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Specificity and Regulation of γ-Aminobutyrate Transport in *Escherichia coli*

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A specific γ-aminobutyrate (GABA) transport system in *Escherichia coli* K-12 cells with a K_m of 12 μM and a V_{max} of 278 nmol/ml of intracellular water per min is described. Membrane vesicles contained D-lactate-dependent activity of the system. Mutants defective in GABA transport were isolated; they lost the ability to utilize GABA as a nitrogen source, although the activities of glutamate-succinylsemialdehyde transaminase (GSST) (EC 2.6.1.19) and succinylsemialdehyde dehydrogenase (SSDH) (EC 1.2.1.16), the enzymes that catalyze GABA utilization, remained as high as in the parental CS101B strain. The ability to utilize L-ornithine, L-arginine, putrescine, L-proline, and glycine as a nitrogen source was preserved in the mutants. The genetic lesions resulting in the loss of GABA transport, *gabP5* and *gabP9*, mapped in the *gab* gene cluster in close linkage to *gabT* and *gabD*, the structural genes of GSST and SSDH, and to *gabC*, a gene controlling the utilization of GABA, arginine, putrescine, and ornithine. The synthesis of the GABA transport carrier is subject to dual physiological control by (i) catabolite repression and (ii) nitrogen availability. Experiments with glutamine synthetase (EC 6.3.1.2)-negative and with glutamine synthetase-constitutive strains strongly indicate that this enzyme is the effector in the regulation of GABA carrier synthesis by route (ii).

γ-Aminobutyrate (GABA) is catabolized in *Escherichia coli* via a specific pathway leading to its conversion in two successive steps to succinate, which is then further handled by the Krebs cycle (5). The synthesis of the two enzymes of the GABA degradative pathway is coordinately regulated by a common control gene and is highly sensitive to catabolite repression. However, unlike other catabolite-sensitive systems, the GABA system resists catabolite repression under conditions of limited nitrogen supply (6). A recent study has shown that the factor responsible for this relief from repression is glutamine synthetase (GS; EC 6.3.1.2) which is derepressed under these conditions (15).

In the present work, we extended these studies to a highly specific GABA transport carrier in the *E. coli* K-12 membrane that is part of the GABA regulatory system.

MATERIALS AND METHODS

Microorganisms. The organisms used in this work were all derivatives of *E. coli* K-12 CS101. They are listed in Table 1.

Isolation of mutants defective in GABA transport. GABA transport-negative mutants S-5 and S-9 used in this work were obtained by "tritium suicide" (10). Strain CS101B was UV irradiated to a survival of 10^{-3} to 10^{-4} of the initial cell count. Aliquots were transferred to glucose-NH_4^+ minimal medium and incubated overnight in the dark at 37°C. The cultures were washed and suspended in glucose-GABA minimal medium to a cell density of 30 Klett units (filter no. 54) and incubated with shaking at 37°C until a cell density of 50 Klett units was reached. The cells were washed twice in basal medium, suspended in 0.5 ml of glucose medium with [^{3}H]GABA (519 μCi/μmol), 1 mM, as the nitrogen source, and incubated for 130 min (one doubling) at 37°C. (The concentration of GABA used allowed logarithmic growth at a constant rate for the duration of the experiment.) The culture was filtered on a sterile membrane filter (0.45 μm, 13 mm; Sartorius, 34 Göttingen, West Germany), suspended in 5 ml of glycerol-NH_4^+ minimal medium, and left in the refrigerator at 4°C for 6 weeks. After that time the viable cell count decreased from 5.3×10^7 to 1.2×10^6 cells per ml. About 5×10^3 cells were plated on MacConkey agar and replicated onto glycerol-GABA minimal agar plates. Fifty-one colonies were unable to grow on the latter. Twelve of these colonies were picked off the master plate, purified, and tested for growth on putrescine and glycine as the sole nitrogen source. All of them retained the ability to utilize both nitrogen sources, but lost the capacity for GABA transport. Two GABA transport-negative mutants thus isolated were chosen for further study.

Growth medium and cultivation of bacteria. The basal medium of Davis and Mingioli (4) from which citrate was omitted was supplemented with 25 μg of L-methionine per ml, with 0.5% glucose, 0.5%

TABLE 1. *E. coli K-12 strains used*

Strain	Utilization of GABA as nitrogen source	Genotype[a]	Source (reference)
CS101A	–	*metB1*	(5)
CS101B	+	*metB1 gabC1*	(5)
S-5	–	*metB1 gabC1 gabP5*	Obtained by [^{3}H]-GABA suicide from CS101B
S-9	–	*metB1 gabC1 gabP9*	Obtained by [^{3}H]-GABA suicide from CS101B
CS101BG	+[b]	*metB1 gabC1 glnA*	(15)
CS101BC	+	*metB1 gabC1 glnA302* (GlnC)	(15)

[a] Genetic symbols used are according to Bachmann et al. (1).

[b] Strain CS101BG can utilize GABA as the major nitrogen source if glutamine is also added to satisfy the specific requirement for it, and only in media where conditions of catabolite repression do not prevail.

glycerol, or 1.0% sodium succinate as the carbon source, and with 0.1% ammonium sulfate, 0.2% L-glutamine, or 0.2% GABA as the nitrogen source, as indicated. Cultures were grown in a shaking water bath (169 3-cm strokes per min) at 37°C, unless otherwise specified.

GABA transport assay with intact cells. Cultures were harvested in the logarithmic phase (turbidity of 80 Klett units, filter no. 54), washed twice, suspended in uptake buffer (60 mM potassium phosphate buffer [pH 7.0], 10 mM ammonium sulfate, 50 mM magnesium sulfate, 0.5% glucose, and 200 μg of chloramphenicol per ml) to a concentration of 88 μg of protein per ml, and incubated at 30°C for 30 min. Uptake was started by the addition of 0.5 ml of cell suspension to 1.5 ml of prewarmed uptake buffer containing the desired concentration of [^{14}C]GABA (10 μCi/μmol). After incubation with aeration for the indicated length of time (Fig. 1) or for 8 min (Fig. 2 and 4 and Tables 2 to 4), the entire reaction mixture was filtered, and the filter was washed twice with 5 ml of uptake buffer, dried, and counted as described (7).

Transport assay with membrane vesicles. Membrane vesicles were prepared according to Kaback (8) except that for spheroplast formation lysozyme at a concentration of 100 μg/ml was used. Membrane vesicles, 5 μl containing 33.5 μg of protein, were diluted to a final volume of 50 μl in the following mixture: 50 mM potassium phosphate (pH 6.6), 10 mM magnesium sulfate, 20 mM D-lactate lithium salt, and [^{14}C]GABA (49.4 μCi/μmol) as indicated. Uptake was measured at 15, 30, 60, and 90 s. Initial rates were determined from the linear portions of the time curves.

Chemicals. [^{14}C]GABA (49.4 mCi/mmol) and [^{3}H]-GABA (35.1 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. L-Glutamine, M.A. reagent, was obtained from Mann Research Laboratories, Orangeburg, N.Y. Putrescine was obtained from Sigma Chemical Co., St. Louis, Mo. L-Amino acids, A grade, were from commercial sources.

RESULTS

GABA uptake by *E. coli* K-12 CS101B. As shown in Fig. 1, the GABA-utilizing strain CS101B, grown in a glucose-minimal medium with GABA as the major source of nitrogen, was capable of rapidly accumulating GABA against a concentration gradient. GABA uptake at 30°C was linear for about 8 min and continued at a decreasing rate up to 30 min, when an intracellular concentration of 10 mM was reached. At least 75% of the radioactivity taken up was recovered from the cells as free GABA, and less than 10% was incorporated into trichloroacetic acid-insoluble material. Figure 2 gives substrate saturation data from which one obtains a K_m value for GABA of 12 μM and a V_{max} of 278 nmol of GABA per ml of intracellular water per min. At an external concentration of 2 μM, the intracellular concentration of free GABA after 30 min of incubation at 30°C was about 600 μM (data not shown).

Specificity of the GABA transport system. The GABA transport system is highly specific, as illustrated in Table 2. Substrate saturation kinetics of GABA uptake with concentrations of GABA from 2 to 40 μM were determined in the absence of inhibitors and in the presence

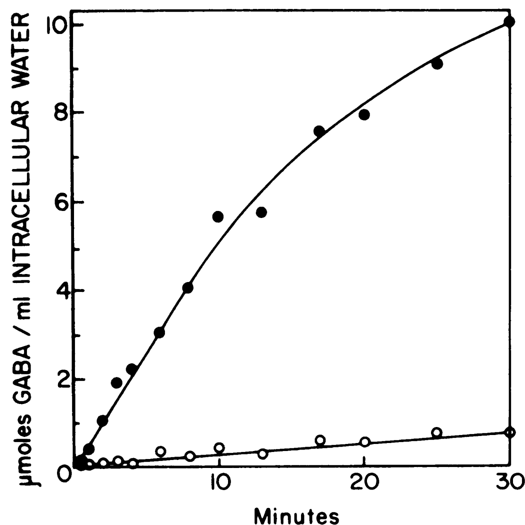

FIG. 1. *Kinetics of GABA uptake in E. coli K-12 CS101B. Bacteria were grown in minimal medium supplemented with 0.5% glucose as a carbon source and 0.2% GABA as a nitrogen source. GABA uptake at 30°C was measured as described in the text. The radioactive material accumulated in the cells after 8 min was analyzed after extraction with boiling water for 10 min and thin-layer chromatography on silica gel using n-propanol–ammonium hydroxide (7:3) as the solvent system.* (○) *Sample treated with 5% trichloroacetic acid for 20 min;* (●) *without trichloroacetic acid treatment.*

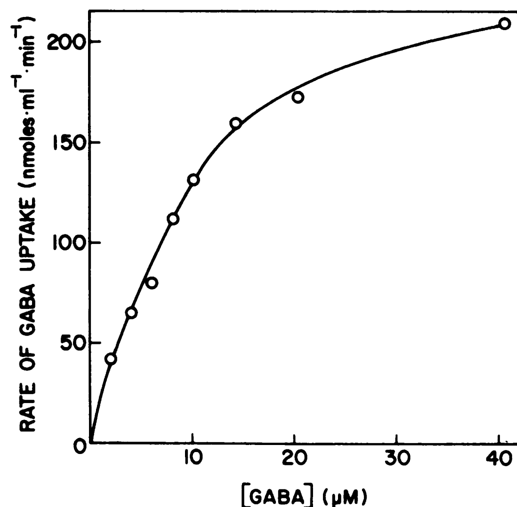

FIG. 2. *Substrate saturation curve of GABA uptake in strain CS101B. Cells were grown in glucose-GABA minimal medium. GABA uptake was measured as described in the text.*

TABLE 2. *Effect of amino acids and analogs on GABA transport in E. coli CS101B*[a]

Addition (concn)	K'_m/K_m	V'_{max}/V_{max}
Putrescine (1 mM)	1.0	1.0
α-Aminoisobutyrate (10 mM)	1.0	1.0
L-Ornithine (1 mM)	1.0	1.0
L-Lysine (1 mM)	1.0	1.0
Glycine (1 mM)	1.0	1.0
L-Leucine (1 mM)	1.0	1.0
L-Isoleucine (1 mM)	1.0	1.0
L-Alanine (5 mM)	4.8	2.0
L-Serine (1 mM)	5.7	2.4
L-Threonine (5 mM)	1.7	0.9
L-Threonine (10 mM)	3.0	1.0

[a] Cells were grown in glucose-GABA minimal medium. Substrate saturation curves of GABA transport within the concentration range of 2 to 40 μM GABA were determined with and without the specified additions. K'_m and V'_{max} are the apparent K_m aand V_{max} determined in the presence of inhibitor; a ratio of 1.0 indicates that the compound added did not affect the parameter in question. For other details, see the text.

of other amino acids in excess. As shown in Table 2, GABA uptake was not inhibited in the presence of a 500-fold molar excess of putrescine, α-aminoisobutyrate, L-arginine, L-ornithine, L-lysine, glycine, L-leucine, and L-isoleucine. L-Threonine was a weak competitive inhibitor with a K_i of 5 to 7 mM, whereas L-alanine and L-serine exerted weak inhibition of the mixed type.

GABA transport in preparations of membrane vesicles. Preparations of membrane vesicles from CS101B cultures grown in glycerol-GABA minimal medium exhibited D-lactate-stimulated transport of GABA with affinity similar to that observed with intact cells (K_m = 20 μM, V_{max} = 2 nmol/mg of protein per min). The results are shown in Fig. 3. (Since 1 mg of protein corresponds to an intravesicular space of about 2.2 μl, the V_{max} may be expressed as 908 nmol/ml per min)

Mutants specifically defective in GABA transport. The specificity of the GABA transport system was further emphasized by the isolation and behavior of GABA transport mutants (Table 3). UV-irradiated suspensions of strain CS101B were exposed to [^{3}H]GABA, and mutants that lost the ability to utilize GABA as a nitrogen source were selected as described above. Most of the mutants thus isolated were found to have lost the ability to accumulate GABA, but retained the parental high levels of GABA transaminase (GSST; EC 2.6.1.19) and succinylsemialdehyde dehydrogenase (SSDH; EC 1.2.1.16), the specific enzymes of the GABA catabolic pathway. The ability to utilize putrescine, L-ornithine, L-arginine, L-proline, and glycine as the sole source of nitrogen was also preserved in the mutants. Genetic analysis of two such mutants (S-5, S-9) disclosed that the mutational lesions (*gabP5*, *gabP9*) occurred within the *gab* gene cluster, which consists of closely linked genes controlling and determining the synthesis of proteins involved in the utilization of GABA (manuscript in preparation). *gabC*, the gene that controls the synthesis of

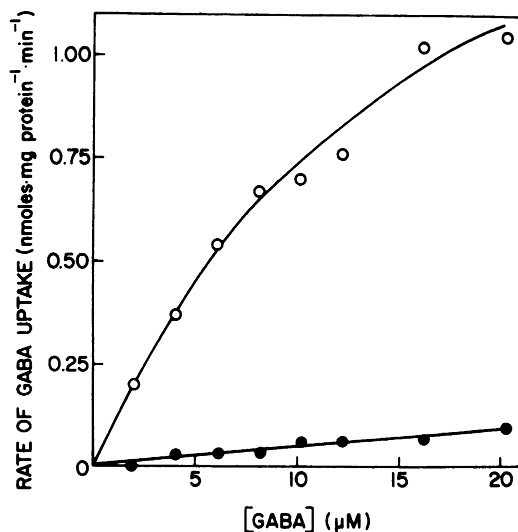

FIG. 3. *GABA uptake by cell-free preparations of CS101B membrane vesicles. Cultures were grown in glycerol-GABA minimal medium. Transport was measured as described in the text, in the absence* (●) *and in the presence* (○) *of D-lactate.*

TABLE 3. *Comparison of GABA transport in wild-type and mutant strains of E. coli CS101*[a]

Strain	Utilization of GABA as nitrogen source	Relevant genotype[b]		GABA transport activity V_{max} (nmol·ml^{-1}·min^{-1})[c]
		gabC	*gabP*	
CS101A	−	+	+	19.3
CS101B	+	c	+	178.6
S-5	−	c	*gabP5*	0[d]
S-9	−	c	*gabP9*	0[d]

[a] Cultures were grown in succinate-ammonia minimal medium at 37°C.

[b] c, Constitutive.

[c] K_m for GABA was the same (12 μM) in strains CS101A and CS101B.

[d] The radioactivity retained on the filter was not significantly different from that measured in the zero-time control mixtures. For other details, see the text.

enzymes in the GABA catabolic pathway, also controls the appearance of GABA transport activity (Table 3). Thus, strain CS101A, with the wild-type allele of *gabC* and low GSST and SSDH activities, also exhibits very low GABA transport activity. A mutation in *gabC*, as in strain CS101B, resulting in derepression of GSST and SSDH synthesis, also derepresses GABA transport.

Physiological regulation of the synthesis of the GABA transport system. The formation of the GABA transport system in the derepressed *gabC* mutant strain CS101B is subject to strong catabolite repression. The rate of GABA uptake by cells grown in a glucose-NH_4^+ medium is only about one-ninth of the rate obtained with succinate-NH_4^+-grown cells (Fig. 4). However, when the nitrogen supply is limited by substituting GABA for $(NH_4)_2SO_4$ as the source of nitrogen, glucose no longer represses the appearance of GABA transport activity. In fact, the GABA transport activity of glucose-GABA-grown cells was some 50% higher than that of cells grown in a succinate-NH_4^+ medium. That the derepression was indeed a result of nitrogen limitation and not of induction by GABA can be seen from data in Table 4, where nitrogen limitation was accomplished by the use of glutamine as the sole source of nitrogen. Although no GABA was present in the growth medium, CS101B grown in glucose-glutamine medium at 30°C exhibited very high GABA transport activity. Addition of $(NH_4)_2SO_4$ to the growth medium severely repressed the appearance of GABA transport activity.

The regulatory function of GS in the formation of the GABA transport system. We have recently shown that GS is responsible for the escape of GSST and SSDH synthesis from repression by glucose in *E. coli* grown in a nitrogen-limited medium (15). To ascertain that GS is also responsible for the appearance of high GABA transport activity under these conditions, we compared the effect of nitrogen supply during growth in a glucose medium on the formation of GABA transport activity in strain CS101B and in two isogenic strains, CS101BG, a glutamine auxotroph devoid of GS activity, and CS101BC, a GS-constitutive mutant in which GS synthesis is not repressed under conditions of nitrogen abundance. As shown in Table 4 and Fig. 4, GABA transport in strain CS101B indeed demonstrated the familiar pattern of escape from catabolite repression under conditions of nitro-

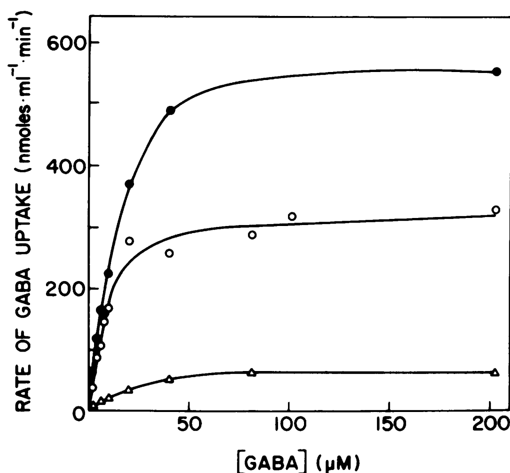

FIG. 4. *Effect of growth conditions on the formation of GABA transport activity in E. coli K-12 CS101B.* (●) *Glucose-GABA minimal medium;* (○) *succinate-NH_4^+ minimal medium;* (△) *glucose-NH_4^+ minimal medium. For other details, see the text.*

TABLE 4. *Effect of nitrogen limitation on GABA transport activity in strain CS101B and in the GS mutants CS101BG and CS101BC*[a]

Strain	Growth medium	GABA transport activity V_{max} (nmol·ml^{-1}·min^{-1})[b]
CS101B	glu, gln, NH_4^+	3.8
CS101B	glu, gln	203.0
CS101BG	glu, gln, NH_4^+	10.3
CS101BG	glu, gln	11.6
CS101BC	glu, gln, NH_4^+	291.7
CS101BC	glu, gln	291.7

[a] Cultures were grown at 30°C with strong aeration. glu, 0.5% glucose; gln, 0.2% L-glutamine; NH_4^+, 0.1% $(NH_4)_2SO_4$. For other details, see the text.

[b] K_m for GABA was the same (12 μM) in all six cultures tested.

gen limitation. However, the GS-less strain CS101BG was not derepressed in a nitrogen-limited medium, whereas in the GS-constitutive strain GS101BC high levels of GABA transport activity were always obtained regardless of whether the nitrogen supply was limited or not.

DISCUSSION

The experiments described here clearly demonstrate the existence in *E. coli* of a highly specific membrane transport carrier for GABA. GABA transport was not inhibited by basic or branched-chain amino acids or by glycine (Table 2) and therefore was not mediated by the LIV-I, LIV-II (11), arginine-specific, lysine-arginine-ornithine (12), or *dag* (9) transport systems engaged in the uptake of these amino acids by *E. coli*. Moreover, the gene that determines GABA transport, *gabP*, is located within the *gab* gene cluster, which also contains *gabT* and *gabD*, structural genes of the GABA pathway enzymes, and *gabC*, a gene which pleiotropically controls all of the GABA pathway (to be published).

The synthesis of the GABA transport carrier in *E. coli* is under dual physiological control by (i) catabolite repression and (ii) nitrogen availability. Our experiments with GS-negative and GS-constitutive mutants strongly indicate that GS is the effector which regulates GABA carrier synthesis by (ii). As demonstrated by Magasanik and co-workers for the regulation of transcription in the *hut* system of *Klebsiella aerogenes* (13), GS probably acts as an activator of transcription in the system of *E. coli*. Activation by GS is not required in situations where no catabolite repression prevails, probably because of the availability of sufficient CRP-cyclic AMP complex to serve as an alternative activator. Another transport system that seems to be regulated by GS is that of glutamine in *Salmonella typhimurium* (2) and possibly also in *E. coli* (14). Catabolite repression as a means of regulating amino acid transport has been indicated in the case of tryptophan permease in *E. coli* (3). Since GABA may serve as a source of both carbon and nitrogen, it is advantageous to the cell to be able to enhance its uptake in response to signals which relay a shortage in either one of them.

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