Tyrosine Biosynthesis in *Sorghum bicolor:* Characteristics of Prephenate Aminotransferase

Daniel L. Siehl, James A. Connelly, and Eric E. Conn

Department of Biochemistry and Biophysics, University of California, Davis, California 95616, U.S.A.

Z. Naturforsch. **41c,** 79–86 (1986); received June 28/August 14, 1985

Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

Prephenate: Glutamate Aminotransferase, Tyrosine Biosynthesis, *Sorghum bicolor,* Shikimate Pathway, Arogenate

A stable activity which transfers the amino group from glutamate to prephenate was extracted from 4-day old etiolated shoots of sorghum. The activity was retained on DEAE cellulose and eluted as a single peak. Prephenate aminotransferase co-eluted with a very abundant α-ketoglutarate: aspartate aminotransferase, but heating at 70 °C resulted in loss of α-ketoglutarate: aspartate activity with nearly full retention of prephenate: glutamate aminotransferase activity. The heated enzyme displayed high affinity and specificity for prephenate. Among 7 donors tested, only glutamate, and aspartate at less than 20% the rate with glutamate, supported prephenate aminotransferase activity. In the reverse direction, a reaction rate comparable to that in the forward direction was unchanged as the concentration of α-ketoglutarate was reduced from 1.0 to 0.09 mM. The apparent K_m for arogenate was 0.8 mM. The forward reaction was unaffected by the inclusion of tyrosine, phenylalanine or tryptophan. Together with the discovery of arogenate dehydrogenase in sorghum [3], these data indicate that, in the sorghum plant, tyrosine derives from prephenate by transamination and aromatization, rather than the reverse sequence.

Introduction

Young sorghum plants produce large quantities of the cyanoglucoside dhurrin, through a series of reactions for which tyrosine serves as precursor (for review, see Conn [1]). There is considerable diversity among species in their routing of metabolite flow from prephenate to tyrosine and phenylalanine, arising from the possibility that the reactions of transamination and aromatization may proceed in either order (for review, see Byng [2]). The order of these reactions has not been defined in sorghum. As reported in the accompanying article [3], an abundant enzymatic activity was found for the dehydrogenation of arogenate, but not of prephenate, to produce tyrosine. This seemed to define the pathway from prephenate to tyrosine as proceeding by transamination followed by aromatization, and presupposed the existence of a prephenate aminotransferase capable of producing arogenate. The present study sought evidence for such an enzyme by pursuing the following specific aims: (1) search for prephenate and phenylpyruvate aminotransferases in crude extracts of sorghum, assaying in the direction of biosynthesis; (2) establish whether the activity is specific for the biosynthetic intermediate, or represents overlapping activity of another, abundant, aminotransferase; (3) investigate the physical, kinetic and regulatory properties of the enzyme.

Reprint requests to Prof. Dr. E. E. Conn.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0100–0079 $ 01.30/0

Methods

Plant material, extraction of enzymes

Seedlings of a *Sorghum bicolor* × *Sorghum sudanensis* hybrid (WAC Forage 99, WAC Seed, Inc., Hereford, Texas 79045) were grown in the dark on a wire mesh platform between double layers of grade 10 cheesecloth. Four to 5 days after inbibition, the 6–10 cm shoots were harvested and immediately immersed in liquid nitrogen in a large mortar. The shoots were ground to a fine powder. The powder was transferred to a mortar at room temperature, an equal volume of extraction buffer (200 mM Tris-HCl, pH 8, 1% β-mercaptoethanol, 20% ethylene glycol) was added, and the mixture was ground together until thawed. This crude extract was squeezed through fine cheesecloth and centrifuged at 15,000 × *g* for 10 minutes. The supernatant was layered over a column of Sephadex G-25 which had been equilibrated with DEAE cellulose loading buffer (50 mM Tris-HCl, pH 8, 20% ethylene glycol). Fractions contain-

ing protein were pooled and loaded onto a column (2.4 cm diameter × 12 cm) of Whatman DE52. The column was washed with 2 column volumes of loading buffer and eluted with a linear gradient of loading buffer containing 0.0 to 0.5 M NaCl in a total of 8 column volumes. The pool of fractions containing prephenate aminotransferase activity was dialyzed against 50 mM Tris-HCl, pH 8, 20% ethylene glycol, and was the source of enzyme for analysis of the properties. Most of the results were obtained from a similar procedure in which ethylene glycol was not present in the extraction or DEAE loading buffers. The presence of ethylene glycol did not affect the concentration of NaCl at which prephenate aminotransferase eluted from DEAE cellulose, but resulted in about a 1.5-fold greater recovery of activity with respect to fresh weight of starting material.

Assay of prephenate aminotransferase

Activity in the direction of arogenate formation was assayed by monitoring the production of ^{14}C labeled α-ketoglutarate from prephenate and L[U-^{14}C] glutamate. Appropriately diluted enzyme mixed with unlabeled glutamate was delivered as a 10 µl aliquot. Ten minutes were allowed for conversion of the cofactor from pyridoxal to pyridoxamine. (When labeled and unlabeled glutamate were added together to an aliquot of enzyme, labeled α-ketoglutarate was produced as a burst, in the presence or absence of any α-keto acceptor.) To start the reaction, prephenate and labeled glutamate were added as a 10 µl aliquot. Reaction mixtures contained 25 mM Tris-HCl, pH 8, 10% ethylene glycol, 0.8 µM pyridoxal phosphate, 8 µM EDTA and various concentrations of amino donors and α-keto acid acceptors as indicated in the legends of figures and tables. Most assays contained glutamate at 1.33 mM and 55.9 Bq./nmol. For determination of the K_m for glutamate, a constant amount of [^{14}C]glutamate (1486 Bq.) was added to each reaction, resulting in various specific activities, depending on the concentration of glutamate. Reactions were stopped by adding 200 µl of 0.05 N HCl. The amount of [^{14}C]-α-ketoglutarate produced was determined as follows: 215 µl of each assay mixture was layered over a 0.5 ml bed of Dowex 50 X8-400, equilibrated with water adjusted to pH 3.5 whith HCl. The column was washed with 2 column volumes of water at pH 3.5. All of the pass-through and washings were collected in a scintillation vial for counting. The column retained 99.7% of the [^{14}C]glutamate applied.

Commercial preparations of [^{14}C]glutamate were contaminated with material, presumably α-ketoglutarate, which did not bind to Dowex 50. This was removed by binding [^{14}C]glutamate to Dowex 50 at pH 3.5, washing with water at pH 3.5, then eluting glutamate with 4 column volumes of 1 N NH_4OH. The eluate was evaporated by a stream of nitrogen gas until nearly dry. The contaminant gradually reappeared, requiring that the purification procedure be repeated monthly.

That arogenate was the amino acid formed in the forward reaction was determined by derivatization with *o*-phthalaldehyde and analysis by HPLC. Reaction mixtures were similar to the above, except that [^{14}C]glutamate was omitted and that Tricine buffer was substituted for Tris, the *o*-phthalaldehyde derivative of which interfered with the analysis. Reactions were stopped by adding 30 µl of 0.4 M Borate-KOH, pH 10.4 and 5.0 µl of *o*-phthalaldehyde reagent, consisting of 10% methanol, 0.3% β-mercaptoethanol and 0.1% *o*-phthalaldehyde (Sigma) in 0.4 M Borate-KOH, pH 10.4. After 5 min, the mixture was chromatographed isocratically on a 0.4 cm × 25 cm octadecyl silane HPLC column (Bio Rad), at a flow rate of 1 ml of 5 mM potassium phosphate, pH 7.2 per min, monitoring the absorbance at 336 nm. Alternatively, reactions were stopped by the addition of 20 µl 0.1 N HCl. After 40 min, the mixture was neutralized with 20 µl 0.1 N NaOH. Derivatization with *o*-phthalaldehyde proceeded, as above, by the addition of 30 µl 0.4 M Borate-KOH, pH 10.4 and 5 µl *o*-phthalaldehyde reagent. Analysis by octadecyl silane HPLC was performed at a flow rate of 1 ml/min according to the following program: 100% 5 mM potassium phosphate, pH 7.2 for 5 min, linear gradient solution from 100% to 70% potassium phosphate, 0% to 30% acetonitrile for 10 min, final solvent conditions for 10 more min.

The activity in the direction of prephenate formation was assayed by monitoring the production of prephenate from arogenate and α-ketoglutarate. Enzyme and α-ketoglutarate were combined at 4-fold their final concentrations and distributed in aliquots of 10 µl. After 10 min, reactions were started by adding 30 µl of arogenate. Reactions were stopped by adding 30 µl of 1 N HCl. After 10 min, sufficient for the acid-catalyzed conversion of prephenate to phenylpyruvate, 130 µl of 1 N NaOH were added

and the optical density at 320 nm was determined immediately. The reaction rate was quantified using the molar extinction coefficient of 17,500 $M^{-1}cm^{-1}$ for phenylpyruvate [4].

Preparation of substrates

Prephenate and arogenate were prepared as described in the accompanying article [3]. Further purification was achieved by preparative HPLC. Prephenate solutions were prepared for HPLC by passing through C-18 Sep-Pak cartridges (Waters, Assoc.) equilibrated with water. The resulting material was applied to a preparative octadecyl silane column (Whatman Partisil, 1 × 50 cm) and eluted with 5 mM potassium phosphate, pH 7.2 at 3 ml/min. The material absorbing at 220 nm appearing after the void volume was collected, and concentrated at 35 °C under vacuum. It was then applied to a preparative cyclodextrin bonded phase column (Cyclobond, Astec, Inc., Whippany, New Jersey 07981) and eluted at 3 ml/min with 5 mM potassium phosphate, pH 7.2: methanol, 9:1. The arogenate obtained as described in the accompanying article was applied directly to the preparative cyclodextrin column and eluted in the same buffer. The peaks of prephenate, identified by acid catalyzed conversion to phenylpyruvate, and arogenate, identified by thin layer chromatography of the dansyl derivative on micropolyamide [5], were collected and concentrated by lyophilization. Concentrations of prephenate were determined spectrophotometrically after acid-catalyzed conversion to phenylpyruvate [6].

Purified arogenate gave a single peak of material absorbing at 220 nm when chromatographed on a cyclodextrin bonded phase (HPLC) column and a single peak after derivatization with *o*-phthalaldehyde when chromatographed on octadecyl silane (HPLC) column. In addition, because acid catalyzes the rapid conversion of arogenate to phenylalanine [7], acid-treatment relocated the *o*-phthalaldehyde derivative of purified arogenate to the retention time of *o*-phthalaldehyde-phenylalanine when run on octadecyl silane with 5 mM potassium phosphate, pH 7.2: acetonitrile, 4:1.

The concentrations of solutions of arogenate known to be free of other *o*-phthalaldehyde reacting compounds and of the gamma-lactam of arogenate were determined by comparing the optical density of the *o*-phthalaldehyde derivative of acid-treated samples with a standard curve generated with phenylalanine. To 20 µl of sample was added 20 µl N HCl. After 10 min, 20 µl 0.1 N NaOH, 140 µl water, 180 µl 0.4 M Borate-KOH, pH 10.4 and 20 µl *o*-phthalaldehyde reagent were added. After 5 min, the optical density at 336 nm was determined. Impure solutions of arogenate could be quantified by chromatography of *o*-phthalaldehyde samples on an octadecyl silane HPLC column, run with 5 mM potassium phosphate, pH 7.2. The area of the *o*-phthalaldehyde-arogenate peak of the unknown solution was compared with that of standard arogenate.

High performance anion exchange chromatography of aminotransferases

An aliquot of prephenate aminotransferase activity obtained by chromatography on DEAE cellulose and Sephadex G-75 was injected on a SynChropak AX300 column (SynChrom, Inc., Linden, Indiana) equilibrated with 20 mM Tris-Acetate, pH 8. The column was washed with starting buffer for 6 min at a flow rate of 3 ml/min, and eluted with a 60 ml gradient of 0.0 to 1.2 M sodium acetate in starting buffer. Fractions (1.3 ml) containing activity were desalted by dialysis against 50 mM Tris-HCl, pH 8, 20% ethylene glycol.

Stability of aminotransferases to heating

Pooled activity from DEAE cellulose chromatography was dialyzed against 50 mM potassium phosphate, pH 7.2, 1 mM EDTA, 0.1 mM pyridoxal phosphate and 20% glycerol, a mixture of agents determined to be effective in stabilizing prephenate aminotransferase from tobacco cells [8]. Aliquots were heated at various temperatures for 17 min in capped microcentrifuge tubes, after which enzyme activities were determined.

Isoelectric focussing was performed on LKB PAG-plates, pH 3.5–9.5, by the procedure supplied by the manufacturer. Activity was recovered from gels as follows: the area of the gel containing protein (determined by staining a parallel lane with Coomassie Blue) was cut into 1 mm slices. To each slice was added 20 µl of 400 mM Tris-HCl, pH 8 in 20% ethylene glycol. After 15 hr at 4 °C, 5 µl were removed for assay of prephenate: glutamate aminotransferase by the method employing [^{14}C]glutamate. The remainder was assayed for α-ketoglutarate: aspartate activity, as indicated in the legend to Fig. 1.

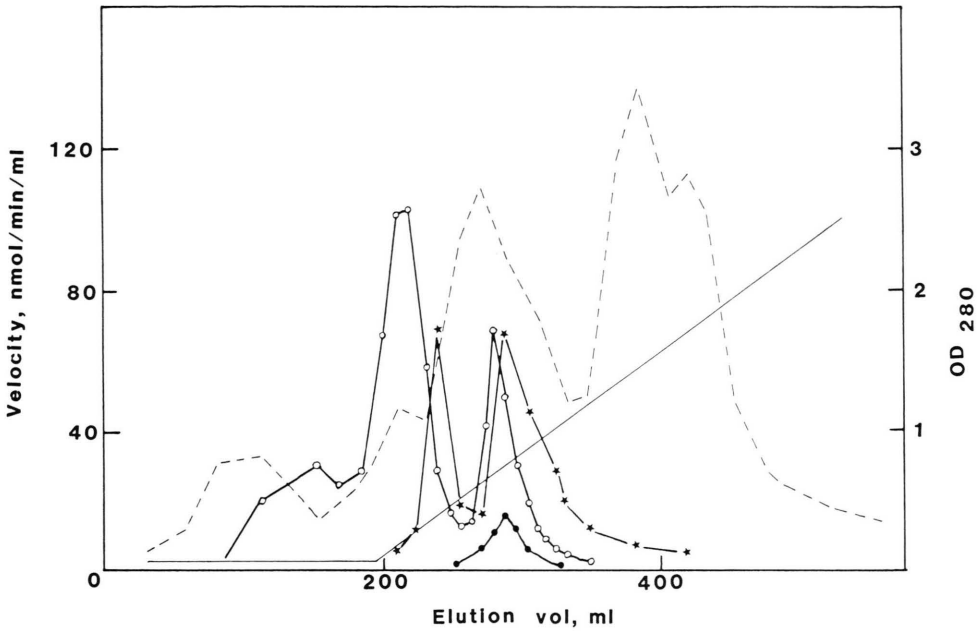

Fig. 1. Chromatography of aminotransferases on DEAE cellulose. 80 ml (160 mg protein) of crude extract recovered from Sephadex G-25 were chromatographed as described under Methods. Glyoxylate/serine and α-ketoglutarate : aspartate aminotransferases were assayed by reactions coupled to the oxidation of NADH, as described by Nakamura and Tolbert [18]. Recovery of prephenate : glutamate aminotransferase was 102%. Thin solid line represents the concentration of NaCl, from 0.0 to 0.5 M. Thin broken line represents the absorbance at 280 nm. Symbols for aminotransferase: ○——○, α-ketoglutarate : aspartate; ★——★, glyoxylate : serine; ●——●, prephenate : glutamate.

Protein concentrations were determined by the method of Lowry [9] using BSA as the standard.

Results

Initial studies sought to evaluate the amounts of aminotransferase activity toward prephenate relative to that with phenylpyruvate and *p*-hydroxyphenylpyruvate in crude extracts of sorghum. Prephenate aminotransferase was the predominant activity (not shown) which, together with the existence of arogenate dehydrogenase, suggested that transamination of prephenate preceded aromatization in tyrosine biosynthesis. Further evidence was obtained by characterizing the activity, as follows.

Chromatography on DEAE cellulose (Fig. 1) resulted in one peak of prephenate : glutamate aminotransferase activity, which co-eluted with peaks of α-ketoglutarate : aspartate and glyoxylate : serine aminotransferases, each of which were resolved into two peaks. Chromatography of the pooled activity toward prephenate on Sephadex G-75 resulted in the separation of prephenate : glutamate from glyoxylate : serine aminotransferase, but not from α-ketoglutarate : aspartate (not shown).

Three lines of evidence indicate that prephenate activity is the property of a protein distinct from α-ketoglutarate : aspartate aminotransferase. First, when aliquots of pooled aminotransferase activity from DEAE cellulose were incubated in the presence of ammonium sulfate, activity of prephenate : glutamate aminotransferase in the 9000 × *g* supernatant fell from 100% of total at 40% saturation, to 63% at 50% saturation, to 0% at 60% saturation. In the case of α-ketoglutarate : aspartate activity, 95%, 90% and 63% of total activity remained in the supernatant at 40%, 50% and 60% saturation, respectively.

Second, chromatography on a high performance anion exchange column resulted in distinct peaks of prephenate : glutamate and α-ketoglutarate : aspartate aminotransferase activities. Because of the abundance of the latter, the slight separation was inadequate for preparing prephenate : glutamate activity free of α-ketoglutarate : aspartate aminotransferase.

Third, as was the case with the enzyme from tobacco cells [8], prephenate : glutamate aminotransferase was remarkably stable to heating. Ninety-five percent of prephenate : glutamate activity remained after heating at 70 °C for 17 min. The value for α-ketoglutarate : aspartate was 8%. Whether this represented residual activity of α-ketoglutarate : aspartate aminotransferase or overlapping activity of another, heat-stable, aminotransferase was not determined.

Identification of product

The amino acid product of prephenate : glutamate aminotransferase was identified as arogenate. In an analysis by HPLC of the *o*-phthalaldehyde derivatives of a standard reaction mixture, a product appeared which co-eluted with *o*-phthalaldehyde-arogenate (not shown). Furthermore, 40 min incubation of an identical reaction mixture with acid, followed by derivatization, resulted in a shift of the product peak to the retention time of *o*-phthalaldehyde-phenylalanine. Arogenate is the only known compound that both reacts with *o*-phthalaldehyde and is converted to phenylalanine by acid catalysis.

Physical properties

The isoelectric point, as determined by recovery of activity from isoelectric focussing gels, was pH 5.2 (not shown). Optimal activity occurred at pH 8.0 to 8.5. Activity was nil at a pH below 6.0 or above 10.5.

Kinetic observations

Kinetic analysis was undertaken to assess the affinities of prephenate and glutamate for the heated aminotransferase isolated from sorghum. The saturation curve for prephenate in the presence of glutamate at a concentration near its apparent K_m (see below) showed a complex interaction (Fig. 2). At very low concentrations of prephenate, the reaction rate rose sharply with increasing substrate. At intermediate concentrations (0.04 to 0.24 mM), the rate still rose with substrate concentration, but the effect of substrate inhibition became apparent. At concentrations above 0.24 mM prephenate, the reaction rate decreased. A rough estimate of the apparent K_m for prephenate at the three lowest concentrations would be 70 μM.

Conventional kinetics were observed when the concentration of glutamate was varied and prephenate was held at the optimal concentration of 0.25 mM (Fig. 3). The double reciprocal plot indicated an apparent K_m of 1.07 mM for glutamate.

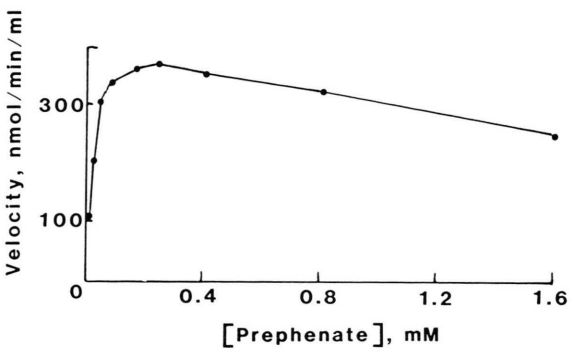

Fig. 2. Activity of prephenate aminotransferase as a function of the concentration of prephenate. Reaction mixtures (20 μl) contained [^{14}C]glutamate at 1.33 mM and 55.9 Bq/nmol, various concentrations of prephenate, 1.33 μg of heated prephenate aminotransferase from DEAE cellulose chromatography and additions as indicated under Methods.

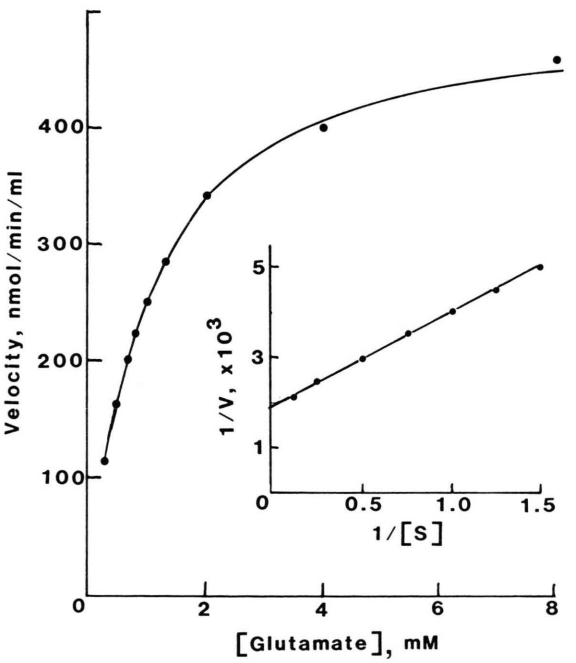

Fig. 3. Activity of prephenate aminotransferase as a function of the concentration of glutamate. Reaction mixtures contained 250 μM prephenate, 1486 Bq of [^{14}C]glutamate, various concentrations of unlabeled glutamate, 1.33 μg of heated prephenate aminotransferase from DEAE cellulose chromatography, and other additions as described under Methods.

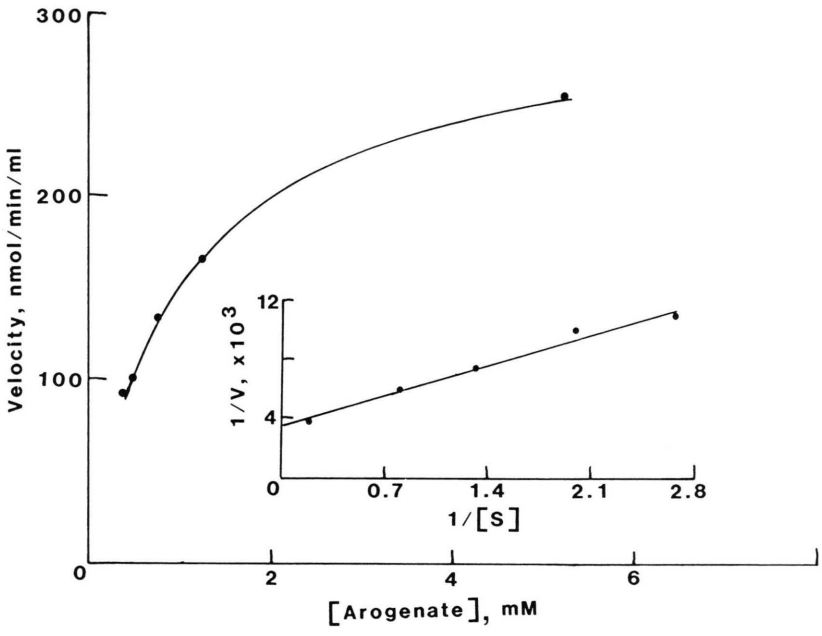

Fig. 4. Activity of prephenate aminotransferase measured in the direction of prephenate formation. Reactions were performed using 1.0 mM α-ketoglutarate, arogenate as indicated and other additions, as described under Methods.

In the direction of prephenate formation, the enzyme showed a similar high affinity for the α-keto acid and lower affinity for the amino acid. When measured at 0.75 mM arogenate, the reaction rate did not depend on the concentration of α-ketoglutarate over the range of 0.088 to 1.14 mM (not shown). On the other hand, when measured at 1 mM α-ketoglutarate, the reaction rate showed conventional saturation kinetics as the concentration of arogenate was increased (Fig. 4). The apparent K_m for arogenate was 0.8 mM.

Substrate specificity

Substrate specificity was assessed in the pooled prephenate : glutamate activity from DEAE cellulose chromatography, heated at 70 °C for 17 min. First, activity was measured for various α-keto acceptors with glutamate as the amino donor (Table I). Concentrations of the keto acids were selected to approximate 1 and 10 times the apparent K_m for prephenate (Fig. 2). Other than with prephenate, appreciable activity was found only in the presence of oxaloacetate, which reacted at 78% of the rate with prephenate at high concentration, but only 18% of the rate with prephenate at the low concentration. This suggests either a lower affinity interaction of oxaloacetate with prephenate : glutamate aminotransferase, or the presence of a separate enzyme. These possibilities were not resolved.

Table I. Activity of prephenate aminotransferase in the presence of various α-keto acids and glutamate. Reaction mixtures (20 µl) contained [^{14}C]glutamate at 1.33 mM and 55.9 Bq/nmol, the α-keto acid indicated, 3.3 µg of heated prephenate aminotransferase from DEAE cellulose chromatography, and other additions as described under Methods. The rate of α-ketoglutarate production at 100% activity was 104 and 90.4 pmol/min for 0.1 and 1.0 mM prephenate, respectively. Substrates supporting less than 1.0% activity at both concentrations included β-phenylpyruvate, β-hydroxypyruvate, glyoxylate, α-ketoisocaproate and α-ketoisovalerate.

α-Keto acid	Concentration [mM]	Activity*
Prephenate	0.1	100
	1.0	100
Oxaloacetate	0.1	18.6
	1.0	78.3
Pyruvate	0.1	1.0
	1.0	9.9
p-Hydroxyphenylpyruvate	0.1	0.4
	1.0	10.1

* Expressed as a percentage of the activity observed with prephenate at the same concentration.

Specificity for the amino donor was assessed in reactions of prephenate with various amino acids

Table II. Activity of prephenate aminotransferase in the presence of various α-amino acids and prephenate. Reaction mixtures (20 μl) contained 250 μM prephenate, 3.3 μg of heated prephenate aminotransferase from DEAE cellulose chromatography, the indicated amino acid, and other additions as described under Methods. Activity was measured as the production of the ^{14}C-labeled α-keto analogue of each amino acid, as described under Methods. Substrates supporting less than 1.0% activity at 1.0 and 4.0 mM included leucine, phenylalanine, glycine, serine and alanine.

Amino acid	Concentration [mμ]	Activity [pmol/min]	[% of glutamate]
Glutamate	0.5	64.8	100
	1.0	99.6	100
	2.0	137	100
	4.0	161	100
Aspartate	0.5	11.9	18.3
	1.0	17.7	17.8
	2.0	19.6	14.3
	4.0	17.3	10.7

(Table II). Other than with glutamate, appreciable activity was observed only in the presence of aspartate.

Regulation

The possibility that prephenate : glutamate aminotransferase is regulated by aromatic amino acids was investigated by including them individually in standard reaction mixtures. The reaction rate with 250 μM prephenate and 1.33 mM glutamate was unchanged by the inclusion of tyrosine, phenylalanine or tryptophan at concentrations up to 2.0 mM.

Discussion

In crude extracts of sorghum seedlings, transaminase activity for prephenate was found to predominate over that for phenylpyruvate and *p*-hydroxyphenylpyruvate. Further purification of the prephenate activity by DEAE cellulose chromatography and heating resulted in an activity that showed high affinity (Fig. 2) and specificity (Table II) for prephenate. These findings, together with the discovery of arogenate, but not prephenate, dehydrogenase [3] strongly suggest that production of tyrosine from prephenate proceeds in sorghum seedlings by transamination followed by dehydrogenation, rather than the reverse sequence. Thus, tyrosine biosynthesis in sorghum appears to be similar to that in tobacco, where arogenate, but not prephenate dehydrogenase [10] and prephenate aminotransferase [8] were found. On the other hand, mung bean had a dual pathway, containing prephenate and arogenate dehydrogenase as well as prephenate and *p*-hydroxyphenylpyruvate aminotransferase activities [11].

The aminotransferase activity toward aromatic α-keto acids observed in crude extracts of sorghum seedlings may represent overlapping activity of the very abundant oxaloacetate : glutamate activity present. Forest and Wightman [12] purified oxaloacetate : glutamate aminotransferase from bushbean and found that aromatic α-keto acids also supported activity, but at less than 10% the rate with oxaloacetate. Similarly, a low affinity aromatic aminotransferase activity was found in oak leaves, but the activity of the preparation toward oxaloacetate was not reported [13]. These and other studies (for review, see Wightman and Forest [14]) performed prior to the discovery of the arogenate pathway [15] sought evidence that fit the paradigm for aromatic amino acid biosynthesis established in enteric bacteria (for review, see Pittard and Gibson [16]).

As was the case with the enzyme from tobacco cells [8], prephenate aminotransferase from sorghum exhibited substrate inhibition. Kinetic data from numerous studies indicate a ping-pong reaction mechanism for aminotransferases, in which the enzyme shifts back and forth between two forms, each of which reacts with only one of the substrates [14]. This mechanism is subject to inhibition by a high affinity substrate [17]. In the case of prephenate aminotransferase, the reaction sequence is proposed to be:

	Preph		Arog	Glu		αKG
E-PAP	↓	E-PLP	↑	↓	E-PAP	↑

in which PAP represents pyridoxamine phosphate and PLP, pyridoxal phosphate. Apparently, prephenate reacts not only with the pyridoxamine form of the enzyme, but also interacts with the pyridoxal form in such a way as to inhibit the reaction with glutamate.

It is not to be assumed that an aminotransferase catalyzes a freely reversible reaction. The glyoxylate : serine reaction of the photorespiration cycle was shown to be kinetically irreversible in spinach [18] and wheat [19]. The data presented in this paper do, however, indicate that the prephenate : gluta-

mate reaction is freely reversible. Directionality for net flux to tyrosine would be conferred by the subsequent aromatization and decarboxylation catalyzed by arogenate dehydrogenase.

The apparent lack of regulation of prephenate aminotransferase by phenylalanine and tyrosine suggests that prephenate is not located at the branch point between these products. Conversely, the inhibition of arogenate dehydrogenase by tyrosine [3] suggests that arogenate is the branch-point. This would imply the existence of an arogenate dehydratase for the production of phenylalanine. We are currently searching for such an activity.

Acknowledgement

This work was supported in part by the U.S. Public Health Service Grant (GM-05301-27).

[1] E. E. Conn, Cyanogenic glycosides, in: The biochemistry of plants: a comprehensive treatise (P. K. Stumpf and E. E. Conn, eds.), **Vol. 7,** Academic Press, New York 1980.
[2] G. S. Byng, J. F. Kane, and R. A. Jensen, C.R.C. Crit. Rev. Micro. **9,** 227–252 (1982).
[3] J. A. Connelly and E. E. Conn, Z. Naturforsch. **41c,** 69–78 (1986).
[4] M. I. Gibson and F. Gibson, Biochem. J. **90,** 248–256 (1964).
[5] A. M. Fazel and R. A. Jensen, J. Bact. **138,** 805–815 (1979).
[6] R. L. Metzenberg and K. K. Mitchell, Arch. Biochem. Biophys. **64,** 51–56 (1956).
[7] L. O. Zamir, R. Tiberio, and R. A. Jensen, Tetrahedron Lett. **24,** 2815–2818 (1983).
[8] C. A. Bonner and R. A. Jensen, Arch. Biochem. Biophys. (1985), in press.
[9] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193,** 265–275 (1951).
[10] C. G. Gaines, G. S. Byng, R. J. Whitaker, and R. A. Jensen, Planta **156,** 233–240 (1982).
[11] J. L. Rubin and R. A. Jensen, Plant Physiol. **64,** 727–734 (1979).
[12] J. C. Forest and F. Wightman, Can. J. Biochem. **50,** 813–829 (1972).
[13] P. Gadal, H. Bouyssou, and J.-P. Barthe, Physiol. Veg. **7,** 69–80 (1969).
[14] F. Wightman and J. C. Forest, Phytochemistry **17,** 1455–1471 (1978).
[15] S. L. Stenmark, D. L. Pierson, and R. A. Jensen, Nature **247,** 290–292 (1974).
[16] J. Pittard and F. Gibson, Cur. Top. Cell. Reg. **2,** 29–63 (1970).
[17] W. W. Cleland, Steady state kinetics, in: The enzymes (P. D. Boyer, ed.), **Vol. II,** Academic Press, New York 1970.
[18] Y. Nakamura and N. E. Tolbert, J. Biol. Chem. **258,** 7631–7638 (1983).
[19] J. King and E. R. Waygood, Can. J. Biochem. **46,** 771 (1968).