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ENZYMOLOGY OF L-TYROSINE BIOSYNTHESIS IN CORN (*ZEA MAYS*)

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Key Word Index—*Zea mays*; Gramineae; corn; arogenate dehydrogenase; chorismate mutase; shikimate dehydrogenase; purification; kinetics.

Abstract—A newly found amino acid, denoted L-arogenate, has been identified as an intermediate in L-tyrosine biosynthesis in corn. Activity for prephenate dehydrogenase was not detected. Arogenate dehydrogenase, chorismate mutase and shikimate dehydrogenase were partially purified from root tissue of *Zea mays* by DEAE-cellulose chromatography. NAD^+ was required for arogenate dehydrogenase activity, while $NADP^+$ was obligatory for shikimate dehydrogenase activity. L-Tyrosine was an effective feedback inhibitor of arogenate dehydrogenase, acting competitively with respect to arogenate and non-competitively with respect to NAD^+. The biochemical diversity to be found in plant systems is illustrated by results previously obtained in mung bean (*Vigna radiata*) shoots where arogenate dehydrogenase requires $NADP^+$ and is sensitive to feedback inhibition by L-tyrosine. In addition, mung bean shoots also possess prephenate/$NADP^+$ dehydrogenase which is sensitive to feedback inhibition by L-tyrosine.

INTRODUCTION

The presence in nature of two different routes for L-tyrosine biosynthesis (i.e. the arogenate pathway or the 4-hydroxyphenylpyruvate pathway) is now becoming more widely recognized as a result of studies on prokaryotic organisms [1–5]. These biochemical alternatives are shown in Fig. 1. The cyanobacteria were the first group of organisms shown to synthesize L-tyrosine exclusively by the arogenate route [3, 5]. A similar enzymological pattern exists in coryneform species of bacteria, where its *in vivo* function has been documented most rigorously through the use of auxotrophic and regulatory mutants [1, 6, 7]. However, the comparative enzymological analysis of tyrosine biosynthesis in higher plants is currently rudimentary. Prephenate dehydrogenase (EC 1.3.1.12), an enzyme indicative of the presence of the 4-hydroxyphenylpyruvate route of tyrosine biosynthesis, has been demonstrated in mung bean [8]. It has since been demonstrated that mung bean shoots from seedlings have the potential to utilize both the 4-hydroxyphenylpyruvate and arogenate routes for L-tyrosine biosynthesis [9].

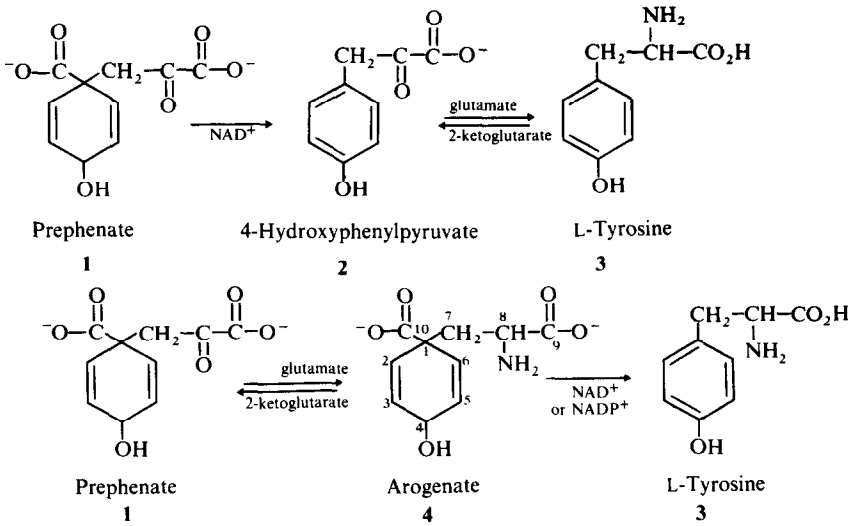

Fig. 1. The 4-hydroxyphenylpyruvate (top) and arogenate (bottom) pathways of L-tyrosine biosynthesis in nature. The enzymes which catalyze the reactions, in clockwise order, are prephenate dehydrogenase, 4-hydroxyphenylpyruvate aminotransferase, prephenate aminotransferase and arogenate dehydrogenase. The numbering system of L-arogenate (**4**), β-(1-carboxy-4-hydroxy-2,5-cyclohexadien-1-yl)alanine, as used in its structure determination by Zamir *et al.* [16], is shown.

We report here the presence and some characteristics of arogenate dehydrogenase (β - (1 - carboxy - 4 - hydroxy - 2,5-cyclohexadiene-1-yl)alanine dehydrogenase) isolated from corn seedling roots.

RESULTS

Root tissue from *Z. mays* was used to prepare crude extracts as described in the Experimental. Excellent activities were found for arogenate dehydrogenase, chorismate mutase (EC 5.4.99.5) and shikimate dehydrogenase (EC 1.1.1.25). Prephenate dehydrogenase activity, assayed for in the presence of either NAD^+ or $NADP^+$, was not detected. The lack of prephenate dehydrogenase activity suggests the absence of the 4-hydroxyphenylpyruvate pathway while the presence of arogenate dehydrogenase indicates the presence of the arogenate pathway to L-tyrosine in corn. All three enzymes studied were stable to partial purification using DEAE-cellulose chromatography (Fig. 2). Shikimate dehydrogenase required $NADP^+$, and the ion-exchange chromatography results clearly show that the two dehydrogenases are separate enzyme species. Under the chromatographic conditions used, the three isoenzymes of chorismate mutase reported by Woodin *et al.* [10] were not resolved. The coincident elution profiles for chorismate mutase and arogenate dehydrogenase are consistent with the possible presence of a multi-functional protein or protein–protein aggregate.

Arogenate dehydrogenase utilized only NAD^+ as cofactor, $NADP^+$ being totally inactive. The apparent K_m for NAD^+ was 57 μM (Fig. 3); the apparent K_m for L-arogenate was 0.30 mM. L-Tyrosine caused a 50% inhibition of enzyme activity at 1.0 mM with non-saturating concentrations of L-arogenate (150 μm). L-Tyrosine was found to inhibit competitively with respect to L-arogenate and non-competitively with respect to NAD^+ (Fig. 3).

DISCUSSION

It has been shown that root tissue of corn seedlings contains the necessary enzymes for the operation of the arogenate route for L-tyrosine biosynthesis. In contrast, shoots of mung bean (*Vigna radiata* (L.) Wilczek) have the ability to use either the arogenate or the 4-hydroxyphenylpyruvate route for L-tyrosine formation. In addition to variation in the route of biosynthesis, there is molecular diversity in terms of the cofactor utilized in the dehydrogenase reaction. Arogenate dehydrogenase from corn requires NAD^+ as cofactor while the prephenate/arogenate dehydrogenase from mung bean uses $NADP^+$. Arogenate dehydrogenase of corn is sensitive to feedback inhibition by L-tyrosine in contrast to the

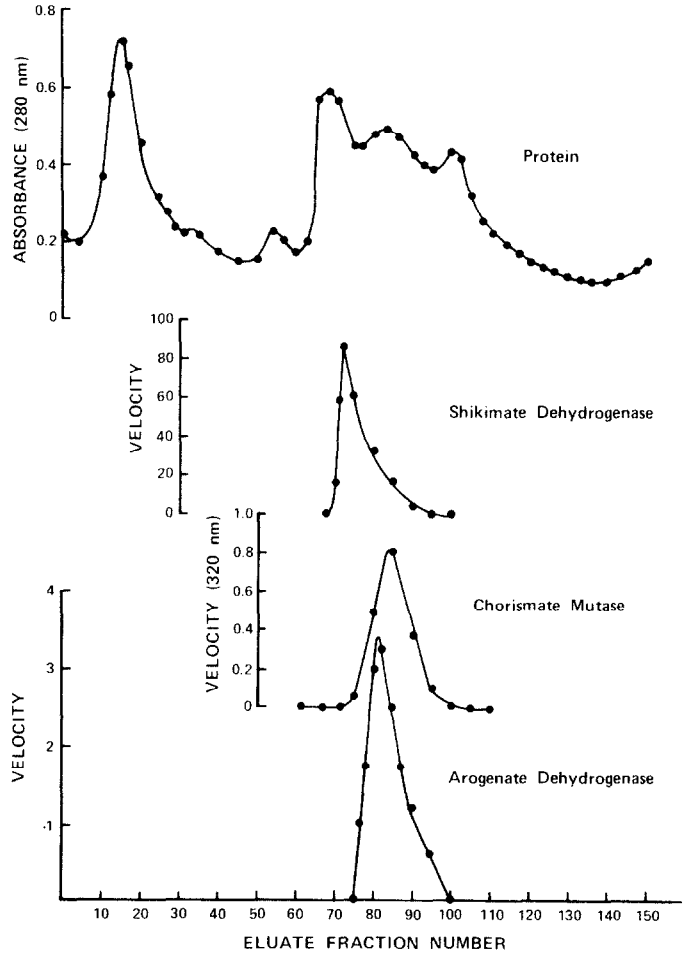

Fig. 2. DEAE-cellulose chromatography of a crude extract of corn seedlings. The velocities of the dehydrogenase enzymes are expressed as change in fluorescence per min (excitation 340 nm, emission 460 nm).

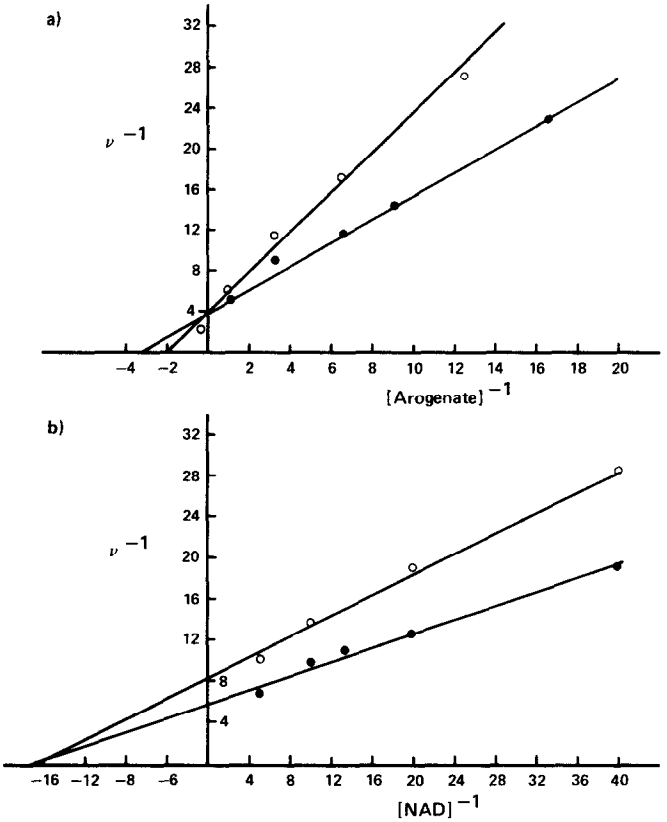

Fig. 3. Double reciprocal plots showing (a) competitive inhibition in the presence of 1 mM L-tyrosine with varying arogenate concentrations at 0.5 mM NAD^+ and (b) non-competitive inhibition in the presence of 1 mM L-tyrosine with varying NAD^+ concentrations and 375 μM arogenate (fixed substrate).

insensitivity of arogenate dehydrogenase from mung bean to feedback inhibition. However, prephenate dehydrogenase of mung bean is sensitive to feedback inhibition [9].

It is clear that micro-organisms are diverse in L-tyrosine biosynthesis with respect to the pathway(s) present, dehydrogenase(s) cofactor specificity and sensitivity of dehydrogenase enzymes present to feedback inhibition by L-tyrosine [11]. It already seems likely that photosynthetic eukaryotes exhibit equal enzymological diversity (Fig. 4). It will be interesting to determine whether expression of enzyme diversity differs with tissue and developmental time and might therefore have developmental implications.

EXPERIMENTAL

Preparation of extracts. Seeds of *Zea mays* (hybrid earliking) were spread on single layers of cheesecloth that covered 8 cm of moistened horticultural perlite in Nalgene tubs. The tubs were covered with Al foil and the seeds germinated at 22°. After 7 days the roots of the seedlings were cut free, rinsed with distilled H_2O, and frozen in liquid N_2. The frozen tissue was ground to a fine powder in liquid N_2 using a Waring blendor. 10% (w/w) PVP was mixed into the frozen powder and the mixture brought to 0°. This was then extracted with 50 mM K–Pi buffer (pH 7.0) containing 1 mM dithiothreitol. The slurry was filtered through cheesecloth and centrifuged for 20 min at 20000 *g*. The extract was then concd by precipitation with 90% $(NH_4)_2SO_4$. The

Organism	Prephenate Dehydrogenase	Arogenate Dehydrogenase
Corn		
Mung Bean		
*Euglena gracilis**		

Key: NAD–dependent; NADP–dependent

Fig. 4. Diversity of L-tyrosine biosynthesis in photosynthetic eukaryotes. *Byng, G. S., Whitaker, R. J., Shapiro, C. and Jensen, R. A. (*Mol. Cell Biol.*, in press).

protein pellet was dissolved in a minimal quantity of buffer and desalted by passage through a Sephadex G-25 column equilibrated with the same buffer. This preparation was denoted crude extract.

Column chromatography. Protein (100 mg) was applied to a DEAE-cellulose (Whatman DE52) column (1.5 × 20 cm) equilibrated with 50 mM Pi (pH 7.0) containing 1 mM dithiothreitol. The column was washed with the equilibration buffer and proteins were eluted in a 300 ml linear gradient (0–0.5 M KCl in the same buffer). Fractions of 2.2 ml were collected.

Analytical techniques and enzyme assays. Prephenate and arogenate dehydrogenase were assayed as previously described [9]. Chorismate mutase was assayed by the method of Patel *et al.* [4]. Protein concn was determined by the method of Bradford [12] as described in Bio-Rad Technical Bulletin (1051).

Biochemicals and chemicals. Barium prephenate was prepared from culture supernatants of a tyrosine auxotroph of *Salmonella typhimurium* [13] and was converted to the K salt with excess K_2SO_4 prior to use. Chorismate was prepared from culture supernatants of a multiple auxotroph of *Klebsiella pneumoniae* according to the method of Gibson [14]. Arogenate was prepared from culture supernatants of a triple auxotroph of *Neurospora crassa* [15]. The purification and isolation was modified according to Zamir *et al.* [16].

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