, linoleic acid. The spin-state exchange of heme iron in Fusarium lipoxygenase from a low to high spin-state by the addition of linoleic acid was observed. The addition of linoleic acid to the enzyme at pH 9.0 gave rise to the appearance of EPR lines at g=5.92 and 3.58, while at pH 12.0, lines at g=6.12 and 3.41 were newly appeared. At the same time, the resonance at g=4.31 was increased both at pH 9.0 and 12.0 in the presence of linoleic acid.

INTRODUCTION

Lipoxygenase(linoleate: oxygen oxidoreductase, EC 1.13.11.12), oxidizing unsaturated fatty acids with a *cis, cis*-1,4-pentadiene structure to isomeric dienoic hydroperoxides, was initially found in soybean and later in a wide range of plant kingdom[1-3]. Several lipoxygenases from animal sources have been also described in the last few years[4,5]. However, none had been reported from microbial sources. Recently, we found a lipoxygenase activity from a fungus, *Fusarium oxysporum*, and the purified enzyme protein was named Fusarium lipoxygenase[6,7]. Fusarium lipoxygenase contains one mole protoheme IX per mole enzyme and requires Co^{2+} as a stabilizing factor[7] in contrast to soybean lipoxygenase 1 which contains one mole non-heme iron per mole enzyme[8-10]. Fusarium lipoxygenase shows maximum activity at pH 12.0 and

* Present address: Tokyo Research Laboratory of Kyowa Hakko Kogyo Co., Ltd., 3-6-6 Asahicho, Machidashi, Tokyo(Japan)

0006-291X/79/020319-06$01.00/0

produces conjugated 9-hydroperoxide isomer fairly selectively. ^{18}O is introduced into the hydroperoxide only from gaseous oxygen[11]. It is a novel example of protoheme IX-containing dioxygenase which has included only tryptophan pyrrolases from several organisms[12].

In this paper, the electron paramagnetic resonance(EPR) measurements of Fusarium lipoxygenase in the presence or absence of substrate were performed at liquid nitrogen temperature in order to clarify the involvement of protoheme IX in the oxygenation reaction by Fusarium lipoxygenase.

MATERIALS AND METHODS

Enzyme Fusarium lipoxygenase was crystallized from the purified preparation obtained according to the method described previously[7]. For EPR measurements, the crystalline Fusarium lipoxygenase was dissolved into 50 mM Tris-acetate buffer(pH 7.5) containing 10^{-3}M EDTA, and dialyzed at 0°C for 24 h against the same buffer using cellulose tube treated with EDTA in order to remove Co^{2+} which was previously added as a stabilizing factor [7]. After extensive dialysis against distilled water, the enzyme solution was lyophilized and dissolved into 0.1 M borate buffer(pH 9.0 or 12.0).

Substrate The solution of linoleic acid was prepared as described previously[7].

EPR spectra EPR spectra were recorded using JASCO Model JES-FE3X electron spin resonance spectrometer. All measurements were carried out at liquid nitrogen temperature(77°K). The g values shown in this paper do not necessarily represents true values but may be given as a means of quickly locating prominent points in the spectrum.

Chemicals Linoleic acid(above 99%) was purchased from Applied Science Lab., Inc., Penna., U. S. A.

RESULTS

At pH 9.0 The EPR spectra of Fusarium lipoxygenase recorded at liquid nitrogen temperature in the presence or absence of linoleic acid(pH 9.0) were shown in Fig. 1. The result shows that heme iron of Fusarium lipoxygenase in the absence of substrate is a form of essentially low spin-character with g values of 2.9 and 2.3(Fig. 1-1). This character is in analogous to the visible absorption spectra[7]. The EPR spectrum of Fusarium lipoxygenase was different from that of cytochrome b_5[13], which also contains protoheme IX. It was also different from that of soybean lipoxygenase 1[14], which contains non-heme iron. On the other hand, the sample frozen at 77°K

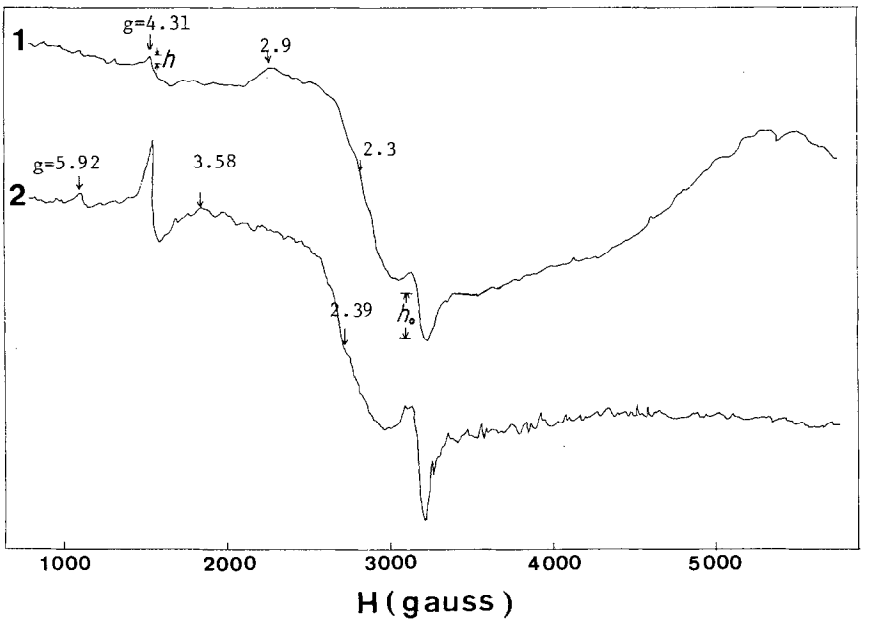

Fig. 1. EPR spectra of Fusarium lipoxygenase in a frozen solution of pH 9.0 at 77°K. Curve 1: $7.6 \cdot 10^{-5}$M Fusarium lipoxygenase in 0.1 M borate buffer (pH 9.0), untreated. Curve 2: Incubated for 5 min at 0°C after addition of 0.3 volume of a 16 mM solution of linoleic acid. The conditions of EPR spectroscopy of curve 1 and 2 were as follows, respectively: microwave power, 50 mW and 20 mW; Gain, $1.6 \cdot 10^3$ and $6.3 \cdot 10^2$; modulation amplitude 8 G and scanning rate 1250 G/min throughout.

after the incubation with linoleic acid(pH 9.0) for 5 min at 0°C shows high spin signals with g=5.92, 4.31 and 3.58(Fig. 1-2). The intensity(h/h_o=0.17) of the peak at g=4.3, which is characteristic signal for the high spin-state of ferric ions in a ligand of rhombic symmetry[14], is significantly increased (h/h_o=0.77) and the considerable changes were observed whithin the low spin region of 4000 to 5000 G.

At pH 12.0 A pronounced narrowing of the low spin signals were observed at pH 12.0 as shown in Fig. 2, thus indicating a considerable changes or rearangements of the ligands to protoheme IX. The spin-state change in Fusarium lipoxygenase caused by the addition of substrate were also observed at pH 12.0 especially with the appearance of increased high spin siganl at g=6.12. The enhancement of the signal at g=4.31(from h/h_o=0.43 to 0.78),

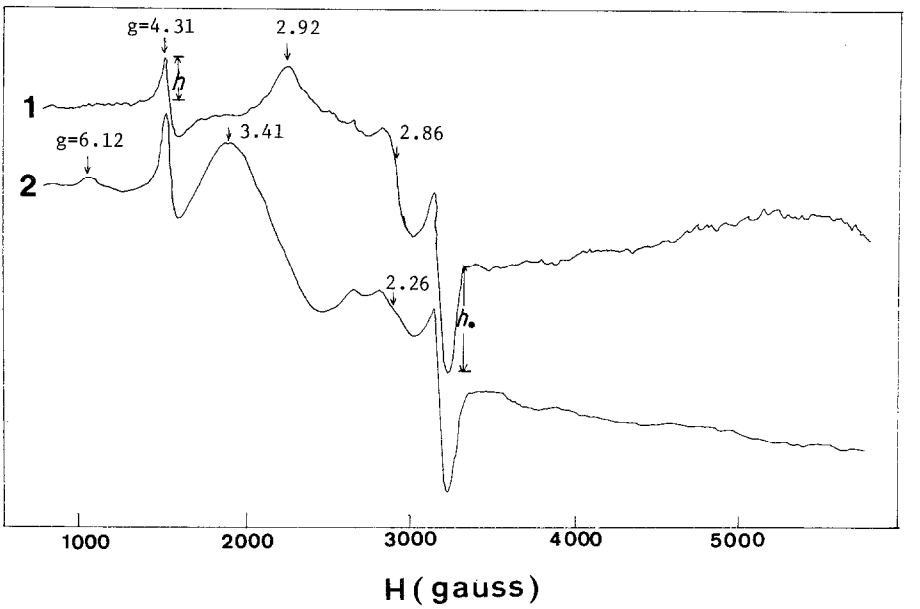

Fig. 2. EPR spectra of Fusarium lipoxygenase in a frozen solution of pH 12.0 at 77°K. Curve 1: $9.2 \cdot 10^{-5}$M Fusarium lipoxygenase in 0.1 M borate buffer(pH 12.0), untreated. Curve 2: Incubated for 5 min at o°C after addition of 0.3 volume of a 16 mM solution of linoleic acid. The conditions of EPR spectroscopy of curve 1 and 2 were as follows: microwave power, 8 mW; Gain, $1 \cdot 10^{3}$; modulation amplitude 8 G and scanning rate 625 G/min throughout.

the change within the region of 2000 to 3000 G, and the change within the region of 4000 to 5000 G are the common phenomena to both samples measured at pH 9.0 and pH 12.0. Therefore, it may be concluded that the protoheme IX is closely interacting with linoleic acid.

DISCUSSION

In both oxidized and reduced forms, the main absorption spectral features of Fusarium lipoxygenase in Ref. 7 is similar to that of cytochrome b_5[15] excepting the position and the structure of the α- and β-bands. This similarity suggests that the heme iron of Fusarium lipoxygenase is in a low spin-state[16] and have internal ligands on the 5th and 6th coordination sites[17,18]. The results obtained by EPR measurements disproved such

a possibility, that is, the protoheme IX of Fusarium lipoxygenase is of a low spin-type. However, the spin-state change of heme iron in Fusarium lipoxygenase from a low to high spin-state by the addition of linoleic acid as substrate was observed. It has been considered that the iron in a high spin-state combines with molecular oxygen more efficiently than the iron in a low spin-state since the heme in a low spin-state can not easily interact with an external ligands due to strong internal interaction with residues of the apoproteins[16]. Thus, it is reasonable to suppose that the heme iron of Fusarium lipoxygenase exchanges its spin-state from a low to high state to facilitate the reaction with molecular oxygen in the presence of substrate. This exchange of spin-state caused by the addition of the substrate is previously reported in $P\text{-}450_{cam}$ by Tsai *et al.*[19]. And the spin-state exchange in accordance with conformational changes in cytochrome oxidase was also considered to facilitate the reaction with molecular oxygen[20]. Therefore, this discovery presented in this paper gives a clue to the understanding of the reaction mechanism and the role of protoheme IX of Fusarium lipoxygenase. Further studies on these points are under investigation.

The pronounced narrowing properties of EPR signals and the dimunition of α-herical structure at pH 12.0[21] will provide some evidence of considerable conformational changes of the enzyme, which results in an unusual sharp peak at pH 12.0 in the pH-activity curve[7] and the change of the positional specificity of hydroperoxidation depending on pH[11].

ACKNOWLEDGEMENTS

The results presented here are part of the doctoral thesis of Y. Matsuda. The authors are indepted to Dr. M. Kohno, Nihon Denshi Co., Ltd., for his elaboration of EPR spectroscopy. Thanks are due also to Dr. T. Satoh, The Tokyo Metropolitan University, for his stimulating guidance.

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