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Control of the Pathway of γ-Aminobutyrate Breakdown in *Escherichia coli* K-12

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Mutants of *Escherichia coli* K-12 isolated for their ability to utilize γ-aminobutyrate (GABA) as the sole source of nitrogen exhibit a concomitant several-fold increase in the activities of γ-aminobutyrate-α-ketoglutarate transaminase (GSST, EC 2.6.1.19) and succinic semialdehyde dehydrogenase (SSDH, EC 1.2.1.16). The increase in rate of enzymatic activity is not accompanied by any changes in the affinities of the mutant enzymes for their respective substrates. The synthesis of the two enzymes is highly coordinate under a great variety of conditions, in spite of the wide range of activities observed. In cultures grown in minimal media with ammonium salts as the source of nitrogen, both GSST and SSDH are severely repressed by glucose. Substitution of ammonia with GABA, glutamate, or aspartate greatly reduces the effect of glucose on the synthesis of the GABA utilization enzymes. This escape from catabolite repression is specific for GSST and SSDH and does not involve other enzymes sensitive to catabolite repression (e.g., β-galactosidase, EC 3.2.1.23, and aspartase, EC 4.3.1.1).

We have recently shown that wild-type *Escherichia coli* K-12 strains unable to utilize γ-aminobutyric acid (GABA) as a sole source of nitrogen exhibit very low activity of γ-aminobutyrate - α - ketoglutarate transaminase (GSST) and succinic semialdehyde dehydrogenase (SSDH). Mutants selected for their ability to grow on GABA as the sole source of nitrogen showed a concomitant six- to ninefold increase in the activities of these two enzymes (1).

The pleiotropic effects of single-step mutations on the activities of the two enzymes specifically involved in GABA catabolism suggest that the mutations occurred at a control locus which regulates the synthesis of these enzymes. However, alternative explanations, for example the possibility that the mutations affected a structural gene specifying a polypeptide common to the two enzymes, have not been ruled out. The experiments described in the present paper argue strongly against the latter interpretation and provide rather convincing evidence that the synthesis of the two GABA-utilizing enzymes is controlled by a common regulatory mechanism.

MATERIALS AND METHODS

Chemicals. L-Aspartic and L-glutamic acids were purchased from British Drug Houses, Ltd., Poole, England. α-Ketoglutaric acid (αKG) and GABA were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio; isopropyl-β-D-thio-galactopyranoside and ortho-nitrophenyl-β-D-galactopyranoside were products of Sigma Chemical Co., St. Louis, Mo.; and nicotinamide adenine dinucleotide phosphate (NADP) was from Boehringer and Sons, Mannheim, West Germany. ^{14}C-GABA and ^{14}C-αKG, both uniformly labeled, were purchased from Calbiochem Inc., Los Angeles, Calif., and The Radiochemical Centre, Amersham, Bucks., England, respectively. Succinic semialdehyde (SSA) was prepared by synthesis and hydrolysis of dimethylformylsuccinate (4). Nessler's reagent was prepared by the method of Johnson (6).

Bacterial strains. *E. coli* K-12 Hfr Cavalli, CS101A, a methionine auxotroph, and its glutamate-utilizing derivative, strain CS8A, both unable to utilize GABA as a carbon or nitrogen source, were used. Mutants capable of utilizing GABA as a sole nitrogen source and secondary mutants capable of utilizing GABA as a source of carbon were obtained by ultraviolet mutagenesis as described earlier (1).

Media and bacteriological techniques. The composition of growth media and the techniques used for growing bacteria and determination of growth rates were as earlier described (1).

Preparation of cell extracts. Cell extracts used for GSST and SSDH determinations were prepared by sonic oscillation as described (1).

Toluene treatment. A cell suspension in 0.1 M

phosphate buffer, pH 7.3, containing 0.001 M $MgCl_2$ and 0.01 M 2-mercaptoethanol, at a cell density equivalent to 4 to 10 mg of protein per ml, was shaken at 37 C for 15 min in the presence of 1% toluene and 0.005% deoxycholate. Toluene-treated cells were used in determinations of β-galactosidase and aspartate-ammonia lyase. As shown earlier in this laboratory, very similar values were obtained when toluene-treated cells or sonically disrupted preparations were used (M. Marcus, Ph.D. thesis, Hebrew Univ., Jerusalem, 1968).

Determination of GSST and SSDH. The assay procedures have been described elsewhere (1). For the determination of K_m of SSDH, the protein concentration was reduced to 10 μg/ml and that of NADP was increased to 0.5 mg/ml.

β-Galactosidase assay. β-Galactosidase was determined by the method of Pardee et al. (10), except that the cell suspension was prepared and treated as described above under "Toluene treatment."

Determination of aspartate-ammonia lyase. The activity of aspartase was measured as described by Halpern and Umbarger (2).

Graphic presentation of data. The data presented in Fig. 3 on the specific activities of GSST and SSDH of cultures grown on various carbon and nitrogen sources and the kinetic data presented in Fig. 1 and 2 were mathematically treated by the linear regression method. The data used for the Lineweaver-Burk plots (Fig. 1 and 2) were appropriately weighted by the method of Wilkinson (11). The mathematical analyses were carried out on a CDC 6400 computer at the Hebrew University of Jerusalem Computation Center.

RESULTS

Saturation kinetics of GSST and SSDH of wild-type and GABA-utilizing mutants of E. coli K-12. If a structural gene were involved in the mutations that enabled the cells to utilize GABA as the sole source of nitrogen, one would reasonably expect the mutant enzymes to show altered affinity towards their substrates. Lineweaver-Burk plots of enzyme saturation data on GSST (for GABA) and SSDH (for SSA) of wild-type and GABA-utilizing mutant strains are presented in Fig. 1 and 2. One can readily see that in both the CS101 and CS8 series there is no observable change in the K_m values for the two enzymes. The K_m of GSST for αKG (4.0×10^{-4} M) and the K_m of SSDH for NADP (5.5×10^{-4} M) also remained unchanged in the mutants (results not shown here). The apparent six- to sevenfold increase in V_{max} in the mutants is taken to represent a corresponding increase in the amount of enzyme formed.

Coordinate synthesis of GSST and SSDH. The specific activities of GSST and SSDH in 76 extracts prepared from cultures grown under different conditions were measured. The results are presented in Fig. 3, in which the activities of the two enzymes in each extract are plotted in relation to each other. One can see that the synthesis of the two enzymes was coordinate under all conditions tested; that is to say, the changes in conditions examined affected the rate of synthesis of GSST and SSDH to the same extent. As pointed out in the legend to Fig. 3, the correlation coefficient was very high, R = 0.93.

Catabolite repression. As had been found for many other catabolic pathways (7), GSST

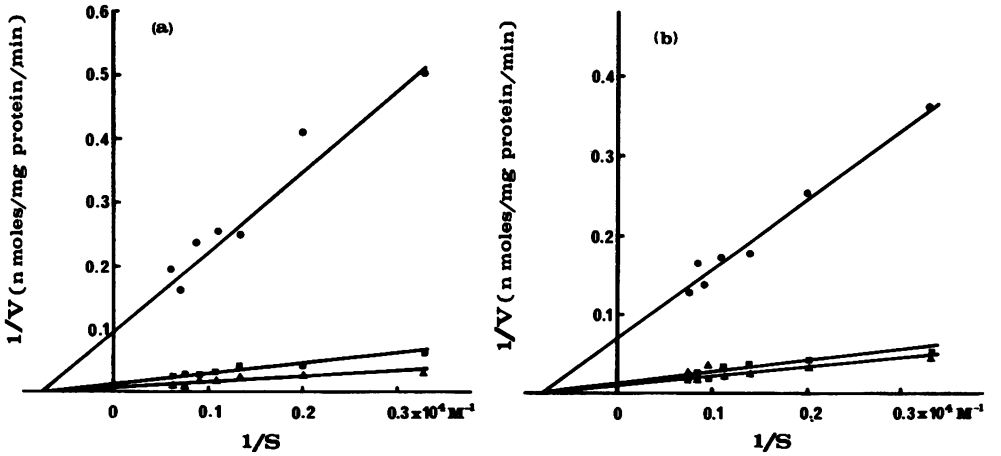

FIG. 1. *Lineweaver-Burk plot of the activity of GSST in extracts of E. coli K-12 wild-type and GABA-utilizing mutant strains. (a) E. coli K-12 CS101 series; (b) E. coli K-12 CS8 series. Symbols: CS101A, CS8A (wild-type strains), ●; CS101B, CS8B (GABA-nitrogen-utilizing mutants), ■; CS101C, CS8C (GABA-carbon-utilizing mutants), ▲. The K_m values for GABA obtained from these data are 1.3×10^{-3} M and 1.25×10^{-3} M, for the CS101 and CS8 series, respectively.*

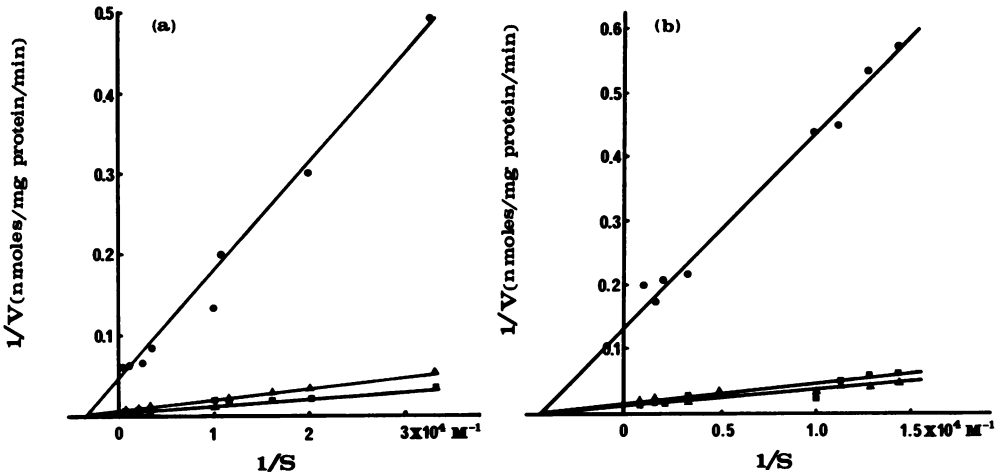

FIG. 2. *Lineweaver-Burk plot of the activity of SSDH in extracts of E. coli K-12 wild-type and GABA-utilizing mutant strains. Designations and symbols are as in Fig. 1. The K_m values for SSA obtained from these data are 2.8 × 10⁻⁴ M and 2.2 × 10⁻⁴ M, for the CS101 and CS8 series, respectively.*

and SSDH, the enzymes of the GABA utilization pathway in *E. coli* K-12, are subject to severe catabolite repression, as shown in Table 1. GABA-nitrogen-utilizing mutants CS101B and CS8B were grown in minimal media with inorganic ammonium salts as the source of nitrogen and succinate or glucose as the source of carbon, with or without GABA. One can see from Table 1 that under these conditions, whether serving as the sole carbon source, or when present in the medium together with succinate or GABA, glucose repressed the synthesis of GSST and SSDH to less than 10% of that found in cells grown in succinate medium. Addition of GABA did not relieve glucose repression, nor did GABA affect the levels of the enzymes of GABA utilization in succinate-ammonia medium in the absence of glucose.

Escape from catabolite repression. Table 2 shows that catabolite repression of GSST and SSDH synthesis exerted by glucose was relieved upon substitution of ammonia with GABA as the sole source of nitrogen. That the escape from repression was not a specific effect of GABA is demonstrated by the data presented in Tables 3 and 4. One can see from these data that escape from repression of GSST and SSDH synthesis may also be brought about by substitution of ammonia with glutamate or aspartate as the sole source of carbon, although the extent of derepression was less marked than in the case of GABA.

The data in Table 2 also indicate that the escape of GSST and SSDH synthesis from glucose repression in the presence of GABA as the sole source of nitrogen was not simply related to the slower growth of this culture as compared to that grown in the glucose-ammonia medium. (Compare growth rates and enzyme activities of succinate-ammonia- and succinate-GABA-grown cells.)

Specificity of escape from repression. Table 4 shows that the escape from glucose repression upon substitution of ammonia with other nitrogen sources was specific for GSST and SSDH, the enzymes of the GABA utilization pathway. β-Galactosidase and aspartate-ammonia lyase were as strongly repressed by glucose in the presence of GABA or aspartate as in the presence of ammonia as the source of nitrogen.

DISCUSSION

Our experiments support the assumption that the mutations obtained in this laboratory, which enable *E. coli* K-12 to utilize GABA as the sole source of nitrogen (1), involve a control locus regulating the synthesis of the two enzymes of the pathway of GABA utilization. An alternative possibility that a structural gene is involved seems unlikely in view of the fact the the K_m values of both GSST and SSDH in the mutants examined were the same as the respective values found for the parental strains (see Fig. 1 and 2). It is noteworthy that the K_m for SSA in the SSDH reaction in *E. coli* K-12, as determined in this study, is approximately two orders of magnitude higher than that found with preparations of pseudomonads (5). The relatively low affinity of the *E. coli* SSDH for SSA ($K_m \approx 2.5 \times 10^{-4}$ M)

probably explains the inability of the GABA-nitrogen-utilizing mutants to grow on GABA as a sole source of carbon, in spite of their high SSDH activities, which are 10-fold greater than those of the wild-type parent strains. The utilization of GABA as a source of carbon is made possible by a second mutation which enhances the permeability of the cell to GABA (1), thus increasing the intracellular concentration of SSA.

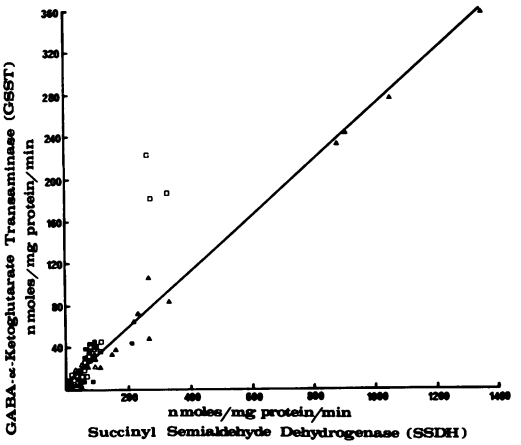

FIG. 3. *Coordinate synthesis of GSST and SSDH in E. coli K-12 grown on different carbon and nitrogen sources. Symbols: strain CS101A, ●; strain CS101B, ■; strain CS101C, ▲; strain CS8A, ○; strain CS8B, □; strain CS8C, △. All the strains tested were grown in minimal medium containing inorganic ammonium salts as the source of nitrogen and glucose, glycerol, succinate, glycerol plus glucose, or succinate plus glucose as the source of carbon, in the presence or in the absence of GABA. The GABA-utilizing mutants were also grown in minimal medium with GABA or glutamate as the sole source of nitrogen, in the presence of glucose or succinate as the carbon source. The GABA-carbon-utilizing mutants only were also grown on GABA as the sole carbon and nitrogen source. The concentrations of carbon and nitrogen sources in the different combinations employed were as indicated in Tables 1 through 4; the concentration of glycerol (where added) was 0.5%. Correlation coefficient, R = 0.93; standard deviation, SD = 23 nmoles/mg of protein/min. The intercept was 8.7 nmoles/mg of protein/min (GSST activity), and the slope was 0.28.*

Further support for our contention that the two enzymes of the pathway of GABA utilization belong to a single regulatory unit is provided by the data in Fig. 3, which clearly show that the synthesis of GSST and SSDH is strictly coordinate under a great variety of conditions. In spite of the wide fluctuations in the activities of the two enzymes observed (1.5 to 358.0 nmoles/mg of protein/min for GSST and 1.0 to 1,350.0 nmoles/mg of protein/min for SSDH), the coordinacy between them was very high, with a correlation coefficient of 0.93. These data are compatible with the idea that the two enzymes of the GABA utilization pathway constitute an operon (3). Genetic analysis of this pathway is now in progress in our laboratory.

The synthesis of the enzymes of GABA utilization of *E. coli* K-12 is highly sensitive to catabolite repression (see Table 1), a phenomenon characteristic of many catabolic pathways (7). It is of particular interest that GSST and SSDH escape from repression by glucose when the ammonia in the growth medium is substituted with GABA, glutamate, or aspartate (Tables 2, 3, and 4). A similar escape from catabolite repression had been described for the histidine utilization enzymes in *Aerobacter aerogenes*, following substitution of the ammonia in the growth medium with histidine (9). On the other hand, in *Salmonella typhimurium*, in which histidine utilization is also subject to catabolite repression, no such escape was observed (8). Nor are we aware of any other reported case of escape from catabolite repression in *E. coli*.

It is of interest that the escape from catabolite repression observed in our experiments

TABLE 1. *Effect of glucose on the synthesis of GSST and SSDH in E. coli K-12*[a]

Carbon source	Specific activity (nmoles/mg of protein/min)			
	E. coli K-12 CS101B		*E. coli* K-12 CS8B	
	GSST	SSDH	GSST	SSDH
Succinate, 1.0%	42.5	72.0	42.5	89.5
Succinate, 1.0% + GABA, 0.2%	39.2	67.0	47.7	78.6
Glucose, 0.5%	2.3	2.8	2.9	3.8
Glucose, 0.5% + GABA, 0.2%	2.5	6.7	4.4	1.0
Glucose, 0.5% + succinate, 0.5%	2.5	6.3	5.4	4.4

[a] NH_4Cl, 0.05% plus NH_4NO_3, 0.01%, served as the major source of nitrogen. For other conditions see text and reference 1. Abbreviations: GSST, γ-aminobutyrate-α-ketoglutarate transaminase; SSDH, succinic semialdehyde dehydrogenase; GABA, γ-aminobutyrate.

TABLE 2. *Escape of GSST and SSDH from catabolite repression in cultures of E. coli K-12 CS101C in the presence of GABA as the sole source of nitrogen*

Nitrogen source	Carbon source	Generation time (min)	Specific activity (nmoles/mg of protein/min)	
			GSST	SSDH
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Succinate, 1.0%	72	39.2	144.0
NH_4Cl, 0.05%, + NH_4NO_3, 0.01% + GABA, 0.2%	Succinate, 1.0%	70	38.0	142.0
GABA, 1.0%	GABA, 1.0%	125	278.0	1045.0
GABA, 0.2%	Succinate, 1.0%	70	244.0	905.0
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	GABA, 1.0%	135	358.8	1350.0
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Glucose, 0.5%	60	4.8	17.8
GABA, 0.2%	Glucose, 0.5%	210	234.4	880.0

TABLE 3. *Escape of GSST and SSDH from catabolite repression in cultures of E. coli K-12 CS8B grown on glutamate as sole source of nitrogen*

Nitrogen source	Carbon source	Generation time (min)	Specific activity (nmoles/mg of protein/min)	
			GSST	SSDH
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Glucose, 0.5%	90	4.9	14.1
L-Glutamate, 0.2%	Glucose, 0.5%	120	91.2	271.0
L-Glutamate, 0.2%, + NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Glucose, 0.5%	75	4.7	5.7

TABLE 4. *Specificity of escape from catabolite repression of GSST and SSDH in cultures of E. coli K-12 CS101C*

Nitrogen source	Carbon source	Generation time (min)	Specific activity (nmoles/mg of protein/min)			
			GSST	SSDH	β-Galactosidase	AAL[a]
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Succinate, 1.0%	72	39.2	147.0	390.0	464.0
NH_4Cl, 0.05%, + NH_4NO_3, 0.01%	Glucose, 0.5%	60	4.8	17.8	24.6	66.0
GABA, 0.2%	Glucose, 0.5%	210	234.4	880.0	19.7	27.0
L-Aspartate, 0.2%	Glucose, 0.5%	155	80.0	220.0	22.1	7.1

[a] Aspartate-ammonia lyase.

was limited to GSST and SSDH and did not involve other enzymes sensitive to catabolite repression, such as β-galactosidase and aspartase. A similar specificity of escape was described in the above-mentioned work on *A. aerogenes*. The escape of the histidine utilization enzymes of *A. aerogenes* from glucose repression when ammonia was removed from the medium was not accompanied by any escape from repression of the catabolic enzyme myo-inositol dehydrogenase (9). The authors suggested that this might indicate that different catabolites acted in controlling the synthesis of different catabolite-repression-sensitive enzymes, perhaps a single nitrogen-containing compound being responsible for the repression of all enzymes acting on nitrogenous compounds, and another compound being responsible for the repression of carbohydrate-degrading enzymes. Our results indicate a still higher degree of specificity, as witnessed by the fact that conditions which allowed the synthesis of the GABA-utilizing enzymes at a high rate failed to relieve the repression of aspartase, an enzyme which also acts on a nitrogenous substrate.

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ADDENDUM

In a recent paper by Prival and Magasanik (J. Biol. Chem. **246**:6288–6296, 1971) the authors dem-

onstrated an escape of histidase *and* proline oxidase from catabolite repression in *Klebsiella aerogenes* grown under conditions of nitrogen limitation. The resistance to catabolite repression of these enzymes was in sharp contrast to the behavior of β-galactosidase, the catabolite repression of which was enhanced during nitrogen-limited growth.

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