Advances in the Biochemistry and Physiology of Plant Lipids,
L. -Å. Appelqvist and C. Liljenberg, editors

THE CONVERSION OF 9-D- AND 13-L-HYDROPEROXYLINOLEIC ACID BY SOYBEAN LIPOXYGENASE-1 UNDER AEROBIC CONDITIONS

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ABSTRACT

A novel pathway for the conversion of hydroperoxylinoleic acid by soybean lipoxygenase-1 is described. During the reaction oxygen is consumed (0.7 mol/mol 13-L-LOOH*; 0.33 mol/mol 9-D-LOOH) which may explain that in the presence of oxygen much more polar material is formed than in its absence. Besides, epoxy-hydroxy- and oxodienoic fatty acids are formed. However, under aerobic conditions no 13-oxo-tridecadienoic acids are produced from 13-L-LOOH.

INTRODUCTION

Lipoxygenase-1 from soybeans catalyses the formation of fatty acid hydroperoxides from poly-unsaturated fatty acids (e.g. linoleic acid) and molecular oxygen. When oxygen is depleted another type of reaction starts viz. the coupled conversion of the fatty acid and the fatty acid hydroperoxide. In recent years the mechanism of these reactions has been studies with various techniques e.g. EPR and optical spectroscopy[1,2]. It has been established that, depending on the reaction conditions, different enzyme species can occur[3]. Following an investigation of the kinetics of the anaerobic reaction[4] a mechanism has been proposed that accounts for both the spectroscopic and kinetic evidence.

Recently, two novel pathways for lipoxygenase-catalysed conversions of hydroperoxylinoleic acid have been discovered[5,6]. When lipoxygenase-1 is incubated with either 13-L- or 9-D-hydroperoxylinoleic acid approximately 40% of the hydroperoxide is converted into oxodienes absorbing at 285 nm. Under such anaerobic conditions also more polar compounds are formed, the main constituent of which was shown to be *threo*-11-hydroxy-*trans*-12,13--epoxy-9-*cis*-octadecenoic acid.

*13-L-LOOH: 13-L-hydroperoxylinoleic acid
9-D-LOOH: 9-D-hydroperoxylinoleic acid

If, however, oxygen is admitted to a system containing lipoxygenase and hydroperoxylinoleic acid the reaction rate is considerably lower and a partly different set of products are formed. This paper describes the latter reaction in more detail.

MATERIALS AND METHODS

Lipoxygenase-1 was isolated from soybeans according to Finazzi-Agrò et al.[7], spec. activity: 230 µmol linoleic acid oxygenated per min per mg. 13-L-LOOH and 9-D-LOOH were prepared as described previously[5] and purified by high performance liquid chromatography[4]. Spectrophotometric measurements were carried out in a 1 cm pathlength cuvette at 25 C. The reaction was started by the addition of 200 µg of enzyme to 2.5 ml of a hydroperoxide solution in airsaturated 0.1 M sodiumborate buffer (pH 9.0). The change in absorbance at 285 nm or 234 nm was recorded with a Cary 118C spectrophotometer. Spectra were taken before and after reaction. Oxygen uptake was measured with a Clark oxygen electrode connected to a GME Oxygraph model KM (GME, Middleton, Wis., U.S.A.). The measuring cuvette was completely filled (2.4 ml) with air-saturated 0.1 M sodiumborate buffer (pH 9.0) and sealed with a rubber cap.

Preparative experiments were carried out by incubating 10 mg 13-L-LOOH or 9-D-LOOH with 32 mg lipoxygenase-1 in 400 ml air-saturated 0.1 M sodiumborate buffer (pH 9.0) at 25 C (LOOH: 80 µM, oxygen: 240 µM, enzyme: 0.8 µM). After completion of the reaction (9-D-LOOH: approx. 2.5 h, 13-L-LOOH approx. 17 h) the solution was acidified to pH 2 and extracted with diethylether. The extract was washed with water, dried and concentrated. The residue was esterified with diazomethane and subjected to thinlayer chromatography on 0.25 mm pre-coated silicagel plates (Silicagel 60 F-254, 20x20 cm, E. Merck, A.G., Darmstadt, Germany). Separation of the products was performed with the solvent system hexane-diethylether (60:40, $^{v}/v$). The compounds were located by spraying with phosphomolybdic acid (5% in ethanol, $^{w}/v$). 2,4-Dinitrophenylhydrazine (0.4% in 2 M HCl, $^{w}/v$) was used to identify carbonyl compounds and an acidified ferrous thiocyanate (0.2 g NH_4SCN in 15 ml acetone plus 10 ml of an acidified solution of $FeSO_4$ in water, 4% ($^{w}/v$))to identify hydroperoxy-compounds.

RESULTS AND DISCUSSION

Fig. 1 shows progress curves for the conversion of 13-L-LOOH by lipoxygenase-1 starting at an oxygen concentration of 5 µM.

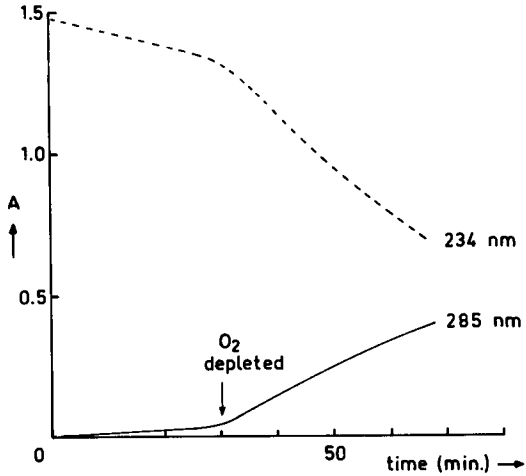

Fig. 1. Progress curves for the conversion of 13-L-LOOH by lipoxygenase-1 recorded at 234 nm and 285 nm. 13-L-LOOH: 60 μM, initial oxygen concentration: 5 μM, enzyme: 0.8 μM, 0.1 M sodiumborate buffer (pH 9.0), t = 25 C. Measurements were carried out as described previously (5).

From these curves recorded at 234 and 285 nm it appears that oxygen inhibits the rate of formation of oxodienoic acids (absorbing at 285 nm) as well as the rate of conversion of hydroperoxide itself. Moreover, the yield of oxodienoic acids per mol converted 13-L-LOOH was found to be reduced in the presence of oxygen. Apparently, oxygen reacts with intermediate radicals which leads to the formation of products other than oxodienoic acids. Therefore, the existence of a lag period in the anaerobic conversion of 13-L--LOOH by lipoxygenase-1 can be attributed to the inhibition by traces of oxygen, which are slowly consumed. In the presence of 240 μM of oxygen the conversion of hydroperoxide as well as the formation of oxodienoic acids were indeed found to proceed much slower than in the absence of oxygen (Table 1).

No reaction was observed with heat-inactivated lipoxygenase-1 (5 min at 85 C in a 2% sodiumdodecylsulphate solution). Fig. 2 shows ultra-violet spectra before and after the conversion of 13-L- and 9-D-LOOH. It appears that from 13-L-LOOH under aerobic conditions considerably less oxodienoic acids are formed than under anaerobic conditions (8% and 41% respectively), whereas with 9-D-LOOH the difference is less pronounced (see Table 1).

TABLE 1

COMPARISON OF THE AEROBIC AND ANAEROBIC CONVERSIONS OF 13-L-LOOH AND 9-D--LOOH BY LIPOXYGENASE-1

Anaerobic measurements were carried out as described previously (5) and aerobic experiments as described in Materials and Methods. 13-L-LOOH or 9-D-LOOH: 80 μM, oxygen in the aerobic experiments: 240 μM, enzyme: 0.8 μM (spec. act.: 230 μmol linoleic acid converted per min per mg) and 1.0 μM (spec. act.: 160 μmol linoleic acid converted per min per mg) in the aerobic and the anaerobic experiments respectively.
Buffer: 0.1 M sodiumborate (pH 9.0), t = 25 C.

	spec. act. nmol LOOH $min^{-1}mg^{-1}$	A_{285} formed %	A_{234} remaining %	O_2 uptake μM	spec. act. nmol O_2 $min^{-1}mg^{-1}$
13-L-LOOH	12	41	16	-	-
9-D-LOOH[a]	266	36	12	-	-
13-L-LOOH	3.0	8	16	56	2.3
9-D-LOOH[b]	20	26	15	26	6.6

[a] anaerobic
[b] aerobic

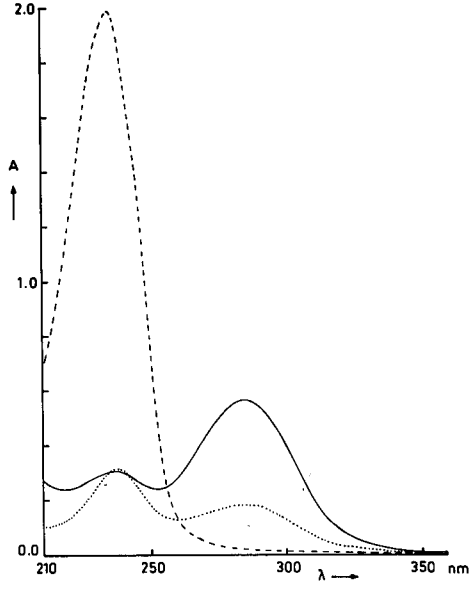

Fig. 2. Ultra-violet spectra before (-----) and after the aerobic conversion of 80 μM 13-L-LOOH (·····) and 80 μM 9-D-LOOH (———) by 0.8 μM lipoxygenase-1 in air-saturated 0.1 M sodiumborate buffer (pH 9.0) at 25 C.

The extent of the oxygen uptake during the reaction was found to be proportional to the concentration of the hydroperoxides, but it appeared to be different for the two isomeric hydroperoxides: 0.7 mol oxygen per mol 13-L--LOOH and 0.33 mol oxygen per mol 9-D-LOOH were consumded (Table 1). Fig. 3 shows thinlayer chromatograms of the products formed in the aerobic conversion of 13-L-LOOH and 9-D-LOOH by lipoxygenase-1.

To obtain information about the relative amounts of the products formed from 13-L-LOOH, incubations were carried out with (U-^{14}C)-labelled 13-L-LOOH as substrate. The results are given in Table 2.

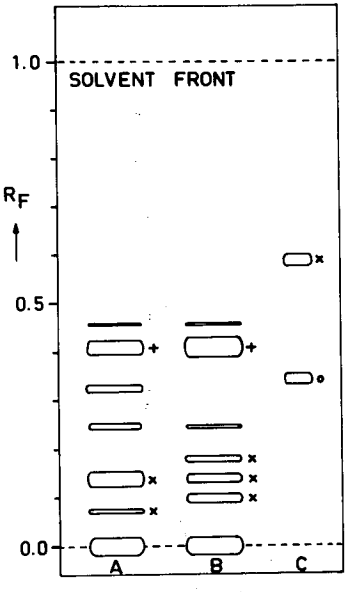

Fig. 3. Thin-layer chromatograms of the products formed in the aerobic conversion of 13-L-LOOH (A) and 9-D-LOOH (B) by lipoxygenase-1.
C: methyl-esters of 13-L-LOOH and 9-D-LOOH (lower band) and linoleic acid (upper band).
The compounds were located by spraying with phosphomolybdic acid. The compounds were visible under UV light unless indicated with: X
+: band coloured orange when sprayed with dinitrophenylhydrazine.
0: band coloured red when sprayed with ferrous thiocyanate.

TABLE 2

CONVERSION OF 13-L-[U-^{14}C]-LOOH BY LIPOXYGENASE-1 UNDER AEROBIC CONDITIONS

Experiments were carried out as described previously for anaerobic conditions[5]. Incubation time: 24 h. [13-L-(U-^{14}C)-LOOH]: 76 µM, [enzyme]: 4 µM, [oxygen]: 240 µM. buffer 0.1 M sodiumborate (pH 9.0). t = 25 C.

R_F value	% of total radioactivity	tentative assignment
origin	63.1	oxidative polymers
0.15	16.9	epoxy-hydroxy-octadecenoic acid
0.24	7.7	unknown
0.32	2.3	unknown
0.42	8.5	13-oxo-octadecadienoic acid
0.46	1.5	unknown

From the absence of 13-oxo-tridecadienoic acids it can be concluded that oxygen interferes with the chain-fission reaction which is observed in the anaerobic conversion. However, 13-oxo-octadecadienoic acid (R_F=0.42) appears to be formed in both the aerobic and the anaerobic reaction. The lower yield under aerobic conditions observed in the spectrophotometric experiments (Table 1) was confirmed by the results of the experiments with (U-^{14}C)-labelled 13-L-LOOH (Table 2). Considerably more material (63%) can be found at the origin under aerobic conditions suggesting that in the presence of oxygen oxidative polymerization might have occurred (0.7 mol oxygen consumed per mol 13-L-LOOH).

In case of 9-D-LOOH the product-pattern observed in the aerobic reaction does not differ markedly from that of the anaerobic reaction, but also here material has accumulated at the origin, probably for the same reason as with 13-L-LOOH as substrate.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. G.P.M. Rijke-Schilder for skilful technical assistance. This investigation was supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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