PUTRESCINE - α-KETOGLUTARATE TRANSAMINASE IN E. COLI.

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There has been considerable interest recently on the physiological role of the polyamines putrescine and spermidine in bacteria and the T phages. (Mora, et.al., 1962; earlier work has been reviewed by Tabor, et.al., 1961.) By contrast, the degradation of these polyamines by bacteria has received relatively little attention with only one report on the enzymatic degradation of putrescine. This was the work of Jakoby and Fredericks who showed that Pseudomonas fluorescens adapted to grow on putrescine as the sole C and N source could convert putrescine to succinate via γ-aminobutyraldehyde, γ-aminobutyrate and succinic semialdehyde. It was assumed that putrescine was oxidized to γ-aminobutyraldehyde by a diamine oxidase. We have now obtained a mutant of E. coli B which can grow on putrescine as the sole C and N source. Cell-free extracts of this mutant actively convert putrescine to succinate in similar manner as reported with Pseudomonas fluorescens (Jakoby and Fredericks, 1959). However, the first step, the conversion of putrescine to γ-aminobutyraldehyde, is not catalyzed by a diamine oxidase, but by a transaminase. The evidence for this new transaminase and some of its properties are briefly reported here.

Materials and Methods

E. coli B was first adapted to grow on glucose and inorganic salts with putrescine (0.2%, neutralized with HCl) as the sole source of N. These adapted cells were then grown on putrescine as the sole source of both C and N. Subsequently, it was found that the putrescine grown cells constituted a mutant selected under the condition of "adaptation". (The evidence for this and the characteristics of this mutant will be reported elsewhere.) All work to be

reported here were carried out with this mutant grown on putrescine (0.2%, neutralized with HCl) and inorganic salts to a cell density of 10^9/ml.

Freshly harvested or frozen cells were homogenized for 10 minutes at 0°C in a VirTis homogenizer with ten parts of phosphate buffer (O.1M, pH 7.0) and twenty parts of fine glass beads. The transaminase activity was found exclusively in the supernatant after centrifuging for 40 minutes at 144,000 x g. This will be referred to hereafter as crude supernatant.

The amounts of γ-aminobutyraldehyde were determined by coupling with o-aminobenzaldehyde and measuring the absorption at 435mμ (Holmstedt, et.al., 1961). This reagent was added directly to the reaction mixture as it has no effect on the enzyme. Glutamate was identified by paper chromatography in two systems (Consden, et. al., 1944; Slotta and Primosigh, 1951), but was not determined quantitatively. γ-aminobutyraldehyde was synthesized according to Jakoby and Fredericks (1959). T-labeled putrescine was purchased from New England Nuclear Corporation.

Results and Discussion

When the crude supernatant was incubated at pH 8-9 with putrescine and α-ketoglutarate, glutamate was formed. If o-aminobenzaldehyde was included in the reaction mixture, the yellow condensation product between γ-aminobutyraldehyde and o-aminobenzaldehyde was also obtained. The formation of both this yellow pigment and glutamate required the presence of putrescine and α-ketoglutarate and was stimulated by pyridoxal phosphate. After partial purification of the enzyme by protamine sulfate treatment and ammonium sulfate fractionation, an absolute requirement for pyridoxal phosphate was observed. This is shown in Table I.

The enzyme has a pH optimum of 9 and is almost completely inactive at pH 7. It is completely inhibited by 5×10^{-4} M. semicarbazide, hydroxylamine or cyanide. Putrescine can be replaced by cadaverine or 1,7-diaminoheptane, but not by 1,3-diaminopropane, spermidine, γ-aminobutanol, histamine, tyramine or β-alanine. Substitution of α-ketoglutarate by oxalacetate or pyruvate resulted in much lower activity.

When this mutant was cultured for many generations in a medium of glucose and inorganic salt (5 successive transfers of one drop into 50 ml) the cells still contained approximately 30% as much transaminase as did cells

TABLE I

Pyridoxal Phosphate Requirement

Enzyme Used	Pyridoxal Phosphate	ΔOD at 435mμ per mg protein per 10 minutes
E. coli B	+	0
crude supernatant	-	0
Mutant crude	+	.094
supernatent	-	.064
Mutant	+	.283
fraction	-	0

The 1 ml of reaction mixture contained 0.1 ml of crude supernatant or corresponding amount of partially purified enzyme, 15 μmoles of putrescine, 4μmoles of α-ketoglutarate, 200 μmoles of Tris buffer pH9.0, 10 μmoles of o-aminobenzaldehyde, and 20 γ of pyridoxal phosphate when used. The mutant fraction was obtained after protamine sulfate and ammonium sulfate treatment. Details will be reported later.

grown on putrescine. It thus appears that the transaminase is constitutive in this mutant.

The subsequent catabolism of γ-aminobutyraldehyde in this strain of E. coli is the same as found in Pseudomonas fluorescens (Jakoby and Fredericks, 1959) and consists of the following three reactions:

$$\gamma\text{-aminobutyraldehyde} + DPN^+ \rightarrow \gamma\text{-aminobutyrate} + DPNH$$

$$\gamma\text{-aminobutyrate} + \alpha\text{-ketoglutarate} \rightarrow \text{succinic semialdehyde} + \text{glutamate}$$

$$\text{succinic semialdehyde} + DPN^+ \rightarrow \text{succinate} + DPNH$$

The first reaction was demonstrated by the reduction of DPN^+ by γ-aminobutyraldehyde. The second reaction was demonstrated by the formation of glutamate from γ-aminobutyrate and α-ketoglutarate as well as by coupling to the third reaction. Thus, when α-ketoglutarate, γ-aminobutyrate and DPN^+ were incubated with the crude supernatant, a rapid reduction of DPN^+ was obtained after a lag of approximately six minutes. Incubation of α-ketoglutarate, γ-aminobutyrate or glutamate alone with DPN^+ and the crude supernatant gave no reduction of DPN^+.

The conversion of putrescine to γ-aminobutyraldehyde followed by the three reactions listed above should lead to the formation of succinate. Experimentally, a DPN^+ and α-ketoglutarate dependent conversion of T-labeled putrescine to T-labeled succinate (identified by paper chromatography, Lugg and Overell, 1947) was readily demonstrated using dialyzed crude supernatant.

Summary

A mutant of E. coli B was obtained which can grow on putrescine as the sole C and N source. This mutant converts putrescine to succinate via γ-aminobutyraldehyde, γ-aminobutyrate and succinic semialdehyde in similar manner as found in Pseudomonas fluorescens. The first reaction, however, was not catalyzed by a diamine oxidase, but by a transaminase which is constitutive in this strain of E. coli.

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References

1. Consden, R., Gordon, A.H., and Martin, A.J.P., Biochem. J., 38, 224 (1944).
2. Holmstedt, B., Lasson, L., and Tham, R., Biochim. Biophys. Acta, 48, 182 (1961).
3. Jakoby, W. B., and Fredericks, J., J. Biol. Chem., 234, 2145 (1959).
4. Lugg, J.W.H., and Overell, B.T., Nature, 160, 87 (1947).
5. Mora, P.T., Young, B.G., and Rizivi, S., J. Bio. Chem., 237, 157 (1962).
6. Slotta, K.H., and Primosigh, J., Nature, 168, 696 (1951).
7. Tabor, H., Tabor, C. W., and Rosenthal, S. M., Ann. Rev. Biochem., 30, 579 (1961)