JOURNAL OF BACTERIOLOGY, Apr., 1967, p. 1409-1415
Vol. 93, No. 4

Genetic Analysis of Glutamate Transport and Glutamate Decarboxylase in *Escherichia coli*[1]

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Received for publication 28 November 1966

The location of the *Escherichia coli* K-12 genes determining or regulating glutamate transport, and the location of the gene determining glutamate decarboxylase synthesis, were established by conjugation. The ability to grow on glutamate as the sole source of carbon and energy was used to select for glutamate transport recombinants. Two genes determining the ability to grow on glutamate as the sole source of carbon and energy were mapped. One (*gltC*) is located near *mtl* (mannitol), and the other (*gltH*) appears to be located between the *gal* (galactose) and *trp* (tryptophan) loci. The glutamate decarboxylase gene (*gad*) is strongly linked to *gltC*. The *gltC*$^+$ recombinants grow on glutamate much faster and accumulate this amino acid to a greater extent than do the *gltH*$^+$ recombinants. The *gltH*$^+$ gene functioned only in one female strain (P678), whereas the *gltC* gene functioned in all the female strains tested (P678, C600, W1).

Most wild-type strains of *Escherichia coli* K-12, *E. coli* W, and *E. coli* H (Harvard strain) do not grow on glutamate as the sole source of carbon and energy. Glutamate-utilizing mutants (Glut$^+$) were isolated from these strains (6, 7).

In previous papers from this laboratory (5–7), evidence was presented showing that a permeability barrier is responsible for the inability of the wild-type strains to grow on glutamate. It was also found (4, 7) that, in *E. coli* H, the ability to form the enzyme glutamate decarboxylase (GAD) in cultures grown on different carbon sources, in the presence and in the absence of glutamate, differed from that of glutamate-utilizing mutants derived from these strains.

A better understanding of the functions of glutamate permease and GAD, and their relationships in *E. coli*, requires genetic analysis of these two traits.

The purpose of this work was to determine the number and location of genes affected by mutations enabling growth on glutamate, and the location of the GAD gene.

MATERIALS AND METHODS

Microorganisms. The strains used throughout this work are listed in Table 1. All of them are derivatives of *E. coli* K-12.

[1] Part of this work was taken from the Ph.D. thesis to be submitted by M. M. to the Senate of the Hebrew University.

Growth media. The liquid minimal medium of Davis and Mingioli (1), from which citrate was omitted, was routinely used for growing the bacteria. The solid minimal medium of Tatum and Lederberg (12) from which the trace-element supplements were omitted, was used to select recombinants. This medium was solidified by addition of 2% agar (Difco) and supplemented with a carbon source, as required (glucose, 0.2%; glutamate, lactose, mannitol, or xylose, 0.5%), amino acids [threonine, leucine, and methionine, where required; 25 μg (per ml) of the L isomer, except where otherwise specified], and thiamine hydrochloride (where required, 1 μg/ml). Eosin-methylene blue (EMB)-mannitol and EMB-xylose were sometimes used to detect recombinants for mannitol and xylose. The EMB medium was prepared as described by Luria et al. (11). Mating broth, containing Casamino Acids and yeast extract, was that described by Hayes (8).

Tryptose Blood Agar Base, Difco (TBA) was used to maintain stocks of strains on slants or petri dishes.

Growth experiments. Growth experiments were performed as described by Halpern and Lupo (6).

Uptake of glutamate-^{14}C. Glutamate uptake was studied by the method of Kessel and Lubin (10), as described by Halpern and Lupo (6).

Selection of Glut$^+$ mutants. Cultures in the logarithmic phase in minimal medium were harvested by centrifugation, suspended in saline to a density of 10^9 cells per milliliter, and irradiated with ultraviolet light to a survival of 10^{-3} to 10^{-4}. Several 5-ml samples from each irradiated culture were transferred to test tubes containing 5 ml of double-strength broth and incubated in the dark for 2 hr at 37 C and then over-

TABLE 1. *Strains used*

Designation	Mating type	Glutamate utilization	Genotype[a]
Hfr CS101	Hfr	−	*met*$^-$ *gltC*$^-$ *gad*$^+$ *str-s* (λ)$^+$
CS1	Hfr	+	*met*$^-$ *gltC1 gad*$^+$ *str-s* (λ)$^+$
CS5	Hfr	+	*met*$^-$ *gltC5 gad*$^+$ *str-s* (λ)$^+$
CS7	Hfr	+	*met*$^-$ *gltC7 gad*$^+$ *str-s* (λ)$^+$
CS8	Hfr	+	*met*$^-$ *gltC8 gad*$^+$ *str-s* (λ)$^+$
Hfr H	Hfr	−	*gltH*$^+$ *thi*$^-$ *gltC*$^-$ *gad*$^-$ *str-s* (λ)$^-$
H1	Hfr	+	*gltH*$^+$ *thi*$^-$ *gltC51 gad*$^-$ *str-s* (λ)$^-$
H5	Hfr	+	*gltH*$^+$ *thi*$^-$ *gltC55 gad*$^-$ *str-s* (λ)$^-$
H6	Hfr	+	*gltH*$^+$ *thi*$^-$ *gltC56 gad*$^-$ *str-s* (λ)$^-$
H7	Hfr	+	*gltH*$^+$ *thi*$^-$ *gltC57 gad*$^-$ *str-s* (λ)$^-$
P678	F$^-$	−	*thr*$^-$ *leu*$^-$ *thi*$^-$ *gltC*$^-$ *gad*$^-$ *mtl*$^-$ *xyl*$^-$ *mal*$^-$ *gltH*$^-$ *lac*$^-$ *str-r lam-r* (λ)$^-$
C600	F$^-$	−	*thr*$^-$ *leu*$^-$ *thi*$^-$ *gltC*$^-$ *gad*$^-$ *lac*$^-$ *str-r* (λ)$^-$
W1	F$^-$	−	*thr*$^-$ *leu*$^-$ *met*$^-$ *gltC*$^-$ *gad*$^-$ *str-r* (λ)$^+$

[a] The genetic nomenclature is based on the proposal of Demerec et al. (2). Genotype symbols: *leu*, *met*, *thi*, *thr*, genes determining and regulating the synthesis of leucine, methionine, thiamine, and threonine, respectively (+ and − indicate the ability and inability to synthesize the specific amino acid or vitamin, respectively); *lac*, *mal*, *mtl*, *xyl*, genes determining and regulating the utilization of lactose, maltose, mannitol, and xylose, respectively (+ and − indicate ability and inability to utilize the given substrate, respectively); *gltC*, gene determining or regulating glutamate permation; *gltC*$^-$, wild-type allele resulting in Glut$^-$ phenotype; *gltC1*, *gltC5*, *gltC7*, *gltC8*, *gltC51*, *gltC55*, *gltC56*, *gltC57*, mutant alleles resulting in Glut$^+$ phenotype; *gltH*$^+$, gene resulting in Glut$^+$ phenotype when introduced to strain P678 (*gltC*$^-$); its allele *gltH*$^-$ results in Glut$^-$ phenotype of wild-type strain P678 (*gltC*$^-$); *gad*, gene determining glutamate decarboxylase synthesis (+ and − indicate the ability to form high (1,000 to 5,000 μliters of CO_2 per mg of protein per hr) and low (0 to 400) levels of decarboxylase activity, respectively); *str*, genes determining response to streptomycin (*s* and *r* indicate susceptibility and resistance, respectively); *lam*, genes determining response to phage λ (*r* indicates resistance); (λ), lysogenicity for phage λ (+ and − indicate lysogenic and nonlysogenic, respectively).

night, for phenotypic expression, in a shaking water bath at 37 C. A 5-ml sample from each test tube was centrifuged, washed, and suspended to a final density of 2×10^7 cells per milliliter in 100-ml conical flasks containing 40 ml of minimal medium supplemented with 0.5% L-glutamate (sodium salt) and the required nutrients. [Hfr CS101 was supplemented with 25 μg (per ml) of L-methionine, and Hfr H with 1 μg (per ml) of thiamine hydrochloride.] The flasks were incubated with shaking at 37 C, until turbidity developed. Glut$^+$ mutants were isolated and purified by streaking twice on solid glutamate medium, supplemented with the requirements of the parent strains. One mutant from each flask was kept for further analysis. Thus, the different mutants represent independent mutational events.

Mating experiments. Male and female strains were maintained on TBA slants and checked for their genetic markers a few days prior to their use in crosses. Single colonies of the parent strains were inoculated into test tubes containing 2 ml of broth and incubated overnight without shaking at 37 C. In the morning, the female strains were diluted in warm broth to 10^8 cells per milliliter, and the male strains to 5×10^7; 5 ml of the diluted male suspension and 10 ml of the diluted female suspension were incubated separately in 100-ml conical flasks. The male bacteria were incubated without shaking and the female bacteria, with shaking at 37 C. The recipient culture was grown logarithmically to a density of approximately 5×10^8 viable cells per milliliter and the donor culture, to approximately 2×10^8. Mating was started by mixing the parental strains in appropriate volume ratios to give final cell densities of 2×10^7 male cells and 4×10^8 female cells per milliliter. The mating was performed in a volume of 5 ml, in 100-ml conical flasks. The flasks were incubated in a water bath at 37 C without shaking. Samples were withdrawn at different times and diluted into phosphate buffer containing 200 μg of streptomycin per ml. The diluted samples were then vibrated for 60 sec on a Super Mixer (Lab-Line Instruments, Inc., Melrose Park, Ill.) at full speed, to interrupt conjugation; 0.1-ml amounts of the vibrated samples, in desired dilutions, were plated on selective media containing 200 μg of streptomycin per ml, to kill the male parent strains, and were incubated at 37 C.

Isolation of recombinants and scoring for unselected markers. Recombinants were isolated by streaking single colonies twice on the appropriate selective media. Unselected markers were scored by the replica method.

Assay of GAD. Cells from fresh TBA slants were inoculated into 40 ml of minimal medium adjusted to *p*H 6.8 and supplemented with 0.25% glucose and growth requirements (40 μg of the L isomers of the required amino acids and 1 μg (per ml) of thiamine hydrochloride), in 100-ml conical flasks. The cultures

were incubated overnight in a New Brunswick reciprocal shaking water bath (120 strokes/min) at 37 C. The bacteria were harvested in the stationary phase (optical density approximately 600 Klett units, filter no. 42; final *p*H of culture, 6.3) and washed in saline by centrifugation. The pellets were resuspended in saline, and 0.01 ml of toluene and 0.01 ml of a 0.5% solution of sodium deoxycholate (per ml of cell suspension) were added. The suspension was vibrated for a few seconds on the Super Mixer and incubated for 15 min at 37 C in a shaking water bath. Enzyme activity was measured manometrically in a Warburg apparatus. The reaction mixture contained (in 2 ml): glutamic acid, *p*H 4.9, 0.06 M; citrate-phosphate buffer, *p*H 4.9 (0.0187 M citric acid and 0.0375 M Na_2HPO_4); and toluene-treated cells (equivalent to 0.35 mg of protein).

RESULTS

Growth of wild-type and mutant strains on glutamate. The growth rates of the wild-type male and female strains and of the Glut$^+$ mutants are given in Table 2. The five wild-type strains grew extremely slowly, if at all, on glutamate as the sole source of carbon and energy. Of 12 Glut$^+$ mutants of Hfr CS101, 7 were tested; they grew on glutamate with a generation time of 1.5 to 3.25 hr. (The growth rates of two mutants of this group, CS7 and CS8, are shown in Table 2.) All four Glut$^+$ mutants isolated from Hfr H grew on glutamate with a generation time of 2.4 to 2.75 hr. (The growth rate of mutant H1 is given in Table 2.)

Mapping of the genes by interrupted mating. Preliminary mating experiments showed that it was possible to obtain Glut$^+$ recombinants, with either Hfr Hayes Glut$^+$ mutants or Hfr Cavalli Glut$^+$ mutants as donors. The results of interrupted-mating experiments with Hfr CS8 and Hfr H1 as donors and P678 as the recipient are shown in Fig. 1 and Table 3. These results indicate the presence of two loci determining the ability to grow on glutamate. One locus is situated near the *mtl* locus (*gltC*); the other (*gltH*) would appear to be located between the *gal* and *trp* loci, on the basis of entry times of approximately 25 min for *gal* and approximately 35 min for *trp*, as shown by Taylor and Thoman (13). As shown in Fig. 1A, the Hfr CS8 × P678 cross gave upward-breaking time curves of recombinant formation. This anomaly has been described by Taylor and Thoman (13), who showed that the initial slopes, when extrapolated to intersect with the abscissa, give the true map distances. The lower number of *gltC*$^+$ recombinants, as compared with the *mtl*$^+$ and *xyl*$^+$ recombinants, was due to the use of minimal glutamate medium to select for *gltC*$^+$ recombinants, whereas *mtl*$^+$ and *xyl*$^+$ recombinants were detected on a rich medium (EMB-mannitol-agar and EMB-xylose-agar, respectively). Selection on a minimal medium interferes with integration and reduces the number of recombinants (3). In experiments in which *mtl*$^+$ and *xyl*$^+$ recombinants were selected on minimal medium, the numbers of recombinants for these two traits were somewhat lower than the number of *gltC*$^+$ recombinants obtained in the same cross.

Similar mating experiments were performed with additional Glut$^+$ mutants, CS1, CS5, CS7, H5, H6, and H7, which served as donors, and two additional Glut$^-$ female strains, C600 and W1, which served as recipients. Hfr CS101 did not form Glut$^+$ recombinants with any of the three female strains used, whereas all of the Glut$^+$ mutants derived from Hfr CS101 formed Glut$^+$ recombinants with these recipients (Table 3). The time of entry of the *glt* locus was the same (approximately 50 min) in all the crosses, corresponding to that of *gltC*. On the other hand, the wild-type Glut$^-$ strain, Hfr H, did form Glut$^+$ recombinants when P678 was the recipient and the time of entry of the *glt* gene was 28 min, corresponding to that of *gltH*. The Glut$^+$ mutants of Hfr H, H5, H6, and H7 gave rise to

TABLE 2. *Growth rates of Escherichia coli K-12 wild-type and mutant strains on glutamate as the source of carbon and energy*

Strain	Doubling time on 0.5% glutamate
	hr
F$^-$ P678	>24.0
F$^-$ C600	22.0
F$^-$ W1	17.0
Hfr CS101	>24.0
Hfr H	>24.0
Hfr CS7	2.3
Hfr CS8	1.8
Hfr H1	2.4

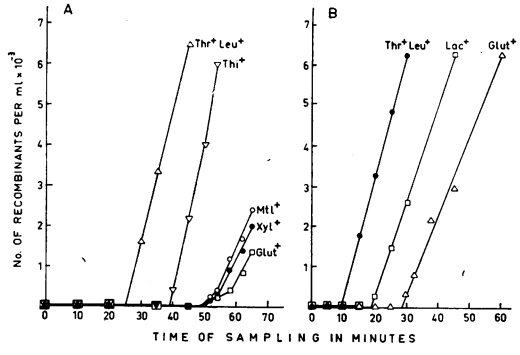

FIG. 1. *Kinetics of chromosome transfer to strain P678. Donor strains: Hfr CS8-A, Hfr H1-B.*

TABLE 3. *Formation of Glut$^+$ recombinants in Escherichia coli K-12 by conjugation*

Mating pair	Glutamate-utilizing ability		Formation of Glut$^+$ recombinants	Time of entry of *glt*$^+$ gene by conjugation
	Donor	Recipient		
				min
Hfr CS101 × P678	−	−	−	—
Hfr CS1 × P678	+	−	+	approx 50
Hfr CS5 × P678	+	−	+	approx 50
Hfr CS7 × P678	+	−	+	50
Hfr CS8 × P678	+	−	+	50
Hfr CS101 × C600	−	−	−	—
Hfr CS8 × C600	+	−	+	approx 50
Hfr CS101 × W1	−	−	−	—
Hfr CS8 × W1	+	−	+	approx 50
Hfr H × P678	−	−	+	28
Hfr H1 × P678	+	−	+	28
Hfr H5 × P678	+	−	+	approx 28
Hfr H6 × P678	+	−	+	approx 28
Hfr H7 × P678	+	−	+	approx 28
Hfr H × C600	−	−	−	—
Hfr H1 × C600	+	−	+	approx 120
Hfr H × W1	−	−	−	—
Hfr H1 × W1	+	−	+	approx 120

TABLE 4. *Growth rates of Glut$^+$ recombinant strains of Escherichia coli K-12 on glutamate as the sole source of carbon and energy*

Mating	Time of entry of the *glt*$^+$ gene	Doubling time of recombinants on 0.5% glutamate
	min	*hr*
Hfr CS101 × P678	—	No Glut$^+$ recombinants obtained
Hfr CS7 × P678	approx 50	3.7–5.2 (7)[a]
Hfr CS8 × P678	50	3.4–5.5 (8)
Hfr H × P678	28	7.8–8.2 (6)
Hfr H1 × P678[b]	28	7.8–8.2 (6)
Hfr H1 × P678[b]	approx 120	3.0–3.5 (4)
Hfr CS101 × C600	—	No Glut$^+$ recombinants obtained
Hfr CS8 × C600	approx 50	2.1–3.3 (4)
Hfr H × C600	—	No Glut$^+$ recombinants obtained
Hfr H1 × C600	approx 120	2.1–3.2 (4)

[a] Numbers in parentheses designate the number of recombinants tested.

[b] The six recombinants that grew on glutamate with a generation time of approximately 8 hr were selected after 60 min of conjugation. The four recombinants with a generation time of 3.0 to 3.5 hr were selected after 140 min of conjugation. These four recombinants also inherited the *mtl*$^+$ and *xyl*$^+$ genes.

Glut$^+$ recombinants, with the same time of entry as Hfr H and H1, when P678 served as the recipient. With C600 and W1 as recipients, Hfr H did not form any Glut$^+$ recombinants; its Glut$^+$ mutant, Hfr H1, formed Glut$^+$ recombinants only after prolonged conjugation (more than 2 hr).

Growth of Glut$^+$ recombinants on glutamate. The growth rates of Glut$^+$ recombinants obtained in different crosses, on glutamate, are shown in Table 4. The recombinants from crosses in which P678 served as the recipient fall into two distinct classes: those with a generation time of approximately 3.4 to 5.5 hr and those with a generation time of approximately 8 hr. The time of entry of the *glt* genes involved in the formation of the two classes of recombinants indicates that the first class inherited the *gltC* gene, situated near the *mtl* gene, whereas the second class received the *gltH* gene, located between the *gal* and *trp* loci (Fig. 3). Glut$^+$ recombinants from crosses in which C600 served as the recipient grew on glutamate with a generation time of 2.1 to 3.3 hr. The time of entry of the *glt* gene in these crosses indicates that the gene transmitted is *gltC* (Fig. 3). The faster growth of these recombinants on glutamate may be due to genetic differences between C600 and P678.

Comparison of glutamate accumulation by re-

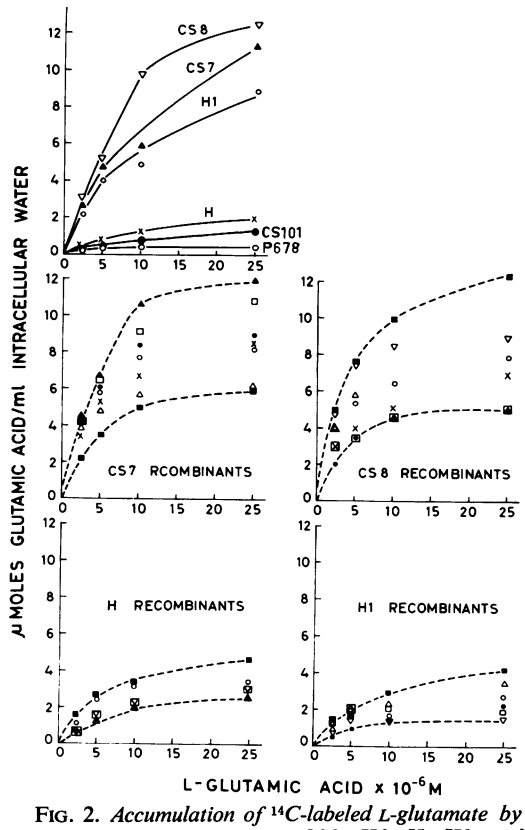

FIG. 2. *Accumulation of ^{14}C-labeled L-glutamate by parent strains, CS101, CS7, CS8, Hfr H, H1, and P678, and by Glut$^+$ recombinants. The Glut$^+$ recombinants tested were isolated from the following crosses: CS7 × P678 (seven recombinants); CS8 × P678 (seven recombinants); Hfr H × P678 (six recombinants); Hfr H1 × P678 (six recombinants). The cultures were grown on 1% succinate as the sole source of carbon and energy. Accumulation experiments were performed in the presence of succinate (1%) as the energy source. The area defined by the broken line gives the range of glutamate accumulation by the different recombinants from each cross tested. Each recombinant is represented by a different symbol.*

combinant and parent strains. Figure 2 shows the accumulation of ^{14}C-labeled L-glutamate by Hfr CS101, Hfr H, Hfr H1, Hfr CS7, Hfr CS8, and P678, and by Glut$^+$ recombinants obtained in crosses between the above donors and P678. Logarithmic-phase cultures in which protein synthesis was arrested by the addition of chloramphenicol (6, 10) were used. All the strains tested accumulated glutamate against a concentration gradient, the intracellular concentration of glutamate at equilibrium in the Glut$^+$ mutants (CS7, CS8, and H1) being 5- to 30-fold higher than that of the parent strains (Hfr CS101, Hfr H, and P678). The accumulation of glutamate by Glut$^+$ recombinants obtained from the crosses CS7 × P678 and CS8 × P678 was approximately equal to that of the donor strains. The capacity of the different recombinants for glutamate accumulation correlated nicely with the respective rates of growth of these recombinants on glutamate. The Glut$^+$ recombinants obtained by crossing Hfr H and Hfr H1 with P678 accumulated, at equilibrium, 4 to 12 times more glutamate than did P678, and most of them also accumulated more than did Hfr H. However, all of these recombinants showed much lower (one-half to one-sixth) glutamate accumulation than that of Hfr H1. The relatively low capacity for glutamate accumulation of the "H" and "H1" recombinants correlates well with the longer generation time (approximately 8 hr; Table 3) of these strains on glutamate.

Mapping of the gltC gene by recombination analysis. The results of interrupted matings with Hfr CS8 as donor and P678 as recipient (Fig. 1) show that the *gltC* gene is very close to the *mtl* gene. Although these results indicate that the *gltC* gene enters before the *mtl* gene, this evidence is not conclusive, since the method of interrupted mating is not accurate enough for ordering the sequence of very close genes (9). Unselected-marker-frequency analysis was therefore performed (Table 5). The observed difference in linkage between each pair of markers, depending on which of the two markers was selected for, is a consequence of the gradient of transmission (9). Consequently, the order of the genes indicated by these results is: *gltC, mtl, xyl.*

Further evidence for this sequence was obtained by three-factor-cross analysis. Hfr CS8 was mated with P678, and *xyl*$^+$ recombinants were selected and partly purified by transfer of single colonies to the same selective medium; *mtl*$^+$ and *gltC*$^+$ recombinants were scored by the replica method. The results (Table 6) confirm the order of genes inferred from the unselected-marker-frequency analysis.

TABLE 5. *Unselected-marker-frequency analysis by conjugation of the linkage relationships among gltC, mtl, and xyl in Escherichia coli K-12*[a]

Selected marker	No. of recombinants	Unselected marker	No. of recombinants	Linkage
				%
gltC$^+$	298	*mtl*$^+$	226	75
gltC$^+$	298	*xyl*$^+$	179	60
mtl$^+$	136	*gltC*$^+$	126	93
mtl$^+$	136	*xyl*$^+$	105	77
xyl$^+$	182	*gltC*$^+$	149	82
xyl$^+$	182	*mtl*$^+$	159	87

[a] Hfr CS8 and F$^-$ P678 served as donor and recipient, respectively.

Mapping of the gad gene. The activity of GAD in the male and female strains is shown in Table 7. The great differences in GAD activity between the donors and the recipient make possible the recognition of inheritance of the donor *gad* gene by the recombinants. Preliminary mating experiments showed that *gltC*$^+$ recombinants obtained in a cross between Hfr CS8 and P678 sometimes inherited the *gad* gene of the male. Since no method was available for the selection of *gad*$^+$ recombinants, mapping of *gad* had to be done by selecting for other markers and scoring *gad* as an unselected marker. Interrupted-mating experiments with Hfr CS8 and Hfr CS7 as donors and P678 as the recipient were performed (Table 8); *gad* is most probably located in the vicinity of the *mtl* and *gltC* genes (Fig. 3).

TABLE 6. *Three-factor-cross analysis of gltC in Escherichia coli K-12 by conjugation*[a]

Selected marker	Unselected markers	No. of recombinants	Percentage of recombinants
xyl$^+$	*mtl*$^+$ and *gltC*$^+$	147	80.8
xyl$^+$	*mtl*$^+$ and *gltC*$^-$	12	6.6
xyl$^+$	*mtl*$^-$ and *gltC*$^-$	21	11.5
xyl$^+$	*mtl*$^-$ and *gltC*$^+$	2	1.1

[a] Hfr CS8 and F$^-$ P678 served as donor and recipient, respectively.

TABLE 7. *Glutamate decarboxylase (GAD) activity of Escherichia coli K-12*

Strain	GAD activity[a]
Hfr CS101	1,800
Hfr CS7	1,800
Hfr CS8	1,000
F$^-$ P678	100–400

[a] Expressed as microliters of CO_2 per milligram of protein per hour.

DISCUSSION

The results clearly show that *gltC* of *E. coli* K-12 is located near *mtl*. Hfr Cavalli Glut$^+$ mutants, CS1, CS5, CS7, and CS8, transferred *gltC1*, *gltC5*, *gltC7*, and *gltC8*, respectively, after 50 min of conjugation, whereas the Hfr Hayes Glut$^+$ mutant H1 transferred *gltC51* after about 2 hr of conjugation. The *gltC* allele of Hfr CS101 (Glut$^-$), was also mapped near the *mtl* gene, by conjugation with a Glut$^+$ derivative of P678 (*unpublished data*).

Conjugation experiments with Hfr H Glut$^+$ mutants and P678 led to the discovery of an additional locus, *gltH*, enabling recombinants to grow on glutamate. By its time of entry, this gene

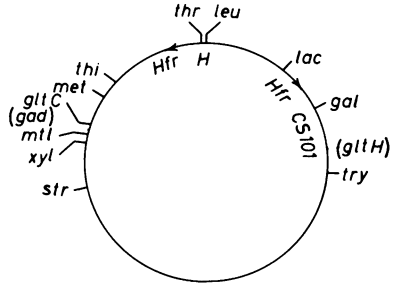

FIG. 3. *Escherichia coli chromosome drawn to scale according to Taylor and Thoman (13), showing the positions of gltC, gltH, and gad.*

TABLE 8. *Mapping of gad (glutamate decarboxylase) in Escherichia coli K-12 by interrupted mating and linkage analysis*[a]

Donor strain	Recipient strain	Selected markers	Genotype of recombinants[b]	No. of recombinants tested	No. of *gad*$^+$ recombinants	GAD activity of *gad*$^+$ recombinants[c]
Hfr CS8	F$^-$ P678	*thr*$^+$ *leu*$^+$	(*thr*$^+$ *leu*$^+$) *thi*$^-$ *met*$^+$ *gltC*$^-$ *mtl*$^-$ *xyl*$^-$	10	0	—
Hfr CS8	F$^-$ P678	*thi*$^+$ *met*$^-$	(*thi*$^+$ *met*$^-$) *gltC*$^-$ *mtl*$^-$ *xyl*$^-$	10	0	—
Hfr CS8	F$^-$ P678	*gltC*$^+$	Not analyzed	60	53	1,000–3,000
Hfr CS7	F$^-$ P678[d]	*gltC*$^+$	(*gltC*$^+$) *mtl*$^-$ *xyl*$^-$	37	30	1,000–5,000
Hfr CS7	F$^-$ P678[d]	*gltC*$^+$ *mtl*$^+$	(*gltC*$^+$ *mtl*$^+$) *xyl*$^-$	40	39	1,600–5,000

[a] The matings were interrupted 2 to 3 min after the beginning of entry of the latest selected marker. The genotypes of the isolated recombinants were determined by the replica method.

[b] Gene symbols within parentheses indicate that the genes were contributed by the donor, with the remaining genes being contributed by the recipient.

[c] Expressed as microliters of CO_2 per milligram of protein per hour; *gad*$^-$ recombinants ranged from 0 to 400.

[d] A revertant of P678 not requiring threonine and leucine.

appears to be located between the *gal* and *trp* loci (Fig. 3). Glut$^+$ recombinants at the *gltH* locus were also obtained when Hfr H, the original wild-type Glut$^-$ strain, served as the donor. The ability of Hfr H to form Glut$^+$ recombinants with P678, and our finding that Hfr H Glut$^+$ mutant H1 transmitted also the *gltC*$^+$ gene (Table 3), show that the mutated *glt* locus in the Hfr H mutant was *gltC* as in the Hfr CS101 mutants, and not the *gltH* locus.

All the *gltH*$^+$ recombinants exhibited lower rates of growth on glutamate and accumulated less ^{14}C-labeled L-glutamate than the *gltC*$^+$ mutants and recombinants (Table 4 and Fig. 2).

Another point relevant to the nature of the *gltH* gene is our finding that *gltH*$^+$ recombinants are obtained only in crosses in which P678 serves as the recipient. No *gltH*$^+$ recombinants were obtained with the recipients C600 and W1. These data may perhaps indicate that *gltH* is a suppressor gene. However, alternative explanations for the behavior of *gltH* have not been excluded.

The *gltC* gene is either the structural gene of glutamate permease or a regulatory gene controlling the expression of the structural gene. Our data do not allow differentiation between these two possibilities. However, at present we prefer the notion that *gltC* is a regulatory gene for the following reasons. (i) All the wild-type strains tested accumulated glutamate against a concentration gradient, albeit to a lower extent than did the glutamate-utilizing mutants, and could not satisfy the cell's requirements for carbon and energy for growth. (ii) The affinity of the permease for glutamate in the wild-type (*gltC*$^-$) and mutant (*gltC*$^+$) strains was practically the same, 10.0×10^{-6} and 7.7×10^{-6} M, respectively (5, 6).

We therefore believe that glutamate permease synthesis in *E. coli* is under genetic control, being partly repressed in the wild-type and derepressed in the *gltC*$^+$ mutants.

In earlier work (7), it has been observed that mutation enabling growth of *E. coli* W and *E. coli* H (Harvard strain) on glutamate was accompanied by partial or complete loss of the capacity for synthesizing GAD. We hope that the close linkage between *gltC* and *gad* in *E. coli* K-12 described here (Fig. 3) will facilitate the study of the regulation of GAD and its relationship to glutamate transport. It is of interest that most of the *gad*$^+$ recombinants isolated from crosses between *gad*$^+$ donors and *gad*$^-$ recipients had much higher GAD activities than the *gad*$^+$ donor strains (Tables 7 and 8). The reason for this is not clear. Studies on the mechanism of regulation of *gad* and on the nature of the *glt* genes are now in progress.

ACKNOWLEDGMENTS

We express thanks to W. Hayes for some of the strains used in this work.

This investigation was supported by Public Health Service grant GM-10044 from the Division of General Medical Sciences.

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