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DISTRIBUTION OF LIPOXYGENASE AND HYDROPEROXIDE LYASE IN THE LEAVES OF VARIOUS PLANT SPECIES

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Key Word Index—Various plant species; leaves; volatile C_6-aldehydes; lipoxygenase; hydroperoxide lyase; distribution.

Abstract—The enzyme activity responsible for volatile C_6-aldehyde formation was accompanied by lipoxygenase and hydroperoxide lyase in the green leaves of 28 plant species tested, but the level of each enzyme's activity varied. Lipoxygenase activity rather than hydroperoxide lyase activity appears to affect the overall C_6-aldehyde formation. There was a positive correlation ($r = 0.712$) between hydroperoxide lyase activity and the chlorophyll content of the green leaves; no correlation was found between lipoxygenase activity and chlorophyll content.

INTRODUCTION

Many higher plants have the ability to produce the C_6-aldehydes, hexanal and *trans*-2-hexenal, which with their alcohols are responsible for the characteristic odour of green leaves [1]. The major biosynthetic pathway for these C_6-aldehydes is one of sequential peroxidation and cleavage of linoleic and linolenic acids through their 13-hydroperoxides as intermediates [2, 3]. Lipoxygenase and hydroperoxide lyase (HPO lyase) are responsible for these reactions. Lipoxygenase is present in many plant tissues, including seeds, vegetables and fruits [2, 4, 5], and HPO lyase also has been found in some plant tissues [6–10].

In a previous paper, we reported that the enzyme activities for C_6-aldehyde formation are found in a wide range of higher plants [11]. No correlations for lipoxygenase, HPO lyase and the overall C_6-aldehyde formation were made. We here describe the distribution of lipoxygenase and HPO lyase in the green leaves of various plants.

RESULTS AND DISCUSSION

Enzyme activities that function in C_6-aldehyde formation were determined for 28 plant species (Table 1). Most species tested showed C_6-aldehyde-forming activity, but the values varied. All the plants had both lipoxygenase and HPO lyase activity. This is evidence that the C_6-aldehyde-forming enzyme system in green leaves in general is composed of lipoxygenase and HPO lyase, as it is in tea [3], watermelon [6], tomato [7] and pear [9].

The amount of lipoxygenase activity varied with the plant species, as also has been reported by Pinsky *et al.* [5]. Cabbage and lettuce, which are used raw as food, had low lipoxygenase activities (Table 1). HPO lyase was widespread in green leaves in relatively high amounts (Table 1), but leaves with low chlorophyll contents had low levels of HPO lyase activity. When a correlation coefficient (r) was calculated between HPO lyase activity and chlorophyll content from the values ($n = 37$) in Table 1 and for the green leaves of *Phaseolus vulgaris* [12], an r value of 0.712 was obtained (Fig. 1). There was no correlation found between lipoxygenase activity and chlorophyll content. This suggests the possibility that the degree of HPO lyase activity depends on some function of the chloroplasts. A linear regression equation, $Y = 2.85X + 0.39$, was obtained by the least square method for HPO lyase activity (Y) and the chlorophyll content (X) (Fig. 1). When the value 0 was extrapolated to X in the equation, Y was 0.39. This value indicates the existence of a chlorophyll-independent HPO lyase. Thus, HPO lyase probably exists in multiple forms; a chlorophyll-dependent form in chloroplasts and a chlorophyll-independent one in nonphotosynthetic organelles and membranes. In fact, HPO lyase activity has been found in various non-green tissues [6, 7, 9, 10, 12, 13].

The C_6-aldehyde-forming activity that resulted from the sequential actions of lipoxygenase and HPO lyase varied widely. Ginkgo (Gymnospermae), edible vegetables and mulberry belong to a group with low C_6-aldehyde-forming activity. The mulberry leaf homogenate was very viscous and turned brown immediately, probably because of a large amount of phenolics which may have

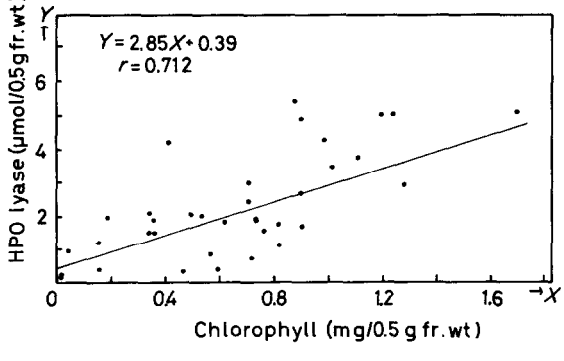

Fig. 1. Correlation between HPO lyase activity and the chlorophyll content of green leaves. Values for HPO lyase activities and chlorophyll contents are taken from Table 1 and ref. [12].

Table 1. Lipoxygenase, HPO lyase and C_6-aldehyde-forming activity in the green leaves of various plants

Plant	Lipoxygenase (μmol O_2/min·0.5 g fr. wt)	HPO lyase (μmol/0·5 g fr. wt)	C_6-aldehyde-forming activity (μmol/0·5 g fr. wt)	Chlorophyll (mg/0·5 g fr. wt)	Date harvested
Ginkgo (*Ginkgo biloba*)	0.10	0.31	0.01	0.474	July
Pumpkin (*Cucurbita maxima*)	0.24	2.08	0.01	0.499	July
Watermelon (*Citrullus vulgaris*)	2.75	4.89	5.21	0.904	July
Cabbage (*Brassica oleracea* var. *capitata*)	0.04	0.18	Trace	0.020	September
Chinese cabbage (*Brassica napus*)	0.14	0.17	Trace	0.030	September
Tea (*Thea sinensis*)	0.30	2.12	1.08	0.343	June
Camellia (*Camellia japonica*)	0.50	1.98	0.83	0.197	June
Sasanqua (*Camellia sasanqua*)	0.20	0.39	0.04	0.160	June
Japanese persimmon (*Diospyros kaki*)	0.10	1.50	0.26	0.343	July
False acacia (*Robinia pseudoacacia*)	0.40	1.71	0.36	0.913	July
Alfalfa (*Medicago sativa*)	0.53	3.07	1.66	0.712	June
White clover (*Trifolium repens*)	1.70	3.00	3.00	1.287	July
Soybean (*Glycine max*)	2.70	2.06	1.08	0.540	July
Kidney bean (*Phaseolus vulgaris*)	0.38	5.01	0.81	1.250	June
Holly (*Ilexintegra thunb*)	0.20	0.89	0.37	0.579	July
Mulberry (*Morus bombycis*)	1.92	0.92	0.03	0.060	July
Sweet gum (*Liquidambar styraciflua*)	0.58	1.55	0.79	0.356	July
Spinach (*Spinacia oleracea*)	0.25	4.25	Trace	0.420	September
Potato (*Solanum tuberosum*)	0.17	2.42	0.03	0.713	June
Sweet potato (*Ipomoea batatas*)	0.40	1.92	0.47	0.364	July
Tobacco (*Nicotiana tabacum*)	1.31	0.72	0.91	0.720	August
Eggplant (*Solanum melongena*)	0.91	1.96	0.19	0.741	June
Tomato (*Lycopersicon esculentum*)	0.37	1.84	0.48	0.630	June
Sunflower (*Helianthus annuus*)	0.05	1.58	0.60	0.770	July
Burdock (*Arctium lappa*)	1.20	1.92	0.48	0.740	July
Lettuce (*Lactuca sativa*)	0.08	1.19	Trace	0.170	September
Rice (*Oryza sativa*)	0.13	0.40	0.22	0.598	July
Corn (*Zea may*)	0.55	1.10	0.43	0.830	June

Table 2. Enzyme activities in the green leaves and fruit of tomato plants

Tissue	Colour	Protein (mg/g fr. wt)	Chlorophyll (μg/g fr. wt)	Lipoxygenase (μmol/min·g fr. wt)	HPO lyase (μmol/g fr. wt)	C_6-aldehyde-forming activity (μmol/g fr. wt)
Leaves	Green	114	1220	0.42	1.16	0.82
Unripe fruit	Green	33	40	0.20	0.68	Trace
Ripe fruit	Red	41	6	1.54	0.32	Trace

reacted with the aldehydes. The leaves in this group showed low lipoxygenase activity rather than low HPO lyase activity, evidence that lipoxygenase activity affects the formation of C_6-aldehydes. A similar phenomenon has been observed in tea plants; seasonal changes in C_6-aldehyde formation are caused by changes in lipoxygenase activity, not by changes in HPO lyase activity [3].

When the enzyme activities in tomato leaves were compared with those in tomato fruits, which are reported to contain the C_6-aldehyde-forming enzyme system [7, 14], a greater C_6-aldehyde-forming activity was found in the green leaves than in the fruit (Table 2). During ripening, HPO lyase activity per g fr. wt of fruit decreased with the decrease in chlorophyll content, but still it was sufficient to produce a considerable amount of the C_6-aldehydes. The C_6-aldehyde-forming activity in tomato fruit was slight, even with increasing lipoxygenase activity and sufficient HPO lyase activity. This may be explained by the fact that the lipoxygenase in tomato fruits favours 9-hydroperoxide formation, as reported by Galliard and Matthew [7].

Thus, the possibility that the lipoxygenase in other plant species favours 9-hydroperoxide formation and that even apparently high lipoxygenase activity does not always result in the high activity for C_6-aldehyde formation cannot be excluded. The product specificity of lipoxygenase and the substrate specificity of HPO lyase must be considered when studying the C_6-aldehyde

formation in addition to the amount of each enzyme's activity.

We do not yet know why green leaves contain HPO lyase. Possibly it is the result of the degradation or detoxication of peroxidized fatty acids formed in chloroplasts by photo-oxidation.

EXPERIMENTAL

Plant leaves were collected in the university campus during summer. Expanded leaves, not too aged, were used for the expts. Fresh leaves were homogenized in a chilled mortar for 3 min with 20 vols. (v/w) 50 mM Pi buffer, pH 6.3. The homogenate then was passed through four layers of gauze to remove large cell debris. Lipoxygenase activity was determined with linoleic acid from the O_2 consumption at 25° [3]. HPO lyase activity (hexanal formation from the 13-hydroperoxide) was measured by the headspace method with 13-hydroperoxylinoleic acid as the substrate [3]. The C_6-aldehyde-forming activity (hexanal formation from linoleic acid) was measured by the headspace method with linoleic acid as the substrate [3]. Chlorophyll was determined by the method of Mackinney [15] and protein by the method of Lowry *et al.* [16].

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