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Partial purification and properties of a transamidinase from *Lathyrus sativus* seedlings

INVOLVEMENT IN HOMOARGININE METABOLISM AND AMINE INTERCONVERSIONS

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A transamidinase was purified 463-fold from *Lathyrus sativus* seedlings by affinity chromatography on homoarginine–Sepharose. The enzyme exhibited a wide substrate specificity, and catalysed the reversible transfer of the amidino groups from donors such as arginine, homoarginine and canavanine to acceptors such as lysine, putrescine, agmatine, cadaverine and hydroxylamine. The enzyme could not be detected in the seeds, and attained the highest specific activity in the embryo axis on day 10 after seed germination. Its thiol nature was established by strong inhibition by several thiol blockers and thiol compounds in the presence of ferricyanide. In the absence of an exogenous acceptor, it exhibited weak hydrolytic activity towards arginine. It had apparent mol.wt. 210000, and exhibited Michaelis–Menten kinetics with K_m 3.0mM for arginine. Ornithine competitively inhibited the enzyme, with K_i 1.0mM in the arginine–hydroxylamine amidino-transfer reaction. Conversion experiments with labelled compounds suggest that the enzyme is involved in homoarginine catabolism during the development of plant embryo to give rise to important amino acids and amine metabolites. Presumptive evidence is also provided for its involvement in the biosynthesis of the guanidino amino acid during seed development. The natural occurrence of arcain in *L. sativus* and mediation of its synthesis *in vitro* from agmatine by the transamidinase are demonstrated.

The molecular and regulatory aspects of the transamidinases (amidinotransferases, EC 2.1.4.–) have been extensively investigated in relation to creatine biosynthesis in vertebrates and to streptomycin biogenesis in certain soil micro-organisms (Walker, 1973). However, similar detailed studies on the analogous enzymes from higher plants are lacking, despite the fact that these enzymes have been implicated in the elaboration of a variety of secondary metabolites (Smith, 1971). During our studies on biosynthesis and regulation of aliphatic amines in the developing seedlings of *Lathyrus sativus* (Ramakrishna & Adiga, 1974; Suresh & Adiga, 1977), we were intrigued to find that incubation of [^{14}C]putrescine with undialysed crude extracts of the seedlings resulted in the significant formation of [^{14}C]agmatine, notwithstanding the irreversibility of the agmatine→putrescine pathway (Smith & Garraway, 1964). The possibility of a transamidinase mediating agmatine formation from the diamine was strengthened by the subsequent observation that cadaverine greatly stimulated [*amidine*-^{14}C]homoagmatine production in the dialysed crude extracts of the plant tissue incubated with [*amidine*-^{14}C]homoarginine under conditions that are suboptimal for the homoarginine decarboxylase reaction (Ramakrishna & Adiga, 1976). In the present paper we describe the partial purification of this transamidinase activity from the higher plant. Several of the physicochemical and functional characteristics of the enzyme were also investigated to gain an insight into its probable physiological role in the amine and amino acid metabolism in the plant during early development.

The seeds of *L. sativus* are rich in L-homoarginine as a free amino acid (Rao *et al.*, 1963; Bell, 1971); however, the biosynthetic route and physiological relevance of this guanido amino acid during plant growth still remain largely enigmatic. Though our earlier studies showed that homoarginine contributes vital amine metabolites such as cadaverine by conversion through homoagmatine (Ramakrishna & Adiga, 1974), the possibility of its participation in other important functions during

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seedling development was considered. In the present paper we show that the transaminidase is involved in the biosynthesis of homoarginine as well as metabolism to yield important metabolites during plant embryo growth.

Materials and methods

The source of *L. sativus* seeds and germination conditions have been described earlier (Suresh *et al.*, 1976). Analytical-grade reagents were used in all experiments. Unlabelled amino acids and aliphatic amines, hydroxylamine hydrochloride, *p*-hydroxymercuribenzoate, *N*-ethylmaleimide, Sepharose CL-4B, dithiothreitol and thioproline were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Canavanine was purified from jack beans by the modified method of Rosenthal (1973). CNBr was synthesized as detailed by Hartman & Dreger (1931). The procedure of Kalyankar *et al.* (1958) was followed to prepare hydroxyguanidine hydrochloride by treating hydroxylamine with *O*-methylisourea. Synthesis of pentacyanoaminoferroate was accomplished by employing aq. NH_3 (sp.gr. 0.880) and sodium nitroprusside (Fearon, 1946). The sources of radiochemicals used are as follows: [1,4-^{14}C]putrescine dihydrochloride (sp. radioactivity 54 mCi/mmol), The Radiochemical Centre (Amersham, Bucks., U.K.); [*amidine*-^{14}C]homoarginine, Calbiochem (Los Angeles, CA, U.S.A.); L-[U-^{14}C]arginine and [G-^{3}H]lysine, Bhabha Atomic Research Centre (Bombay, India).

Infiltration of labelled compounds into *L. sativus* shoots was performed as detailed elsewhere (Ramakrishna & Adiga, 1974).

Extraction of labelled homoarginine and the amines

After infiltration, *L. sativus* shoots were homogenized in 0.3 M-$HClO_4$ at 0°C, the extract was neutralized with KOH and precipitated $KClO_4$ was removed by centrifugation. After addition of 200 nmol of unlabelled homoarginine as the carrier, the clear extract (pH 6.0) was passed through a small Dowex 50 X-12 (NH_4^+ form) column (3 cm × 10 cm) and washed successively with deionized water and 1 M-NH_3. Homoarginine selectively eluted with 2 M-NH_3 was not contaminated with other basic amino acids, which were earlier removed by the 1 M-NH_3 wash. The 2 M-NH_3 eluate was evaporated *in vacuo* at 50°C. For recovery of ^{14}C-labelled amines and their derivatives, the reaction mixture was passed through a small column of Dowex 50 X-12 (H^+ form), and the labelled amines were eluted as described by Inoue & Mizutani (1973). To calculate the specific radioactivity, homoarginine was quantified as described previously (Ramakrishna & Adiga, 1973).

Chromatographic separation and radioactivity measurements

Homoarginine and amines were resolved and located on paper chromatograms, and associated radioactivities were measured as described previously (Suresh & Adiga, 1977) with a Beckman LS-100 liquid-scintillation spectrometer.

Isolation and identification of arcain

This was accomplished by a slight modification of the procedure of Audit *et al.* (1967). Briefly, 7-day-old etiolated seedlings were homogenized in cold 0.3 M-$HClO_4$, and neutralized clear supernatant was passed through a column (3.5 cm × 10 cm) of Amberlite IRC-50 (pyridinium form). After extensive washing of the column with distilled water, agmatine and most of the polyamines were removed by washing with 150 ml of 1 M-pyridine/acetate buffer, pH 3.5, and arcain was eluted with a similar buffer at pH 3.0. The eluate, concentrated to a small volume, was subjected to ascending paper chromatography with butan-1-ol/pyridine/acetic acid/water (4 : 1 : 1 : 2, by vol.) as the solvent, and the resolved diguanidobutane was detected with Sakaguchi reagent (Audit *et al.*, 1967).

Preparation of homoarginine–Sepharose

The procedure of March *et al.* (1974) was employed for Sepharose activation and subsequent washing. The CNBr-activated Sepharose was stirred with 50 mM-L-homoarginine in 0.2 M-$NaHCO_3$, pH 9.0, for 20 h at 4°C. After extensive washing, the resultant homoarginine–Sepharose gave an intense orange–red colour with Sakaguchi reagent, showing that the coupling was through the α-amino group. On acid hydrolysis, 4 μmol of homoarginine was liberated/ml of Sepharose.

Assay methods

(*a*) *Arginine–hydroxylamine transamidinase.* The general transamidinase assay method of Walker & Walker (1970) was used. The reaction mixture (1.0 ml) contained 100 mM-potassium phosphate buffer, pH 7.5, 2 mM-dithiothreitol, 10 mM-arginine, 100 mM-hydroxylamine hydrochloride and 0.1–2 mg of enzyme protein, and was incubated at 37°C for 1 h. For the blank, arginine was replaced by 100 mM-ornithine. Termination of the enzyme reaction and quantification of hydroxyguanidine formed with pentacyanoaminoferroate were performed as described by Walker & Walker (1970).

(*b*) *Canavanine–lysine transamidinase.* The reaction mixture (1.0 ml) contained 100 mM-potassium phosphate buffer, pH 8.0, 2 mM-dithiothreitol, 25 mM-lysine, 10 mM-canavanine and 0.1–2 mg of enzyme protein, and was incubated at 37°C for 2 h. The corresponding blank contained all the above components except that boiled enzyme protein was

used. The reaction was stopped and homoarginine formed was determined by the procedure of Akamatsu & Watanabe (1961).

Urea liberated by the hydrolytic activity of transamidinase was measured by the method of Prescott & Jones (1969).

Enzyme unit

One unit of transamidinase is defined as 1.0nmol of product formed/h per mg of protein.

Other methods

Protein was determined by the procedure of Lowry *et al.* (1951), with crystalline bovine serum albumin as the standard. Polyacrylamide-gel electrophoresis was performed as described by Davis (1964). Gel filtration was performed at 4°C on a column (1.8cm × 88cm) of Sephacryl S-200 with 30mM-potassium phosphate buffer (pH 7.5)/2mM-2-mercaptoethanol as the eluent. The enzyme protein (1.5mg) dialysed overnight at 4°C against the buffer was applied, and the elution volume was determined by activity measurement. The molecular weight was computed by reference to elution volumes of the following standards: catalase (232000), bovine serum albumin (68000 monomer, 136000 dimer) and horseradish peroxidase (45000).

Purification of the enzyme

The initial steps in the purification of the enzyme from 7-day-old etiolated seedlings of *L. sativus* employed a protocol essentially the same as described previously (Suresh *et al.*, 1976), except that 50mM-potassium phosphate buffer, pH 8.0, containing 2mM-2-mercaptoethanol replaced sodium phosphate buffer. For affinity chromatography, 5ml of homoarginine–Sepharose was packed into a small column (3.5cm × 11cm) and equilibrated with the above buffer [20mM-potassium phosphate buffer (pH 7.5)/2mM-2-mercaptoethanol]. After removal of nucleoproteins by precipitation with $MnCl_2$, the supernatant was adjusted to pH 7.5 and passed through the affinity matrix at a flow rate of 1ml/min. The unadsorbed non-specific proteins were removed by extensive washing with the equilibration buffer until the A_{280} value of the eluent was less than 0.05. Substrate-specific elution of the enzyme was achieved by using 4mM-homoarginine in the above buffer. The fractions containing the enzyme protein were pooled, dialysed against the buffer to remove homoarginine and concentrated over Aquacide to a small volume.

Results

Infiltration experiments: additional evidence for a transamidinase

In addition to the experiments with crude extracts referred to above, further evidence for the transamidinase in *L. sativus* seedlings was obtained when certain labelled amino acids were infiltrated into and allowed to be metabolized in the excised shoots of the plant. When either labelled arginine or lysine was given, significant quantities of radioactivity were incorporated into homoarginine subsequently isolated by chromatography. Similarly, the radioactivity from [*amidine*-^{14}C]homoarginine was also incorporated into free arginine as well as that incorporated into acid-insoluble protein, suggesting the reversibility of the amidino-transfer reaction *in vivo*. The presence of the label in homoarginine after administration of [U-^{14}C]arginine indicates that the latter amino acid functions *in vivo* as the amidine donor. In agreement with this is the finding that [^{3}H]lysine (acceptor) was also utilized for the production of the labelled homoarginine. In fact, the relative specific radioactivity (d.p.s./μmol) of the labelled homoarginine produced after administration of these two precursors is consistent with the premise that the amidine group of arginine and the entire lysine moiety are involved in the synthesis of homoarginine (Table 1).

Table 1. *Incorporation of labelled precursors into homoarginine and arginine by L. sativus seedlings*

Batches of ten excised shoots were supplied with 20μCi of [^{14}C]arginine or [^{3}H]lysine or 2μCi of [^{14}C]homoarginine, and the corresponding guanido amino acids were extracted after 20h. For further details see the text.

		Homoarginine		Arginine	Radioactivity in acid-precipitable fraction	
Precursor	Specific radioactivity (mCi/mmol)	10^{-6} × Total radioactivity (d.p.s.)	10^{-5} × Sp. radioactivity (d.p.s./μmol)	10^{-5} × Total radioactivity (d.p.s.)	10^{-4} × Total radioactivity (d.p.s.)	Radioactivity (d.p.s./mg of protein)
[U-^{14}C]Arginine	66	7.84	4.90	—	—	—
[G-^{3}H]Lysine	1500	35.3	50.5	—	—	—
[*amidine*-^{14}C]-Homoarginine	5	—	—	23.3	2.6	3540

Distribution of transamidinase in the seedlings

The unequivocal presence of the transamidinase activity in the crude extracts of the seedlings was established by using the two transamidinase assay systems with different sets of donor/acceptor combination. The arginine–hydroxylamine amidino-transfer assay method was used to study the relative distribution of the enzyme in different parts of the plant embryo over a 12-day growth period (Fig. 1). The activity was barely detectable on day 1, but it increased steeply subsequently. The highest specific activity was localized in the embryo axis, in which it progressively increased by 8.5-fold to reach the maximum on day 10. The cotyledonary tissue exhibited low transamidinase in terms of specific activity, but the total contribution was roughly equivalent to that of the embryo axis. In the whole seedlings, the maximum activity was reached around day 7 of growth and plateaued off thereafter. The localization of the highest specific activity of the enzyme in the rapidly growing part of the plant embryo is significant.

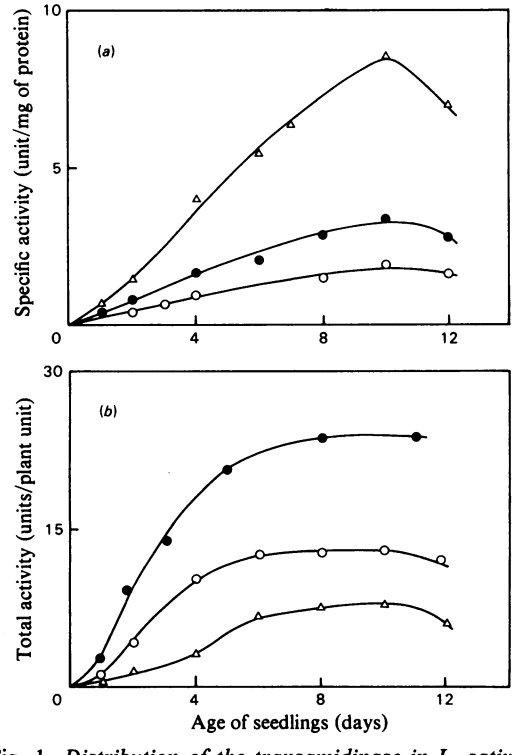

Fig. 1. *Distribution of the transamidinase in L. sativus seedlings during growth*

The enzyme was assayed in the crude extracts of the different parts of the seedlings by the arginine–hydroxylamine amidino-transfer assay as detailed in the text. Plant unit represents a whole seedling or a pair of cotyledons or an embryo axis. Data are the means of triplicate values. (*a*) Specific activity; (*b*) total activity. ●, Whole seedlings; ○, cotyledons; △, embryo axis.

Partial purification of the enzyme

A small-scale purification procedure evolved for this purpose afforded a 463-fold increase in the specific activity of the enzyme, with a yield of 32%. Substantial purification resulted from the step involving affinity chromatography on homoarginine–Sepharose as the insoluble matrix. Fractionation with $(NH_4)_2SO_4$ in the sequence of steps was omitted, since it proved to be deleterious to the enzyme activity. The two assay methods, namely (1) a general transamidinase procedure involving arginine–hydroxylamine amidino transfer to give hydroxyguanidine and (2) a specific assay (canavanine–lysine amidino transfer) leading to homoarginine synthesis, were utilized to follow the progress of purification (Table 2). The constancy of the ratios of the enzyme specific activities obtained with the two assay procedures indicates that a single enzyme with a wide substrate specificity is involved. Fig. 2 shows the elution profile of the transamidinase from the homoarginine–Sepharose column. Washing of the affinity column with 0.1 M-KCl in the equilibration buffer failed to dislodge the enzyme, which could be subsequently

Table 2. *Partial purification of transamidinase from L. sativus seedlings*

For experimental details see the text.

		Arginine–hydroxylamine transamidinase			Canavanine–lysine transamidinase				
Fraction	Protein (mg)	Total activity (units)	Specific activity (units/mg of protein) (*A*)	Purification (fold)	Total activity (units)	Specific activity (units/mg of protein) (*B*)	Purification (fold)	Recovery (%)	Ratio *A*/*B*
Crude extract	5400	5022	0.93	1.0	594	0.11	1.0	100	8.4:1
$MnCl_2$ supernatant	4500	4950	1.1	1.1	585	0.13	1.0	98	8.4:1
Homoarginine–Sepharose affinity step	4.5	1948	433	463	194	42	381	32	10:1

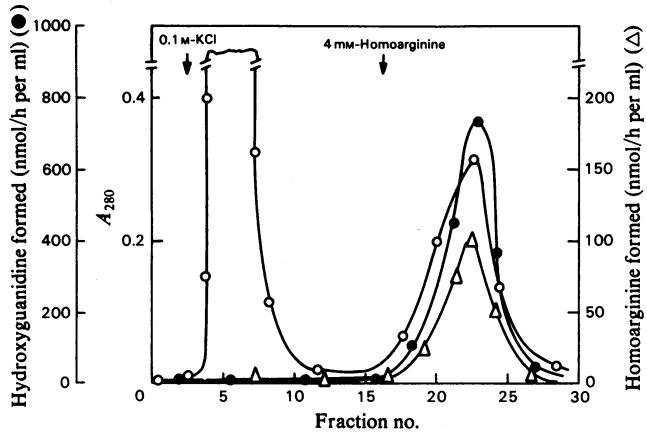

Fig. 2. *Affinity chromatography of transamidinase of L. sativus seedlings on a homoarginine–Sepharose column.* The column (3.5 cm × 11 cm) was equilibrated with 20 mM-potassium phosphate buffer, pH 7.5, containing 2 mM-2-mercaptoethanol. After the removal of nucleoproteins by $MnCl_2$ precipitation, the clear supernatant of the crude extracts of the whole seedlings (300 ml) was applied on the column and washed with equilibration buffer and 0.1 M-KCl in the above buffer successively. The enzyme was eluted (indicated by arrow) by including 4 mM-homoarginine in the equilibration buffer (20 ml). Fractions of volume 2 ml were collected and the enzyme activity was determined by arginine–hydroxylamine transamidinase (●) and canavanine–lysine transamidinase (△) assays as described in the text. O, A_{280}.

eluted by substrate-specific elution (4 mM-homoarginine in the equilibration buffer). Polyacrylamide-gel electrophoresis, on a 7.5% gel, of the concentrated enzyme protein at this stage showed two stainable major protein bands of approximately equal intensity and two minor components (results not shown).

General properties

The transamidinase exhibited a single broad pH optimum at 7.5–8.0 in both the assay systems. In terms of the affinity for its substrates, maximal activity was obtained with arginine concentrations in the range 1–5 mM in the presence of a fixed concentration (0.1 M) of hydroxylamine in the arginine–hydroxylamine amidinotransferase reaction. A Lineweaver–Burk plot gave a K_m of 3.0 mM for arginine. The transamidinase activity was eluted as a single sharp symmetrical peak during the gel filtration on Sephacryl S-200 (superfine grade), with an elution volume corresponding to a mol.wt. of 210000 (results not given).

Reactions catalysed by the enzyme

In view of the wide substrate specificity of animal transamidinases (Walker, 1973), several relevant donor/acceptor combinations were tested as the substrates with this plant enzyme and the products identified (Table 3). The finding that, in addition to the arginine/hydroxylamine and canavanine/lysine combinations used in the assays above, the amidino transfer occurs between the guanidino amino acid and diamines confirms the earlier observations with regard to the amine interconversions. It is noteworthy that significant amounts of arcain were synthesized when agmatine itself functioned as the acceptor, an observation that is in line with the isolation and characterization of the diguanidoamine as a naturally occurring metabolite in this higher-plant system. Of special significance is the mediation of the labelled homoarginine synthesis in the reaction mixtures containing either labelled arginine/unlabelled lysine or vice versa, as well as with unlabelled homoagmatine/labelled lysine, as the donor/acceptor system. Besides supporting the results of the infiltration experiments (Table 1), these observations strongly suggest the involvement of transamidinase in the biosynthesis of homoarginine *in vivo* and point to the probable donor/acceptor combination involved therein. Furthermore, preferential synthesis of homoarginine *vis-à-vis* arginine mediated by the enzyme with canavanine as the common amidine donor and the expected acceptor amino acids is also consistent with this premise. Thus 80 nmol of homoarginine but only 10 nmol of arginine were produced with lysine and ornithine respectively as the amidine acceptors. The plant enzyme, however, did not catalyse the glycine–arginine amidino transfer, as revealed by the failure to produce detectable amounts of ornithine in the assay (Chinard, 1952).

Table 3. *Substrate combinations and the products of L. sativus transamidinase*
The labelled (0.1 μCi) amidino donor or the acceptor was employed. The unlabelled substrate used was at 2 μmol/assay mixture. The different substrate combinations were incubated in the presence of the partially purified enzyme preparation (100 μg) under the assay conditions described in the text. The labelled products were isolated and identified as described in the Materials and methods section.

Donor	Acceptor	Labelled product identified
Arginine	[U-^{14}C]Lysine	Homoarginine
[U-^{14}C]Arginine	Lysine	Homoarginine
Homoagmatine	[U-^{14}C]Lysine	Homoarginine
[*amidine*-^{14}C]Homoarginine	Ornithine	Arginine
[*amidine*-^{14}C]Homoarginine	Cadaverine	Homoagmatine
Homoarginine	[1,5-^{14}C]Cadaverine	Homoagmatine
[U-^{14}C]Arginine	Putrescine	Agmatine
Arginine	[1,4-^{14}C]Putrescine	Agmatine
[U-^{14}C]Arginine	Agmatine	Arcain

Table 4. *Effects of thiol blockers and thiol compounds on the transamidinase activity*
The enzyme activity was assayed by the arginine–hydroxylamine amidino-transfer reaction as described in the text.

Thiol blockers/thiol compounds added (μmol/ml)	Inhibition (%)
p-Hydroxymercuribenzoate (0.5)	55
N-Ethylmaleimide (0.5)	60
Iodoacetate (0.5)	20
Thiolhistidine (0.55)	0
Thioproline (0.55)	0
Thiouracil (0.55)	0
Potassium ferricyanide (2.2)	0
Thiolhistidine (0.55) + ferricyanide (2.2)	90
Thioproline (0.55) + ferricyanide (2.2)	83
Thiouracil (0.55) + ferricyanide (2.2)	71

Effects of thiol compounds and blockers

Since a cysteine residue was shown to participate in the active site of the transmidinase in general (Walker, 1973), the influence of thiol compounds, namely 2-mercaptoethanol, reduced glutathione and dithiothreitol, on the catalytic efficiency of the *L. sativus* enzyme was investigated in the concentration range 1–10 mM. Among these, addition of dithiothreitol (2 mM) to the reaction mixture had the maximum beneficial effect, the stimulation of the enzyme activity being 2-fold (results not shown).

The thiol nature of the plant transamidinase was further exemplified by strong inhibition of the activity on the inclusion of well-known thiol blockers (Table 4). Furthermore, some other thiol compounds, namely thiolhistidine, thioproline and thiouracil, though inactive by themselves, exerted strong deleterious effects on the activity of the enzyme when incubated in the presence of ferricyanide, which, however, by itself had no influence (Walker, 1958).

Hydrolytic activity of the plant transamidinase

In the absence of an appropriate exogenous acceptor, the donor was previously shown to be slowly hydrolysed by the transamidinase preparation (Walker, 1973), with water acting as the endogenous acceptor; the amidine donor was presumed to cleave to urea and the corresponding derivatives. The partially purified *L. sativus* enzyme similarly catalysed arginine hydrolysis, but with a rate as low as 1% of that of the transferase activity. Thus with hydroxylamine (100 μmol) as acceptor hydroxyguanidine (280 nmol) was produced. The possibility that arginase contamination in the enzyme preparation might account for this observation appears unlikely, since the addition of Mn^{2+} resulted in only marginal (25%) stimulation in urea production, in comparison with the other systems wherein exogenous Mn^{2+} was shown to stimulate arginase activity by severalfold (Greenberg, 1951).

Analogue inhibition

Fig. 3 depicts the pattern of the inhibition of the enzyme activity by different substrate analogues, as determined by the arginine–hydroxylamine transamidinase assay. Interestingly, ornithine competitively inhibited both the canavanine–lysine transamidinase and the arginine–hydroxylamine transamidinase activities, with a K_i of 1.0 mM for the latter reaction. Diaminobutyrate and lysine were weak inhibitors, whereas glycine and norvaline caused only a marginal inhibition. The strong inhibition by ornithine, which is also a product, is in accordance with previous observations with the animal transamidinases (Walker, 1973).

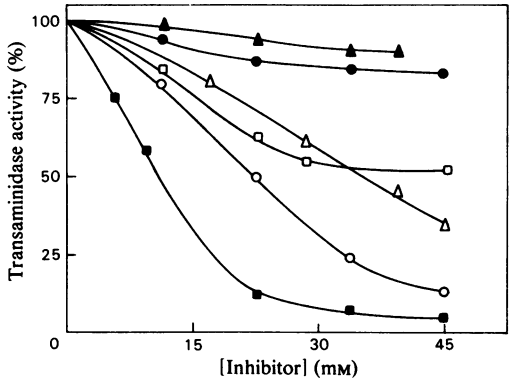

Fig. 3. *Inhibition of transamidinase by different analogues*

The enzyme activity was assayed by the arginine–hydroxylamine amidino-transfer reaction in the presence of the inhibitors at the indicated concentrations: ○, L-ornithine; △, L-2,4-diaminobutyrate; □, L-lysine; ●, glycine; ▲, norvaline. The effect of ornithine (■) on the enzyme activity was also determined in canavanine–lysine amidino-transfer reaction.

Discussion

In the present investigation, a simple affinity procedure employing homoarginine–Sepharose was adopted to achieve substantial purification of a transamidinase from *L. sativus* seedlings, and represents the first attempt at the partial purification, elucidation of some properties and functions of a transamidinase from a higher plant. The observation that the activity was not detectable in the seeds and the seedlings up to day 1, but that both total and specific activities registered a co-ordinated increase during subsequent development, suggests that the enzyme is synthesized *de novo* to subserve embryonic growth. However, activation of pre-existing inactive enzyme protein during seedling development cannot be ruled out at this stage. That it may be intimately associated with the nutrient mobilization at the very site of rapid cellular proliferation is suggested by the preferential and progressive enhancement in its specific activity in the embryo axis up to day 10 of growth. Parallel changes in both specific and total activities are also commensurate with the above premise.

In terms of general properties, the plant enzyme exhibits several characteristics in common with the mammalian and microbial transamidinases, particularly with regard to wide substrate specificity, the reversibility of the reactions catalysed, inhibition by various analogues and the thiol nature. An important feature of the present findings is the evidence for the probable mode of biosynthesis of homoarginine in *L. sativus*, a pathway that has remained elusive so far. In the past, several investigators (Kleczkowski, 1966; Jasiorowska & Kleczkowski, 1970; Bell, 1971) have speculated that this unusual guanidino amino acid might arise by a pathway analogous to that functioning in arginine synthesis; the involvement of either the urea-cycle enzymes or a separate set of similar enzymes was implicated in the process, but this has not been proved so far. The speculation (Brown & Al-Baldawi, 1977) that the unusual dehydroxylation of γ-hydroxyhomoarginine functions as a probable route of homoarginine synthesis has not yet been substantiated. The direct involvement of the transamidinase in the biogenesis of the guanidino amino acid is clearly suggested by the results given in Table 3, showing that lysine functions as an efficient acceptor of the amidino carbon from arginine. Additional support for this premise stems from the infiltration experiment (Table 1) with labelled lysine and arginine to give rise to labelled homoarginine with specific radioactivities consistent with their being the physiological acceptor and donor respectively of amidino function. In accordance with the above are the findings that (*a*) the plant enzyme has a specific affinity for homoarginine–Sepharose, (*b*) canavanine/lysine combination as the substrate generates homoarginine as the product, and (*c*) the enzyme preferentially utilizes lysine *vis-à-vis* ornithine as the acceptor under the conditions of assay. It is conceivable that the relatively weak homoarginine-synthetic activity during the seedling growth reflects its role more as a mediator of homoarginine catabolism rather than its biosynthetic function to generate lysine and arginine (Table 3). This is also supported by the incorporation of the label from homoarginine into arginine in the infiltration experiments (Table 1). This implies that ornithine is the physiological amidine acceptor from homoarginine under these conditions; the earlier demonstrations that, during germination and seedling development among the several leguminous and other plants, ornithine concentrations steeply increased in cotyledons and/or embryo axis (Boulter & Barber, 1963; Lawrence & Grant, 1963; Boulter & Davis, 1968; Chou & Splittstoesser, 1972) are consistent with this postulate. On the basis of this argument, during the fruiting stage, when homoarginine accumulates in the seeds, the reverse situation should prevail, with the transamidinase preferentially catalysing the biosynthesis of the guanido amino acid. What physiological signal triggers this reversal in enzyme function awaits further study.

It is also clear from the present findings that the transamidinase has other important functions during seedling growth, namely the involvement in amine

interconversion (Table 3). Since the partially purified enzyme also catalyses agmatine synthesis from putrescine, it would appear that the transamidinase plays a significant role in polyamine production by regulating the diamine concentration. Additionally, since putrescine at higher concentrations is toxic to *Anacystis nidulans* (Guarino & Cohen, 1979) and to higher plants (Coleman & Richards, 1956; Wielgat & Kleczkowski, 1971), the enzyme might assume a detoxifying function, particularly under conditions of K^+ deficiency with associated high intracellular concentration of the diamine (Smith, 1970). Lastly, though the biological role of arcain in plants is unclear, the transamidinase involvement in its biosynthesis in *L. sativus* is unequivocal (Table 3); the demonstration of the natural occurrence of the biguanidino compound in this plant system is in line with the above findings.

From the foregoing, it would appear that the transamidinase plays many important roles in *L. sativus*, these being attributable to its capacity to function with a variety of donor/acceptor combinations as the substrates. The earlier findings that homoarginine is actively transported to the embryo axis during seedling development (Ramakrishna & Adiga, 1973), and the present observation that the enzyme can efficiently utilize this guanidino amino acid as a substrate to yield, depending on the acceptor, a variety of metabolites including amino acids such as arginine and lysine, supports our earlier conclusion that homoarginine in *L. sativus* is not just an inert nitrogen-rich storage product/secondary metabolite, but provides essential metabolites for proper development of the plant embryo (Ramakrishna & Adiga, 1974, 1976).

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