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SHORT COMMUNICATION

α-ALANINE AMINOTRANSFERASE FROM TOMATO FRUIT

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Abstract—The α-alanine aminotransferase, L-alanine:2-oxoglutarate aminotransferase EC 2.6.1.2, has been demonstrated in tomato fruit. The enzyme was found in both the supernatant and the mitochondrial preparations; under the experimental conditions used, activity in the mitochondria decreased with development of fruit. The α-alanine aminotransferase was more active than leucine aminotransferase from the same source.

INTRODUCTION

THE $(NH_4)_2SO_4$ precipitates (40–55 per cent saturation) obtained from tomato extract have been shown to exhibit leucine aminotransferase (L-leucine:2-oxoglutarate aminotransferase EC 2.6.1.6) activity.[1] This was the first demonstration of such an enzyme in tomato fruit. The mitochondrial preparations, however, were shown to have little activity. Although α-alanine aminotransferase has been known to occur widely, it has not been shown definitely in tomato fruit. The $(NH_4)_2SO_4$ precipitates and the mitochondria prepared from tomato fruit in the same way as reported previously[1] were tested for α-alanine aminotransferase activity and both were found to be highly active. Their activities were generally several-fold higher than those of leucine aminotransferase from the same source.

RESULTS

The α-alanine aminotransferase activity was first demonstrated by spectroscopy. The 2,4-dinitrophenylhydrazone (DNPH) formed from the products of the enzyme reaction mixture (Table 1) had an absorption maximum at 400 nm that coincided with authentic pyruvic acid–DNPH. Although the absorption spectra of the DNPHs of both pyruvic and 2-oxoisocaproic acids were similar to each other, the possibility that the latter might be formed in the reaction system was ruled out by TLC analyses in four solvents.[1] Under the experimental conditions used, the difference in absorbance between the reaction mixture with and without added alanine usually ranged from about 0·5 to 1 unit with 1 cm cells.

The transamination reaction was confirmed by isotopic studies with L-alanine-U-^{14}C. Regardless of enzyme source pyruvic acid was the most highly labeled product carbonyl compound. About 10 per cent of the radioactivity of the added L-alanine-^{14}C was incorporated.

Both the mitochondrial preparation and the $(NH_4)_2SO_4$ precipitates were active catalyzing the reaction (Table 1). About 2–10 per cent of the added substrate was transaminated in a

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[1] M. H. YU and MARY SPENCER, *Phytochem.* 8, 1173 (1969).

10 min incubation period, the rate being dependent on the enzyme source. High activities were exhibited by preparations from green fruit. The activity associated with the mitochondria tended to decrease with development of the fruit, but no clear-cut pattern of changes could be observed with the supernatant fraction.

DISCUSSION

The α-alanine and leucine aminotransferases from tomato fruit differed from each other markedly. Whereas the site of leucine aminotransferase was predominantly in the supernatant,[1] the α-alanine aminotransferase was present in both supernatant and mitochondria (Table 1). Such a difference in the cellular distribution of enzymes may suggest how and where each of the amino acids is metabolized in tomato tissue. Bone and Fowden[2] suggested that α-alanine aminotransferase might be predominantly mitochondrial and that such a distribution would allow for the easy utilization of pyruvate produced by the mitochondria enzymes catalyzing the Krebs cycle reactions.

TABLE 1. α-ALANINE AMINOTRANSFERASE FROM TOMATO FRUIT

Enzyme source	Enzyme activity (mμmole pyruvate/mg protein/10 min)	
	$(NH_4)_2SO_4$ precipitation	Mitochondria
Young green fruit, 1·5 in. diam.	780*	885
Mature green fruit, 2–3 in. diam.	1012	562
Pink fruit	194	342
Red fruit	427	294

* An assay mixture contained 45 μmoles of Tris-HCl buffer (pH 8·4), 10 μmoles of L-alanine, 10 μmoles of 2-oxoglutaric acid, 0·1 μmole of pyridoxal phosphate, and enzyme (0·6–1·1 mg), in a final volume of 1·5 ml. Incubation was at 30° for 10 min. The values are averages of two experiments.

The α-alanine aminotransferase activity was always much higher than that of leucine aminotransferase from the same preparation.[1] With mature green fruit preparations, about a five-fold difference was found, and a greater difference was exhibited by preparations from ripe fruit. Under the conditions used, the rate of the conversion of L-α-alanine to pyruvic acid was 2–10 per cent within a 10 min incubation period, compared with a maximum of about 3 per cent for leucine aminotransferase.[1] Wilson *et al.*[3] reported that while aminotransferases associated with the branched-chain amino acids were moderately active, those with alanine and aspartic acid were highly so, and attributed this to their stability. We found that the dialysate of the $(NH_4)_2SO_4$ precipitates obtained from tomato fruits showed an increased enzyme activity for α-alanine transamination while the leucine aminotransferase activity was lost almost completely.[1] This may reflect stability differences, although other factors may also be involved. The diminishing activity of the mitochondrial α-alanine aminotransferase activity with ripening of the fruit is similar to that found with leucine aminotransferase reported previously.[1] Among other causes, this may be attributed to the degradation of

[2] D. H. BONE and L. FOWDEN, *J. Exp. Botany* **11**, 104 (1960).
[3] D. G. WILSON, K. W. KING and R. H. BURRIS, *J. Biol. Chem.* **208**, 863 (1954).

enzymes or to the production of certain inhibitors. (Nor can one ignore the differences in texture, acidity, etc. of the original tissue, which are bound to influence the yields and properties of the preparations. Comparisons can be regarded as primarily qualitative in nature.) According to Dickinson and Hanson[4] the capacity of tomato mitochondria to oxidize malate and pyruvate diminishes progressively during ripening. They suggest that this decline might result from the appearance of an inhibitor.

EXPERIMENTAL

The materials and the methods used for the preparation of enzymes, the assay conditions, and the analyses of the reaction products were the same as reported previously,[1] with the exception that 10 μmoles of L-α-alanine was used as substrate in the enzyme assay. A standard curve for pyruvic acid–DNPH was constructed with solutions of known concentrations that were carried through the same procedure.

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[4] D. B. Dickinson and J. B. Hanson, *Plant Physiol.* **40**, 161 (1965).