Concentration of Hydroperoxide Lyase Activities in Root of Cucumber Seedlings

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A study on distribution of hydroperoxide lyase activities in cucumber seedlings was performed. Hydroperoxide lyase activities were at elevated level in root, and in the upper and greener sections the lower activities were observed.

Introduction

Hydroperoxide lyases cleave hydroperoxide of linoleic and linolenic acid to form C_6-aldehyde and/or C_9-aldehyde. These enzymes are widespread in plants [1] but there exist various types of substrate specificity. HPO lyase solubilized from tea chloroplasts can act on 13-HPO but not on 9-HPO [2] and *vice versa* in the case of the enzyme partially purified from pear fruits [3]. Extracts of *Phaseolus vulgaris* leaves can cleave both 9-HPO and 13-HPO [4]. To elucidate the physiological role of these enzymes and products, *i.e.* C_6- and C_9-aldehydes, we have been searching for suitable enzyme sources. In this way we found that extracts of cucumber seedlings could cleave both 9- and 13-HPO at various efficiencies peculiar to each organ.

Materials and Methods

Cucumber (*Cucumis sativus* L. cv. Suyo) seeds were soaked overnight in tap water and germinated under fluorescent light (7700 lx) with a 14 h-photoperiod at 25 °C. Nine day-old seedlings were homogenized in a chilled mortar with 2 volumes of 0.1 M phosphate buffer, pH 6.4, containing 1 mM EDTA. The homogenate was filtered through 4 layers of cheese cloth, and the filtrate was used as the enzyme solution.

9-HPO (containing 15% of geometrical and positional isomers of the hydroperoxides) and 13-HPO (containing 10% of geometrical and positional isomers of the hydroperoxides) were prepared from linoleic acid (purified once on a SiO_2 column, Wako Chemicals, Osaka, 95% pure) as previously described, using potato lipoxygenase from potato tuber [5] and soybean lipoxygenase (Sigma, type I) [6], respectively.

Hydroperoxide lyase activity was determined from the quantity of the 2,4-dinitrophenylhydrazone derivatives of the products by HPLC (Hitachi 655 A-11) equipped with a Zorbax-ODS column (4.6 mm ∅ × 150 mm, Dupont) with detection at 350 nm. As a carrier solvent acetonitrile-tetrahydrofuran-water (80:1:19, flow rate, 1 ml/min) was used. Hydroperoxide (4 μmol) or linoleic acid (20 μmol) dissolved in ether was poured into a 50 ml-Erlenmeyer flask and after evaporation of the solvent with N_2 flow, 1 ml of the enzyme solution and 4 ml of homogenization buffer were mixed. After 30 min of incubation at 25 °C, 15 ml of 0.1% 2,4-dinitrophenylhydrazine in ethanol containing acetic acid (0.5 M) was added to reaction mixtures. The resultant hydrazone derivatives were extracted with hexane and concentrated *in vacuo*. The concentrate was dissolved in chloroform and an aliquot of the chloroform solution was used for HPLC analysis. For quantification, calibration curves constructed with authentic hexanal and (*E*)-2-nonenal (Wako Chemicals, Osaka) were used. Protein was determined by the Lowry method [7].

Results and Discussion

We have reported previously that etiolated cucumber seedlings have HPO lyase activities [8]. To investigate the activity distribution of HPO lyase within cucumber seedlings, 9-day-old seedlings were divided into four sections, *i.e.* cotyledons, hypocotyl, 1-cm section at the junction of root and hypocotyl, and root, and HPO lyase activities in each section were analyzed by HPLC quantification (Table I). The specific activities of both 9-HPO lyase and 13-HPO lyase were found to be the highest in the root. Cotyledons had the lowest activities of both lyases. The activities of C_6- and C_9-aldehyde formation from linoleic acid, which involve two enzymes of lipoxy-

Abbreviations: 13-HPO, 13-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid; 9-HPO, 9-hydroperoxy-(10*E*,12*Z*)-octadecadienoic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography.

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Table I. Hydroperoxide lyase activity distribution within cucumber seedlings (only specific activities are given).

Sections	Hexanal		(*E*)-2-nonenal	
	[nmol/mg protein/30 min]			
whole seedling	65.7	(155)*	82.8	(44.6)
cotyledons	60.9	(380)	20.6	(9.40)
hypocotyl	183	(421)	134	(76.4)
hypocotyl-root junction	324	(544)	201	(117)
root	339	(549)	424	(338)

Crude extract of each section was reacted with 13-HPO (4 μmol; for detection of hexanal) or 9-HPO (4 μmol; for detection of (*E*)-2-nonenal) for 30 min at 25 °C.
* Numbers given in parentheses represent the amount of each aldehyde formed by the addition of 20 μmol of linoleic acid.

genase and HPO lyase, varied almost parallel to those from the respective HPO. The amount of (*E*)-2-nonenal formed from 9-HPO was about a third of that of hexanal formed from 13-HPO, although, the amount of (*E*)-2-nonenal formed from linoleic acid in cotyledons was only one-fortieth of that of hexanal formed from the same substrate. This characteristic partiality for hexanal formation may account for product specificity of lipoxygenase localized in this tissue. On the other hand, the low level of the activity in whole seedlings was evident, so that an inhibitory factor localized in a certain tissue was expected. For example, the amount of (*E*)-2-nonenal formed from linoleic acid by a mixture of equal amount of the enzyme solutions extracted from root and cotyledons was only 40 to 70% of the sum of the amount formed individually. An inhibitory factor like this has been observed in cotyledons of tea seedlings [9].

It is the first time to detect 9-HPO lyase in root (Table I). Vick and Zimmerman [10] observed high specific activity of 13-HPO lyase in root of watermelon seedlings, though they detected exclusively high activity in hypocotyl-root junction.

The proportion of the activity of 9-HPO lyase to that of 13-HPO lyase is not the same within each section, and these proportions peculiar to each section were almost constant from 3-day-old to about 10-day-old seedlings (results not shown). These results indicate that the enzyme system which generates volatile aldehydes is not a simple but a complex one including isozymes differing in substrate specificities. The data presented in Table I were obtained by examinations performed at one pH of 6.4. Thus, changes of activities at various pHs were examined as shown in Fig. 1. The pH-dependence curve of 9-HPO lyase in root has a distinct peak around pH 7.5 to 8.0. To our knowledge HPO lyase having the optimal pH in the alkaline region has not been known so far. The optimal pH at 6.4 was reported for 9-HPO lyase in cucumber fruits [11]. This difference in optimal pHs between tissues suggests that 9-HPO lyase in root of cucumber seedlings is differs from that in cucumber fruits. 13-HPO lyase in cotyledons provides a blunt pH-dependence curve having a peak at around 6.0 to 6.5 and a shoulder at around 7.5 to 8.0. The pH-dependence curve obtained for 9-HPO lyase in cotyledons does not show a distinct peak, which means 9-HPO lyase in cotyledons is not the same enzyme localized in root.

With these results, the concentration of HPO lyase, especially 9-HPO lyase, in root of cucumber was

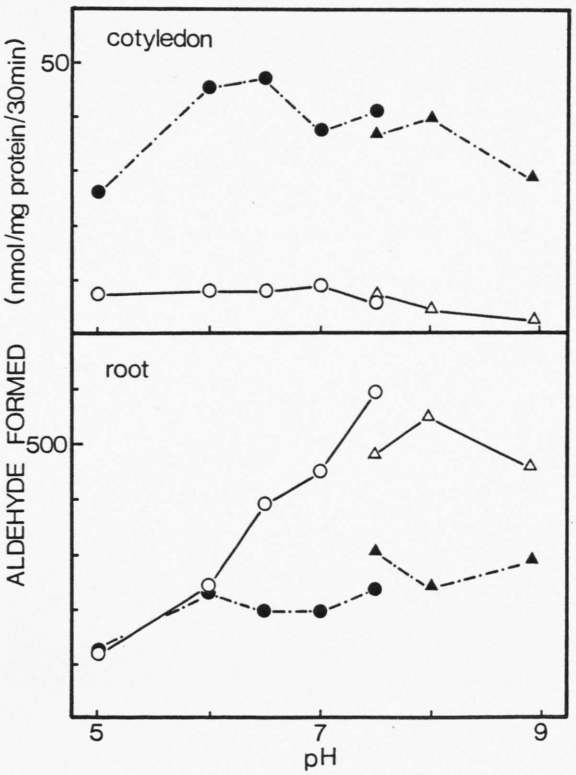

Fig. 1. pH-Dependence curves of the activities of 13-HPO lyase and 9-HPO lyase. (●), 13-HPO lyase activity detected at pH 5.0 to 7.5 (McIlvaine's buffer); (▲), 13-HPO lyase activity detected at pH 7.5 to 8.8 (50 mM Tris-HCl); (○), 9-HPO lyase activity detected at pH 5.0 to 7.5 (McIlvaine's buffer); (△), 9-HPO lyase activity detected at pH 7.5 to 8.8 (50 mM Tris-HCl).

ascertained even at various pHs ranging 5 to 9. This concentration of HPO lyase in root of cucumber seedlings may have important physiological and pathological roles. For example, anti-fungal effect of volatile aldehydes [12] and hormonal effect of oxo-acids (counterparts of hydroperoxides cleaved by HPO lyases) [13] have been observed under natural conditions. The investigations to obtain further evidences of these roles together with isozymes relationships are now under way.

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