EVIDENCE FOR THE INVOLVEMENT OF SERINE TRANSHYDROXYMETHYLASE IN SERINE AND GLYCINE INTERCONVERSIONS IN *SALMONELLA TYPHIMURIUM*

GEORGE V. STAUFFER AND JEAN E. BRENCHLEY

Department of Microbiology, The Pennsylvania State University, University Park, Pennsylvania 16802

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ABSTRACT

Salmonella typhimurium can normally use glycine as a serine source to support the growth of serine auxotrophs. This reaction was presumed to occur by the reversible activity of the enzyme, serine transhydroxymethylase (E. C. 2. 1. 2. 1; L-serine: tetrahydrofolic-5, 10 transhydroxymethylase), which is responsible for glycine biosynthesis. However, this enzyme had not been demonstrated to be solely capable of synthesizing serine from glycine *in vivo*. The isolation and characterization of a mutant able to convert serine to glycine but unable to convert glycine to serine supports the conclusion that a single enzyme is involved in this reversible interconversion of serine and glycine. The mutation conferring this phenotype was mapped with other mutations affecting serine transhydroxymethylase (*glyA*) and assays demonstrated reduced activities of this enzyme in the mutant.

SERINE and glycine biosynthesis proceed by the following reactions from 3-phosphoglycerate (UMBARGER and UMBARGER 1962; UMBARGER, UMBARGER and SIU 1963; PIZER 1963; SCRIMGEOUR and HUENNEKENS 1962):

1. 3-phosphoglycerate + $NAD^+ \overset{serA}{\rightleftharpoons}$ 3-phosphohydroxypyruvate + NADH + H^+
2. 3-phosphohydroxypyruvate + glutamate $\overset{serC}{\rightleftharpoons}$ 3-phosphoserine + α-ketoglutarate
3. 3-phosphoserine $\overset{serB}{\rightarrow}$ serine + Pi
4. serine + tetrahydrofolate $\overset{glyA}{\rightleftharpoons}$ glycine + 5, 10-methylenetetrahydrofolate.

The fourth reaction not only produces glycine from serine during biosynthesis, but also forms 5, 10-methylenetrahydrofolate which is involved in the synthesis of the majority of one-carbon fragments (MUDD and CANTONI 1964). Some other reaction capable of converting glycine to serine can also occur since glycine can support the growth of serine auxotrophs. Serine transhydroxymethylase has been assumed to be responsible for both of these reactions since *in vitro* assays demonstrate that this enzyme activity is reversible and can catalyze the interconversion of serine and glycine when the appropriate substrates are available (SCRIMGEOUR

and HUENNEKENS 1962; TAYLOR and WEISSBACK 1965). However, the possibility remained that other enzymes might be involved in these reactions since glycine auxotrophs, lacking the serine transhydroxymethylase activity, had not been tested for their ability to synthesize serine from exogenous glycine.

The phenotype of a mutant isolated from a serine-auxotroph of *Salmonella typhimurium* suggested either the existence of another route for the synthesis of serine from glycine, or an alteration in serine transhydroxymethylase which impairs only that function. This mutant still requires serine, but is now incapable of using exogenous glycine as a serine source. Thus, it can synthesize enough glycine *from* serine to allow growth, but can no longer convert sufficient glycine *to* serine. Characterization of this and other glycine-requiring mutants indicates that serine transhydroxymethylase alone is involved in these reversible reactions.

MATERIALS AND METHODS

Strains: Bacterial strains used are listed in Table 1.

Media: Both the glucose minimal medium and the Luria Broth complex medium were as described previously (BRENCHLEY 1973). Supplements were added at the following concentrations: amino acids and dipeptides, 200 μg/ml; purines and pyrimidines, 10 μg/ml; vitamins, 2 μg/ml.

Mutagenesis: Mutagenesis of strain JB507 (*serA13*) with N-methyl-N′-nitro-N-nitrosoguandine at 100 μg/ml was as described by ADELBERG, MANDEL and CHEN (1965). The penicillin counterselection was in glucose minimal medium plus 200 μg/ml of glycine and 20,000 units of penicillin G per ml. Surviving colonies were scored for their ability to grow on glucose minimal agar supplemented with either serine or glycine. One mutant capable of growth with serine but not glycine was isolated and is designated strain JB561.

Transductions: Transductions were performed either with the P22 (*int-4*) mutant, L4 (SMITH and LEVINE 1967), or KB1 phage (BORO and BRENCHLEY 1971). Normally, 0.1 ml of a phage lysate (10^{11} phage/ml) was used to infect 0.9 ml of a late-log or stationary-phase culture (about 5×10^9 cells/ml) of the recipient grown in Luria Broth. The cells and phage were immediately plated on the appropriate media for selection of transductants.

Growth: Cultures were grown overnight at 37° in minimal medium containing 0.02% glucose and the appropriate supplements. These cultures were used to inoculate fresh 0.4% glucose

TABLE 1

Bacterial strains

Strain	Genotype	Relevant phenotype	Source
JL781	+	Wild type	J. INGRAHAM
JL600	*strB57*	Resistant to low levels of streptomycin and requires niacinamide and thiamine	B. AMES
JB507	*serA13*	Requires L-serine or glycine	K. SANDERSON
JB561	*serA13, gly-951*	Requires L-serine, no longer grows on glycine	Mutagenesis
JB562	*serA13, strB57*	Requires L-serine or glycine. Other requirements as JL600	Transduction
JB563	*serA13, glyA10*	Requires L-serine and glycine	Transduction
JB564	*serA13, glyA20*	Requires L-serine and glycine	Transduction
JB565	*glyA10*	Requires glycine	K. SANDERSON
JB566	*glyA20*	Requires glycine	J. GOTS
JB567	*gly-951*	Partial requirements for glycine	Transduction

minimal medium with the appropriate supplements. The inoculum was added to give a reading of less than 10 Klett units in order to allow a number of generations of growth to occur before harvesting. Growth was monitored with a Klett-Summerson Spectrophotometer using a blue number 42 filter.

Cell extracts: A 50-ml volume of cells at a Klett reading of 100 ± 5 units was chilled on ice. The cells were harvested by centrifugation at 12,100 × *g* and washed 2 times in cold 0.85% NaCl. The cells were stored as frozen pellets and assays were performed within 24 hours. Under these conditions, the serine transhydroxymethylase was stable but started to lose activity during longer storage periods. Extracts were prepared by resuspending the pellets in 0.75 ml of a 1.0-M phosphate buffer (pH 7.5) and sonifying 3 times for 15 seconds with 30-second cooling intervals on a Bronwill Biosonik IV sonicator at a setting of 60% using a microprobe. Debris was removed by centrifugation at 17,300 × *g* for 20 minutes on a Sorvall RC2-B centrifuge. All procedures were carried out below 4°. Extracts were used immediately for enzyme assays.

Enzyme assays: Serine transhydroxymethylase activity was determined by the method of SCRIMGEOUR and HUENNEKENS (1962), except that the phosphate buffer was changed to 1.0 M and pH 7.5. A solution of formaldehyde was used to prepare a standard curve. Absorbancies were read on a Beckman DU-2 Spectrophotometer at 420 nm. A second procedure for determining serine transhydroxymethylase activity was that of TAYLOR and WEISSBACH (1965) that monitors the conversions of 3-^{14}C-L-serine to glycine.

Measurement of glycine transport: The method of COSLOY (1973) was followed with slight modification. A 50-ml volume of cells at a Klett reading of 100 ± 2 units was chilled on ice. The cells were harvested by centrifugation at 12,100 × *g*, washed once in cold 0.02% glucose minimal medium and resuspended in 5 ml of cold 0.4% glucose minimal medium. Chloramphenicol (300 μg/ml) was added and the cells were stored in the cold until ready for use (not longer than 1 hr). For measurement of glycine transport a 0.5-ml sample of cell suspension was warmed in 1.5 ml of 0.4% glucose minimal medium at 37° for 5 minutes. The assay was started by adding 0.5 ml of ^{14}C-glycine (specific activity, 0.5 μCi/μmol; 1 μmol/ml). Samples of 0.2 ml were withdrawn at appropriate intervals, rapidly diluted 1 to 10 in prewarmed 0.4% glucose minimal media, and immediately filtered on a millipore filter (0.45 μ, 25 mm). The filters were rapidly washed with 5 ml of prewarmed glucose minimal medium. The competition experiment contained a 100-fold excess of cold glycine. Samples of the cell suspension were taken for determining the wet weight of cells added to the reaction mixture prior to the addition of chloramphenicol.

The radioactivity on the filters was determined by placing the dry filters in 10 ml. of scintillation fluid; 100 ml absolute methanol, 7.0 g of 2,5-diphenyloxazole, 250 mg of p-bis [2-(5-phenyloxazolyl)]-benzene, 125 g of naphthalene, made to 1 liter in 1,4-dioxane. The samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3375.

Protein determination: Protein determinations were made by the method of LOWRY *et al.* (1951).

Chemicals: L-alanine, L-aspartate, L-glutamate, L-methionine, DL-serine, L-serine, glycine, glycyl-glycine, dl-L-tetrahydrofolate, pyridoxal-5′-phosphate, acetylacetone, glycyl-DL-serine, sodium glyoxylate, pyruvate, dimedon, chloramphenicol, niacinamide, thymine, adenine, and streptomycin were from Sigma Chemical Co. (St. Louis, Mo.). 2-Mercaptoethanol was from Eastman (Rochester, New York). Guanosine was from California Corporation for Biocehmical Research (Los Angeles, Calif.). Toluene, sodium formate, sodium citrate, glycerol, trichloroacetic acid and ammonium succinate were from Baker Chemical Co. (Phillipsburg, N.J.). Paraformaldehyde was obtained from Fisher Scientific Co. (Fair Lawn, N. J.). 3-^{14}C-L-serine and ^{14}C-glycine were from Schwarz/Mann (Orangeburg, New York). Naphthalene, 1,4-dioxane and sodium acetate were from Mallinckrodt Chemical Works (St. Louis, Mo.). 2,5-diphenyloxazole and p-bis [2-(5-phenyloxazolyl)]-benezene were from New England Nuclear (Boston, Mass.). All other chemicals were reagent grade and commercially available.

RESULTS

The mutant strain JB561 was isolated from a serine auxotroph and still requires serine but has lost the ability to grow on glycine (Figure 1). This phenotype

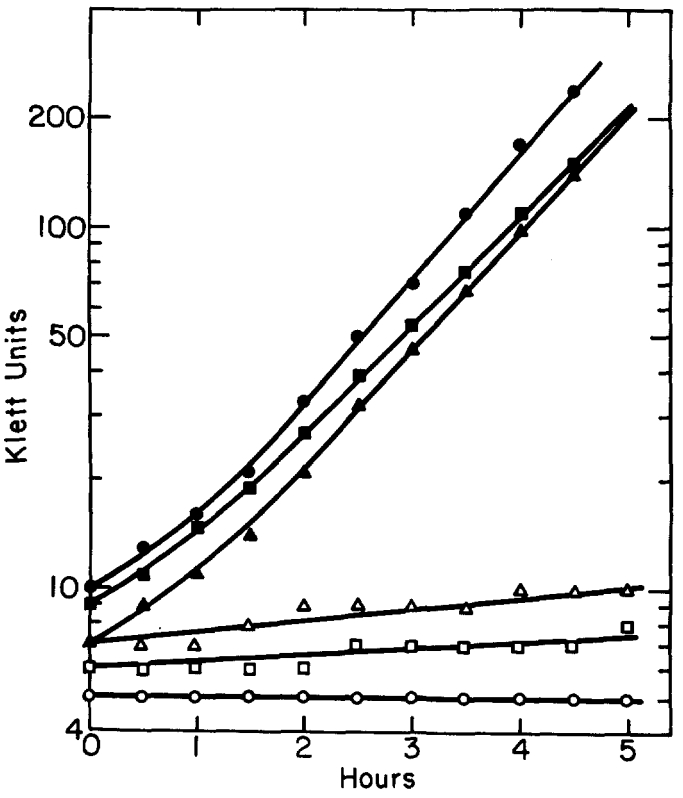

FIG. 1.—Growth response of JB561. Cells were grown in glucose minimal media supplemented as follows: No additions (○); glycine (□); glycyl-glycine dipeptide (△); L-serine (▲); L-serine + glycine (●); and glycyl-DL-serine dipeptide (■). All amino acids and dipeptides were added to a final concentration of 200 μg/ml.

differs from the parent strain JB507 (*serA13*), which can grow when supplemented with either serine or glycine. Since the new mutation introduced into the *serA13* mutant apparently allowed synthesis of glycine from serine but not the reverse reaction, the possibility that two separate enzymes might be involved in this interchange was suggested. Therefore, this mutant was further characterized to determine whether two enzymes existed or whether the serine transhydroxymethylase had been altered.

An initial series of transductions was carried out to establish that the mutant retained the original *serA13* lesion and that the new phenotype resulted from a second mutation. Transductions using KB1 phage grown on strain JB561 (*serA13, gly-951*) failed to yield serine-independent transductants with strain JB507 (*serA13*) as the recipient. The reciprocal transduction with phage grown on the original JB507 strain produced transductants from strain JB561 capable of growth on glycine, but all transductants required serine, indicating that the *serA13* lesion was still present. Control transductions with phage grown on a prototrophic strain JL781 yielded a high number of serine-independent trans-

ductants when either strain JB507 or strain JB561 was the recipient. As would be expected if strain JB561 contained two mutations, transductions with a prototrophic strain as donor into strain JB561 gave all Ser^+ transductants when the selection was on minimal medium, but gave both serine prototrophs and serine auxotrophs when the selection medium included glycine (where glycine could be used as a serine source for transductants retaining the *serA13* lesion). These results are consistent with strain JB561 retaining the original *serA13* lesion and having a separate mutation causing the inability to grow on glycine. They do not, however, determine whether this second mutation (*gly-951*) is linked by transduction to the *serA* site since the Ser^+ transductants could be either of two genotypes, ones that have received only the wild-type DNA for the *serA* gene, or ones that have received the wild-type DNA for both the *serA* and *gly-951* sites. The JB561 strain was further characterized to determine the physiological result and genetic identity of the *gly-951* mutation. The effects of these two mutations could have caused the inability to grow on glycine in a number of ways: (1) by rendering the cell sensitive to glycine in some way so that growth was inhibited, (2) by altering glycine transport so that it no longer entered the cell, (3) by altering the serine transhydroxymethylase activity for only the conversion of glycine to serine, or (4) by eliminating another route normally responsible for producing serine from glycine.

Evidence against glycine being inhibitory to the growth of strain JB561 is presented in Figure 1. Although the culture does not grow with glycine alone it grows well with the addition of either serine or the glycyl-DL-serine dipeptide, which supplies both glycine and serine. Thus, if glycine is inhibitory, serine overcomes this inhibition. A number of other compounds, including all common amino acids, purines, and pyrimidines, were also tested and none allowed growth of strain JB561 on minimal medium plus glycine.

The phenotype does not result from an alteration in glycine transport. Figure 1 shows the response of strain JB561 cultures to the two similar dipeptides, glycyl-glycine and glycyl-DL-serine; growth occurs only with glycyl-DL-serine. Since free amino acids and dipeptides have different transport systems (SUSSMAN and GILVARG 1971; NEWMAN and MAGASANIK 1963; LEVINE and SIMMONDS 1960; SIMMONDS and MILLER 1957), it is unlikely that strain JB561 would have simultaneously lost the ability to transport both glycine and glycyl-glycine. A direct measure of glycine transport for strain JB561 and the parent strain JB507 confirms these observations (Figure 2). For both strains, uptake of ^{14}C-glycine is rapid within the first few minutes of addition, followed by a decreasing rate after 3 or 4 minutes. Addition of a 100-fold excess of cold glycine to the reaction mixture with strain JB561 substantially decreases the uptake of radioactive glycine. These and the above growth results suggest that strain JB561 transports glycine as well as strain JB507 and that altered glycine transport does not explain the phenotype of strain JB561.

Since glycine did not inhibit the growth of strain JB561 and appears to enter the cell normally, the possibility that strain JB561 could be blocked in a separate reaction for converting glycine to serine, not involving serine transhydroxy-

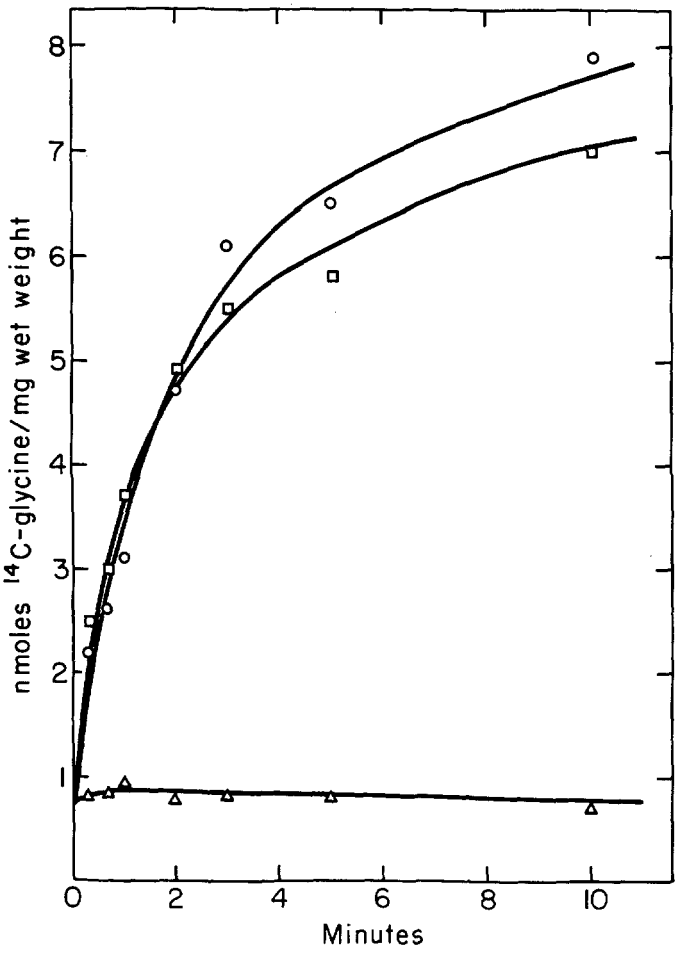

FIG. 2.—Uptake of ^{14}C-glycine by strains JB507 and JB561. JB507 (○); JB561 (□); and JB561 plus a 100-fold excess of cold glycine (△).

methylase, was considered. One possibility is that the biosynthesis of serine from glycine proceeds through glyoxylate and formate. The formate could then form 10-formyltetrahydrofolate which can be reduced to the oxidation level of formaldehyde and donated to another molecule of glycine to yield serine (SIEKEVITZ and GREENBERG 1949; SAKAMI 1949; NEWMAN and MAGASANIK 1963; PIZER 1965). This pathway probably plays a minor role except under conditions where cells are forced to use glycine to synthesize serine (serine auxotrophs supplemented with glycine).

If this pathway were normally responsible for converting glycine to serine, a loss of one of these enzyme activities could explain the phenotype of strain JB561. Thus, the growth of strain JB561 with glycine and either formate or glyoxylate (both supplemented at 1 mg/ml) was examined. Neither compound supported growth either on plates or in liquid cultures even after 3 days' incubation at 37°.

This suggested that in strain JB561 an alteration in an enzyme involved in the formation of formate from glycine was not responsible for the phenotype of strain JB561. Two *serA*, *glyA* double mutants, strains JB563 (*serA13*, *glyA10*) and JB564 (*serA13*, *glyA20*), known to have mutations in the gene encoding serine transhydroxymethylase were also tested, and neither could grow with a supplement of glycine and glyoxylate or glycine and formate. Since the *serA*, *glyA* double mutants were also unable to grow, it seems likely that the final enzyme for the condensation of the one-carbon unit with glycine is serine transhydroxymethylase.

Other possible routes to serine were considered. Since ULANE and OGUR (1972) reported that in Saccharomyces a catabolite-repressible pathway to glycine and serine existed, the growth of serine and glycine auxotrophs was examined with several carbon sources other than glucose. Of the carbon sources tested, galactose and arabinose supplemented with 0.4% glyoxylate allowed some growth after about a 12-hour lag, but the growth rates were very slow with generation times greater than 24 hours. One other compound found to allow slow growth of serine- or glycine-requiring mutants when added as a supplement, is threonine. This is presumably due to an enzyme which degrades threonine to acetaldehyde and glycine (MELTZER and SPRINSON 1952; PIZER 1965). None of these possible reactions appears to support rapid enough growth to be considered a major pathway to either serine or glycine in Salmonella.

Since there was no evidence for a second major pathway, further characterization of strain JB561 focused on its genetic and biochemical properties. Genetic evidence for the involvement of the serine transhydroxymethylase would be provided if the second mutation in strain JB561 (*serA13*, *gly-951*) could be mapped with other known *glyA* mutations. However, preliminary interrupted mating experiments and transductions with strain JB561 (*serA13*, *gly-951*) as the recipient and donor strains with wild-type DNA for the *serA* and *glyA* genes gave a preponderance of serine prototrophs when the selections were made directly on minimal plus glycine medium. Further experiments suggested that conjugants and transductants from strain JB561 could not express the function of the $glyA^+$ gene when the selections were made only in the presence of glycine. Thus, a method using a *strB* mutant to avoid selecting $glyA^+$ transductants in the presence of glycine alone was developed.

The *strB57* mutation confers both a resistance to low levels of streptomycin and a requirement for niacinamide and thiamine. Therefore, selection procedures using *strB57* can make use of either the streptomycin resistance or the niacinamide and thiamine requirements. Since the *strB* and *glyA* loci are adjacent on the Salmonella linkage map and could be co-transducible (SANDERSON 1972), transduction of two glycine-requirers, strain JB565 (*glyA10*) and strain JB566 (*glyA20*), into strain JL600 (*strB57*) were performed and selection was made for prototrophy of the niacinamide and thiamine requirement. For both strains, the *glyA* markers co-transduced with the *strB57* mutation (Table 2, lines 1 and 2).

These results suggested that the *strB* marker could be useful in transductions

TABLE 2

Results of transductions with various glyA *and* strB *strains*

Donor	Recipient	Scoring media				Percent co-transduction*
		Minimal	Serine	Glycine	Serine + Glycine	
JB565 (*glyA10*)	JL600 (*strB57*)†	69	69	76	76	9
JB566 (*glyA20*)	JL600 (*strB57*)†	69	69	76	76	9
JB561 (*serA13, gly-951*)	JB562 (*serA13, strB57*)‡	0	228	189	228	17
JB565 (*glyA10*)	JB562 (*serA13, strB57*)‡	0	32	32	35	9
JB566 (*glyA20*)	JB562 (*serA13, strB57*)‡	0	82	82	102	19

* The percent co-transduction refers to the percent of the transductants that have received both the *strB*+ and the *glyA* lesion from the donor. Transductants were selected on the medium indicated and then scored for their ability to grow on the scoring medium. The numbers under the Serine + Glycine column represent the total number of colonies scored.

† Transductants selected on medium supplemented with glycine.

‡ Transductants selected on medium supplemented with both serine and glycine.

with strain JB561. Therefore, KB1 phage were grown on strain JL600 (*strB57*) and used to construct a *serA13, strB57* double mutant by transducing the *strB57* marker into strain JB507 (*serA13*). Transductants were selected on minimal medium supplemented with serine, niacinamide, thiamine, and 100 μg/ml streptomycin. One transductant, strain JB562, carrying both the *serA13* and *strB* mutations, was isolated. Because of the niacinamide and thiamine requirements of this mutant, further selections for *strB*+ transductants could be made in the presence of both serine and glycine but without the addition of the vitamins.

Phage lysates from strains JB561 (*serA13, gly-951*), JB565 (*glyA10*), and JB566 (*glyA20*) were used in transductions with strain JB562 (*serA13, strB57*) as the recipient. (Because strain JB562 was lysogenic for the KB1 phage, these three transductions were all performed with the L4 phage.) As shown in Table 2, transduction of the known *glyA10* and *glyA20* mutations into a serine auxotroph gives 9% and 19% co-transduction respectively, and leads to a dual requirement of serine and glycine. The transduction using strain JB561 (*serA13, gly-951*) as a donor demonstrated that the *gly-951* lesion, like the *glyA10* and *glyA20*, was co-transducible with the *strB57* mutation.

Another result of the above transduction was the construction of two new serine-glycine dual auxotrophs, strain JB563 and strain JB564, which now allowed a direct examination of the linkage of *gly-951* with the *glyA10* and *glyA20* lesions. Since the JB561 genotype still allowed conversion of serine to glycine, phage grown on strain JB561 (*serA13, gly-951*) could be used to transduce the double mutants JB563 (*serA13, glyA10*) and JB564 (*serA13, glyA20*) to glycine prototrophy on medium containing serine. Transductants receiving either the *gly-951* lesion or wild-type DNA for the *glyA* region could grow on this medium, but they could be distinguished by examining their ability to grow on medium supplemented with glycine. As shown in Table 3, all the transductants with strain JB561 as donor received the donor phenotype and were unable to use glycine as a serine source. These results make it likely that the *gly-951* mutation is really *glyA951*. When strain JB507 (*serA13*) was used as the donor in control

TABLE 3

Results of transductions with JB561, JB563 and JB564

Donor	Recipient	Scoring media			Percent of transductants with donor phenotype
		Minimal	Glycine	Serine	
JB561 (*serA13, gly-951*)	JB563 (*serA13, glyA10*)*	0	0	114	100
JB561 (*serA13, gly-951*)	JB564 (*serA13, glyA20*)*	0	0	114	100
JB507 (*serA13*)	JB563 (*serA13, glyA10*)*	0	76	76	100
JB507 (*serA13*)	JB564 (*serA13, glyA20*)*	0	70	70	100

* Transductants selected on medium supplemented with serine.

transductions, all transductants received the donor (wild-type) phenotype and were able to use either serine or glycine to supply the required serine.

Further genetic evidence that the *gly-951* lesion is coincident with the *glyA* region is provided by a complementation test using abortive transductants. In the above transduction in which strain JB561 (*serA13, gly-951*) is the donor and strain JB564 (*serA13, gly20*) the recipient, at least twice as many abortive transductants as normal transductants were observed when the selection was on serine-supplemented medium. However, when the selection was on glycine-supplemented media (where a *serA13, gly-951* strain cannot grow), neither transductants nor abortive transductants were found. If the *gly-951* lesion had been contiguous with, but separate from, the *glyA* region, there might have been few normal transductants produced in the cross because the majority of those becoming $glyA^+$ also would become *gly-951* by co-transduction and fail to grow on the glycine medium. However, if the gene functions were separate it should have been possible to obtain abortive transductants by complementation. The absence of abortives further indicates that the *gly-951* lesion is in *glyA*. Control transductants with strain JB507 (*serA13*) into strain JB564 (*serA13, glyA20*) did yield abortive transductants, showing that they can grow on the glycine medium.

To support the above genetic data, enzyme assays of the serine transhydroxymethylase activity were performed on extracts from these mutants. Results obtained by measuring the conversion of glycine to serine are given in Table 4. The values are expressed as percent of the wild-type activity from cells grown in glucose minimal medium and as specific activity (μg HCHO consumed/mg protein/min) and represent the averages of at least three assays. The two *glyA* mutants have a low level of enzyme activity (12% or less of the wild-type activity), while strain JB561 has a reduced but still appreciably high level (34% of the wild-type activity). This activity was higher than expected for strain JB561 (*serA13, gly-951*) since glycine will not support growth of this mutant. To eliminate the possibility that the high activity could be due to the assay method, the reaction was also measured by determining the amount of serine converted to glycine by the procedure of TAYLOR and WEISSBACH (1965) (Table 5). Again, the enzyme activity in the strain JB561 extract was less than 40% of that for wild-type cultures grown on glucose minimal medium. Control experi-

TABLE 4

Results of serine transhydroxymethylase assay measuring the conversion of glycine to serine

Strain	Growth media* Serine	Glycine	Serine + Glycine	Minimal
JL781 (wild type)	75†(5.25)	113 (7.91)	47 (3.29)	100 (7.00)
JB561 (*serA13, gly-951*)	34 (2.38)	—‡	29 (2.03)	—
JB565 (*glyA10*)	—	8 (0.56)	12 (0.84)	—
JB566 (*glyA20*)	—	12 (0.84)	<1 (0.07)	—
JB567 (*gly-951*)	19 (1.33)	13 (0.91)	N.D.§	44 (3.08)

* The growth media were glucose minimal containing the indicated supplements at 200 μg/ml.
† Values are expressed as percent of the activity found with the wild-type strain (JL781) grown on glucose minimal medium. Numbers in parentheses are specific activities (μg HCHO consumed/mg protein/min).
‡ A dash means that the particular medium would not support growth of the indicated strain.
§ Not determined.

ments with extracts prepared from the parent strain, JB507 (*serA13*), demonstrated that this lesion alone did not cause a reduction in the serine transhydroxymethylase activity, as this strain had wild-type levels.

Although these assays substantiated the reduced serine transhydroxymethylase activity for strain JB561, they did not fully explain this strain's inability to grow on glycine-supplemented medium since 30% to 40% enzyme activity might be expected to allow some growth. The enzyme level for strain JB561 in glycine medium could not be tested directly because of the strain's failure to grow. However, the construction of a Ser^+ transductant of strain JB561 (designated strain JB567) allowed further examination of the effects of the *gly-951* lesion in the absence of the *serA13* mutation. Strain JB567 (*gly-951*) grows in glucose minimal medium, glucose minimal plus serine, or glucose minimal plus glycine with generation times of 86, 67 and 54 minutes, respectively (compared with a wild-type strain having a generation time of about 57 minutes in glucose minimal.) Thus, this Ser^+ derivative of strain JB561 can synthesize its own serine but still has a partial requirement for glycine. When a culture of strain JB567 was grown in glucose minimal medium supplemented with glycine, the serine transhydroxymethylase activity was lowered to 13% (Table 4, last line), as compared with

TABLE 5

Results of serine transhydroxymethylase assay measuring the conversion of serine to glycine

Strain	Growth media* Serine	Serine + Glycine	Minimal
JL781 (wild type)	N.D.†	N.D.	100‡
JB507 (*serA13*)	80	67	—§
JB561 (*serA13, gly-951*)	40	28	—

* The growth media were glucose minimal containing the indicated supplements at 200 μg/ml.
† Not determined.
‡ The value of 100 for JL781 represents 1.58 μg of serine consumed/mg protein/min.
§ A dash means that the particular medium would not support growth of the indicated strain.

44% for cells grown in glucose minimal alone. The reduction of serine transhydroxymethylase activity in a strain carrying the *gly-951* lesion could explain the inability of strain JB561 to grow on glycine. A wild-type strain does not show a reduction in enzyme level when grown with glycine (Table 4, line 1).

Since these results could suggest that the *gly-951* lesion has altered the regulation of serine transhydroxymethylase, it was important to substantiate the genetic evidence that *gly-951* lies within the gene encoding serine transhydroxymethylase and effects this enzyme directly rather than being a mutation in a regulatory gene which affects enzyme levels.

One method often used to demonstrate a direct effect on a protein is to provide evidence that the enzyme activity is temperature-sensitive. In order to examine the effects of temperature on serine transhydroxymethylase activity, strain JL781 and two mutant strains, JB561 (*serA13, gly-951*) and JB567 (*gly-951*), were grown at 27° and the serine transhydroxymethylase activity assayed on extracts at 27° and 42°.

When the specific activities from the *gly-951* strains were compared with those observed with the JL781 extract, it was found that the mutant strains had higher relative activities at 27° than at 42° (Table 6). Thus, the *gly-951* lesion resulted in a heat-sensitive serine transhydroxymethylase activity. This heat-sensitive effect on enzyme activity was further reflected in the growth phenotype of strain JB567. This strain grows with a doubling time that is slightly longer than that of JL781 in glucose minimal medium at 27° (114 min and 84 min, respectively) but has more than twice the generation time at 42°.

DISCUSSION

The results of experiments with strain JB561 and other serine-glycine-requiring mutants constructed during this work are consistent with serine transhydroxymethylase being solely responsible for the interconversion of serine and glycine. Strain JB561 requires serine for growth but has lost the ability to utilize glycine as a serine source. This inability to grow with a glycine supplement is not caused by a growth inhibitory effect of glycine nor by an altered cell per-

TABLE 6

Measurements at 27° and 42° of serine transhydroxymethylase activity from wild-type and mutant strains

Strain	Growth media*	Serine transhydroxymethylase activity 27°†	42°†
JL781 (wild type)	minimal	100‡	100‡
JB567 (*gly-951*)	minimal	52	24
JB561 (*serA13, gly-951*)	minimal + serine	33	19

* Cells grown at 27° in the indicated medium.
† Temperature at which the enzyme was assayed.
‡ Specific activity for JL781 was 3.05 μg HCHO consumed/mg protein/min at 27° and 5.78 μg HCHO consumed/mg protein/min at 42°. These activities were put at 100% for comparing the relative activities obtained with the mutants.

meability to glycine. Both genetic and physiological characterizations of this strain suggest that it has a mutation in the *glyA* gene.

The genetic experiments demonstrated that the lesion (*gly-951*) preventing growth with glycine is co-transducible with a *strB57* locus, as are other *glyA* mutations. Furthermore, transductions of *gly-951* into strains having *glyA10* and *glyA20* mutations showed 100% linkage of *gly-951* to the *glyA* region. These and the abortive transduction data suggest that *gly-951* is *glyA951*. In addition, enzyme assays for the *glyA* gene product, serine transhydroxymethylase, showed a reproducible reduction of activity for strain JB561 to 34% of that observed for the wild type. Evidence that the serine transhydroxymethylase in mutants carrying the *gly-951* lesion is more heat-labile than the enzyme from the parent strain supports the genetic information that the serine transhydroxymethylase is directly affected (Table 6). These enzyme results correlate well with this mutation, severely limiting the growth of the mutants at 42° while having little effect at 27°.

The above evidence obtained by the characterization of the JB561 mutant indicating that serine transhydroxymethylase is the only enzyme involved in the interconversion of serine and glycine is substantiated by the phenotype of the *serA*, *glyA* double mutants constructed in this study. Each of the *glyA* mutations (*glyA10* and *glyA20*) alone confers only a requirement for glycine; but when either *glyA* mutation is transduced into a strain containing a *serA13* mutation the strain requires both serine and glycine (Table 2). If a separate route from glycine to serine were functional, these transductants should have required only glycine and have been able to synthesize serine from the glycine supplement. Since the transductants require both amino acids, the loss of serine transhydroxymethylase activity caused by the *glyA10* and *glyA20* mutations prevents this interconversion.

Although all of the information is consistent with an alteration in serine transhydroxymethylase preventing the growth of JB561 on glycine, it does not immediately explain why the observed 34% activity is not sufficient to support at least slow growth. Since the results with the transductant containing only the *gly-951* lesion (JB567) show a further decrease in serine transhydroxymethylase activities (13%) when this strain is grown in medium containing glycine (Table 4), it may be that this enzyme is further repressed in strains carrying the *gly-951* lesion. This 13% activity is about the amount found with the glycine auxotrophs (8% to 12%) and could be sufficient to explain the inability of strain JB561 to grow. It is not clear why the strain carrying only the *gly-951* lesion has this reduced serine transhydroxymethylase activity when grown with glycine, while the wild-type strain shows no reduction and may even exhibit a slight increase (113%). However, other results (STAUFFER and BRENCHLEY, unpublished data) demonstrate that the regulation of serine transhydroxymethylase activity is complicated and that a number of compounds are indirectly involved, perhaps because of their influence on one carbon metabolism. It may be that the altered regulation with the *gly-951* mutation is caused by a change in a normal regula-

tory function of the serine transhydroxymethylase protein itself or by a change in the production of one-carbon units (or derivatives) involved in its regulation.

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LITERATURE CITED

ADELBERG, E. A., M. MANDEL and G. C. C. CHEN, 1965 Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Com. **18:** 788–795.

BORO, H. and J. E. BRENCHLEY, 1971 A new generalized transducing phage for *Salmonella typhimurium* LT2. Virology **45:** 835–836.

BRENCHLEY, J. E., 1973 Effect of methionine sulfoximine and methionine sulfone on glutamate synthesis in *Klebsiella aerogenes*. J. Bacteriol. **114:** 666–673.

COSLOY, S. D., 1973 D-serine transport system in *Escherichia coli* K-12. J. Bacteriol. **114:** 679–684.

LEVINE, E. M. and S. SIMMONDS, 1960 Metabolite uptake by serine-glycine auxotrophs of *Escherichia coli*. J. Biol. Chem. **235:** 2902–2909.

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

MELTZER, H. L. and D. B. SPRINSON, 1952 The synthesis of 4-C^{14},N^{15}-L-thereonine and a study of its metabolism. J. Biol. Chem. **197:** 461–474.

MUDD, S. H. and G. L. CANTONI, 1964 Biological transmethylation, methyl-group neogenesis and other "one-carbon" metabolic reactions dependant upon tetrahydrofolic acid. Pp. 1–42, *Comprehensive Biochemistry, XV*. Edited by M. FORKIN and E. H. STOLZ.

NEWMAN, E. B. and B. MAGASANIK, 1963 The relation of serine-glycine metabolism to the formation of single-carbon units. Biochim. Biophys. Acta **78:** 437–448.

PIZER, L. L., 1963 The pathway and control of serine biosynthesis in *Escherichia coli*. J. Biol. Chem. **238:** 3934–3944. ——, 1965 Glycine synthesis and metabolism in *Escherichia coli*. J. Bacteriol. **89:** 1145–1150.

SAKAMI, W., 1949 The conversion of glycine into serine in the intact rat. J. Biol. Chem. **178:** 519–520.

SANDERSON, K. E., 1972 Linkage map of *Salmonella typhimurium*, edition IV. Bacteriol. Rev. **36:** 558–586.

SCRIMGEOUR, K. G. and F. M. HUENNEKENS, 1962 Serine hydroxymethylase. Pp. 838–843. In: *Methods in Enzymology V*. Edited by COLOWICK and KAPLAN.

SIEKEVITZ, P. and D. M. GREENBERG, 1949 The biological formation of serine from glycine. J. Biol. Chem. **180:** 845–856.

SIMMONDS, S. and D. A. MILLER, 1957 Metabolism of glycine and serine in *Escherichia coli*. J. Bacteriol. **74:** 775–783.

SMITH, H. O. and M. LEVINE, 1967 A phage P22 gene controlling integration of prophage. Virology **31:** 207–216.

SUSSMAN, A. J. and C. GILVARG, 1971 Peptide transport and metabolism in bacteria. Ann. Rev. Biochem. **40:** 397–407.

TAYLOR, R. T. and H. WEISSBACH, 1965 Radioactive assay for serine transhydroxymethylase. Anal. Biochem. **13**: 80–84.

ULANE, R. and M. OGUR, 1972 Genetic and physiological control of serine and glycine biosynthesis in *Saccharomyces*. J. Bacteriol. **109**: 34–43.

UMBARGER, H. E. and M. A. UMBARGER, 1962 The biosynthetic pathway of serine in *Salmonella typhimurium*. Bochim. Biophys. Acta **62**: 193–195.

UMBARGER, H. E., M. A. UMBARGER and P. M. L. SIU, 1963 Biosynthesis of serine in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **85**: 1431–1439.

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