POSITIONAL SPECIFICITY OF CORN GERM LIPOXYGENASE AS A FUNCTION OF pH.

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SUMMARY

The positional specificity of partially purified corn germ lipoxygenase was investigated as a function of pH with linoleic acid as the substrate. At pH 9.0 predominantly 13-hydroperoxy-9-cis,11-trans-octadecadienoic acid (85 %) was formed whereas at pH 6.6 the specific formation of 9-hydroperoxy-10-trans, 12-cis-octadecadienoic acid (80 %) by the same preparation was observed. The 13-hydroperoxide which is formed on incubation at pH 9.0 has predominantly L-configuration.

INTRODUCTION

Crude soybean lipoxygenase (Linoleate: oxygen oxido-reductase; E.C. 1.13.1.13) contains at least two iso-enzymes (Guss et al[1]; Christopher et al[2]) which display different positional specificities in the oxygenation of linoleic acid (Christopher & Axelrod[3]). Soybean lipoxygenase-1 was reported to be almost 100 % specific for the formation of 13-hydroperoxy-octadecadienoic acid from linoleic acid at pH 9.0 whereas soybean lipoxygenase-2 formed a mixture of equal amounts of the 9- and 13-isomers at pH 6.6. As the pH-optima for lipoxygenase-1 and -2 differ considerably, incubation of unfractionated soybean lipoxygenase preparations with linoleic acid at either pH 9.0 and pH 6.6 should bring about a shift in the 9:13 ratio due to the different activities of the constituent iso-enzymes at pH 9.0 and 6.6 respectively. This has indeed been observed by Galliard & Phillips[4] and by us[5].

Lipoxygenase from corn germs has been investigated recently by Gardner & Weisleder[6] and by Hamberg[7] and the enzyme was found to be specific (83-89 %) for the formation of 9-D-hydroperoxy-10-trans, 12-cis-octadecadienoic acid from linoleic acid at pH 6.5 and 7.4.

In order to establish whether the product ratio formed from linoleic acid by corn germ lipoxygenase varies with the experimental conditions in a similar way as has been found with soybean lipoxygenase, a partially purified corn germ lipoxygenase preparation was incubated with linoleic acid at both pH 6.6 and 9.0 and the reaction products were analysed with respect to structure and configuration.

were recorded with a Unicam-SP800-B or a Zeiss-PMQII spectrophotometer and IR-spectra with a Beckman IR-8 instrument.

RESULTS AND DISCUSSION

Incubation at pH 6.6. The TLC-pattern of the mixture of fatty acid methyl esters obtained after treatment of the reaction products with $NaBH_4$ in methanol is given in Fig. 1A. The main spot in the hydroxyoctadecadienoate region was isolated. After hydrogenation the mass spectrum showed characteristic peaks of methyl 9-hydroxystearate at m/e 187, 158 and 155. Optical rotation measurements of the hydroxyoctadecadienoate yielded $[\alpha]_{546}^{25} = +4.2^{o}$ (c, 4.6; CH_3OH) which is in agreement with the results reported earlier by Gardner & Weisleder[6], pointing to the presence of the 9-D-isomer, and with those obtained by Hamberg[7] following an independent route. Both approaches demonstrated a high degree of specificity for the formation of the 9-D-hydroperoxide from linoleic acid by corn germ lipoxygenase at low pH-values. Mass spectrometry of the mixture of unfractionated methyl hydroxystearates indicated that it consisted of 80 % 9- and 20 % 13-hydroxystearates.

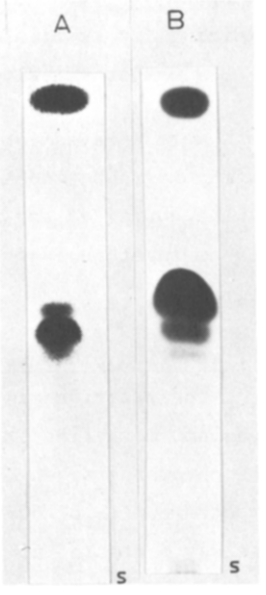

Fig. 1.

TLC-patterns obtained after $NaBH_4$-treatment of the reaction products of corn germ lipoxygenase and linoleic acid at pH 6.6 (A) and pH 9.0 (B). Solvent system: light petroleum (b.p. 60-80°C): diethylether, 3:2 (v/v).

MATERIALS & METHODS

Linoleic acid (purity > 99 %) was a gift from the Unilever Research Laboratories, Vlaardingen/Duiven, The Netherlands.

Lipoxygenase preparation. A corn germ (Zea mays, var. Caldera) lipoxygenase was prepared from an extract of acetone-powders of ground corn germs by collecting the fraction precipitating between 30-60 % $(NH_4)_2SO_4$-saturation and subsequent fractionation on a Sephadex-G 100 (Superfine) column (80 cm x 2.5 cm) with 0.05 M pyridine-acetate buffer (pH 4.9) as eluent. Pooled samples of the active fractions did not contain hydroperoxide-isomerase activity[8] as on incubation with purified linoleic acid hydroperoxides no conversion into oxo-hydroxy-compounds was detectable.

Incubation procedure. Incubations of lipoxygenase with linoleic acid were carried out at 0° C in an oxygen atmosphere. The NH_4^+-salt of the substrate was dissolved in either 0.04 M NH_4OH-NH_4Cl buffer solution (pH 9.0) or a 0.1 M KH_2PO_4-K_2HPO_4 buffer solution (pH 6.6) to a final substrate concentration of 1.8 mM. Neither emulsifying agents nor Ca^{2+}-salts were added. 3 ml of enzyme preparation was capable of oxidizing 100 mg linoleic acid for 82 % and 67 % in 90 min. at pH 6.6 and pH 9.0 respectively.

Lipoxygenase-assay. Preparations were assayed for lipoxygenase activity with a Clark-oxygen-electrode in a GME-Oxygraph, model KM, with 1.8 mM. NH_4^+-linoleate-solutions. In preparative work the course of the reaction was followed by measuring the absorbancy at 234 nm in ethanol of small aliquots from the reaction mixture.

Isolation and identification of reaction products. The reaction products were recovered after acidification of the reaction mixture by extraction with diethylether and were converted into methyl-esters with CH_2N_2. The mixture of fatty acid methyl esters was chromatographed on 0.3 mm silicagel-G plates with the solvent system light petroleum (b.p. 60-80° C): diethylether, 7:3 (v/v). Reduction of the hydroperoxides was performed at 0° C with $NaBH_4$ in methanol. The unsaturated hydroxy-fatty acid methyl esters were hydrogenated with PtO_2 as catalyst in methanol. Thin-layer chromatography of the hydroxy-fatty acid methyl esters was carried out on silicagel-G with the solvent system light petroleum (b.p. 60-80° C): diethylether, 3:2 (v/v). Proton-magnetic resonance (PMR)-spectra were recorded with a Varian XL-100 instrument in $CDCl_3$ with tetramethylsilane as internal reference and mass spectra with an AEI-MS9 instrument. Optical rotations were measured with a Perkin Elmer 141 polarimeter (1 dm tube). UV-spectra

Incubation at pH 9.0. The TLC-pattern of the reaction mixture of corn germ lipoxygenase with linoleic acid at pH 9.0 after $NaBH_4$-treatment is given in Fig. 1B.

After isolation and hydrogenation of the main product the mass spectrum showed peaks of high intensity at m/e 211, 214 and 243 demonstrating that the hydroxyl-group is located at C-13.

In order to establish the configuration of the double bonds the 100 Mc. PMR-spectrum was recorded and found to be identical with that from 13-D-hydroxy--9-cis,11-trans-octadecadienoic acid methyl ester (D-coriolic acid)[9].

Optical rotation measurements before hydrogenation yielded: $[\alpha]_{578}^{25} = +13.3^{o}$ (c, 2.6; CH_3OH) and after complete hydrogenation: $[\alpha]_{578}^{25} = 0.36^{o}$; $[\alpha]_{546}^{25} = +0.44^{o}$ (c, 2.5; CH_3OH). On the basis of these data we conclude that at pH 9.0 this corn germ preparation forms predominantly 13-L-hydroperoxy-9-cis, 11-trans-octadecadienoic acid from linoleic acid.

Mass spectra of an unfractionated mixture of methyl hydroxystearates obtained from an incubation at pH 9.0 indicated that 85 % was 13-hydroxystearate and the remaining 15 % 9-hydroxystearate. IR-spectroscopy of the hydroxydienoates occurring as by-products from the reaction at pH 9.0 (spots with somewhat lower R_f-values in the hydroxydienoate region, Fig. 1B) indicated the presence of both cis,trans- and trans,trans-conjugated double bonds.

It thus appears that the positional specificity in the oxygenation of linoleic acid by corn germ lipoxygenase is extremely dependent on the pH of the reaction medium.

This phenomenon either reflects the influence of pH on the relative activities of two iso-enzymes present in this preparation or a sensitivity of the active site of one single enzyme for the pH of the reaction medium, resulting in the observed change in product specificity.

However, in view of the results reported on the different positional specificities of soybean lipoxygenase iso-enzymes[3] it seems more likely that also corn germ lipoxygenase consists of a mixture of lipoxygenase-isoenzymes with different catalytic properties.

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