OCCURRENCE OF D-AMINO ACID AMINOTRANSFERASE IN PEA SEEDLINGS

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Summary : Aminotransferase reacting between D-amino acids and α-keto acids was found in the germinating pea seedlings. With partial purification, it was evident that the enzyme preparation contained no racemase and L-amino acid aminotransferase and was only specific for D-amino acid transamination. Large amounts of D-alanine found in the germinating pea seedlings were assumed to be formed by this enzyme action.

During the studies on the conjugated amino acids in pea seedlings (Pisum sativum, var. Alaska), one of the most predominant nihydrin negative alanine conjugates was proved to be N-malonyl-D-alanine (1,2). On the other hand, the configuration studies elucidated that γ-glutamylalanine, which was known to be extensively produced during the early growth of pea (3), consisted of L-glutamic acid and D-alanine (4). These D-alanine conjugates were actively synthesized and in 8 days of germination D-alanine contents in pea seedlings increased up to 3 μmoles / seedling (5). These findings, to our knowledge, are the first reports on the natural occurrence of D-alanine at high concentration in higher plants. In the course of the investigation of D-alanine metabolism in pea seedlings, we found a significant activity of the enzymatic transamination between D-amino acids and α-keto acids. Thus, this enzyme was partially purified in order to elucidate

its properties and the relationship to D-alanine biosynthesis in pea seedlings.

Experimental. Pea seeds (Pisum sativum, var. Alaska) were sterilized with 0.1 % benzalkoniumchloride and were germinated in the dark at 25° for 6 days, as described previously (6). The purification of the enzyme extracted from decotylized pea seedlings was carried out by ammonium sulfate precipitation and DEAE-Sephadex chromatography. Purification steps were summerized in Table I. Details in the purification will be published elsewhere. D-Amino acid aminotransferase activity was measured by using D-alanine and α-ketoglutarate as the substrates in a coupled system containing lactate dehydrogenase and NADH (7). Throughout the process of the purification, L-alanine - α-ketoglutarate aminotransferase and alanine racemase activities were also determined (8). Configuration of the amino acids obtained from the enzymatic reaction were determined enzymatically and chromatographically as described priviously (4). Substrate specificity of the enzyme was measured with various combinations of D-amino acids and α-keto acids. Quantitative analyses of the amino acids formed by the enzymatic transamination were carried out by using an automatic amino acid analyzer, Yanagimoto LC-5S.

Results and Discussion. At the purification process by DEAE-Sephadex chromatography D-amino acid aminotransferase was clearly separated from L-alanine aminotransferase. No detectable alanine racemizing activity was found in any fraction throughout the purification process. The final preparation (80-fold) was confirmed to be fairly specific for the transamination between D-amino acids and α-keto acids.

Substrate specificity of the enzyme measured with various amino donors and pyruvate or α-ketoglutarate as the acceptor is

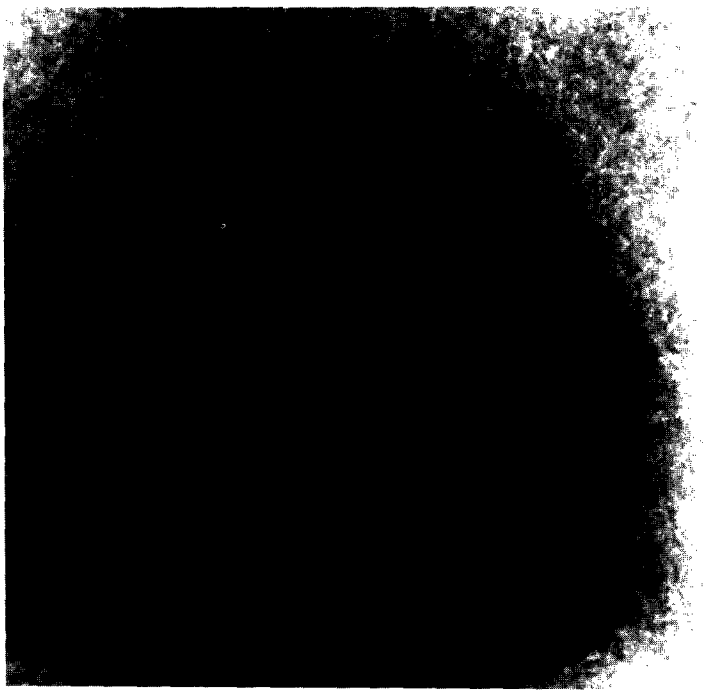

Figure 1 (continued).

c. Colony of cells formed from the same cell in b after 6 days, surrounded by an area of complete fibrinolysis.

cell death. Advantage was taken of this observation to prepare partially purified enzyme. After cells were grown in 40 to 80 - 10 cm plates, the medium was aspirated and the plates were rinsed with a small volume of serum-free medium. Three ml of serum-free medium then was added to each plate and they were incubated for 48 hrs. The medium was collected, concentrated by lyophilization, dissolved in a small volume of PBS and dialyzed for 6 hrs. against one liter of the same to eliminate excess salt. Denatured precipitated protein was eliminated by centrifugation.

Assay of fibrinolytic activity. When concentrated conditioned serum-free medium (CSFM) was assayed for fibrinolysin in fibrin-agar plates as described under methods there was a linear relationship between protein concentration and ring diameter over a wide range (Fig. 4).

TABLE II. Specificity of Amino Acids as Amino Group Donor

Amino donor	Keto acid acceptor	
	Pyruvate (Ala formed, mμmoles)	α-ketoglutarate (Glu formed, mμmoles)
D-Glu	61.9	-
D-Ala	-	44.0
D-α-Aminobutyrate	64.4	16.3
D-Asp	54.2	75.3
D-Try	25.7	14.8
D-Met	10.9	3.9
D-Phe	3.6	3.2
D-Lys	8.8	0
D-Leu, D-Val	0	0
L-Ala, L-Glu, L-Asp	0	0

The assay was carried out in a final volume of 3 ml containing 40 μmoles of amino acid, 40 μmoles of keto acid, 300 μmoles of Tris buffer, pH 8.3, 20 μg of pyridoxal phosphate and 0.002 units of enzyme at 37° for 30 min. The amino acids formed by the enzymatic transamination were measured by an automatic amino acid analyzer.

G-100, this enzyme was estimated to have a molecular weight of 65,000 $\pm$ 5,000. Some properties of this enzyme were similar to that of D-alanine - D-glutamate aminotransferase reported by Carrion and Jenkins (9). Details in comparison are now under investigation.

If the transamination involving D-amino acids as suggested in this paper is participating in the metabolism of D-alanine in pea seedlings, it, undoutedly, requires some other D-amino acids as a source which may be synthesized by another mechanism. In our experiments, alanine racemase was not found in the extract of pea

seedlings, but the possible existence of other amino acids racemases may be expected. It is of interest to investigate whether the biosynthetic system of D-amino acids, as known to exist in various microorganisms, occurs in higher plants. Subsequent studies are necessary to understand the physiological significances of D-amino acids in higher plants.

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