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DISTRIBUTION OF HYDROXYCINNAMOYL-CoA : ω-HYDROXYPALMITIC ACID *O*-HYDROXYCINNAMOYLTRANSFERASE IN HIGHER PLANTS

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Abstract—The occurrence of hydroxycinnamoyl-CoA : ω-hydroxypalmitic acid *O*-hydroxycinnamoyltransferase in roots of plants from various families is reported. Among the 21 species investigated, 17 plants representing 11 different families of angiosperms and gymnosperms showed extractable activity. In intact tobacco plants, the enzyme which was undetectable in leaves and stems, was apparently restricted to roots. Relatively high enzymatic activity was found however in cell suspension cultures grown from tobacco pith.

INTRODUCTION

We recently characterized in wound-healing potato tuber discs a transferase—hydroxycinnamoyl-CoA:ω-hydroxypalmitic acid *O*-hydroxycinnamoyltransferase (HHT)—which transfers ferulic acid from feruloyl-CoA to ω-hydroxypalmitic acid and to several 1-alkanols [1]. Both the time course of induction and the tissue distribution of HHT in the wound periderm of potato tuber discs were found to be coincident with the deposition of suberin [1, 2]. ω-Feruloyloxypalmitic acid, the main product formed *in vitro* by HHT, is a putative precursor of the ferulic acid esters of long-chain 1-alkanols which make up part of the waxes associated with potato tuber skin [1]. The correlation between HHT induction and suberization was further strengthened by the finding that HHT is induced by abscisic acid [3], as previously reported for several enzymes involved in suberin biosynthesis in potato tubers [4–7].

Since suberin naturally covers the underground organs of plants such as roots or tubers, and since ferulic acid esters of 1-alkanols have often been detected among the waxes associated with suberin [8], we studied the distribution of HHT in roots of plants belonging to various families in order to determine whether HHT activity would be commonly detectable in suberizing tissues. We report here the wide distribution of HHT in plants from different families of angiosperms and gymnosperms. Among the 21 species investigated, 17 plants representing 11 different families of angiosperms and gymnosperms showed extractable HHT activity (Table 1). The highest specific activities were found in roots from *Nicotiana tabacum* and *Allium porrum*. It is important, however, to underline that the roots used in this study were taken from plants or seedlings at different developmental stages. This probably explains part of the differences observed between the plants investigated. HHT activity was measured spectrophotometrically and was in several cases difficult to detect. The identity of the products formed during the reaction was therefore systematically checked for each plant tested by analysing an aliquot of the reaction mixture by HPLC. ω-Hydroxypalmitic acid was almost always the best substrate with regard to 1-tetradecanol as previously reported for the enzyme from potato [1], except in the case of *Petroselinum crispum*, *Brassica oleracea* and the two Coniferae tested (Table 1). Relative activities in the presence of various cinnamoyl-CoA derivatives were also determined by HPLC as previously described [1]. Feruloyl-CoA was always the best substrate but sinapoyl- and *p*-coumaroyl-CoA were also conjugated in the extracts of all the plants listed in Table 1. By contrast no product formation was detected with cinnamoyl- and caffeoyl-CoA except in extracts of *Phoenix dactylifera*, in which caffeic acid was conjugated to ω-hydroxypalmitic acid.

HHT was undetectable in leaves and stems of tobacco plants, which contained a relatively high HHT activity in their roots (Table 1). Low HHT activity (10 pkat per mg protein) was detected in callus cultures grown from tobacco pith but cell suspensions grown in liquid medium from these calli also contained a relatively high activity, reaching 64 pkat per mg protein eight days after subculture. By contrast no activity was detected in potato callus cultures grown on agar from tuber parenchyma unless abscisic acid was added to the culture medium, confirming previous results [3]. HHT was also undetectable in

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Table 1. Distribution of HHT activity in some higher plants

	Species	Families	HHT activity (pkat per mg protein) 16-OH-HDA*	1-Tetradecanol
Angiosperms				
Dicotyledons	*Nicotiana tabacum* L.	Solanaceae	48.0	33.3
	Solanum tuberosum L.	Solanaceae	17.1	14.0
	Taraxacum officinale L.	Compositae	12.6	8.3
	Petroselinum crispum L.	Umbelliferae	2.0	10.3
	Spinacia oleracea L.	Chenopodiaceae	9.1	6.1
	Cucurbita maxima (L.) Duch.	Cucurbitaceae	5.1	1.3
	Raphanus sativus L.	Cruciferae	2.4	0.2
	Brassica oleraceae L.	Cruciferae	2.2	5.7
	Pisum sativum L.	Leguminosae	0.2	0
	Vicia faba L.	Leguminosae	0.1	0
Monocotyledons	*Allium porrum* L.	Liliaceae	37.3	23.8
	Allium cepa L.	Liliaceae	10.1	0.8
	Phoenix dactylifera L.	Palmae	7.6	1.8
	Zea mays L.	Gramineae	4.7	1.4
	Triticum aestivum L.	Gramineae	2.1	0.6
Gymnosperms	*Picea abies* (L.) Karst.	Pinaceae	7.0	14.4
	Picea pungens (L.) Engelm.	Pinaceae	6.8	11.7

*16-OH-HDA: 16-hydroxyhexadecanoic acid. HHT activity was assayed using 16-OH-HDA or 1-tetradecanol as lipidic substrate, and feruloyl-CoA as phenolic substrate.

potato cell suspension cultures grown in liquid medium in the presence of 2,4-D.

The recent identification of a 'caffeoyl-hydroxyfatty acid-glycerol ester' from the waxes associated with green cotton fibres suberin [9] suggests that transferases such as HHT, which conjugate ω-hydroxyfatty acids to hydroxycinnamic acids, could play a role not only in the biosynthesis of ferulic acid esters of 1-alkanols, but also in the formation of suberin precursors [9]. From this point of view, the fact that HHT is widespread in roots of higher plants is interesting. Although the significance of the occurrence of HHT in tobacco cell suspension cultures is presently difficult to assess, our results suggest that such cultures may prove useful to undertake part of the biochemical studies which are necessary to unravel the role of ferulic acid esters of ω-hydroxyfatty acids in suberin biosynthesis.

EXPERIMENTAL

Plant material. Seeds were treated with $Ca(OCl)_2$ ($15\ g\ l^{-1}$) for 30 min and then thoroughly washed with sterile water before being grown in plastic Petri dishes on water soaked paper at 28° in the dark in a controlled growth room. Roots were harvested 3–10 days after sowing when they had reached a length of *ca* 2 cm. They were then frozen in liquid N_2 and stored at −80° before analysis. Seeds of *Picea abies* and *Picea pungens* were sown in pots in a greenhouse. Roots were taken when the aerial parts had reached *ca* 1.5 cm. Tobacco and potato roots were taken from intact plants grown in a greenhouse for 7 weeks.

Cell suspension cultures. These were grown from callus cultures initiated from *Nicotiana tabacum* cv Xanthi nc pith tissue. They were grown in Murashige and Skoog (MS) medium in the presence of $2\ mg\ l^{-1}$ 2,4-D at 25° under continuous white light. A 50 ml (*ca* 10 g fr. wt) sample of the suspensions were transferred to 250 ml fresh medium at 10 day intervals. Callus cultures of potato (*Solanum tuberosum* L. cv Bintje) were initiated from tuber parenchyma as previously described [3]. To test the effect of ABA on HHT induction, calli were subcultured on MS medium supplemented with both $1.5\ mg\ l^{-1}$ 2,4-D and 10^{-4} M ABA. Growing callus was transferred to 250 ml of MS liquid medium containing $1.5\ mg\ l^{-1}$ 2,4-D on a rotary shaker to form suspension cultures which were then subcultured every 10 days.

Chemicals and substrates. ω-Hydroxypalmitic acid and 1-tetradecanol were purchased from Sigma. Hydroxycinnamoyl-CoA thioesters were prepared by transesterification of hydroxycinnamoyl *N*-hydroxysuccinimide esters with coenzyme A as previously described [1].

Enzyme preparation and assays. All work was carried out at 4°. Plant material was ground in 0.2 M Tris–HCl pH 7.5 containing 2% ascorbic acid, 1 mM EDTA and 10 mM ME. For conifers, protein extraction was done with the same buffer containing 1% PEG 20000. After centrifugation, proteins were precipitated with solid $(NH_4)_2SO_4$ (65% saturation) centrifuged and the pellet was dissolved in extraction medium ($1\ ml\ g^{-1}$ fr. wt) and dialysed against 10 mM Tris–HCl buffer pH 8.5 containing 10 mM ME. HHT was measured in 0.1 M K-P_i buffer pH 7 at 360 nm using feruloyl-CoA and ω-hydroxypalmitic acid or 1-tetradecanol as substrates [1]. The char-

acterization of the reaction products was carried out by spectrophotometry, TLC and HPLC as previously described [1]. Good correspondence was observed between the photometric measurements of HHT activity and the quantification of the esters formed *in vitro* by HPLC, providing that the incubation time did not exceed 10 min, as previously reported in the case of potato tuber extracts [1].

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