NOVEL TYPE OF CATABOLITE REPRESSION IN THE PATHWAY OF γ-AMINOBUTYRATE BREAKDOWN IN *ESCHERICHIA COLI* K-12

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1. Introduction

The pathway of γ-aminobutyrate (GABA) catabolism in *E. coli* K-12 consists of two steps catalyzed by γ-aminobutyrate-α-ketoglutarate transaminase (GSST, EC 2.6.1.19) and succinic semi-aldehyde dehydrogenase (SSDH, EC 1.2.1.16), respectively. We have shown earlier that the synthesis of these two enzymes is subject to catabolite repression. In contrast to other *E. coli* enzymes, which also succumb to catabolite repression (β-galactosidase, EC 3.2.1.23, and aspartate-ammonia lyase (AAL, EC 4.3.1.1), the synthesis of the GABA pathway enzymes is specifically relieved from catabolite repression upon growth in a glucose medium in which the ammonium salts had been substituted by GABA, aspartate or glutamate [1].

In the work of Prival and Magasanik [2] a situation has been described in which the sythesis of histidase (EC 4.3.1.3) and of proline oxidase (EC 1.4.3.2) in *Klebsiella aerogenes* escapes from catabolite repression in a glucose medium when the nitrogen supply is being limited, while the synthesis of other catabolite-repression-sensitive enzymes (*myo*-inositol dehydrogenase, EC 1.1.1.18, β-galactosidase) remains repressed. The authors suggested that enzymes which catalyze the breakdown of nitrogenous compounds serving as the nitrogen source for the cells are released from catabolic repression under conditions of nitrogen limitation. The ability of these enzymes to escape from catabolite repression in a nitrogen-limited medium was shown to be related to the level of glutamine synthetase in the cells [3, 4]. The authors also showed that in addition to the release of histidase and proline oxidase synthesis from catabolite repression by nitrogen limitation, these enzymes, in common with many other enzymes whose synthesis is sensitive to catabolite repression, are derepressed in the presence of cyclic 3′,5′ adenosine monophosphate (cAMP). Under conditions of nitrogen limitation Prival and Magasanik also succeeded in demonstrating release of histidase synthesis from catabolite repression in a cAMP requiring mutant in the absence of cAMP (2).

In the present communication we intend to show that the escape of the synthesis of the GABA-pathway enzymes in *E. coli* K-12 from catabolite repression also depends on the availability of nitrogen, but is not effected by the addition of cAMP to the medium. From experiments in which the effect of L-glutamine on the extent of relief from repression was examined, we conclude that glutamine does not cause depression. However, since glutamine is a good source of ammonia for *E. coli* the level of the GAVA-enzymes in cells grown in a glucose–glutamine medium is as low as in cells from a glucose–ammonia medium.

2. Materials and methods

2.1. *Chemicals*

L-Aspartic acid, D-glucose and sodium succinate were purchased from British Drug Houses, Ltd., Poole, England. L-Glutamine was a product of Mann Research Laboratories, Inc., New York; GABA was obtained from Nutritional Biochemicals, Corp., Cleveland, Ohio. Isopropyl-β-D-thio-galactopyranoside, *ortho*-nitrophenyl-β-galactopyranoside and cyclic 3′,5′-adenosine monophosphate were products of Sigma Chemical Co., St. Louis, Mo. L-Methionine and 2-mercapto-

ethanol were purchased from E. Merck AG Darmstadt, Germany and nicotinamide adenine dinucleotide phosphate (NADP) was from Boehringer and Sons, Mannheim, West Germany. ^{14}C-α-ketoglutarate was obtained from The Radiochemical Centre, Amersham, Bucks., England. Succinic semi-aldehyde (SSA) was prepared by synthesis and hydrolysis of dimethylformylsuccinate [5]. Nessler's reagent was prepared by the method of Johnson [6].

2.2. *Bacterial strain*

Strain CS101B, a mutant capable of utilizing GABA as a sole nitrogen source was used in the following experiments. This mutant was obtained, by ultraviolet mutagenesis, from *E. coli* K-12 Hfr Cavalli, strain CS101A, a methionine auxotroph, as described earlier [1, 7].

2.3. *Media and bacteriological techniques*

The composition of growth media and techniques used for growing bacteria and determination of growth rates were as earlier described [7]. MM designates minimal medium and (MM-N) means minimal medium without ammonium salts.

2.4. *Preparation of cell extracts*

Cell extracts used for testing GSST, SSDH, β-galactosidase and AAL activities were prepared by sonic oscillation as described [7].

2.5. *Determination of GSST and SSDH*

The assay procedures have been described elsewhere [7], except that the concentration of NADP used in the SSDH assay was increased to 0.5 mg/ml.

2.6. *β-Galactosidase determination*

β-galactosidase was determined by the method of Pardee et al. [8]. The protein concentration used was about 4 μg/ml.

2.7. *Determination of AAL*

The activity of aspartase was measured as described by Halpern and Umbarger [9].

Table 1

Catabolite repression and the effect of cAMP on the synthesis of GSST, SSDH, β-galactosidase and AAL in CS101B.

	Addition of 2mM cAMP	Specific activity (nmoles/mg protein/min)			
		GSST	SSDH	β-galactosidase	AAL
MM+succinate,1%	–	61.9	161.0	6.7	300
MM+succinate,1%	+	46.8	198.0	7.0	304
MM+glucose,0.5%	–	6.1	6.0	1.5	122
MM+glucose,0.5%	+	8.6	27.0	4.7	202
(MM-N)+GABA, 0.4%+glucose,0.5%	–	102.1	390.0	1.1	112
(MM-N)+GABA, 0.4%+glucose,0.5%	+	83.1	358.0	9.0	240

All the media were supplemented with 25 μg/ml L-methionine

3. Results

3.1. *Effect of cAMP on the levels of GSST, SSDH, β-glactosidase and AAL in the presence of succinate and glucose as the source of carbon*

We have previously shown [1] that specific release of the synthesis of the GABA-enzymes from catabolite repression can be obtained by substituting GABA for the ammonium salts in the medium. The synthesis of other catabolite-repression-sensitive enzymes (β-galactosidase, AAL) remains repressed under these conditions. Since the synthesis of all the other known catabolite-repression-sensitive enzymes is depressed by the addition of cAMP to the glucose medium, we examined the effect of cAMP on the synthesis of the GABA-pathway enzymes in *E. coli* K-12. As shown in table 1, cAMP has no effect on the level of GSST and SSDH in a glucose-ammonia medium, while causing derepression of β-galactosidase and AAL synthesis. Addition of cAMP had also no effect on the synthesis of GSST and SSDH in a medium in which GABA served as the source of nitrogen. As expected, the synthesis of β-galactosidase and AAL was relieved from catabolite repression under these conditions.

Table 2
Effect of growth in the presence of various concentrations of ammonium salts on the synthesis of GSST and SSDH in CS101B.

Total concentration of ammonium salts (mM)	Generation time (min)	Specific activity (nmoles/mg protein/min) GSST	SSDH
80	120	89.7	159.8
4	192	109.4	219.2
0.4	210	322.2	510.0

Bacteria were grown in MM medium in which the ammonium salts were reduced to the levels indicated in the table, with sodium succinate (1%) as carbon source, supplemented with L-methionine (25 μg/ml).

3.2. *Effect of ammonia limitation on the synthesis of the enzymes of the GABA pathway*

The escape of the synthesis of the GABA-pathway enzymes from catabolite repression in a medium in which the ammonium salts were entirely substituted by GABA [1] raised the possibility that release from repression would also take place if the concentration of the ammonium salts in the medium were reduced. Indeed, as shown in table 2, growth in a succinate medium in which the concentration of ammonium salts was reduced to 0.4 mM resulted in a 3–4-fold increase in the levels of GSST and SSDH activity.

3.3. *Effect of varying concentrations of nitrogen sources other than ammonia on the extent of escape from catabolite repression*

From the above results (table 2) one could assume that the reason for the release of the synthesis of the GABA enzymes from catabolite repression in a medium in which GABA or aspartate were used as the nitrogen source instead of ammonia salts [1], was due to the limited rate of ammonia supply to the cell. If this were so, one would expect that increasing the concentration of GABA or aspartate in the medium would bring about a decrease in the rate of synthesis of these enzymes. Indeed, one can see in table 3, that the concentration of nitrogen sources other than ammonia affects the synthesis of the enzymes of GABA catabolism. The nature of the nitrogen source also influences the rate of enzyme synthesis.

Table 3
Effect of growth in the presence of different concentrations of GABA and aspartate as the nitrogen source on the synthesis of GSST and SSDH in CS101B.

Carbon source	Nitrogen source	Generation time (min)	Specific activity (nmoles/mg protein/min) GSST	SSDH
Succinate,1%	GABA,0.2%	245	140.8	733.0
Succinate,1%	GABA,0.4%	217	86.6	279.0
Glucose,0.5%	GABA,0.2%	260	234.4	880.0
Glucose,0.5%	GABA,0.4%	230	101.8	390.0
Glucose,0.5%	Aspartate,0.2%	155	80.0	220.0
Glucose,0.5%	Aspartate,0.4%	95	26.5	96.9

Bacteria were grown in minimal medium in which the ammonium salts were substituted by the indicated nitrogen source at the concentration specified, with succinate or glucose as carbon source.

3.4. *Effect of L-glutamine on the synthesis of the enzymes of the GABA-degradative pathway*

The finding that the enzymes involved in GABA catabolism are released from catabolite repression only in situations in which growth of the culture is limited by nitrogen indicates that this escape is somehow related to control mechanisms operating at the junction of carbon and nitrogen metabolic pathways, where glutamine occupies a prominent position [10]. A correlation between the escape from catabolite repression and the activity of glutamine synthetase has indeed been found in the case of histidase synthesis is *Klebsiella aerogenes* [3, 4]. We therefore studied the effect of the addition of L-glutamine to the growth medium on the extent of synthesis of the enzymes of the GABA pathway in *E. coli* K-12.

One can see from table 4 that L-glutamine added to a medium containing an excess of ammonium salts, does not result in derepression of GSST and SSDH synthesis (compare lines 1 and 2, and lines 3 and 4 in table 4). In media in which the enzyme levels are high because of escape from catabolite repression (glucose–GABA medium), the synthesis of GSST and SSDH is greatly reduced upon the addition of L-glutamine. This can be explained by L-glutamine being a good source of ammonia (via glutaminase action), as shown by the fact that the rate of growth of *E. coli* K-12 in a glucose medium in which L-glutamine served as the sole source of nitrogen, was much faster than on GABA

Table 4
Effect of L-glutamine on growth and synthesis of GSST and SSDH in CS101B

Growth medium	Addition of 0.2% L-glutamine	Generation time (min)	Specific activity (nmoles mg protein/min) GSST	SSDH
MM+succinate, 1%	-	126	89.2	167.6
MM+succinate, 1%	+	150	82.2	130.7
MM+glucose, 0.5%	-	66	3.5	9.7
MM+glucose, 0.5%	+	66	4.1	6.5
(MM-N)+GABA, 0.4% + glucose, 0.5%	-	165	67.2	281.0
(MM-N)+GABA, 0.4% + glucose, 0.5%	+	72	8.1	8.1
(MM-N)+glucose, 0.5%	+	85	4.4	11.0

as the sole nitrogen source and only somewhat lower than in a glucose–ammonia medium (compare lines 4, 5, 6 and 7 in table 4).

4. Discussion

The synthesis of the enzymes of the GABA catabolic pathway in *E. coli* K-12 is highly sensitive to catabolite repression [1]. Contrary to many other enzymes whose synthesis is released from catabolite repression upon the addition of cAMP to the growth medium [11], the synthesis of GSST and SSDH in *E. coli* K-12 is not affected by this compound (table 1). However, the synthesis of these enzymes can be made to escape from catabolite repression when one substitutes the ammonium salts by GABA, aspartate or glutamate [1], or when growth is limited by low concentrations of ammonium salts in the medium (table 2). Both of these treatments reduce the availability of nitrogen to the cell as manifested by the slowing down of the rate of growth (tables 2 and 3). Under conditions of nitrogen limitation the nature of the carbon source does not greatly affect the levels of GSST and SSDH (table 3).

In their work with *Klebsiella aerogenes*, Prival and Magasanik showed that when growth is limited by the use of poor nitrogen sources, the synthesis of catabolic enzymes responsible for the supply of both carbon and nitrogen (histidase and proline oxidase) is released from catabolite repression, while the synthesis of other catabolic enzymes (*myo*-inositol dehydrogenase and β-galactosidase) is even more strongly repressed than in a glucose–ammonia medium [2]. These authors showed that the synthesis of histidase can be derepressed by the addition of cAMP even in media containing an excess of ammonium salts.

The synthesis of GSST and SSDH, which catalyze the breakdown of GABA in *E. coli* K-12, escapes from catabolite repression under conditions of nitrogen limitation, whereas the synthesis of other catabolic enzymes which catalyze the breakdown of carbon (β-galactosidase) and nitrogen (AAL) compounds, remains repressed (table 1 and ref. [1]). On the other hand, addition of cAMP to the growth medium, which abolishes the catabolite repression of many other enzymes in *E. coli* K-12, including that of AAL, and which causes derepression of histidase and proline oxidase synthesis in *Klebsiella aerogenes*, does not depress the synthesis of the GABA-catabolic enzymes.

As shown in table 4, addition of L-glutamine does not result in any release from catabolite repression of GSST and SSDH synthesis in *E. coli* K-12. Moreover, addition of L-glutamine to a glucose–GABA medium reduces the levels of these enzymes to those found in cells grown in a glucose–ammonia medium. This repression is due to the fact that glutamine is a good nitrogen source as shown in this table (see lines 5, 6 and 7). This is well demonstrated by the quite rapid growth of the culture and the very low levels of GSST and SSDH (similar to those obtained in a glucose–ammonia medium) in media with glutamine as the sole source of nitrogen.

One can therefore conclude that the repression of

the synthesis of GSST and SSDH upon addition of L-glutamine to a glucose GABA medium, is due to increased availability of nitrogen. Under these conditions escape synthesis of GABA enzymes would serve no useful purpose and is therefore curtailed.

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