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CIRCULAR DICHROISM OF FUSARIUM LIPOXYGENASE FROM *Fusarium oxysporum*

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SUMMARY

In the Soret region, a large negative trough at 427.5 nm of the oxidized Fusarium lipoxygenase between large positive peaks at 400 nm and 490 nm was observed. But the CD spectrum of the reduced enzyme was considerably changed and the many complex peaks with positive amplitudes were observed. From these results, the protoheme IX in Fusarium lipoxygenase might be distinguished from that in cytochrome *b* in its function. The spectral conversion between pH 6.0(α-helix form) and pH 9.0(β form) in the far-ultraviolet region were observed and this conversion was entirely reversible. At pH 12.0, however, the negative CD band in the far-ultraviolet region was greatly diminished, suggesting a great change in backbone structure. This considerable conformational change of the enzyme at pH 12.0 will explain well the fact the change of the positional specificity of hydroperoxidation depending on pH and will also explain the fact that the enzyme has the highest activity at pH 12.0 might due to the accessibility of substrate to protoheme IX.

INTRODUCTION

Lipoxygenase(linoleate: oxygen oxidoreductase, EC 1.13.11.12), oxidizes unsaturated fatty acids with *cis, cis*-1,4-pentadiene structure to isomeric dienoic hydroperoxides and was initially found in soybean and later in a wide range of other plants[1]. Several lipoxygenases from animal sources have been also described in the last few years[2,3]. Recently, we found a lipoxygenase from a extracts of a fungus, *Fusarium oxysporum*, and the purified enzyme protein was designated as Fusarium lipoxygenase[4,5]. In contrast to soybean lipoxygenase 1, Fusarium lipoxygenase has a high alkaline pH optimum, requirement for Co^{2+}, and the presence of protoheme IX in spite

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of non-heme iron in the plant lipoxygenases. In this communication, circular dichroism(CD) spectra of Fusarium lipoxygenase from *Fusarium oxysporum* were measured to obtain more information about protoheme IX in Fusarium lipoxygenase.

MATERIALS AND METHODS

Fusarium lipoxygenase was purified from *Fusarium oxysporum* to a homogeneous protein according to the method described previously[5]. The CD spectra were measured at 25°C by JASCO Model J-20 automatic recording spectropolarimeter. Quarts cells with either 1 or 3 mm path length were used. The enzyme was dissolved into each of these following buffers containing 0.25 mM Co^{2+}: 50 mM phosphate buffer(pH 6.0), 50 mM Tris-HCl buffer(pH 9.0), and 50 mM borate buffer(pH 12.0). A sample in a cuvette was reduced by the addition of solid sodium dithionite. Base line were recorded before and after each scan, and subtracted from the recorded spectra. In the figures presented, $[\theta]_R$ and $[\theta]_M$ represent the molecular ellipticity based on the average residue weight(assuming the number of amino acid residues per mole as 129[5]) and the molecular weight(assuming the molecular weight as 12500 [5]), respectively.

RESULTS AND DISCUSSION

In the Soret region, a large negative trough at 427.5 nm of the oxidized Fusarium lipoxygenase between large positive peaks at 400 nm and 490 nm was observed(Fig. 1). This spectral profile suggests so called a "couplet type" caused by heme-heme interaction, which is observed, for instance, in heme octapeptide[6]. But the spectrum showed no considerable change even in the presence of 0.25 M imidazole, thus indicating this "couplet type" of spectrum was not brought about by heme-heme interaction. The CD spectrum of the reduced Fusarium lipoxygenase was significantly different from that of the oxidized enzyme. The large negative trough in the Soret region disappeared, and the many complex peaks with positive amplitudes were observed. Their positions were slightly shifted to the red when compared with those of the oxidized form. It had been posturated that a *b*-type cytochrome has a large negative Cotton effect in the Soret region irrespective to be oxidized or reduced, while protoheme-proteins(hemogrobin, myoglobin, peroxidase, etc.) have a large positive one[7,8]. Based on this hypothesis, the proto-

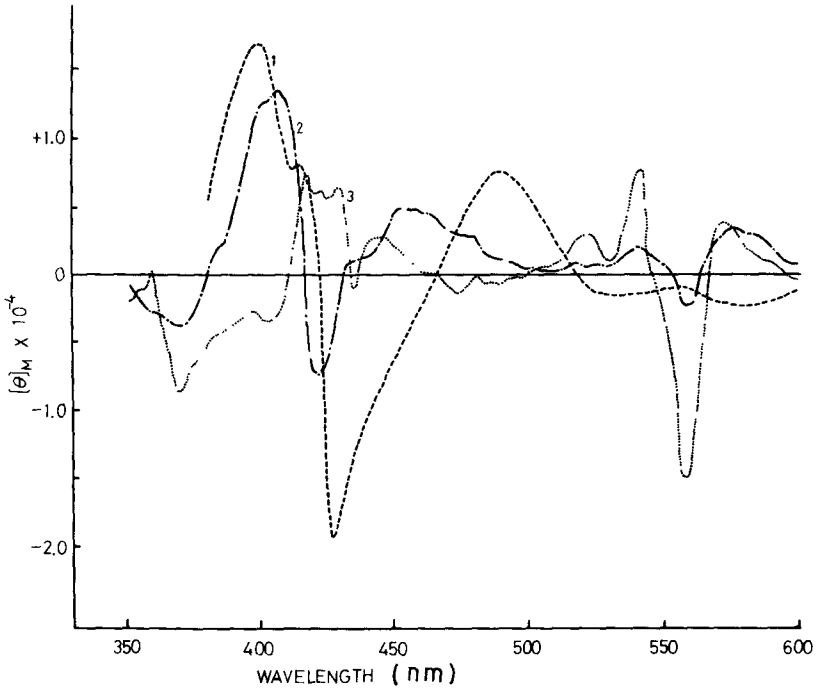

Fig. 1. Circular dichroic spectra of Fusarium lipoxygenase in 50 mM phosphate buffer(pH 6.0) at 25°C. The enzyme was reduced with sodium dithionite. Curve 1(- - - - -): oxidized, 50 mM phosphate buffer containing 0.25 mM Co^{2+} (pH 6.0); enzyme concentration, $1.5 \cdot 10^{-4}$ M; path length, 3 mm. Curve 2(— · —): partially reduced. Curve 3(·····): completely reduced.

heme IX in Fusarium lipoxygenase might be distinguished from that in cytochrome *b* in its function.

The CD spectra of Fusarium lipoxygenase as a function of pH were also measured. As shown in Fig. 2, in the far-ultraviolet region, the CD spectrum at pH 6.0 showed negative troughs at 208 and 215 nm, suggesting the presence of α-helix form in the protein moiety of Fusarium lipoxygenase. When the α-helix content in the enzyme was calculated from the height of $[\theta]_R$ at 222 nm using the value $[\theta]_{222nm}$=-4.0 x 10^4 for helical poly L-glutamic acid as reference[9], a value of 15.1% for Fusarium lipoxygenase was obtained. At pH 12.0, however, the negative CD band at around 222 nm was greatly diminished, suggesting a great change in backbone structure(Fig. 2). The α-helix content of Fusarium lipoxygenase at pH 12.0 decreased to 8.7%. Moreover, the CD spe-

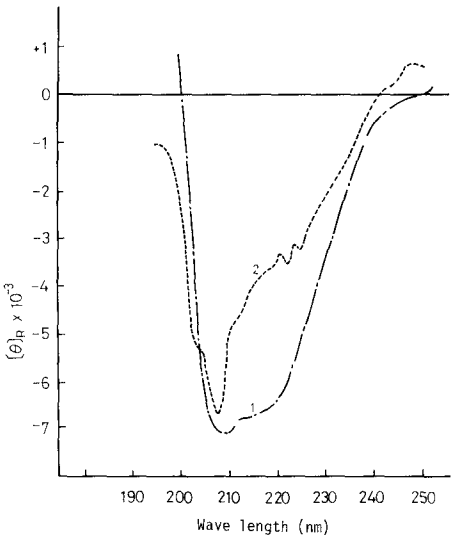

Fig. 2. Circular dichroic spectra of the oxidized form of Fusarium lipoxygenase measured at different pH's. Curve 1(——·——): 50 mM phosphate buffer containing 0.25 mM Co^{2+} (pH 6.0); enzyme concentration, $1.6 \cdot 10^{-5}$ M; path length, 1 mm. Curve 2(- - - - - -): 50 mM borate buffer containing 0.25 mM Co^{2+} (pH 12.0); enzyme concentration, $8.1 \cdot 10^{-6}$ M.

ctrum at pH 9.0 was different from both spectra at pH 6.0 and 12.0. As shown in Fig. 3, the spectrum at pH 9.0 shifted entirely 7-8 nm toward the red when compared with that at pH 6.0. The shifted spectra have a maximum at 202 nm and a minimum at 215 nm, which represents the presence of β structure of protein moiety of Fusarium lipoxygenase[10]. This spectral change between pH 6.0 and 9.0 in the far-ultraviolet region, which was caused by pH values, were entirely reversible. On subsequent dialysis of the enzyme against 50 mM phosphate buffer (pH 6.0) after being kept for 48 hr in 50 mM Tris-HCl buffer(pH 9.0), the CD spectrum was restored to its original α-helix form. But the reversibility of the conformational change of the enzyme between pH 9.0 and 12.0 was not clearly recognized.

We previously noted that Fusarium lipoxygenase had an unusual sharp peak at pH 12.0 with a shoulder around at pH 10.0 and almost no activity at pH 6.0 in the pH-activity curve[5]. Considering the pH-CD spectral change in the far-ultraviolet region observed here, it may be concluded that the enzyme activit

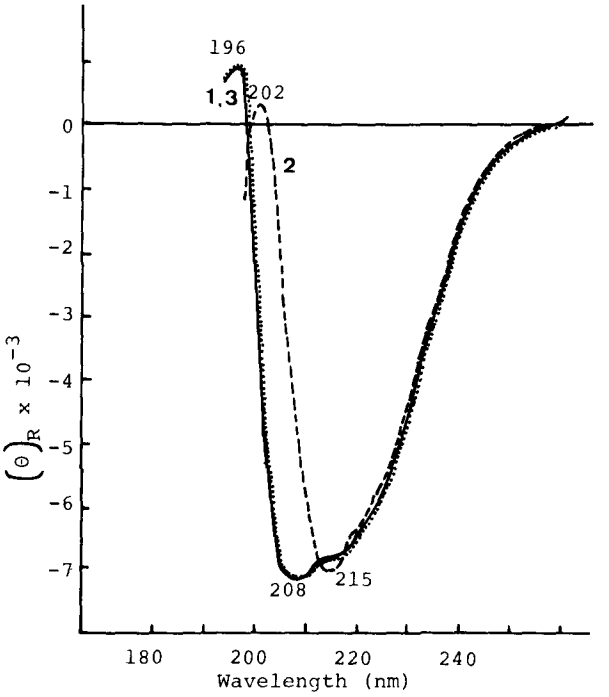

Fig. 3. Circular dichroic spectral changes of Fusarium lipoxygenase depending on pH of the environment. Curve 1(————): 50 mM phosphate buffer containing 0.25 mM Co^{2+} (pH 6.0); enzyme concentration, $6.1 \cdot 10^{-6}$ M; path length, 3 mm. Curve 2(------): 50 mM borate buffer containing 0.25 mM Co^{2+} (pH 9.0); enzyme concentration, $8.2 \cdot 10^{-6}$ M. Curve 3(·····): Dialyzed against 50 mM phosphate buffer containing 0.25 mM Co^{2+} (pH 6.0) after allowing to stand at pH 9.0 for 48 hr; enzyme concentration, $8.2 \cdot 10^{-6}$ M.

might be influenced by the conformational change of the enzyme as follows;

pH 6.0	pH 9.0	pH 12.0
α-helix form $\rightleftharpoons$	β structure $\underset{(?)}{\rightleftharpoons}$	Destruction of a conformation
(no activity)	(active form)	(the highest activity)

The highest activity obtained at pH 12.0 might be due to the accessibility of substrate to protoheme IX, which was caused by the destruction of the conformation of the enzyme protein. This considerable conformational changes of the enzyme at pH 12.0 and the pronounced narrowing properties of EPR signals at pH 12.0(Matsuda, Y., Beppu, T. and Arima, K., unpublished data) will also explain well the fact the change of the positional spacificity of hydroperoxidation depending on pH[11]. Perlmann[12] reported that the activation of pepsi-

nogen to pepsin was accompanied by the decrease of the content of an α-herical structure. Imahori *et al.* also reported that the herical content of carboxypeptidase A was decreased in the presence of Brij 35(polyoxyethylene lauryl ether), although the enzyme activity rather increased[13]. The reversible conformational change of Fusarium lipoxygenase between α-herical structure (not active form) at pH 6.0 and β structure(active form) at pH 9.0 might fit for the enzyme protein active in alkaline region, since β structure seems to be more stable in alkaline aolution than α-herical structure[14-16].

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