SOME PROPERTIES OF A D-ALANINE D-GLUTAMATE AMINOTRANSFERASE PURIFIED FROM *Bacillus subtilis*

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Many transaminases specific for the L amino acids have been purified (9) and the mechanism by which they operate partially solved (2), but no report has appeared of the isolation of a transaminase that would act specifically for the D isomers of the amino acids although their existence was shown by Thorne *et al.* (10) (11) in extracts of *B. subtilis* and *B. anthracis*, as well as by other workers in *Rhodospirillum rubrum* (1), *B. sphericus* (6), and *B. licheniformis* (5). It would be of interest to know if transamination of the D amino acids occurs by the same mechanism as the L isomers, with Vitamin B_6 as a cofactor, and to determine if in the case of the D amino acids there is as narrow specificity with respect to substrates as in the case of L amino acid aminotransferases. Accordingly we have purified 570 fold the enzyme D-alanine D-glutamate aminotransferase from *B. subtilis* in order to study its mode of action and substrate specificity.

Experimental. *B. subtilis* was grown as described by Thorne *et al.* (10) and disintegrated in a Raytheon sonic oscillator. The purification of the enzyme is summarized in Table I. Details on the purification procedure and physicochemical properties of the enzyme will be published elsewhere.

Substrate activities were compared (Table II) on the basis of glutamate formation. Glutamate was determined by the quantitative nynhydrin procedure of Kay *et al.* (4) after paper electrophoresis.

Table I

Purification of D-alanine D-glutamate aminotransferase

step	vol,ml	protein mg/ml	specific activity	yield
Initial extract	1320	37	0.06	100
Protamine sulfate	1600	22	0.066	---
Ammonium sulfate	200	68	0.172	79
Hydroxylapatite eluate	810	1.14	1.2	38
DEAE-sephadex	132	0.60	10.0	27
Sephadex	20.5	0.78	28.8	16
Ammonium sulfate	1.4	9	34.0	14

Specific activity was defined as μmoles of pyruvate formed/min/mg of protein at 37° in an assay mixture containing 100 μmoles D-Ala, 100 μmoles α-ketoglutarate, 50 μmoles phosphate buffer pH 8, 20 μgm of pyridoxal phosphate in a final volume of 2 ml.

Spectrophotometric studies were performed on a Cary Spectrophotometer model 14.

Starch gel electrophoresis was done according to Smithies (8) in phosphate buffer pH 7.5 and by this criteria the enzyme was about 50% 'pure'.

Results and Discussion. A broad pH optimum (8-9) and a twofold stimulation by pyridoxal phosphate were found with D-alanine as amino donor and α-ketoglutarate as substrate. The comparison (Table II) was therefore made at pH 8 in the presence of added pyridoxal phosphate. It is evident that transamination in the case of this enzyme is fairly specific for D-alanine. D-aspartic acid, D-serine, and D-methionine react at a much slower rate. The inactivity with L-alanine indicates not only specificity for the D isomers but also complete absence of alanine racemase in our preparation.

The purified enzyme at pH 7 is yellow and exhibits an absorption spectra with maxima at 415 mμ and 330 mμ (Fig. 1). This spectra is different from the one reported for the L-alanine L-glutamate aminotransferase from pig heart (3). Addition of D-alanine produces a decrease of absorption at 415 mμ that is asso-

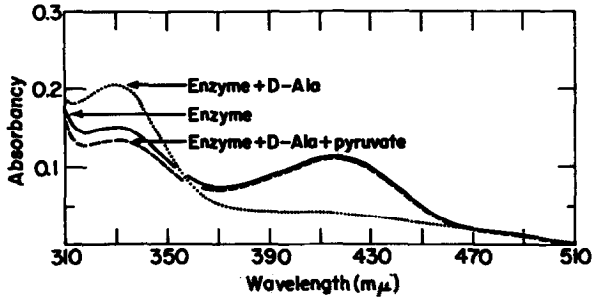
Table II

Specificity of the enzyme

Activity with different amino donors using α-ketoglutarate as acceptor

amino donor	glutamate formed μmoles
D-alanine	6.5
D-aspartic acid	2.25
D-serine	0.75
D-methionine	0.1
D-Leu, D-Val, D-Thr., D-Phe	0
Glycine	0
L-Ala, L-Asp, L-Val	0
DL-aminopimelic, DL-aminoadipic	0

Solutions contained 0.2M amino acid, 0.2M α-ketoglurate, 0.2M phosphate buffer pH 8, 20 μgm pyridoxal phosphate in a final volume of 0.5 ml. After addition of 1 μliter of enzyme (specific activity = 34), they were incubated at 37° for 20 minutes.



ciated with an increase of absorbancy at 330 mμ. This shift in the absorption can be reversed by the addition of pyruvate. These changes on the absorption spectra of the enzyme upon the addition of substrates are similar to those described for the absorption spectra of other transaminases. The absorption maximum at 415 mμ, which is a characteristic feature of pyridoxal phosphate enzymes, may be ascribed to a pyridoxal phosphate aldimine form (7). The absorption maximum at 330 mμ could be a pyridoxal phosphate Schiff base deriva-

tive that does not have a double bond in conjugation with the pyridine ring of pyridoxal (3).

These spectrophotometric observations and our observation of association of activity with a yellow fraction during purification, lead us to conclude that the enzyme that is specific for the pair D-alanine D-glutamate, as in the case of the transaminases of the L amino acid series, has as prosthetic group pyridoxal phosphate bound through the aldehyde to a protein amino group. Although the mechanism of the reaction appears to be similar to that of the L amino acids, it is of interest that the spectra, and hence the binding of the pyridoxal phosphate to the protein, differs from the pig heart alanine transaminase which is specific for the L series. Both enzymes in turn differ spectrally from the pig heart glutamic aspartic transaminase and the beef liver glutamic aspartic transaminases.

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