

Substrate Specificity of Tea Leaf Hydroperoxide Lyase

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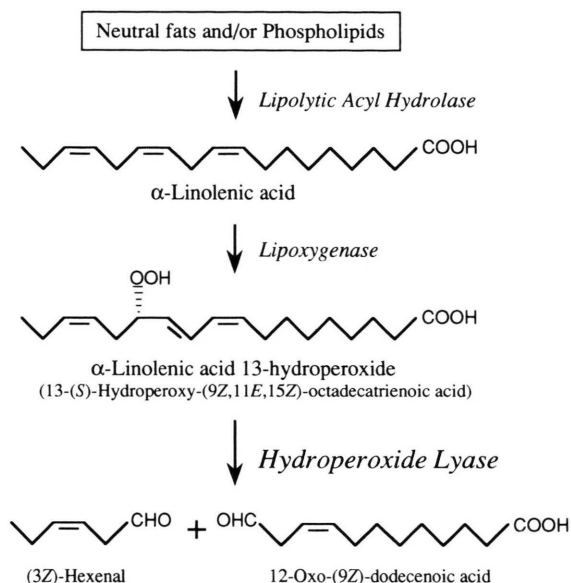
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Aldehyde, Fatty Acid Hydroperoxide, Hydroperoxide Lyase, Substrate Specificity, Tea Leaf

Substrate specificity of tea leaf fatty acid hydroperoxide lyase was systematically investigated using an entire series of ω 6-(*S*)-hydroperoxy- C_{14} - C_{24} dienoic and trienoic acids as substrates. Unexpectedly, the hydroperoxides of C_{22} but not natural substrates, *i.e.*, those of C_{18} , showed the highest reactivities for the lyase. The reactivities of the hydroperoxides of trienoic acids were always four to ten times higher than those of the dienoic acids.

Introduction

Flavors described as grassy, beany and leafy have been attributed to *n*-hexanal, (2*E*)-, (3*E*)- and (3*Z*)-hexenals which evolve from plant tissues upon wounding or homogenization. We have already revealed that these volatile aldehydes are synthesized from neutral fats or phospholipids by an enzyme system containing lipolytic acyl hydrolase, lipoxygenase and fatty acid hydroperoxide lyase (HPO lyase) as shown in Scheme 1 [1]. Although cleavage reaction catalyzed by HPO lyase is of considerable importance to food technologists, the properties of this enzyme have been scarcely reported. Recently, the HPO lyase was purified to a homogeneous state from tea leaves and its properties were elucidated [2]. The leaf HPO lyase catalyzes cleavage of 13-hydroperoxy fatty acid (13-HPO) to form C_6 -aldehydes and C_{12} -oxo acid. The reactivity of α -linolenic acid 13-HPO was about 10 times higher than that of linoleic acid 13-HPO, but the positional isomer, γ -linolenic acid 13-HPO was a relatively poor substrate. The structural requirements of substrates for tea leaf HPO lyase might be that they contain a *Z,E*-conjugated diene system and that the *E* double bond is adjacent to the (*S*)-hydroperoxy-methine [3]. In this study, we intended to elucidate the substrate recognition mechanism of tea leaf HPO lyase on the basis of comparison of reactivities of ω 6-HPOs prepared from the entire series of (ω 6*Z*, ω 9*Z*)- C_{14} - C_{24} -dienoic acids and (ω 3*Z*, ω 6*Z*, ω 9*Z*)- C_{14} - C_{24} -trienoic acids.



Scheme 1. Biosynthesis of green odor in tea leaves. Biosynthetic pathway *via* α -linolenic acid is shown. In this pathway *via* linoleic acid, *n*-hexanal is formed instead of (3*Z*)-hexenal (in detail, see ref. [1]).

Materials and Methods

Materials

Linoleic acid, (11*Z*,14*Z*)-icosadienoic acid, α - and γ -linolenic acids, and (13*Z*,16*Z*,19*Z*)-docosatrienoic acid were purchased from Sigma Chemical Co. (ω 6*Z*, ω 9*Z*)- C_{14} - C_{24} -dienoic and (ω 3*Z*, ω 6*Z*, ω 9*Z*)- C_{14} - C_{24} -trienoic acids were synthesized by the methods described in previous paper [4, 5].

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Preparation of fatty acid ω 6-hydroperoxides

Soybean lipoxygenase-1 (300 U) purified as described in ref. [6] was added to a fatty acid (50 mg) suspended with 50 mM borate buffer, pH 9.0 (300 ml) and incubated for 10 h at 4 °C under O₂ atmosphere. The reaction mixture was acidified with 2 N hydrochloric acid, and was extracted with ether (100 ml \times 3). The extracted HPO was purified by a silica gel column chromatography (*n*-hexane/ether gradient) and stored as a solution of ethanol below -20 °C.

Analyses of hydroperoxides

Ethereal diazomethane was added to HPO (1 mg) in ether (3 ml) below -10 °C. Triphenylphosphine (1 mg) was added to the methyl ester, and the mixture was stirred for over 1 h at room temperature. Compositions of positional and geometrical isomers of HPOs were analyzed in the form of the hydroxymethyl ester by HPLC essentially as described in ref. [4, 5]. Configurations at ω 6-carbon of HPOs were analyzed by gas chromatography of the reductive ozonolysis products of α -MTPA derivatives of the hydroxymethyl ester [7]. The steric purities of HPOs used as substrates for HPO lyase, were summarized in Table I.

Enzyme assay

HPO lyase was purified from tea leaves according to the previous report [2]. Reactivities of the substrates were assayed spectrophotometrically by following decrease of the absorbance at 234 nm. The substrate solution (10 μ l, 5 mM in ethanol) was added to 0.1 M potassium phosphate buffer, pH 7.5 containing an appropriate amount of the purified hydroperoxide lyase to start the reaction at 25 °C. Products were identified by GC, GC-MS and HPLC analyses essentially as described in ref. [8].

Results and Discussion

The entire series of substrates, ω 6-(*S*)-HPOs, used here were prepared by soybean lipoxygenase-1-catalyzing oxygenation of the corresponding fatty acids regio- and enantiospecifically (Table I, [4, 5]). However, some variations in the specificity was also inevitable and the further purification of HPOs were failed.

With both the dienoic and trienoic acid HPOs, tea leaf HPO lyase showed broad substrate specificity. Elongation from C₁₈ (natural substrate) to C₂₂ of the carbon chain between the terminal car-

Table I. Geometrical and optical purity of ω 6-hydroperoxy-(ω 7*E*, ω 9*Z*)-dienoic acids and ω 6-hydroperoxy-(ω 3*Z*, ω 7*E*, ω 9*Z*)-trienoic acids.

Total carbon number	ω 6-(<i>S</i>)-Hydroperoxy-dienoic acid*		ω 6-(<i>S</i>)-Hydroperoxy-trienoic acid**	
	Geometrical purity [%]	Optical purity [%e.e.]	Geometrical purity [%]	Optical purity [%e.e.]
14	86.8	60.0	85.3	91.6
15	94.4	70.8	88.6	90.4
16	99.3	51.4	87.2	98.0
17	95.3	89.6	89.1	83.2
18	96.3	84.0	99.7	72.4
19	95.0	90.2	88.2	75.2
20	93.6	93.0	92.7	98.0
21	90.2	86.6	91.3	88.8
22	92.6	85.0	97.1	85.4
23	86.1	82.0	78.4	84.2
24	75.6	71.0	91.7	72.6

* ω 6-(*S*)-Hydroperoxy-(ω 7*E*, ω 9*Z*)-dienoic acid.

** ω 6-(*S*)-Hydroperoxy-(ω 3*Z*, ω 7*E*, ω 9*Z*)-trienoic acid.

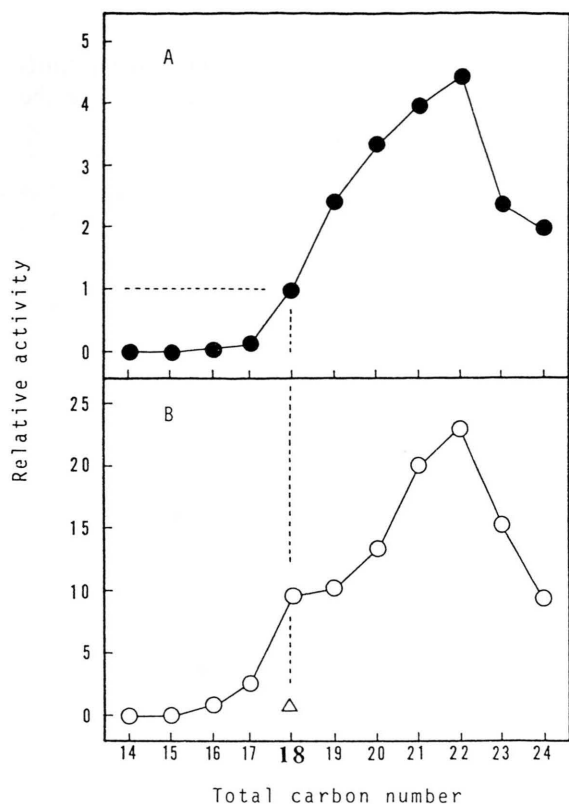


Fig. 1. Substrate specificity of tea leaf fatty acid hydroperoxide lyase. The reactivity of a substrate relative to that of linoleic acid 13-hydroperoxide is plotted against total carbon number. Panel A: relative activities of ω 6-hydroperoxy-(ω 7E, ω 9Z)-dienoic acids; panel B: ω 6-hydroperoxy-(ω 3Z, ω 7E, ω 9Z)-trienoic acids. Reactivity of ω 6-hydroperoxy-(ω 7E, ω 9Z, ω 12Z)-octadecatrienoic acid (γ -linolenic acid 13-hydroperoxide) is also plotted in panel B (Δ). Note that the scales in panels A and B are different.

boxyl group and the hydroperoxy group caused enhancement of the activity for tea leaf HPO lyase. However, more elongation than C₂₂-HPO decreased the activity. It is noticed that reactivities of the trienoic acid HPOs were always four to ten times higher than those of the dienoic acid HPOs having the same carbon number. These indicate that introduction of a double bond between C_{ω3} and C_{ω4} positions was very effective to increase the activity. It is assumed that the compact turning of the side arm at the ω -terminal end caused by a double bond would facilitate the recognition by HPO lyase. While, γ -linolenic acid 13-HPO was catalyzed at a rate of only about 2% of α -linolenic acid 13-HPO (Fig. 1).

In summary, the recognition of the chain length ranging from the ω 10-carbon to the terminal carboxyl group was not so strict for tea leaf HPO lyase, particularly when the length is longer than that of linoleic or linolenic acid. But introduction of a double bond into this side arm decreased the activity strikingly.

Acknowledgements

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