Properties and Subcellular Localization of L-Alanine: Aldehyde Aminotransferase: Concept of an Ubiquitous Plant Enzyme Involved in Secondary Metabolism

Coralie Wink and Thomas Hartmann

Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Pockelsstr. 4, D-3300 Braunschweig

Z. Naturforsch. **36 c**, 625–632 (1981); received April 13, 1981

Spinacia oleracea, Arum maculatum, Alanine: Aldehyde Aminotransferase, Amine-Biosynthesis, Subcellular Localization, Mitochondria

L-Alanine: aldehyde aminotransferase occurs ubiquitously in higher plants. The enzyme catalyzes the reaction: L-alanine + monoaldehyde → monoamine + pyruvate; it is responsible for the formation of aliphatic plant amines and involved in the biosynthesis of hemlock alkaloids as shown by Roberts. A continuous coupled photometric test was developed to determine the low activities of the transaminase. The enzyme from the "amine-free" plant *Spinacia oleracea* was purified 77-fold and separated from other aminotransferases. A comparison of the *Spinacia* enzyme with that isolated from spadix-appendices of the amine-producing *Arum maculatum* during anthesis revealed very similar characteristics in pH-dependence, K_m-values for alanine and aliphatic aldehydes, and inhibition by 2-oxoacids. In contrast to the *Spinacia* enzyme the *Arum* aminotransferase is rapidly inactivated in the absence of pyridoxal-5′-phosphate. The enzymes of *S. oleracea, A. maculatum* and *Mercurialis perennis* are localized in mitochondria, but not in chloroplasts or peroxisomes. The results are discussed in relation to the function of alanine: aldehyde aminotransferase in secondary metabolism. It is suggested that some enzymes may be expressed in plants at low levels, even in the absence of any metabolic function.

Introduction

Aldehyde amination is the common biosynthetic pathway for the formation of simple aliphatic monoamines in higher plants [1, 2]. The enzyme responsible is an aminotransferase which catalyzes the reaction

L-alanine + monoaldehyde → monoamine + pyruvate.

It is remarkable that the capacity to synthesize aliphatic monoamines seems to be universally distributed in higher plants, irrespective whether a plant contains amines or not [2]. First enzymatic studies indicated that the enzyme has very low activity, and the aldehyde aminating activity could not be resolved from one isoenzyme of aspartate aminotransferase. Therefore it has been suggested that the aldehyde aminating activity might be considered a "minor activity" of aspartate aminotransferase [3].

Recent experiments indicate that the formation of the alkaloid γ-coniceine in hemlock (*Conium maculatum*), which proceeds via an alanine-dependent transamination of 5-oxooctanal, is a function of the alanine: aldehyde aminotransferase, too [4]. The *Conium* – enzyme could be separated from aspartate aminotransferase activity [5]. Up to now no other major transaminating activity which co-purifies with the aldehyde aminating activity could be detected. Therefore it seems likely that the activity now called L-alanine: aldehyde aminotransferase constitutes a specific enzyme.

In this communication we report on kinetic properties and subcellular localization of ubiquitous alanine: aldehyde aminotransferase isolated from a typical "amine plant" (*Arum maculatum*) and an "amine-free" plant (*Spinacia oleracea*). We discuss some implications concerning the role of a universally distributed enzyme in plant secondary metabolism.

Reprint requests to Prof. Dr. Th. Hartmann.

0341-0382/81/0700-0625 $ 01.00/0

Materials and Methods

Plant material

Spinach (*Spinacia oleracea*) leaves were purchased from the local market, dog's mercury (*Mercurialis perennis*) leaves and lords-and-ladies (*Arum maculatum*) leaves and spadices were collected in the woods near Braunschweig in April–June, tea (*Thea sinensis*)

seeds were sown in peat compost at 22 °C and the seedlings harvested after 3 weeks.

Purification of alanine: aldehyde aminotransferase

Enzyme was prepared from acetone powders (method A) or isolated cell organelles (method B).

Method A: Acetone powders were prepared as described previously [3]; 15 g acetone powder were suspended in 250 ml 0.05 mol/l Tris-HCl buffer pH 7.5, containing 1 mmol/l dithioerythritol (DTE) and 10 mmol/l diethyldithiocarbamic acid (DIECA), and were stirred for 50 min. The suspension was squeezed through nylon cloth and was centrifuged at $16000 \times g$ for 20 min. The supernatant was subjected to fractionated $(NH_4)_2SO_4$ precipitation using a saturated $(NH_4)_2SO_4$ solution adjusted to pH 8.0. The 45–65% precipitate was recovered and suspended in a minimum of Tris-HCl buffer and passed through a Sephacryl S-200 column (2.6 × 100 cm; 500 ml). The fractions containing transaminase activity were applied to a Blue Sepharose CL 6B column (2.6 × 14.5 cm; 50 ml); the fractions containing alanine: aldehyde aminotransferase activity were subjected to ion exchange chromatography on a DEAE-cellulose column (2.6 × 14.5 cm; 50 ml). After application, the column was washed with four times the sample volume of buffer before a linear gradient of 0–0.15 mol/l KCl in 300 ml buffer was applied. Alanine: aldehyde aminotransferase containing fractions were pooled and chromatographed on a small AH-Sepharose 4B column (1 × 7 cm; 3 ml). The gel was washed with buffer, and transaminase activity was then eluted with buffer containing 0.2 mol/l KCl.

The final enzyme solution was stabilized by adding DTE (1 mmol/l) and ethyleneglycol (10% v/v). The enzyme could be stored at 0 °C without loss of activity for at least two weeks.

Method B: Crude mitochondria were prepared by differential centrifugation as given below and disrupted by freeze-thawing. The extract was filtered and further purified by passage through a Sephadex G-25 column. The protein fraction was applied to a Blue Sepharose CL 6B column (2.6 × 60 cm; 200 ml). Fractions containing transaminase activity were pooled and stored at −20 °C.

Enzyme from *Spinacia* (leaves) and *Thea* (cotyledons) was prepared by method A; the enzymes from *Arum* and *Mercurialis* by method B. All enzymatic studies were performed with 0.05 mol/l Tris-HCl buffer, pH 7.5.

Organelle isolation

Crude mitochondria and crude chloroplasts were prepared by differential centrifugation according to Jacobi [6]. The isolation buffer (pH 7.8) consisted of 10 mmol/l sodium diphosphate-HCl, pH 7.8, 600 mmol/l mannitol, 10 mmol/l DIECA, 1 mmol/l DTE. Precooled plant material (50 g fresh weight) was chopped and homogenized in a Waring blendor with 200 ml isolation buffer. The homogenate was filtered through nylon cloth and cotton wool and centrifuged at $1000 \times g$ for 7 min. The pellet was suspended in 5 ml isolation buffer, recentrifuged and resuspended in either isolation buffer or in hypotonic 0.05 mol/l Tris-HCl buffer pH 7.5 containing 10 mmol/l DIECA and 1 mmol/l DTE ("crude chloroplasts"). The first supernatant was recentrifuged at $12000 \times g$ for 10 min. The pellet was resuspended as above ("crude mitochondria").

If organelles were to be further purified by gradient centrifugation 1 ml "crude organelles", suspended in isolation buffer, was layered on a continuous sucrose gradient (30–60% sucrose in 10 mmol/l diphosphate buffer pH 7.8; 32 ml). Centrifugation was performed in a Sorvall SS-90 vertical rotor at 18000 rpm for 30 min.

Enzyme assays

Alanine: aldehyde aminotransferase: In earlier studies transaminase activity was followed by quantitative amine estimation according to the methods given in ref. [7]. The amine was recovered from the reaction mixture by alkaline steam distillation. Following reaction with 2,4-dinitrofluorobenzene the resulting 2,4-dinitrophenyl derivative was measured photometrically after thin-layer chromatographic separation.

Instead of this rather laborious assay a rapid coupled photometric assay was developed, in which the production of pyruvate from alanine could be followed continuously by adding lactate dehydrogenase. The reaction mixture (total volume 1.0 ml) contained 0.16 mol/l sodium diphosphate-HCl, pH 7.8; 4 mmol/l *n*-hexanal (or other aldehydes); 0.1 mol/l L-alanine; 0.13 mmol/l NADH; 1 mmol/l pyridoxal-5′-phosphate (PLP) if necessary; 10 µl lactate dehydrogenase (83.3 nkat) and 0.05–0.2 ml enzyme solution. Enzyme activity was recorded continuously at 334 nm and 30 °C.

Amination of 5-oxooctanal was determined by measuring the γ-coniceine produced. Assays were performed with sodium nitroprusside as given by Roberts [4].

Other enzymes: Glutamate dehydrogenase (EC 1.4.12) was determined according to Ehmke and Hartmann [8], aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) and alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) as given by Bergmeyer and Bernt [9], and hydroxypyruvate reductase (EC 1.1.1.81) by the method of Tolbert *et al.* [10].

Protein determination

The protein content was estimated by a modified Lowry method [11].

Chemicals

Lactate dehydrogenase (EC 1.1.1.28) and malate dehydrogenase (EC 1.1.1.37) were from Boehringer (Mannheim); Sephadex G-25; Sephacryl S-200; Blue Sepharose CL 6B; Aminohexyl-Sepharose 4B (AH-Sepharose) from Pharmacia Chemicals (Freiburg); DEAE-cellulose (Servacel DEAE 23 SH, 0.93 meq/g) from Serva (Heidelberg).

Results

Partial purification of alanine: aldehyde aminotransferase

Table I shows the results of the purification of alanine: aldehyde aminotransferase from acetone powders of spinach leaves. It is noticeable that the activity of the enzyme in crude extracts is about two orders lower than that of aspartate and alanine aminotransferases. The final enzyme preparation has a specific activity of 700 pkat/mg protein and is completely resolved from aspartate and alanine aminotransferases. Both these contaminating activities were mainly removed by chromatography on DEAE-cellulose using a shallow KCl gradient. Contrary to findings in *Conium* [5] only one isoenzyme of alanine: aldehyde aminotransferase could be detected in *Spinacia* (Fig. 1), which was eluted at the same KCl-concentration as isoenzyme A in *Conium* [5]. Chromatography on AH-Sepharose, which was suitable to concentrate the final enzyme preparation, is supposed to be of hydrophobic nature. Attempts to elute transaminase activity biospecifically (*i. e.* with substrates or products like hexanal or hexylamine) failed.

Affinity chromatography on Blue Sepharose was necessary to remove the interfering NADH-oxidase and alcohol dehydrogenase (Table I), and thus made the direct photometric test of alanine: aldehyde aminotransferase activity possible.

Alanine: aldehyde aminotransferase from *Arum maculatum* could not be purified with reasonable yields by an analogous procedure since the enzyme was too unstable. Isolation of mitochondria from flowering spadix-appendices, solubilization, gel filtration and Blue Sepharose chromatography yielded a more stable enzyme preparation with a specific activity of up to 330 pkat/mg protein.

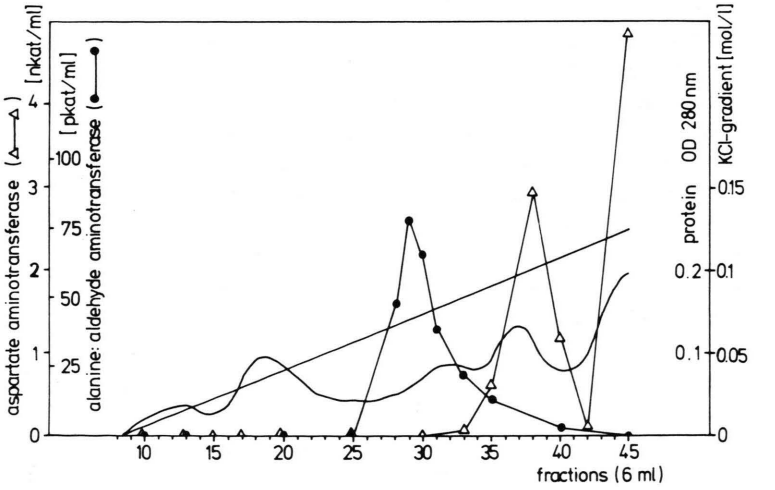

Fig. 1. Separation of alanine: aldehyde aminotransferase and aspartate: 2-oxoglutarate aminotransferase from *Spinacia oleracea* on DEAE-cellulose.

Table I. Purification of L-alanine: aldehyde aminotransferase from acetone powders of spinach leaves.

Purification step	Alanine: aldehyde aminotransferase [a]			Total activities [nkat] of some other enzymes			
	Total activity [nkat]	Specific activity [pkat/mg protein]	Purification (fold)	Alanine aminotransferase	Aspartate aminotransferase	NADH oxidase	Alcohol dehydrogenase
Crude extract	10.4	9	1	949	1654	172	2244
$(NH_4)_2SO_4$ precipitation (45–65%)/Sephacryl S-200	7.8	42	4.6	732	962	25	867
Blue Sepharose	7.8	58	6.4	672	955	7.5	15.5
DEAE-cellulose	7.1	443	49	3.3	0.7	0	0
AH-Sepharose	5.0	694	77	n. d.	< 0.2	0	0

Preparation was started from 15 g acetone powder.
n. d. = not determined.
[a] Hexanal amination.

Some properties of alanine: aldehyde aminotransferase

Enzyme preparations obtained by the methods described above were employed in the following comparative studies.

pH-optimum: The activities of the enzymes from *Spinacia, Arum* and *Thea* show almost identical pH dependence with optimal activity at pH 7.8–8.2.

Substrate specificity: Substrate specificity of both *Arum* and *Spinacia* enzyme is similar to previous reports [1, 3]. L-Alanine is the preferred amino donor and aliphatic monoaldehydes, with the exception of formaldehyde, function as amino acceptors. No amination activity could be measured with various other aldehydes and ketones including benzaldehyde, phenylacetaldehyde, *p*-anisaldehyde, cinnamic aldehyde, *trans*-2-hexenal, acetone. 5-Oxooctanal is the only compound known so far which is aminated in addition to the homologous monoaldehydes.

Substrate kinetics: The K_m values obtained for L-alanine and the aldehydes of the homologous series from ethanal to hexanal indicate a remarkable likeness between the *Spinacia* and *Arum* enzymes (Table II). In general, the aldehydes show a decrease of K_m with increasing length of the carbon chain. Exceptions are ethanal and the branched-chain aldehyde 2-methylpropanal.

Influence of pyridoxal-5'-phosphate (PLP): In contrast to the *Spinacia* enzyme the *Arum* aminotransferase is strongly dependent on PLP (Table III). This indicates that the *Spinacia* enzyme contains tightly bound PLP similar to the enzyme from *Mercurialis* [1], whereas the *Arum* enzyme easily dissociates into apoenzyme and coenzyme. Further support is given by the findings that the *Spinacia* enzyme is only partially inactivated by preincubation with L-alanine in the absence of PLP [3], whereas the *Arum* enzyme is completely inactivated under these conditions. The high instability of the *Arum* aminotransferase in crude acetone powder extracts may be explained by the lability of the apoenzyme-coenzyme complex.

Inhibition by 2-oxoacids: Both *Mercurialis* and *Conium* aminotransferase are inhibited by 2-oxoacids [1, 5]. *Spinacia* and *Arum* enzymes show a similar inhibition by glyoxalate, pyruvate and 2-oxoglutarate (Table IV).

Table II. Michaelis constants for various substrates of alanine: aldehyde aminotransferase.

Substrate	K_m [mmol/l]		
	Spinacia oleracea	*Arum maculatum* [a]	*Thea sinensis*
Amino donor [b]:			
L-Alanine	29	27	28
Amino acceptor:			
Ethanal	17	15	n. d.
n-Propanal	40	31	n. d.
n-Butanal	18	5	n. d.
2-Methylpropanal	22	32	n. d.
n-Pentanal	7	2	n. c.
3-Methylbutanal	7	4	n. d.
n-Hexanal	1.6	1	n. d.
5-Oxooctanal	1.2	1.4	n. d.

[a] Assays contained 1 mmol/l PLP.
[b] Tested with hexanal as amino acceptor.

Table III. Effect of pyridoxal-5'-phosphate (PLP) on alanine: aldehyde aminotransferase from *Spinacia oleracea* and *Arum maculatum*.

Assay	% of hexanal amination	
	Spinacia oleracea	*Arum maculatum*
minus PLP	101	27
plus PLP (1 mmol/l)	100	100

Subcellular localization of alanine: aldehyde aminotransferase

Aminotransferases occur in various compartments of the plant cell; *e. g.* in *Spinacia* four isoenzymes of aspartate aminotransferase have been reported: one each in mitochondria, chloroplasts, peroxisomes and the cytosol [12]. It had to be considered therefore that the ubiquitous alanine: aldehyde aminotransferase could be represented by one isoenzyme and that amine plants would contain a second isoenzyme in a specific cell compartment.

In a first approach mitochondria and chloroplasts were prepared by differential centrifugation and tested for alanine: aldehyde aminotransferase activity. The photometric test could not be applied directly with the lysed organelle fractions since they contained interfering enzymes. Therefore these fractions were purified by method B to eliminate those enzymes. Aminating activity could then be demonstrated in the mitochondrial preparations (Table V).

To confirm that alanine: aldehyde aminotransferase was really located in mitochondria and not peroxisomes, which sediment at similar velocities in differential centrifugation, crude *Spinacia* mitochondria were purified on a 30–60% sucrose gradient (Fig. 2). Glutamate dehydrogenase [13] and hydroxypyruvate reductase [10] were chosen as marker enzymes for mitochondria and peroxisomes, respectively. It is obvious that alanine: aldehyde aminotransferase ac-

Table IV. Inhibition of alanine: aldehyde aminotransferase by 2-oxoacids (sodium salts).

	% of 5-oxooctanal amination	
	Spinacia oleracea	*Arum maculatum*
Control	100	100
Glyoxalate		
0.5 mmol/l	75	70
2.5 mmol/l	43	17
Pyruvate		
0.5 mmol/l	63	72
2.5 mmol/l	47	47
2-Oxoglutarate		
4.5 mmol/l	95	n. d.
22.5 mmol/l	n. d.	78

Table V. Alanine: aldehyde aminotransferase activity in organelle fractions of different plants.

	Alanine: Aminotransferase [pkat/ml]	
	Mitochondria	Chloroplasts
Hexanal amination:		
Arum maculatum		
Leaves[a]	45	0
Spadices[a]	165	–
Spinacia oleracea[b]	41.7	0
Mercurialis perennis[a]	64.4	0
5-Oxooctanal amination:		
Mercurialis perennis[b]	45.1	0

[a] Organelle preparation by differential centrifugation.
[b] Organelle preparation by differential and gradient centrifugation.

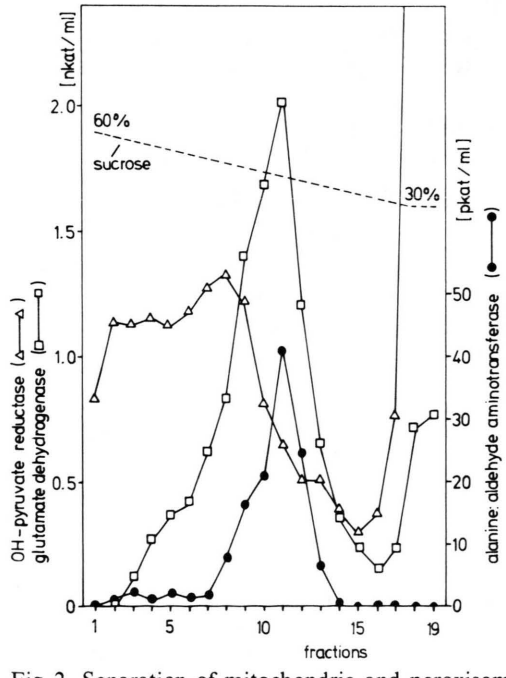

Fig. 2. Separation of mitochondria and peroxisomes from *Spinacia oleracea* on a continuous sucrose gradient.

tivity shows the same distribution as does glutamate dehydrogenase, and thus presence of aminating activity in peroxisomes can be ruled out. Gradient centrifugation of the mitochondrial fraction obtained from *Arum* spadix-appendices during thermogenesis revealed identical results. Here mitochondrial alanine: aldehyde aminotransferase was shown to be accompanied by other aminating enzymes such as aspartate and alanine aminotransferases.

No alanine: aldehyde aminotransferase activity could be detected in chloroplasts prepared either by differential centrifugation or gradient centrifugation (Table V).

Discussion

Aliphatic monoamines are simple but typical secondary plant products. They occur in rather small quantities in a certain number of plants. In some species they are present in nearly all tissues (*e.g. Mercurialis perennis*), in others they are synthesized restricted in time and space. Thus amines are often produced in flowers at anthesis as odour components and attractants for pollinating insects. Well known examples are the flowers of several Rosaceae (*Crataegus, Prunus spinosa, Sorbus*) and Araceae (*Arum maculatum*). L-Alanine: aldehyde aminotransferase, the enzyme responsible for amine formation, is present in all higher plants, however [2]. So the enzymatic potential to produce amines can be assumed for plants, although amine production is found in a limited number of species only. This phenomenon gives rise to two questions:

1) Which are the factors controlling amine formation in plants?
2) How can the universal occurrence of an enzyme which appears to be without function in many species be explained?

As concerns the first question it might be argued that the potential to produce amines, *i.e.* the presence of alanine: aldehyde aminotransferase, is uniformly distributed, but was optimized in amine-producing species during evolution. This optimization may concern kinetic properties or subcellular compartmentation. This possibility is disproved by the present results, however. The properties of alanine: aldehyde aminotransferase from "amine-free" *Spinacia* and "amine-producing" *Arum* are very similar and the *Arum* enzyme does not seem to be specially adapted in its kinetic properties. Moreover the enzymes from both sources share the same subcellular compartment.

Recent work by Roberts [14] on γ-coniceine formation shows that in *Conium maculatum* alanine: aldehyde aminotransferase (called L-alanine: 5-ketooctanal transaminase by Roberts) occurs both in mitochondria and chloroplasts. The two isoenzymes correspond to transaminase A and transaminase B, which had been separated previously [5]. The mitochondrial enzyme (transaminase A) seems to be very similar to the mitochondrial enzymes isolated from *Spinacia* and *Arum*, whereas the chloroplast enzyme (transaminase B) differs significantly from the mitochondrial enzymes (Table VI). It is suggested that the chloroplast enzyme is responsible for alkaloid formation and this view is supported by a number of other facts [14]. In addition to lupin alkaloid biosynthesis [15] γ-coniceine formation seems to be another example of a chloroplast-localized alkaloid synthesis in plants.

One might speculate that in *Conium* alanine: aldehyde aminotransferase has been optimized and integrated into the alkaloid-specific pathway in the course of evolution.

On the contrary product formation seems only to be controlled by the availability of the respective

Table VI. Comparison of some characteristics of alanine: aldehyde aminotransferase in *Conium maculatum* (data from Roberts [5]), *Spinacia oleracea*, and *Arum maculatum*.

	Conium maculatum		*Spinacia oleracea*	*Arum maculatum*
	Transam. A	Transam. B		
K_m L-Alanine (mmol/l)	27	55	29	28
K_m 5-Oxooctanal (mmol/l)	1.6	0.14	1.2	1.4
pH-Optimum	7.8–8.5 (broad)	8.5 (sharp)	7.8–8.0	7.8–8.2
Inhibition by pyruvate	yes	no	yes	yes

substrates, *i. e.* aldehydes, in amine plants. Unfortunately little is known on the physiology of aldehyde metabolism in plants [16]. Unbranched aliphatic aldehydes, such as hexanal, are derived by hydroperoxide cleavage of unsaturated fatty acids [17], mainly during senescence and wounding of plant tissues. The respective enzyme systems are associated with plasmalemma, dictyosomes and endoplasmatic reticulum membranes [18] or chloroplasts [19]. Branched aldehydes, which are the precursors of the most prominent plant amines 2-methylpropylamine and 3-methylbutylamine, are assumed to be degradation products of valine and leucine [20].

As aldehydes easily penetrate membranes they may well enter the mitochondria as the sites of amine formation. For example the ethylamine content is significantly increased in sliced apples kept in anaerobic conditions (N_2-atmosphere), which favour the formation of ethanal [21]. The situation may be similar in leaves of *Mercurialis perennis*, which contain a pattern of various aliphatic aldehydes [1]. In *Arum maculatum* anthesis is accompanied by heat production (thermogenesis) and concomitant exhalation of odoriferous products. It is reasonable to assume that during this metabolic flare-up, which is restricted to the spadix-appendix, the mitochondrial transaminase is supplied with newly produced aldehydes [22].

Finally the question of the universal occurrence of alanine: aldehyde aminotransferase remains to be discussed. There are two possibilities: a) alanine: aldehyde aminotransferase activity represents a minor activity of a functionally important transaminase involved in primary metabolism; b) it represents a distinctive enzyme. The first possibility, suggested by earlier results (see introduction), could be disproved by Roberts [5] and the results presented here. It seems quite likely that alanine: aldehyde aminotransferase is a distinctive enzyme ubiquitously present at low activities in higher plants. There are some similar examples. The cyanide metabolizing enzyme β-cyanoalanine synthase [23] and lysine decarboxylase [24] are distributed in a variety of plants although their biosynthetic function is restricted to a limited number of species. Additionally many biotransformation experiments performed with plant cell cultures indicate the presence of enzyme activities not expected in the species tested. Examples are the synthesis of β-carboline alkaloids upon feeding of tryptophan in *Phaseolus* cultures [25], the transformations of sparteine in *Conium* and *Symphytum officinalis* cell cultures [26], or the transformation of thebaine into codeine in tobacco cultures [27].

Fowden isolated the toxic amino acid acetidine-2-carboxylic acid and other non-protein amino acids from the nitrogenous fraction in the refining of sugar beet [28]. Quite sizable amounts of acetidine-2-carboxylic acid may be obtained, although it is only present in beet extracts at about one fiftieth the concentration of proline, and so would not be detected in ordinary chromatographic screening.

From these findings Fowden put forward the idea that the genetic potential of plants may be more uniform than has been suspected. The various patterns of product accumulation from trace amounts to massive quantities may reflect differences in the degree to which particular genes are "switched on". We would add that some enzymes, *e. g.* alanine: aldehyde aminotransferase, may be expressed at very low levels even in the absence of any function. Such "sleeping enzyme activities" may be one basis for the selection of biosynthetic pathways in secondary metabolism during evolution, such as hemlock alkaloid biosynthesis.

Acknowledgements

We would like to thank Dr. M. F. Roberts (London) for her stimulating discussion, and for supplying a sample of 5-oxooctanal. Prof. M. Umezu (Tottori) kindly sent us seeds of *Thea sinensis*. A grant of the Studienstiftung des Deutschen Volkes to C. W. and financial funds of the Land Niedersachsen are gratefully acknowledged.

[1] T. Hartmann, D. Dönges, and M. Steiner, Z. Pflanzenphysiol. **67,** 404–417 (1972).
[2] T. Hartmann, H.-I. Ilert, and M. Steiner, Z. Pflanzenphysiol. **68,** 11–18 (1972).
[3] W. Unger and T. Hartmann, Z. Pflanzenphysiol. **77,** 255–267 (1976).
[4] M. F. Roberts, Phytochemistry **16,** 1381–1386 (1977).
[5] M. F. Roberts, Phytochemistry **17,** 107–112 (1978).
[6] G. Jacobi, Biochemische Cytologie der Pflanzenzelle, p. 72, Thieme-Verlag, Stuttgart 1974.
[7] H.-I. Ilert and T. Hartmann, J. Chromatogr. **71,** 119–125 (1972).
[8] A. Ehmke and T. Hartmann, Phytochemistry **15,** 1611–1617 (1976).

[9] H. U. Bergmeyer and E. Bernt, in: Methoden der enzymatischen Analyse, (H. U. Bergmeyer, ed.), **Vol. 1,** pp. 769–775, pp. 785–791, Verlag Chemie, Weinheim 1974.
[10] N. E. Tolbert, P. K. Yamazaki, and A. Oeser, J. Biol. Chem. **245,** 5129–5136 (1970).
[11] V. H. Potty, Anal. Biochem. **29,** 535–539 (1969).
[12] A. H. C. Huang, K. D. Liu, and R. J. Youle, Plant Physiol. **58,** 110–113 (1976).
[13] W. Nauen and T. Hartmann, Planta **148,** 7–16 (1980).
[14] M. F. Roberts, Plant Cell Reports (in press).
[15] M. Wink, T. Hartmann, and L. Witte, Z. Naturforsch. **35 c,** 93–97 (1980).
[16] E. Schauenstein, H. Esterbauer, and H. Zollner, in: Aldehydes in Biological Systems, pp. 180–185, Pion Ltd., London 1977.
[17] T. Galliard and H. W.-S. Chan, in: The Biochemistry of Plants (P. K. Stumpf and E. E. Conn, eds.), **Vol. 4,** pp. 131–161, Academic Press, New York 1980.
[18] D. A. Wardale, E. A. Lambert, and T. Gaillard, Phytochemistry **17,** 205–212 (1978).
[19] A. Hatanaka, J. Sekiya, and T. Kajiwara, Phytochemistry **17,** 869–872 (1978).
[20] M. H. Yu and M. Spencer, Phytochemistry **8,** 1173–1178 (1969).
[21] T. Hartmann, Z. Pflanzenphysiol. **57,** 368–375 (1967).
[22] B. J. D. Meeuse, Ann. Rev. Plant Physiol. **26,** 117–126 (1975).
[23] J. M. Miller and E. E. Conn, Plant Physiol. **65,** 1199–1202 (1980).
[24] T. Hartmann, M. Wink, G. Schoofs, and S. Teichmann, Planta Med. **39,** 282 (1980).
[25] J. A. Veliky and U. M. Barber, Lloydia **38,** 125–130 (1975).
[26] M. Wink, T. Hartmann, and L. Witte, Planta Med. **40,** 31–39 (1980).
[27] K. D. Grützmann and H.-B. Schröter, Abhdl. Deutsch. Akadem. Wiss. (Berlin), Kl. Chemie, **3,** 347 (1966).
[28] L. Fowden, Phytochemistry **11,** 2271–2276 (1972).