β-ALANINE AMINOTRANSFERASE(S) FROM A PLANT SOURCE*

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A β-alanine aminotransferase has been identified in a subcellular particulate fraction from bean cotyledons and has been rendered soluble. The enzyme or enzymes utilized oxaloacetate or pyruvate but not α-ketoglutarate as an amino acceptor. This is the first demonstration of such an enzyme in plant tissue.

An enzyme that converts β-alanine to malonate semialdehyde (MSA)[1] via an aminotransferase reaction has been investigated in microorganisms (Roberts et al., 1953; Goldfine and Stadman, 1960; Hayaishi et al., 1961) and in various animal tissues (Roberts and Bregoff, 1953; Kupiecki and Coon, 1957; Baxter and Roberts, 1958). In plants the enzyme has not been satisfactorily identified previously. Roberts and Bregoff (1953), using α-KG as an amino acceptor, were unable to show β-alanine aminotransferase (L-glutamate:malonate-semialdehyde aminotransferase, E.C. 2.6.1) activity in the pellet of 18,000 X *g* centrifugation of pepper or ripening avocado homogenates. Negative results with β-alanine were also obtained by Kupiecki and Coon (1957) with ethanol extracts of spinach leaves when α-KG was used as an amino acceptor.

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[1]Abbreviations used: MSA, malonate semialdehyde; α-KG, α-ketoglutarate; TES, N-Tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid; TCA, trichloracetic acid; OAA, oxaloacetate; Triton X-100, octylphenoxypolyethoxyethanol.

Kretovich et al. (1967) reported on the presence and properties of an aminobutyrate aminotransferase (4-aminobutyrate: 2-oxoglutarate aminotransferase E.C. 2.6.1) from several plant species but β-alanine was not investigated as a possible substrate. The animal enzymes and all but one of the bacterial enzymes exhibit almost equal reactivity with either γ-aminobutyrate or β-alanine as substrate. Miettinen and Virtanen (1953) reported the presence of an active aminobutyrate aminotransferase in pea roots, but found no transamination between β-alanine and α-KG in this tissue.

Hatch and Stumpf (1962) were able to show the presence of β-alanine-^{14}C in several plant species after administration of propionate-1-^{14}C to tissue slices and speculated on the presence of a transamination involving MSA. Tsai and Axelrod (1965) demonstrated the qualitative formation of glutamate after a 90 minute incubation of β-alanine, α-KG and a rape seedling extract.

Methods

A subcellular fraction from 3½ day old etiolated wax bean cotyledons (Phaseolus vulgaris L. var. Kinghorn) was prepared by grinding this tissue in mannitol (0.3 M), TES buffer (0.05 M), pH 7.4 at 0 C (2 ml buffer/g cotyledon). Unbroken cells etc. were removed at 5,000 X g and the pellet from a 32,000 X g centrifugation was suspended in 0.01 M TES buffer (pH 7.6 at 0 C) and freeze-dried. Solubilization was achieved by treating 2.0 g of the dry powder with 40 ml of 0.4% Triton X-100 (Applied Science Laboratories) in 0.01 M TES, pH 7.6 at 0 C. Mild stirring at 0 C for 1 h was followed by a 30 min centrifugation at 100,000 X g. The pellet was resuspended in Triton buffer and stirred overnight. Centrifugation was repeated and the supernatant layers from both spins were chromatographed separately on Sephadex G-25. The protein fractions obtained were combined and used immediately. The lyophilized cytoplasmic proteins were dissolved in water and chromatographed as above.

The enzyme assay was similar to that of Hayaishi and Nishizuka (1962). The standard assay at pH 7.2 or 7.4 contained 60 μmoles of both β-alanine and

amino acceptor, 150 μmoles TES, and approximately 2.0 mg protein in 3.0 ml. Incubation was at 33 C in a shaker bath, the reaction was stopped with 40% TCA, the precipitated protein removed, and two 1 ml aliquots of the supernatant were brought to pH 5.2 by the addition of 0.2 ml of 2 M sodium acetate in 0.9 N NaOH. The formazan of malonate semialdehyde (MSA) was made quantitatively for both samples. The yellow orange formazan was quantitatively extracted and determined as outlined in the above reference. The 2,4-dinitrophenyl hydrazones were made by adding excess 2,4-dinitrophenyl hydrazine to the protein-free sample, followed by extraction with $CHCl_3$:ethanol (4:1). Authentic MSA used for comparison was synthesized as outlined by Robinson and Coon (1963).

Results

Figure 1 gives the aminotransferase (L-alanine:malonate-semialdehyde ami-

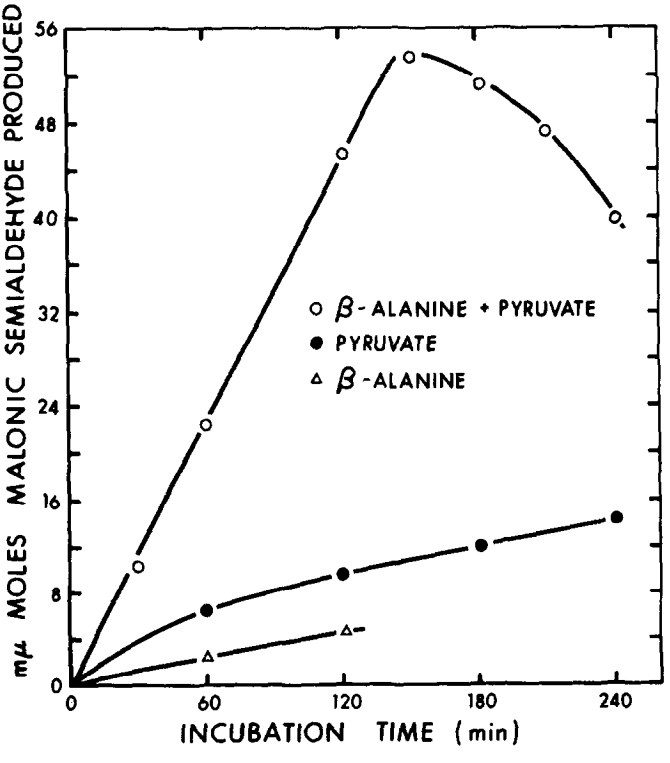

Figure 1. β-alanine transaminase activity with the indicated substrates. Samples contained 60 μmoles of each substrate, approximately 2 mg protein, and 150 μmoles of TES in a final volume of 3.0 ml (pH 7.2). Incubation was at 33 C.

notransferase, E.C. 2.6.1) activity with β-alanine and pyruvate as substrates. Small amounts of malonate semialdehyde (MSA) were formed in the reaction mixtures without β-alanine or pyruvate but the majority of the activity required both substrates. A sharp reduction in enzyme activity combined with the lability of the product, MSA, may account for the drop in product formation after 2½ h is unknown. In the presence of β-alanine and pyruvate MSA production with time normally fell off gradually rather than abruptly as indicated in Figure 1.

The formation of MSA in the β-alanine-pyruvate reaction mixture was substantiated by spectroscopy; the extracted formazan of this reaction mixture and authentic MSA formazan both exhibited maximum absorbance at 437 mμ. The formazan formed in the β-alanine-OAA reaction mixture did not exhibit maximum absorbance at 437 mμ. However, as the formazan was diluted, the wavelength of

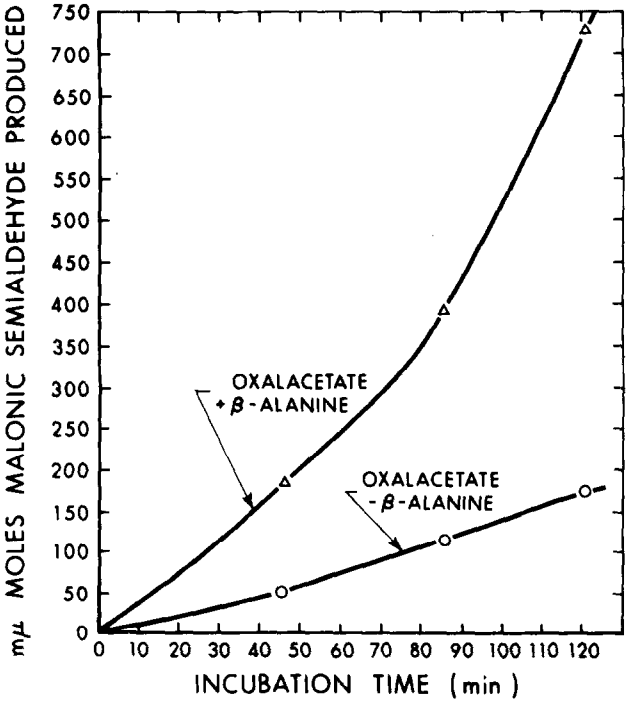

Figure 2. β-alanine transaminase activity with the indicated substrates. Samples contained 60 μmoles of each substrate, approximately 2 mg of protein, and 150 μmoles of TES in a final volume of 3.0 ml (pH 7.4). Incubation was at 33 C.

maximum absorbance approached 437 mμ. Interaction of the formazans of OAA and MSA may have been responsible for this shift. The enzyme did not show any reactivity with α-KG as an amino acceptor.

When OAA was used as an amino acceptor, very large increases in absorbance were obtained over a control with no β-alanine (Figure 2). OAA like MSA will form a formazan by virtue of its active methylene group, and this compound partially interferes with the MSA assay. Thin-layer chromatography confirmed the presence of MSA in the OAA reaction mixture (Figure 3). The digestion was heated before hydrazone formation to decarboxylate the MSA to acetaldehyde, and the hydrazone of acetaldehyde rather than that of MSA appeared on the thin-layer chromatogram (Figure 3). If it is assumed that the increase in

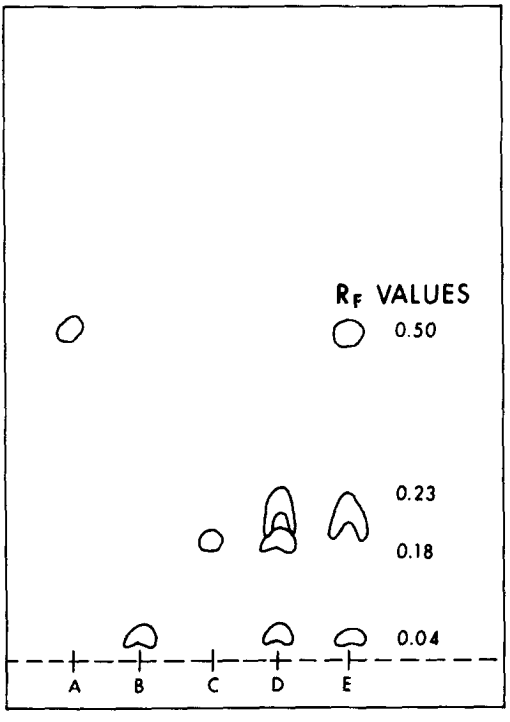

Figure 3. Silica gel thin-layer chromatogram of the 2,4-dinitrophenylhydrazones from the oxaloacetate reaction mixture. Development was in benzene:acetic acid (19:1). A, B, and C are authentic samples of hydrazones of acetaldehyde, oxaloacetate, and malonate semialdehyde, respectively. D and E represent an unheated and heated digestion, respectively. The formation of acetaldehyde in E confirms the presence of malonate semialdehyde in D.

absorbance obtained when β-alanine is added to the OAA reaction mixture is a result of formation of MSA formazan, then the aminotransferase is 15 times more reactive with oxaloacetate as an amino acceptor than with pyruvate. It may be significant that L-aspartate:2-oxoglutarate aminotransferase (E.C. 2.6.1.1) (GOT) is 7.5 times more active than L-alanine:2-oxoglutarate aminotransferase (E.C. 2.6.1.2) (GPT) in this soluble system from a subcellular particulate fraction.

Pyridoxal phosphate appears to be essential in view of the fact that a 40% stimulation of activity was obtained on addition of the cofactor (1×10^{-4} M), even after the enzyme had previously been incubated with saturating amounts of pyridoxal phosphate, and the unbound portion removed by Sephadex G-25 gel filtration. Under the experimental conditions used, it is unlikely that a metal catalyzed nonenzymic transformation could account for the increased activity when pyridoxal phosphate is added.

The supernatant aminotransferase would use neither pyruvate nor α-ketoglutarate as an amino acceptor, but a preliminary experiment indicated that OAA might function in this capacity.

Discussion

An enzyme that converts β-alanine to malonate semialdehyde (MSA), and thereby links β-alanine to the modified β-oxidation scheme described by Giovanelli and Stumpf (1958) is well demonstrated by these experiments. The results obtained suggest a reason why certain investigators have been unable to detect β-alanine aminotransferase activity in plant tissue, namely, the most commonly used amino acceptor, α-KG, did not function in this plant system. Oxaloacetic acid failed to support the β-alanine aminotransferases of Pseudomonas (Hayaiski et al., 1961), beef brain (Baxter and Roberts, 1958) and hog kidney (Kupiecki and Coon, 1957) but has not been previously investigated in a plant system. The results reported herein show that, of the compounds investigated, OAA was the best amino acceptor for the conversion of β-alanine to malonate semialdehyde. Both pyruvate (Figure 1) and OAA (Figure 2) were shown to serve

as amino acceptors for a plant β-alanine aminotransferase. The two substrates may react with two separate enzymes. MSA-^{14}C was identified by extensive thin-layer chromatography in a reaction mixture that contained only enzyme and β-alanine-2-^{14}C.

The necessity of pyridoxal phosphate for maximum activity has been shown for both the bacterial (Hayaishi et al., 1961; Durham et al., 1964) and animal aminotransferases (Baxter and Roberts, 1958) and is shown here for the plant enzyme. The inability of the β-alanine-pyruvate aminotransferase to bind sufficient amounts of the cofactor for maximum activity was also demonstrated.

Since the β-alanine aminotransferase is so closely associated with the mitochondrial β-oxidation of lipids in higher plants, it is perhaps reasonable to assume that its distribution in the cytoplasm may be restricted. Dixon and Fowden (1961) reported that an aminobutyrate aminotransferase (an enzyme that is present in animal preparations, and will transaminate β-alanine) was "concentrated in the mitochondria". A cytoplasmic enzyme that would utilize pyruvate or α-KG as an amino acceptor for a β-alanine aminotransterase could not be detected, although other amino acids may function in this role. Since a β-alanine aminotransferase is the only known metabolic route that leads to catabolism of β-alanine, it is possible that the cellular distribution of the enzyme may play a very important role in determining how and where β-alanine is metabolized in plant tissue.

The demonstration of β-alanine aminotransferase activity in this enzyme system has further implications. This enzyme preparation has also been found to derive ethylene from β-alanine (Stinson and Spencer, unpublished). The presence of an enzyme that could convert β-alanine to malonate semialdehyde gives indirect support for the involvement of malonate semialdehyde in the enzymic conversion of β-alanine to ethylene.

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