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Two Succinic Semialdehyde Dehydrogenases Are Induced when *Escherichia coli* K-12 Is Grown on γ-Aminobutyrate

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When *Escherichia coli* K-12 was grown on γ-aminobutyrate, a second succinic semialdehyde dehydrogenase, dependent upon oxidized nicotinamide adenine dinucleotide or oxidized nicotinamide adenine dinucleotide phosphate and distinct from that induced by γ-aminobutyrate, was gratuitously induced by succinic semialdehyde.

Escherichia coli strains B, C, and W, but not K-12, can use *para*-hydroxyphenylacetate (HPA) as the sole source of carbon and energy and *E. coli* C has been shown to degrade HPA by a sequence of reactions identical to that described for strains of *Pseudomonas* (3, 13). The last reaction of the pathway is the oxidation of succinic semialdehyde to succinate, a reaction catalyzed in *E. coli* B by a succinic semialdehyde dehydrogenase which acts preferentially with NAD^+ (M. I. Donnelly and R. A. Cooper, Eur. J. Biochem., in press).

The oxidation of succinic semialdehyde is also the final reaction in the pathway for the bacterial degradation of γ-aminobutyrate (GABA; 4, 8, 12). In their studies of GABA degradation in *E. coli* K-12, Halpern and colleagues have described only $NADP^+$-linked succinic semialdehyde dehydrogenase activity (4-6, 9, 14).

In an earlier report, we established that *E. coli* B elaborates two distinct succinic semialdehyde dehydrogenases when grown on GABA (Donnelly and Cooper, in press). The amount of one enzyme, specific for $NADP^+$, is highly correlated with that of α-ketoglutarate:γ-aminobutyrate transaminase, the enzyme which initiates GABA degradation. The other enzyme, which functions in HPA degradation, was purified and partially characterized. It acts preferentially with NAD^+ and is induced when cells are exposed to succinic semialdehyde. As a consequence of this mechanism of induction, the NAD^+-dependent enzyme is also present in cells grown on GABA owing to gratuitous induction by succinic semialdehyde (Donnelly and Cooper, in press).

Since the gene encoding the NAD^+-linked dehydrogenase of *E. coli* B appears to be functionally isolated from the other structural genes of HPA metabolism, we decided to determine whether this enzyme could be detected in *E. coli* K-12, in spite of the fact that K-12 cannot degrade HPA.

We report here that *E. coli* K-12 also possesses an NAD^+-dependent succinic semialdehyde dehydrogenase. The enzyme is similar in both its size and its mechanism of induction to that which functions in the metabolism of HPA by *E. coli* B. It is induced during growth of *E. coli* K-12 on GABA.

For our experiments, we used *E. coli* K-12, strain K10, a methionine-positive derivative (1) of the strain used by Dover and Halpern (4). When extracts prepared from *E. coli* K-12 grown on succinate with GABA as the sole source of nitrogen were fractionated by gel filtration on Sephacryl S-200 (Donnelly and Cooper, in press), two distinct succinic semialdehyde dehydrogenases were found. The larger enzyme was specific for $NADP^+$, whereas the smaller acted with both NAD^+ and $NADP^+$. This pattern was the same as that observed for *E. coli* B grown under identical conditions (Donnelly and Cooper, in press). When the NAD^+-dependent enzyme purified from *E. coli* B grown on HPA (Donnelly and Cooper, in press) was passed through this column, it eluted at almost exactly the same position as the NAD^+-linked enzyme from *E. coli* K-12.

To clarify the physiological role of the two enzymes in *E. coli* K-12, we compared their activities in extracts prepared from cells grown under various nutrient conditions. In addition to the wild type, we included in our study an *E. coli* K-12 mutant which could grow on GABA as the sole source of carbon as well as of nitrogen. Such mutants arose spontaneously when wild-type cells were spread onto GABA plates and incubated for several days at 30°C. We also assayed the activity of α-ketoglutarate:γ-aminobutyrate transaminase. These experiments revealed that the amount of the $NADP^+$-specific

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dehydrogenase, but not of the NAD^+-preferring enzyme, is highly correlated with the amount of the transaminase (Table 1). Furthermore, when *E. coli* K-12 was grown on succinate plus ammonia in the presence of succinic semialdehyde, the NAD^+-dependent activity was strongly induced. Under these conditions, the $NADP^+$-specific enzyme and the transaminase were not induced. The apparent moderate increase in the level of $NADP^+$-linked activity was undoubtedly due to the $NADP^+$-dependent activity of the NAD^+-preferring enzyme.

The presence of elevated amounts of the NAD^+-dependent dehydrogenase in cells grown on GABA is, therefore, due to the gratuitous induction of this enzyme by succinic semialdehyde generated during the degradation of GABA. In accord with this hypothesis, less NAD^+-linked activity was observed in the mutant strain in which the very high $NADP^+$-dependent activity presumably leads to a lower intracellular concentration of succinic semialdehyde.

The occurrence of a second succinic semialdehyde dehydrogenase in *E. coli* K-12 grown on GABA does not in itself seriously alter the interpretation of Halpern's data regarding the regulation of GABA degradation. However, it may well explain his inability to obtain GABA mutants defective only in the dehydrogenase reaction (6). Clearly, *E. coli* K-12 possesses a second dehydrogenase which, owing to its mechanism of induction, will be present and function in place of a defective $NADP^+$-specific enzyme. The presence of this second enzyme may also explain why the dehydrogenase mutant which Halpern eventually obtained grew normally on GABA as the sole nitrogen source (9). Although this mutant was said to have no succinic semialdehyde dehydrogenase activity, the assay was conducted at pH 9.9 in Tris buffer; we observed that the NAD^+-preferring dehydrogenase purified from *E. coli* B was maximally active in phosphate buffer at pH 8.2 (Donnelly and Cooper, in press).

A more difficult and interesting question posed by the above data concerns the function of the NAD^+-dependent enzyme. In both strains K-12 and B, the enzyme was induced by its substrate, a compound which has no effect on the expression of enzymes of either the HPA pathway or the GABA pathway, both of which generate succinic semialdehyde (Donnelly and Cooper, in press). *E. coli* K-12 does not grow on HPA, nor does it give rise to mutants which can. None of the enzymes of HPA degradation is induced when cells are exposed to HPA or to the first intermediate of the HPA pathway, 3,4-di-

TABLE 1. *Enzymatic activities in extracts of E. coli K-12*[a]

Strain	Growth condition		Sp act (nmol min^{-1} mg of $protein^{-1}$)		
	Carbon source	Nitrogen source	SSDH (NAD)	SSDH[b] (NADP)	αKG:GABA transaminase
Wild type	Succinate	NH_3	6	17	15
	Succinate	GABA	162	81	93
	Succinate + succinic semialdehyde	NH_3	194	40	8
$GABA^+$ mutant	Succinate	NH_3	6	75	59
	Succinate	GABA	77	463	298
	GABA	GABA	79	398	272

[a] Cells were grown at 30°C with shaking in 100 ml of M63 medium (10) or ammonium sulfate-free M63 medium supplemented with 15 mM succinate or GABA or both, and with 1 ml of nutrient broth. For induction with succinic semialdehyde, an overnight culture of wild-type cells grown on succinate plus ammonia was diluted into fresh medium supplemented to give concentrations of succinate and succinic semialdehyde of 14 and 5 mM, respectively. The culture was allowed to grow for 3 h before harvesting. Cells were harvested during exponential growth and were suspended in 4 ml of 0.09 M sodium-potassium phosphate buffer (pH 7.0), containing 9% glycerol and 1 mM dithiothreitol. Extracts were prepared by ultrasonication, as described previously (Donnelly and Cooper, in press). The crude extracts so obtained were ultracentrifuged at 120,000 × *g* for 90 min to remove NADH-oxidizing activity. Succinic semialdehyde dehydrogenase (SSDH) activity was estimated at pH 8.0 and 30°C as described previously (Donnelly and Cooper, in press), α-ketoglutarate:γ-aminobutyrate transaminase (αKG:GABA transaminase) was assayed by the method of Zaboura and Halpern (Donnelly and Cooper, in press; 14). Protein was determined by the method of Hartree (7).

[b] Not corrected for $NADP^+$-dependent activity of the NAD^+-preferring dehydrogenase.

hydroxyphenylacetate, compounds known to induce the pathway's enzymes in *Pseudomonas putida* (2). Thus, *E. coli* K-12 appears not to be simply an HPA^- mutant (M. A. Skinner, personal communication). Also, *E. coli* strains K-12 and B cannot grow on γ-hydroxybutyrate, a compound metabolized to succinic semialdehyde by a strain of *Pseudomonas* (11). Thus, the true or ancestral function of the NAD^+-dependent succinic semialdehyde dehydrogenase of *E. coli* K-12 remains to be established.

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