A SINGLE TRANSAMINASE FOR 1,4-DIAMINOBUTANE AND 4-AMINOBUTYRATE IN A PSEUDOMONAS SPECIES

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Summary A Pseudomonas species uses a single transaminase to catalyze the transamination of 1,4-diaminobutane and 4-aminobutyrate.

Introduction

In a Pseudomonas species putrescine is converted to succinate and utilized for cellular growth (1) via two transaminations which convert putrescine to 4-aminobutanal and 4-aminobutyrate to succinic semialdehyde. We wish to present evidence that these two transamination reactions appear to be catalyzed by the same enzyme.

Materials and Methods

Putrescine transamination was measured essentially according to the method of Kim (2) with the exception that carbonate-bicarbonate buffer (pH 9.5) replaced tris-HCl (pH 9.0). 4-Aminobutyrate transamination was assayed according to the procedure of Jacoby and Scott (3). In both assays a unit of activity is defined as the amount of enzyme necessary to form one nmole of product per minute.

The Pseudomonas species was isolated in this laboratory and reported earlier (4). Cells were grown, harvested, and suspended in 4 parts of 0.01M phosphate buffer (pH 7) with 0.005 M mercaptoethanol (hereafter referred

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to as buffer), sonicated and centrifuged (10,000 xg, 10 min.) as previously described (4). The resultant supernatant was treated with ribonuclease (RNase A, type 1A, 5X crystallized, Sigma Chemical Company) and deoxyribonuclease (crude, from beef pancreas, Sigma Chemical Company) (0.1 mg each/ml) at room temperature for 30 minutes and further centrifuged at 105,000 xg for one hour, yielding a supernatant which was dialyzed overnight against buffer. This dialyzed solution, labeled crude extract, could be assayed directly for enzyme activity.

125 ml of crude extract from cells grown on 0.1% putrescine as carbon and nitrogen source were fractionated with ammonium sulfate. The fraction precipitated between 40-55% saturation with ammonium sulfate yielded 509 mgs of protein with 85% of the transaminase activity in the crude extract. 494 mgs of this ammonium sulfate fraction were layered onto a DEAE Sephadex A-50 column (4 x 47 cm) and eluted with a linear NaCl gradient (1500 ml of buffer without NaCl in the mixing chamber and 1500 ml of buffer with 0.5 M NaCl in the reservoir). The flow rate was 30 ml per hour. Ten ml fractions were collected. Fractions with transaminase activity were pooled and concentrated with ammonium sulfate, giving 3.4 ml of a solution with 14 mg of protein per ml.

A portion of this solution with 35 mg of protein was mixed with 60% sucrose solution (final sucrose concentration 20%) and subjected to preparative electrophoresis with the apparatus described by Smith and Moss (5) (5 cm height, 5% gel column, 400 V, 42 ma, 12 hours). The upper cathode and lower anode chambers both contained 0.125 M tris-glycine buffer (pH 9.0). 0.25 M tris-glycine buffer (pH 9.0) was passed through the elution chamber at a flow rate of 0.75 ml/min and 4.6 ml fractions were collected.

Results and Discussion

The results of DEAE-Sephadex column chromatography and preparative polyacrylamide gel electrophoresis are shown in Figures 1 and 2. In both procedures good separation of proteins was obtained while the transaminase activity for 4-aminobutyrate and for putrescine appeared together. This led us to consider the possibility that, in spite of the differences in charge of putrescine and 4-aminobutyrate, they may be transaminated by the same enzyme.

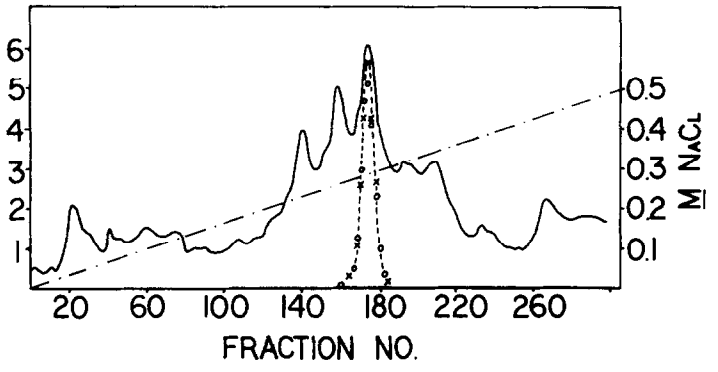

Figure 1. DEAE-Sephadex Column Chromatography Conditions described in text. For the scale on the left, one unit represents: (i) protein concentration (solid line) as OD_{280} of 0.1/ml; (ii) putrescine transaminase (circles) as ΔOD_{435} of 0.1/min/ml (ϵ = 1.86 x 10^3 l./mole/cm; and (iii) 4-aminobutyrate transaminase (crosses) as ΔOD_{340} of 4.0/min/ml (ϵ = 6.22 x 10^3 l./mole/cm.

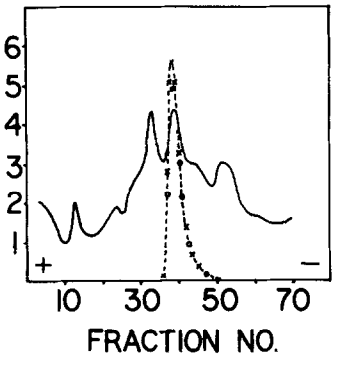

Figure 2. Preparative Gel Electrophoresis Conditions described in text. For the scale on the left one unit represents: (i) protein concentration (solid line) as OD_{280} of 0.05/ml; (ii) putrescine transaminase (circles) as ΔOD_{435} of 0.1/min/ml (ϵ = 1.86 x 10^3 l./mole/cm); and (iii) 4-aminobutyrate transaminase (crosses) as ΔOD_{340} of 5.0/min/ml (ϵ = 6.22 x 10^3 l./mole/cm). This figure shows only the first 70 out of a total of 200 fractions collected. The later fractions contained slightly less than half of the total protein and no enzyme activity.

To test this possibility, we determined the ratios of these two transaminase activities in cells grown in different media where one would normally expect to find differential induction of these two activities. The media chosen were: (i) putrescine (0.1%) as C and N source, (ii) glucose (0.1%) as C source and putrescine (0.1%) as N source, (harvested in early log phase when putrescine was not utilized as C source) (4,6) and (iii) glucose

(0.1%) as C source and 4-aminobutyrate (0.1%) as N source. If there were two separate transaminases, one would expect differential induction in these three media: both transaminases in medium (i),putrescine transaminase in (ii) and 4-aminobutyrate transaminase in (iii). The results (Table I) show clearly that these two transaminase activities are not induced differentially. Although the specific activities varied with the different growth media, the ratio of the two transaminase activities was constant.

TABLE I

EFFECT OF GROWTH CONDITIONS ON TRANSAMINASE

Medium	Transaminase Units/Mg Protein				Activity Ratios 4-Aminobutyrate/ Putrescine	
	Putrescine		4-Aminobutyrate			
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Putrescine	18	12	304	170	17	14
Glucose + Putrescine	9	5	143	86	16	17
Glucose + 4-amino-butyrate	33	28	588	419	18	15

Other preliminary results are also in agreement with the hypothesis of one enzyme catalyzing both reactions. (i) The enzyme shows comparable activity with ornithine as substrate as compared to putrescine. (ii) Putrescine transaminase activity assayed in the presence of 5×10^{-3} M putrescine (K_m 1.9×10^{-3} M) is inhibited 54% and 75% respectively by the presence of 5×10^{-4} M and 5×10^{-3} M 4-aminobutyrate (K_m 4.3×10^{-4} M).

Although putrescine transaminase activity has been observed in a number of microorganisms (1), more detailed study of putrescine and 4-aminobutyrate transaminases has been carried out in only two of them. In Pseudomonas fluorescens (4), Scott and Jacoby reported on a 4-amino-butyrate transaminase which is inactive towards ornithine (putrescine was

not tested). In an Escherichia coli mutant (2), unpublished results from this laboratory (McDonald and Tchen) indicate that there are two separate transaminases for putrescine and for 4-aminobutyrate. In higher organisms 4-aminobutyrate transaminase has long been known to be associated with particulate fractions of brain (7, 8) but, to the best knowledge of the authors, it is not known whether it is active towards putrescine. The diamine transaminase of plants is probably inactive towards 4-aminobutyrate (9). The Pseudomonas species discussed here is apparently unique in having one transaminase catalyzing the transamination of both putrescine and 4-aminobutyrate.

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