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ON THE POSITIONAL SPECIFICITY OF THE OXYGENATION REACTION CATALYSED BY SOYBEAN LIPOXYGENASE-1

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Summary

Lipoxygenase-1 from soybeans is incubated with an isomer of linoleic acid, 13-*cis*, 16-*cis*-octadecadienoic acid. Analysis of the oxygenation products indicates that molecular oxygen is stereospecifically introduced mainly at C-17 ($n-2$) of the fatty acid (in the L$_S$-configuration), and only to a minor extent at C-13 ($n-6$).

These findings contradict previous suggestions about the positional specificity of lipoxygenase-1.

Holman et al. [1] studied the fatty acid substrate specificity of soybean lipoxygenase-1 (EC 1.13.11.12) by incubating the enzyme with the whole series of isomeric octadecadienoic acids, in which the pentadiene system shifts from 2-*cis*, 5-*cis* to 14-*cis*, 17-*cis*. They found, that apart from the natural substrate linoleic acid (9-*cis*, 12-*cis*-octadecadienoic acid) also the 13-*cis*, 16-*cis* isomer is oxygenated by the enzyme at a considerable rate. When the oxygenation rate for linoleic acid was set at 100%, the rate for the 13,16-isomer was 50%, while the oxygenation rates for all other isomers were less than 25%.

The exceptionally high conversion rate for the 13,16-isomer was suggested by Holman et al. to arise from the fact, that this isomer contains a vinylic carbon at C-13, just as linoleic acid does.

This was taken as a further support [1,2] for the concept that lipoxygenase-catalysed oxygenation almost exclusively occurs at carbon atom 13 ($n-6$) of the unsaturated fatty acid substrates.

However, it was not verified via analysis of the oxygenation products whether indeed oxygenation at C-13 occurs in the case of the 13, 16-isomer.

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Since it would have interesting stereochemical consequences if the 13,16-isomer is oxygenated mainly at C-13, the coversion of this compound was studied in more detail.

Lipoxygenase-1 (purified according to Finazzi-Agrò et al. [3]) and 13-*cis*, 16-*cis*-octadecadienoic acid (synthesized and generously provided by Ir. R. Klok, Unilever Research Laboratories, Vlaardingen/Duiven, The Netherlands; purity 98.5% by gas-liquid chromatography) were incubated at 25°C and at pH 9.0 under conditions as previously described [4]. After approx. 90% conversion of the substrate 13,16-isomer, the incubation medium was acidified to pH 3 by adding 2 M HCl and was extracted three times with diethyl ether. The combined extracts were washed once with redistilled water and dried over anhydrous sodium sulphate.

Reaction products and unconverted substrate were converted into the corresponding methyl esters by treatment with diazomethane at 0°C and were separated by thin-layer chromatography on 0.25 mm slica gel plates ("Fertig platten", 20 × 20 cm, 60F254, E. Merck, Darmstadt, G.F.R.), developed with light petroleum (b.p. 60—80°C)/diethyl ether (3:2, v/v). The reaction products, appearing on the thin-layer plates as one main band (R_F 0.65) were scraped from the plates, eluted from the silica with diethyl ether and were identified as *cis-trans* conjugated hydroperoxy fatty acid methyl esters by spectroscopic (ultraviolet, infrared) and chemical analyses.

The hydroperoxides were directly converted into the corresponding hydroxystearates by PtO_2-catalysed hydrogenation in methanol [4] and were again subjected to thin-layer chromatography. (0.25 mm silica gel plates, developed with light petroleum (b.p. 60—80°C)/diethyl ether, 3:2, v/v). After treatment with I_2 vapour two bands appeared on the chromatogram; a minor fraction, which coincided with a standard of 13-hydroxystearate, and a large fraction, which was identified by mass spectrometry as the 17-hydroxystearate (Fig. 1). The ratio of the 17- and 13-hydroxystearates was found to be 85:15, respectively. From measurements of the optical rotation of the 17-hydroxystearate ($[\alpha]_D$ = + 3.1; *c*, 1.9 in methanol at 25°C; cf. ref. 5: $[\alpha]_D$ = + 4.6; *c*, 7.3 in methanol) it was derived, that mainly the 17-L$_S$-hydroperoxide was formed by the enzyme from 13-*cis*, 16-*cis*-octadecadienoic acid and O_2. Optical rotation measurements of the 13-hydroxystearate were inconclusive due to the minute amount of material available.

Thus, contrary to previous suggestions [2], oxygenation of 13-*cis*, 16-*cis*-octadecadienoic acid by lipoxygenase-1 at pH 9.0 leads mainly to the formation of the *cis-trans* conjugated 17-L$_S$-hydroperoxide, while a small amount of 13-hydroperoxides is formed, just as small amounts of largely racemic 9-hydroperoxides are formed from linoleic acid during formation of the 13-L$_S$-hydroperoxide [6].

This result indicates, that the enzymic (stereospecific) oxygenation is not restricted to C-13 ($n-6$) of the fatty acid substrates. Comparing the stereochemistry of the two oxygenation reactions and the type of products formed, it must be concluded that the pentadiene systems of both the 13,16-isomer and linoleic acid are oriented identically on the enzyme molecules and not in a head/tail-reversed orientation (cf. ref. 2). This suggests that hydrophobic interaction between the methylene groups outside the pentadiene system and

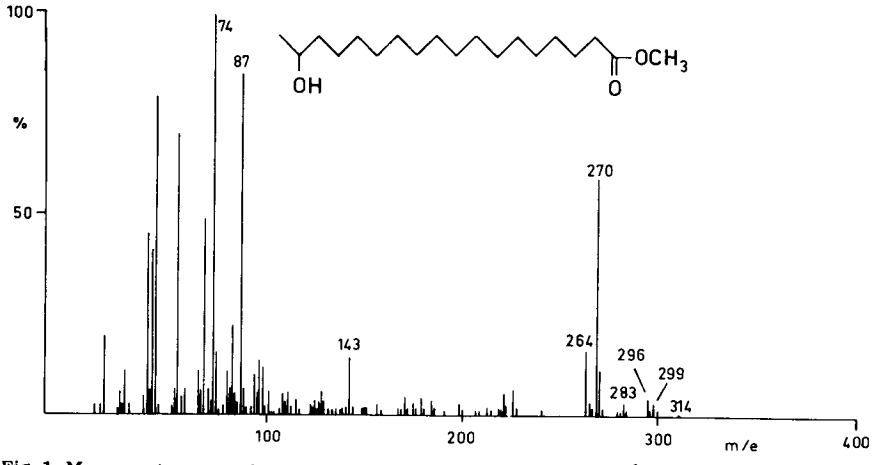

Fig. 1. Mass spectrogram of methyl 17-hydroxystearate (70 eV, 100°C).

the enzyme has no prominent effect on the specific interaction between the pentadiene system and the enzyme. It must be noted, that formation of the conjugated 17-L_S-hydroperoxide implies that a hydrogen is abstracted from C-15 (n-4) of the 13,16-isomer. Thus, also hydrogen abstraction is not restricted to a fixed C atom in the chain. (e.g. in the case of linoleic acid the L_{proS} H atom* is abstracted from C-11 (n—8) of the molecule [7].

The low oxygenation rates found by Holman et al. [1] for the other isomeric octadienoates, especially for those having a long terminal group attached to the pentadiene system (i.e. more than 6 C atoms long), may indicate that a long terminal group — in contrast to a long proximal group — affects the reaction rate, possibly by preventing binding of the pentadiene system to the active site of the enzyme molecule.

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*The methylene groups of linoleic acid and its positional isomers are pro-chiral. Consequently, H atom of a methylene group is either pro-S or pro-R [8]. In addition, the Fischer convention is used (L or D) to designate the positions of the H atoms.