

## The Role of Cyclic Adenosine 3',5'-Monophosphate in the Synthesis of the Enzymes of $\gamma$ -Aminobutyrate Breakdown in *Escherichia coli* K-12

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When *Escherichia coli* K-12 (W 3001) is grown on glucose the synthesis of the enzymes of the pathway of  $\gamma$ -aminobutyrate breakdown is repressed. The specific activity of the NADP-specific succinate semialdehyde dehydrogenase is greater than that of the NAD-specific enzyme.

The synthesis of the enzymes of  $\gamma$ -aminobutyrate breakdown is induced when *E. coli* K-12 is grown on  $\gamma$ -aminobutyrate. In this case, however, the NAD-specific succinate semialdehyde dehydrogenase has more activity than the NADP-specific enzyme. This may indicate the existence of two different enzymes.

When *E. coli* K-12 is grown on  $\gamma$ -aminobutyrate, each of glucose, 2-oxoglutarate, glutamate, and succinate repress both  $\gamma$ -aminobutyrate oxoglutarate transaminase and succinate semialdehyde dehydrogenase. Cyclic AMP does not prevent the repression caused by glucose, which does not therefore operate simply by depletion of endogenous cyclic AMP.

In 1968, cyclic adenosine 3',5'-monophosphate (cyclic AMP) was found to prevent glucose induced "catabolite repression"<sup>1</sup> of  $\beta$ -galactosidase in *E. coli*.<sup>2,3</sup> The ability of cyclic AMP to prevent the glucose repression of enzyme synthesis is not restricted to  $\beta$ -galactosidase. Cyclic AMP has now been shown to stimulate the synthesis of many enzymes in various bacteria.<sup>4,5</sup> It is supposed that catabolite repression is a consequence of the depletion of intracellular cyclic AMP by glucose.<sup>6-11</sup>

The activities of the enzymes of  $\gamma$ -aminobutyrate breakdown are low when *E. coli* is grown on glucose; the enzymes are repressed

by glucose.<sup>12-15</sup> However, Dover and Halpern<sup>15</sup> have shown for *E. coli* K-12 that these enzymes specifically can escape from this catabolite repression when  $\gamma$ -aminobutyrate, glutamate, or aspartate are used as nitrogen source instead of ammonium salts.

In this work the effect of exogenous cyclic AMP on the repression by glucose of the enzymes of  $\gamma$ -aminobutyrate breakdown in *E. coli* K-12 (W 3001) was studied.

### MATERIAL AND METHODS

*Cultivation of E. coli.* A wild strain of *E. coli* K-12 (*E. coli* K-12, W 3001) was transferred with a platinum wire from an agar slant to an inoculum medium (10 ml) containing 1% Difco yeast extract, 1% Difco tryptone, and 0.5% dipotassium monohydrogen phosphate, that had been autoclaved at 115 °C for 7 min. After 4 h incubation at 37 °C, the whole medium was poured aseptically into 250 ml of sterilized inoculum medium. After 16 h incubation at 37 °C without shaking, the cells were centrifuged (4 000 g, 10 min) at room temperature, washed twice with cold 0.9% sodium chloride and suspended in a minimal medium containing 0.1% ammonium chloride, 0.7% disodium hydrogen phosphate, 0.3% potassium dihydrogen phosphate, 0.5% sodium chloride, 0.01% magnesium sulfate heptahydrate, and 0.2% D(+)-glucose. The suspension was shaken (250 rpm) in a rotatory shaker (New Brunswick G-10) at 37 °C, and the turbidity was measured with a Klett-Summerson colorimeter using filter No. 62 (590–660 nm). When the turbidity of the culture was about 150 colorimeter scale units, the washing and centrifugation were performed as described above. The cells were suspended in a minimal medium together with ammonium chloride as the nitrogen source and

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$\gamma$ -aminobutyrate as the only carbon source. Additions to the culture (divided into three) were made as indicated on the figure legends. Cultivation and washing were performed as described above. The cells for the enzyme assays were harvested by centrifugation (5 ml, 7 000 *g*, 5 min) and washed twice with 0.9 % sodium chloride (+10 °C). The cell pellets were stored at 3 °C until subjected to ultrasonic vibration.

**Ultrasonication.** The samples were suspended in 2 ml of 20 mM sodium phosphate buffer (pH 7.0) containing 0.1 % (v/v) 2-mercaptoethanol, and ultrasonicated (MSE Ultrasonic Disintegrator, 60 W) for 6 min (2 microns peak to peak) at 0 °C. The suspensions were then centrifuged at 7 000 *g* for 10 min.

**Enzyme and protein assays.**  $\gamma$ -Aminobutyrate oxoglutarate transaminase and succinate semialdehyde dehydrogenase were measured by the methods described before.<sup>16</sup>

$\beta$ -Galactosidase was determined by the method published earlier,<sup>17</sup> except that the cells were disrupted by ultrasonication and not by toluene treatment. The two methods were compared and shown to give similar results.

The protein contents of the extracts were estimated by the Folin-Ciocalteu method.<sup>18</sup>

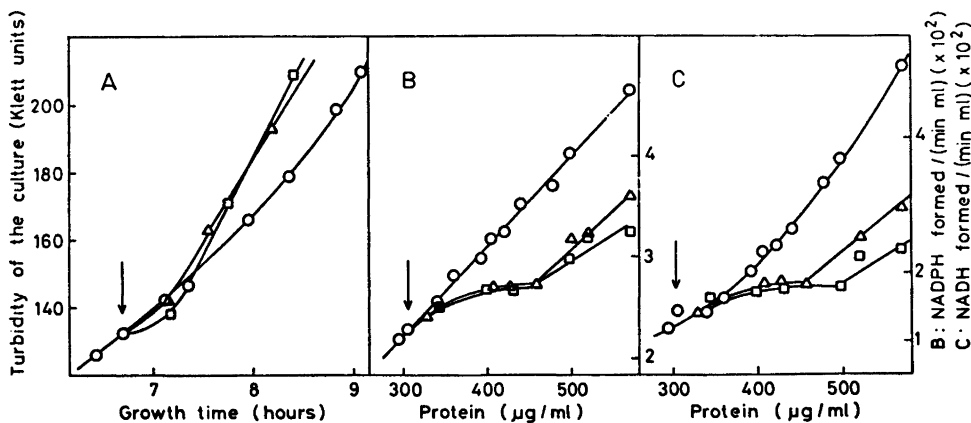
## RESULTS AND DISCUSSION

When *E. coli* K-12 (W 3001) is grown on glucose the specific activities of the enzymes of  $\gamma$ -aminobutyrate breakdown are low (Table 1). The NADP-specific succinate semialdehyde dehydrogenase is clearly much more active

**Table 1.** The specific activities of the enzymes of the pathway of  $\gamma$ -aminobutyrate breakdown [ $\mu\text{mol NADH}$  or  $\text{NADPH}$  formed  $\text{min}^{-1}$  ( $\text{mg protein}^{-1}$ )] in *E. coli* K-12 (W 3001) cells grown on glucose and  $\gamma$ -aminobutyrate. The cells for the assays were harvested in the middle of the exponential growth phase.

Enzyme	Carbon source	
	Glucose	$\gamma$ -Aminobutyrate
$\gamma$ -Aminobutyrate oxoglutarate transaminase (NADPH)	0.009	0.789
Succinate semialdehyde dehydrogenase (NADPH)	0.025	0.250
Succinate semialdehyde dehydrogenase (NADH)	0.003	0.602

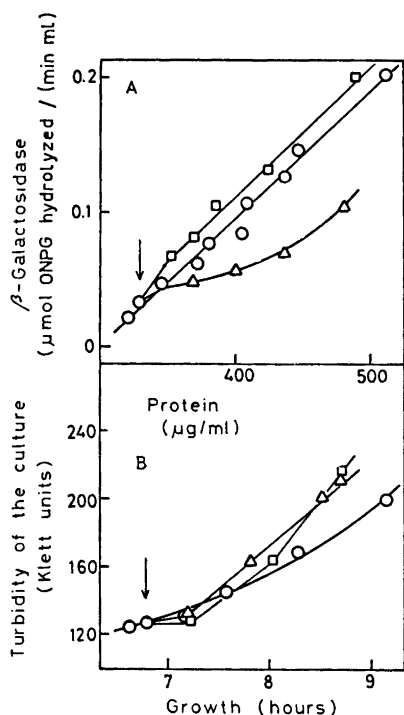
than the NAD-specific enzyme. On the other hand, when  $\gamma$ -aminobutyrate acts as carbon source, the activities of both these enzymes and that of  $\gamma$ -aminobutyrate oxoglutarate transaminase are high. It appears that the synthesis of these three enzymes is induced. In this cultivation, however, the specific



**Fig. 1.** Effect of glucose and cyclic AMP on the synthesis of  $\gamma$ -aminobutyrate oxoglutarate transaminase (B) and NAD-specific succinate semialdehyde dehydrogenase (C). The activities are expressed in ( $\mu\text{mol NAD(P)H}$  formed  $\text{ml}^{-1} \text{min}^{-1}$ ). *E. coli* K-12 (W 3001) culture growing on  $\gamma$ -aminobutyrate (0.25 %, O) was divided into three. The following additions were made at the arrow: none (O); glucose (10 mM,  $\Delta$ ); and glucose (10 mM) plus cyclic AMP (5 mM) ( $\square$ ). In A, growth curves are marked by corresponding symbols.

activity of the NAD-specific succinate semialdehyde dehydrogenase is higher than that of the NADP-specific enzyme.

As mentioned above, several examples of catabolite repression caused by glucose are prevented by cyclic AMP. In the classic case of  $\beta$ -galactosidase,<sup>2</sup> glucose represses the synthesis of induced  $\beta$ -galactosidase, whereas exogenous cyclic AMP eliminates this repression. It is of interest whether a similar situation exists for the control of  $\gamma$ -aminobutyrate oxoglutarate transaminase and succinate semialdehyde dehydrogenase. Glucose causes the repression of these enzymes in *E. coli* K-12



**Fig. 2.** Effect of glucose and cyclic AMP on the differential rate of  $\beta$ -galactosidase synthesis (A) in the culture growing on  $\gamma$ -aminobutyrate as the only carbon source (0.25%, O) and corresponding growth curves (B). To induce  $\beta$ -galactosidase isopropyl- $\beta$ -D-thiogalactoside (IPIG) (1.25 mM) was added to the growth medium 15 min before the arrow. At the arrow the culture was divided into three and the following additions were made: none (O); glucose (10 mM,  $\Delta$ ); and glucose (10 mM) plus cyclic AMP (5 mM) ( $\square$ ). In B, growth curves are marked by corresponding symbols.

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(W 3001) grown on  $\gamma$ -aminobutyrate (Fig. 1). Cyclic AMP added together with glucose might prevent the repression if it were caused by depletion of endogenous cyclic AMP. When cyclic AMP is added with glucose, however, the differential rates of synthesis of the enzymes of  $\gamma$ -aminobutyrate breakdown, do not exceed the repressed values. Accordingly, the repression is not similar to the repression of  $\beta$ -galactosidase: it is unaffected by exogenous cyclic AMP, although exogenous cyclic AMP does prevent the repression by glucose of  $\beta$ -galactosidase in *E. coli* K-12 W 3001 grown on  $\gamma$ -aminobutyrate (Fig 2). As shown in Fig. 3, 2-oxoglutarate, glutamate, and succinate each repress  $\gamma$ -aminobutyrate oxoglutarate transaminase and succinate semialdehyde dehydrogenase.

The metabolism of  $\gamma$ -aminobutyrate is associated with nitrogen metabolism *via* the transamination of 2-oxoglutarate, and also provides succinate, whose role in the citric acid cycle is both energetic and biosynthetic. The control of this metabolism is therefore likely to be complex. We have shown here that repression of  $\gamma$ -aminobutyrate-oxoglutarate transaminase and succinate semialdehyde dehydrogenase is caused by glucose and several Krebs cycle intermediates. It is particularly interesting that the repression by glucose appears not to be mediated merely by lowering the level of endogenous cyclic AMP. Dover and Halpern have previously shown that glucose repression of these enzymes is unusual in that the enzymes can escape from this control when  $\gamma$ -aminobutyrate is the sole nitrogen source.<sup>18</sup>

It has long been known that some enzymes of the citric acid cycle in bacteria are repressed when the culture is grown on glucose. It has also been reported that glutamate is an efficient corepressor.<sup>19-20</sup> In this case catabolite repression requires some other metabolite. This could explain why the enzymes of the citric acid cycle, like the enzymes of  $\gamma$ -aminobutyrate breakdown are not found to be under the control of cyclic AMP. Glutamate, however, is not necessarily the metabolite responsible for control of the enzymes in the metabolism of  $\gamma$ -aminobutyrate, although it is the dominant free amino acid in *E. coli*.<sup>22</sup>

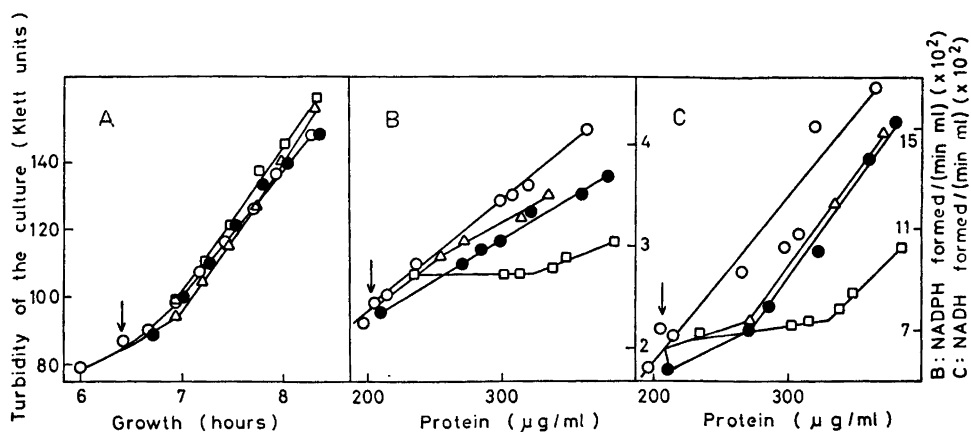


Fig. 3. Differential rate of synthesis of  $\gamma$ -aminobutyrate oxoglutarate transaminase (B) and NAD-specific succinate semialdehyde dehydrogenase (C). The activities are expressed in [ $\mu\text{mol NAD(P)H}$  formed]  $\text{ml}^{-1} \text{min}^{-1}$ . The growth curves are presented in A. *E. coli* K-12 (W-3001) culture growing on  $\gamma$ -aminobutyrate (0.25 %, O) was divided into four. The following additions were made at the arrow: none (O); 2-oxoglutarate (10 mM,  $\Delta$ ); glutamate (10 mM,  $\square$ ); and succinate (10 mM,  $\circ$ ).

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