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Genetic Analysis of the γ-Aminobutyrate Utilization Pathway in *Escherichia coli* K-12

SHABTAY DOVER AND YEHESKEL S. HALPERN

Department of Molecular Biology, Institute of Microbiology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

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The control mutation that results in a concomitant severalfold 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), leading to the acquisition of the ability to utilize γ-aminobutyrate (GABA) as the sole source of nitrogen by *Escherichia coli* K-12 mutants, was mapped by mating and transduction with P1*kc*. The locus affected, *gabC*, is approximately 48% co-transduced with the *thyA* gene, located at min 55 of the *E. coli* K-12 chromosome. The structural gene of the first enzyme in the GABA pathway, GSST, was mapped by interrupted mating, using one of the GSST-less mutants, DB742, isolated in this work. The mutated locus, *gabT*, is situated at about min 73 of the *E. coli* chromosome, close to the *gltC* gene. Genetic evidence concerning the sensitivity of the enzymes of the GABA pathway to catabolite repression under different physiological conditions suggests that the two structural genes of the GABA regulon do not constitute one operon.

We have shown previously that the activities of the two enzymes of the γ-aminobutyric acid (GABA) catabolic pathway, GABA-α-ketoglutarate transaminase (GSST, EC 2.6.1.19) and succinic semialdehyde dehydrogenase (SSDH, EC 1.2.1.16), in GABA-utilizing mutants of *Escherichia coli* K-12, are six- to ninefold higher than the corresponding activities of the wild-type parent strains (3). We have shown also that the affinities of these enzymes for their respective substrates are similar in mutant and wild-type preparations. Furthermore, the synthesis of the two enzymes is highly coordinate under a variety of growth conditions (4). These findings indicate that the mutation enabling *E. coli* K-12 to utilize GABA as a nitrogen source occurred in a control gene.

In the present work, we mapped the control element (*gabC*) of the GABA-utilization pathway and the structural gene of GSST (*gabT*) and found them to be located at a distance of about 15 min from each other on the *E. coli* K-12 chromosome. The evidence obtained does not support the notion that the two structural genes of the GABA-system in *E. coli* K-12 constitute one operon.

MATERIALS AND METHODS

Chemicals. GABA, uracil, L-arginine, and D-(+)-xylose were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; thymine and thiamine-hydrochloride were obtained from Calbiochem, Los Angeles, Calif.; and nicotinamide adenine dinucleotide phosphate (NADP) was a product of Boehringer and Sons, Mannheim, West Germany. D-Glucose and sodium succinate were purchased from British Drug Houses, Ltd., Poole, England; and L-methionine and 2-mercaptoethanol were from E. Merck AG, Darmstadt, Germany. Sucrose was obtained from Mallinckrodt Chemical Works, St. Louis, Mo., and sodium penicillin G was a product of Rafa Laboratories Ltd., Jerusalem, Israel. [^{14}C]α-ketoglutaric acid (sodium salt) was obtained from the Radiochemical Centre, Amersham, Bucks., England. Succinic semialdehyde (SSA) was prepared by synthesis and hydrolysis of dimethylformylsuccinate (6).

Bacterial strains. The strains employed in the experiments reported in this work are listed in Table 1. All of them are derivatives of *E. coli* K-12.

Media and bacteriological techniques. Minimal medium with and without ammonium salts, as described earlier (3), was supplemented with a carbon source and strain requirements as detailed in the text. Mating medium was prepared by the method of Hayes (5), and transduction medium was prepared by the method of Luria (9). Nutrient broth (Oxoid) was used to allow phenotypic expression of the mutants. Agar-agar (2%) was added when solid media were required. The cultures were maintained on MacConkey agar (Difco) plates. The techniques used for growing bacteria and determination of growth rates were as described previously (3).

Isolation of mutants. For selection of spontaneous mutants, cultures in the logarithmic phase in mini-

TABLE 1. *Bacterial strains used*[a]

Strain	Mating type	Utilization of GABA nitrogen		Relevant genotype	Origin	Comments and references
		37 C	42 C			
CS101A	Hfr	–	–	*metB1*	Hfr Cavalli	Point of origin at 15 min, counterclockwise transfer (1)
CS101B	Hfr	+	–	*metB1, gabC1*	UV-induced mutant of CS101A	
CS101C	Hfr	+	–	*metB1, gabC1*	UV-induced mutant of CS101B	Utilizes GABA as the major carbon source
CS8A	Hfr	–	–	*metB1, gltC8*	UV-induced mutant of CS101A	
CS8B	Hfr	+	–	*metB1, gltC8, gabC51*	UV-induced mutant of CS8A	
CS8C	Hfr	+	–	*metB1, gltC8, gabC51*	UV-induced mutant of CS8B	Utilizes GABA as the major carbon source
CS101B/*met*$^+$	Hfr	+	–	*gabC1*	Spontaneous mutant of CS101B	
CS8B/*met*$^+$	Hfr	+	–	*gltC8, gabC51*	Spontaneous mutant of CS8B	
SC23	Hfr	+	–	*metB1, gabC1*	UV-induced mutant of CS101C	Does not utilize GABA-nitrogen in the presence of glucose
RC22	Hfr	+	–	*metB1, gabC1*	UV-induced mutant of SC23	Capable of utilizing GABA-nitrogen in the presence of glucose
DB74	Hfr	–	–	*gabC1, gabT101*	UV-induced mutant of CS101B/*met*$^+$	
DB742	Hfr	–	–	*gabC1, gabT101, str*	Spontaneous mutant of DB74	
AN2	Hfr	+	+	*metB1, gabC2*	UV-induced mutant of CS101A	
AN3	Hfr	+	+	*metB1, gabC3*	UV-induced mutant of CS101A	
AN4	Hfr	+	+	*metB1, gabC4*	UV-induced mutant of CS101A	
AN7	Hfr	+	+	*metB1, gabC7*	UV-induced mutant of CS101A	
AN8	Hfr	+	+	*metB1, gabC8*	UV-induced mutant of CS101A	
AN19	Hfr	+	+	*metB1, gabC19*	UV-induced mutant of CS101A	
OR12	Hfr	–	–		Derivative of WT X494	Point of origin at 61 min, counterclockwise transfer[b]
OR121	Hfr	+	–	*gabC1*	Transductant of OR12 with a P1*kc* lysate from CS101B	
OR38	Hfr	–	–		Derivative of WT X602	Point of origin at 61 min, clockwise transfer[b]
OR383	Hfr	+	–	*gabC1*	Transductant of OR38 with a P1*kc* lysate from CS101B	
KL141	F$^-$	–	–	*pyrE41, argG6, thyA37, thi-1, strA1*	*thyA* derivative of AT22	Reference 8
P42	F$^-$	–	–	*thi-1, str-135, xyl*	Spontaneous revertant of P678	Reference 1
W14	F$^-$	–	–	*str*	Spontaneous revertant of W1485	Reference 1

[a] All the organisms described in this table are *Escherichia coli* K-12 strains.
[b] These strains were obtained from the Institut Pasteur, Paris.

mal medium were harvested by centrifugation, washed, and suspended in saline to a density of 6×10^9 cells per ml; 0.1-ml samples were plated on the selective medium. Ultraviolet (UV)-induced mutants were selected after irradiation of logarithmic-phase cultures to a survival of 10^{-3} to 10^{-4}. The bacteria were transferred to nutrient broth and incubated overnight at 37 C for phenotypic expression, centrifuged, washed, and inoculated directly into the appropriate selection medium, or were treated first with penicillin for counterselection (2).

Mating experiments. The mating experiments were performed by a modification of the method of Hayes (5), with streptomycin (200 μg/ml) as the counterselecting agent. Mating was started by mixing the parental strains in appropriate volume ratios to give final cell densities of 2×10^7 male cells and 2×10^8 female cells per ml. The mating was performed in 10-ml volumes in 125-ml conical flasks. Samples of 1 ml were withdrawn at different times and diluted in 9 ml of saline containing 200 μg of streptomycin per ml. The diluted samples were then vibrated for 60 s on a Vortex-Genie mixer (Scientific Industries Inc., Springfield, Mass.) at full speed, to interrupt conjugation, filtered, washed with 30 ml of the same prewarmed (37 C) saline on membrane filters (Millipore Corp.), and suspended in 1 ml of saline. Samples (0.1 ml) of this suspension were plated on selective media containing 200 μg of streptomycin per ml, and incubated at the appropriate temperature. When an Hfr strain was used as the recipient in the mating, we altered it to behave temporarily like a female

phenocopy, by the method of Maas and Maas (10).

Transduction experiments. Lysates of P1*kc* were prepared by the method of Lennox (7). Transduction experiments were performed, by a modification of the method of Luria (9), as follows. Recipient cultures were grown overnight with shaking at 37 C in transduction medium, centrifuged, washed with saline, and suspended in one-tenth of the original volume of saline. $CaCl_2$ was added to a final concentration of 2.5 mM, and phage lysate was added at a multiplicity of infection of 0.1. After 20 min of incubation at 37 C, the unadsorbed phage particles were removed by centrifugation and washed with saline. The washed bacteria were suspended in saline to a final concentration of 2×10^9 cells per ml, and 0.1-ml samples of this suspension were plated on selective media containing 2.5 mM $CaCl_2$ and incubated at the appropriate temperature.

Preparation of cell extracts. Cell extracts used for the determination of GSST and SSDH were prepared by sonic oscillation as described previously (3).

Determination of GSST and SSDH. The assay procedures for GSST and SSDH were as described elsewhere (3), except that the NADP concentration in the SSDH assay was increased to 0.5 mg/ml.

RESULTS

Mapping of the gabC mutation of strain CS101B. Strain CS101B is a mutant of Hfr Cavalli, with the point of origin at min 15 on Taylor's map (15), transferring the chromosome in an anticlockwise direction. Preliminary mapping by interrupted mating of strain CS101B and the female strain KL141 has shown that *gabC* is located between min 50 and 70 on the *E. coli* K-12 chromosome (Fig. 1). This segment is about one-half of the chromosome away from the point of origin of HfrC. To locate more precisely the *gabC* mutation, we used GABA-utilizing transductants of the Hfr strains OR12 and OR38 which have a common point of origin at min 61, but transfer the chromosome in opposite directions. Interrupted mating between strains OR383 (derived from Hfr OR38) and F⁻P42 yielded no GABA-utilizing recombinants even after 60 min of mating, although xylose-utilizing recombinants appeared at the expected time. Interrupted mating of strain OR121 (derived from Hfr OR12) with the female strain KL141 gave GABA-nitrogen-utilizing recombinants which appeared after about 14 min of conjugation between the *argG* and *thyA* markers (Fig. 2). These results place the *gabC* gene between min 55 and 61 of the *E. coli* K-12 chromosome.

Fine mapping by transduction, with *thyA* and *argG* as selected markers, and scoring for transfer of *gabC1* as the unselected marker revealed that the *gabC* locus was 49% co-transduced with *thyA*, but showed no linkage to *argG*

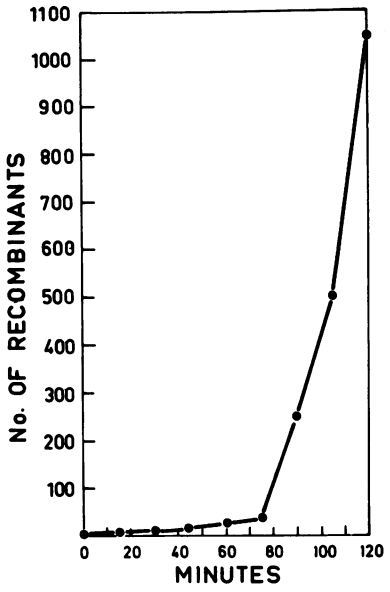

FIG. 1. *Kinetics of gabCl transfer to strain F⁻ W14. The donor strain is Hfr CS101C.*

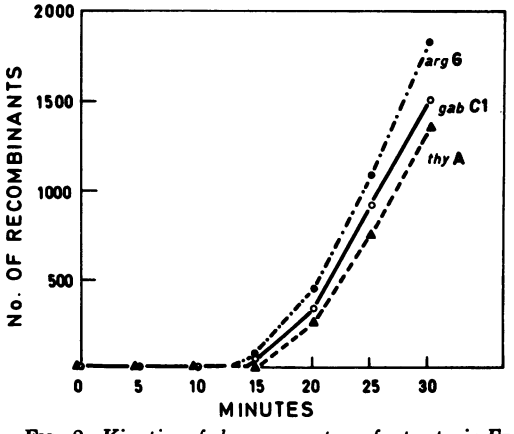

FIG. 2. *Kinetics of chromsome transfer to strain F⁻ KL141. The donor strain is Hfr OR121.*

(Table 2). In Table 3 are shown the results of another transduction experiment in which single transductants for each of the three markers, *argG*, *thyA*, and *gabC*, and double transductants, *thyA gabC* and *argG gabC*, were scored directly on appropriate media. In this experiment, *gabC* was 46% co-transduced with *thyA* and very weakly, if at all, linked to *argG*.

On the basis of these findings, we conclude that the control gene *gabC* is located at min 55, close to *thyA*, on the side proximal to *argG*.

GSST and SSDH activities of GABA-utilizing transductants and exconjugants. GABA-utilizing transductants of strain KL141, carry-

TABLE 2. *Co-transduction of mutation gabC1 with thyA and argG as selected markers*[a]

Selection medium	No. of transductants	No. of transductants capable of utilizing GABA as the nitrogen source	Co-transduction (%)
Complete medium minus L-arginine	142	0	0
Complete medium minus thymine	120	59	49

[a] Strain KL141 was used as recipient, with lysates of P1*kc* grown on CS101B. For other details see Materials and Methods. Complete medium consisted of minimal medium with succinate (1%) as the major carbon source, supplemented with thiamine (1 μg/ml), L-arginine (25 μg/ml), uracil (20 μg/ml), and thymine (20 μg/ml). Cells (2×10^8) were plated on each of the selective media. The same number of uninfected cells was plated as a control for spontaneous mutation. The figures shown in the table were obtained after the subtraction of the control values (four to eight colonies per plate). The presence of the nonselected marker was tested by replica plating onto media with GABA as the nitrogen source.

TABLE 3. *Comparative frequencies of transduction of gabC, thyA, and argG, selected in pairs or as single markers*[a]

Selection medium	No. of transductants
Complete medium minus thymine	5,472
Complete medium minus arginine	4,608
Complete medium with GABA as the nitrogen source	5,600
Complete medium minus thymine with GABA as the nitrogen source	2,560
Complete medium minus arginine with GABA as the nitrogen source	10

[a] The medium, phage, and bacterial strains used were those described in Table 2. For other details see text.

ing the *gabC1* allele, grow slowly in a medium with GABA as the source of nitrogen, in contrast to GABA-utilizing recombinants, obtained by mating of strain KL141 with the male donor CS101B, which exhibit faster growth in the GABA-medium similar to that of the donor strain (Table 4). The differences in the rate of growth on GABA between recombinants obtained by transduction and those obtained by conjugation are accompanied by similar differences in the activities of the enzymes of the GABA-utilization pathway. These findings seem to indicate that an additional locus transferred by the HfrC donor during conjugation, before the control element *gabC*, affects the expression of the structural genes of the GABA system. The nature of this locus and its precise position have not been studied any further.

Rates of growth on GABA at 37 and 42 C and the activities of GSST and SSDH of different E. coli K-12 Cavalli strains grown at the two temperatures. Wild-type and GABA-utilizing *E. coli* K-12 Cavalli strains were grown in succinate-minimal medium with GABA as the nitrogen source for eight generations at 37 C and then transferred to 42 C. The growth rates at 42 C were determined upon the establishment of the new steady state 2 h after transfer to the new temperature (Table 5). Our results show that the ability of GABA-utilizing mutants to grow on GABA is greatly reduced at 42 C, owing to the low levels of GABA-utilizing enzymes formed at this temperature (Table 6).

Isolation of mutants unimpaired in the utilization of GABA at 42 C. Mutants capable of rapid growth on GABA at 42 C were isolated as follows. Strain CS101A was UV-irradiated and colonies were isolated on a GABA-succinate medium at 37 C. Forty-four independent isolates were tested for their ability to grow on GABA as the nitrogen source at 42 C. Six mutants showing rapid growth were saved for further studies. The enzyme activities of these mutants grown at different temperatures were examined. Table 7 shows that the activities of GSST and SSDH in these mutants grown at

TABLE 4. *Range of GSST and SSDH activities in recombinants capable of utilizing GABA as the nitrogen source, obtained by mating and transduction*[a]

Means of genetic transfer	Time before appearance of visible colonies on GABA plates (days)	Sp act (nmol/mg of protein/min)	
		GSST	SSDH
Hfr × F^- mating	2	24.0–60.0	129.0–337.0
Transduction with phage P1*kc*	4	20.2–31.9	45.2–64.6

[a] The strains used for mating and transduction were as in Fig. 2 and Table 2. *thyA*$^+$ recombinants were selected, picked, and transferred onto plates of succinate-minimal medium with GABA as the only source of nitrogen. Four GABA-nitrogen-utilizing recombinants from each type of cross were tested for enzyme activities.

TABLE 5. *Growth rates of wild-type and GABA-utilizing mutants at 37 and 42 C on GABA as the sole source of nitrogen*[a]

Strain	Generation time (min)	
	37 C	42 C
CS101A	840	>1,100
CS101B	218	808
CS101C	180	498
CS8A	840	1,100
CS8B	188	660
CS8C	166	220

[a] The bacteria were grown on minimal medium in which the ammonium salts were substituted by GABA (0.4%).

TABLE 6. *GSST and SSDH activities*[a] *of wild-type and GABA-utilizing mutants, grown in succinate-ammonium salts medium at 37 and 42 C*

Strain	Sp act at:			
	37 C		42 C	
	GSST	SSDH	GSST	SSDH
CS101A	10.3	17.8	2.1	24.2
CS101B	61.5	96.6	4.9	36.3
CS101C	59.2	235.6	6.4	24.2
CS8A	5.6	22.6	6.3	37.1
CS8B	35.6	269.5	6.2	45.2
CS8C	26.9	174.4	10.3	38.7

[a] Activities are expressed as nanomoles per milligram of protein per minute.

TABLE 7. *GSST and SSDH activities*[a] *of mutants capable of utilizing GABA as the nitrogen source at 37 and 42 C, after growth in a succinate-ammonium salts medium at the respective temperature*

Strain	Sp act at:			
	37 C		42 C	
	GSST	SSDH	GSST	SSDH
AN2	55.8	214.0	31.9	193.5
AN3	48.5	85.5	24.4	84.0
AN4	57.5	196.7	15.8	48.4
AN7	22.4	71.0	24.8	48.4
AN8	37.5	77.4	27.2	169.4
AN19	72.0	548.0	19.2	119.4

[a] Activities were expressed as nanomoles per milligram of protein per minute.

42 C are much higher than the respective activities of mutant CS101B grown under similar conditions (Table 6).

Mapping of the mutation that enables growth on GABA as the nitrogen source at 42 C. In preliminary mating experiments with strain AN4 as the donor and strain KL141 as the recipient, recombinants capable of utilizing GABA-nitrogen for growth at 42 C appeared after 60 min of conjugation (Fig. 3). Precise mapping by transduction, with a P1*kc* lysate of strain AN4 and strain KL141 as the recipient, disclosed a 51% linkage of the ability to utilize GABA-nitrogen at 42 C to the *thyA* marker. We therefore concluded that the mutation occurred at, or very close to, the *gabC* locus.

Recombinant colonies on succinate-GABA medium at 42 C appeared 48 h after plating of the conjugation mixture, whereas in transduction experiments visible colonies appeared only after 4 days. It seems, therefore, that the expression of the mutation that enables rapid growth on GABA at 42 C is also enhanced by another gene located between the point of origin of HfrC and the *gabC* locus.

Isolation of mutants lacking GSST activity. To map the structural genes of the GABA system, mutants defective in the activities of GSST and SSDH were required. GABA-utilizing strains (CS101B, CS101C) were irradiated with UV-light and, after overnight growth in nutrient broth, the cultures were subjected to three cycles of penicillin treatment in a GABA-succinate medium. Among the survivors, we expected to find mutants defective in GSST and mutants defective in SSDH activity. In-

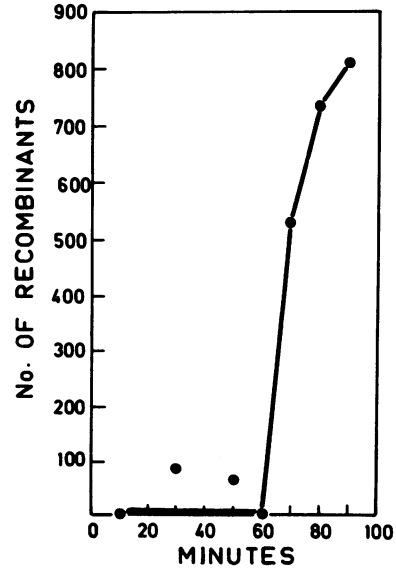

FIG. 3. *Kinetics of gabC4 transfer to strain F⁻ KL141. The donor strain is AN4, a derivative of strain HfrC.*

deed, a great number of independent GSST mutants was obtained, but not a single SSDH-less mutant was found among approximately 150 mutants examined.

Our failure to isolate an SSDH-defective mutant could have been due to the toxic effect of SSA accumulating in such a strain when grown in a GABA medium. This interpretation is supported by the results shown in Table 8, which demonstrate that SSA is indeed toxic to the cells at concentrations of 5 mM and higher. To overcome this difficulty, we isolated SSA-resistant mutants from which we tried to select SSDH-less strains. Selection of SSA-resistant mutants was made on MacConkey plates with a drop of 0.1 M SSA or on minimal medium with SSA (15 mM) as the sole source of carbon to eliminate mutants defective in SSA transport. Among the SSA-resistant mutants, we expected to find some with increased SSDH activities. These could be used directly for mapping the SSDH structural gene. However, no such mutants were found among the many SSA-resistant strains isolated in these experiments. Neither did we succeed in selecting an SSDH-less mutant from SSA-resistant cultures.

Mapping of the GSST structural gene. Mapping of the GSST locus (*gabT*) was carried out by interrupted mating between *E. coli* K-12 Hfr CS8B as the donor and a GSST-less mutant, HfrCS DB742 (*gabT101*, *str*), converted to a female phenocopy by the method of Maas and Maas (10), as the recipient. From the results presented in Fig. 4, we see that *gabT101* is located in the vicinity of min 70, at a distance of approximately 15 min from the control element *gabC*.

TABLE 8. *Effect of various concentrations of SSA, in minimal medium in the presence and in the absence of sodium succinate as the source of carbon, on the growth of wild-type and GABA-utilizing mutants*

SSA concentration (mM)	Bacterial growth[a]					
	Without succinate			With 0.5% succinate		
	CS101A	CS101B	CS101C	CS101A	CS101B	CS101C
20	8	6	7	8	13	20
5	40	28	49	7	7	18
2	25	17	24	74	62	63
0.7	10	8	10	80	62	80
0.2	6	5	6	75	78	72
0.07	5	5	5	65	80	66

[a] Bacteria were grown overnight in minimal medium supplemented with L-methionine (25 μg/ml) at 37 C in 16 mm-diameter tubes. Growth was determined turbidimetrically (expressed in Klett units; one Klett unit is equivalent to 4.8×10^6 cells per ml). When succinate was absent, the bacteria used the SSA as the carbon source.

Do the two structural genes of the GABA pathway constitute a single operon? As previously shown (4), the synthesis of GSST and SSDH in GABA-utilizing strains escapes from catabolite repression in glucose media when GABA serves as the nitrogen source. Nine independent mutants of *E. coli* K-12 CS101C that lost the ability to synthesize the two GABA enzymes at derepressed rates in GABA-glucose medium have been isolated. One such mutant, SC23, was studied further. Revertants capable of growth on GABA-glucose plates were obtained from strain SC23 at a frequency of approximately 5×10^{-7}. One of these revertants, RC22, showed derepressed synthesis of SSDH in GABA-glucose medium, whereas GSST was synthesized at a very low rate in this medium (Table 9). Among 44 revertants of strain SC23 examined, not a single one showed derepression of GSST only. This is probably due to the high toxicity of SSA to *E. coli* (Table 8). A revertant capable of synthesizing GSST, but not SSDH, at a high rate in a GABA-glucose medium would accumulate high concentrations of SSA. Hence, the experimental conditions used for the selection of revertants were counterselective against this particular type of revertants. Another mutant derepressed for SSDH synthesis only, in a GABA-glucose medium was found among methionine prototroph revertants of strain *E. coli* K-12 CS8B (Table 9).

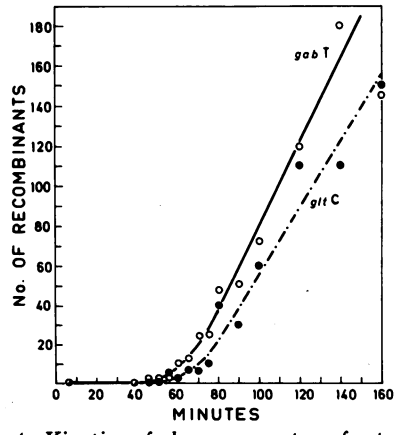

FIG. 4. *Kinetics of chromosome transfer to strain Hfr DB742, transformed into a female phenocopy by the method of Maas and Maas (10). The donor strain is Hfr CS8B.*

TABLE 9. *GSST and SSDH activities[a] in different media of strains which lost the capacity for specific escape from catabolite repression of GSST synthesis*

Strain	Activities in growth media					
	1% succinate + NH_4		0.5% glucose + NH_4		0.5% glucose + 0.4% GABA	
	GSST	SSDH	GSST	SSDH	GSST	SSDH
CS8B/*metI*$^+$	30.4	292.0	4.0	19.4	14.2	239.0
RC22	31.3	125.8	5.9	18.0	2.9	311.0
CS8B (control)	35.6	269.5	6.1	16.1	83.1	390.0

[a] Enzyme activities were expressed as nanomoles per milligram of protein per minute. Bacteria were grown in minimal medium with succinate or glucose as the carbon source and with L-methionine (25 μg/ml). When GABA was used as the nitrogen source, ammonium salts were omitted from the medium.

These results suggest that the two structural genes of the GABA regulon do not constitute a single operon.

DISCUSSION

E. coli K-12 cannot grow in media in which GABA serves as the major carbon or nitrogen source, or both. Mutants capable of utilizing GABA have been isolated in our laboratory (11; M. Marcus, Ph.D. thesis, Hebrew Univ., Jersualem, 1968). Enzymological comparison of these mutants with the wild-type strains led to the suggestion that mutations enabling the cell to grow on GABA as the nitrogen source affected a control gene that regulates the synthesis of the two enzymes of the GABA catabolic pathway, GSST and SSDH (3, 4). This was indicated by the fact that the mutants showed a concomitant six- to ninefold increase in the activities of the two enzymes, but the affinities for the respective substrates remained the same as in the parent strains. That the synthesis of the two enzymes is under a common control has been also indicated by the high degree of coordination between the activities of these two enzymes in cultures grown under a great variety of physiological conditions (4).

Genetic experiments described in this paper strongly support the regulatory nature of the mutation that enables the utilization of GABA-nitrogen for growth (*gabC*). First, mapping experiments showed that the *gabC1* mutation, which resulted in a severalfold increase in the activities of GSST and SSDH, is located at a great distance (at least 15 min) on the *E. coli* K-12 chromosome from the structural gene of GSST, *gabT* (Fig. 5). Second, the GABA-nitrogen utilizing mutants, CS101B and CS8B, grew rapidly on GABA and produced high levels of GSST and SSDH at 37 but not at 42 C (Tables 5 and 6). However, other GABA-utilizing mutants grew equally well and synthesized the GABA-pathway enzymes at high rates both at 37 and 42 C (Table 7). One mutant of the latter class, AN4 (*gabC4*), was genetically analyzed, and the mutation was found to occur at, or very close to, the *gabC1* locus. These data can be explained on the assumption that the *gabC* locus determines the synthesis of a protein that is required for the expression of the structural genes of the GABA system. The protein made by wild-type *E. coli* is inactive, and the activity of the protein in strains carrrying the *gabC1* and *gabC51* mutations is temperature-sensitive, whereas the protein of other *gabC* mutants (e.g., *gabC4*) is stable at both temperatures, 37 and 42 C.

Comparison of the behavior of *gabC* recombinants obtained by transduction and conjugation suggests the involvement of yet another genetic element, located between the point of origin of HfrC and gene *gabC*, in the regulation of the GABA pathway. This is indicated by our finding that in mating experiments recombinant colonies appeared on GABA plates after 2 days of incubation, whereas it took 4 days for GABA-utilizing transductants to appear. Transductants tested for GSST and SSDH showed lower enzyme activities than did GABA-utilizing exconjugant colonies picked after 2 days of incubation (Table 4). If an additional element were indeed involved in maximal expression of the GABA-structural genes, by picking the fast growing colonies in mating experiments we

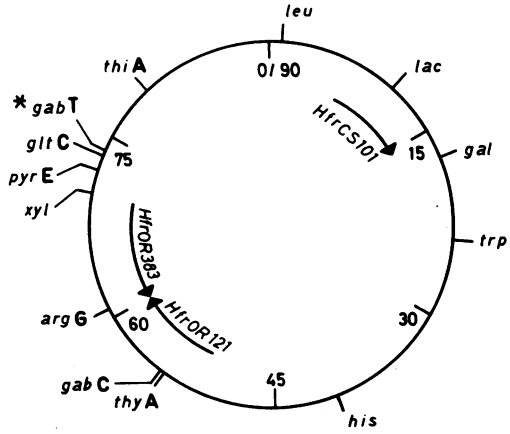

FIG. 5. *Escherichia coli K-12 chromosome drawn to scale according to Taylor and Trotter (14), showing the positions of the gabC and gabT loci, based on the results reported here.*

recovered only those recombinants that also integrated this hypothetical locus and selected against all those in which the two loci were separated by a recombinational event. This possibility would explain the higher enzyme activities in recombinants obtained by mating than those in transductants in which the hypothetical auxiliary locus was missing.

The high degree of coordination in the synthesis of the two enzymes of the GABA pathway (4) seemed to indicate that the two structural genes of the GABA system belong to the same operon. To test this assumption, we set out to isolate mutants defective in the structural genes of the GABA system and map them in relation to each other and to the control gene. GSST-less mutants were indeed readily isolated, mapped as described in the text, and found not to be linked to the control locus, *gabC*. However, in spite of repeated efforts we did not succeed in isolating any mutants altered in the gene coding for SSDH. Our failure to select mutants lacking SSDH activity was most probably due to the lethal effect of such a mutation in GABA-containing media. Such mutants would accumulate SSA which is toxic to the bacteria (Table 8). However, our attempts to select mutants with altered SSDH from SSA-resistant strains isolated for this purpose were also unsuccessful. Neither did we find among the SSA-resistant strains any mutant with greatly increased SSDH activity, which could be directly used for mapping the SSDH locus.

As mentioned in an earlier work (4), the synthesis of the GABA pathway enzymes in *E. coli* K-12, which is under the control of catabolite repression, becomes derepressed under conditions of nitrogen limitation. In the present study two independent mutants have been isolated in which the synthesis of SSDH *only is* derepressed in glucose media under conditions of nitrogen limitation (Table 9). The synthesis of GSST in these mutants is repressed in glucose media even under conditions of nitrogen limitation, but in succinate media high levels of GSST activities are obtained. These mutants may be altered in the promoter locus (12, 13) of the GSST gene. Our finding that the mutation did not affect the ability of SSDH to escape from catabolite repression perhaps indicates that the transcription of the two structural genes is initiated at two separate promoters. This would then argue against the two GABA-enzymes constituting a single operon.

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