

# Glycine Synthesis and Metabolism in *Escherichia coli*

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## ABSTRACT

PIZER, LEWIS I. (University of Pennsylvania, Philadelphia). Glycine synthesis and metabolism in *Escherichia coli*. *J. Bacteriol.* 89:1145-1150. 1965.—A correlation was demonstrated between a nutritional requirement that can only be satisfied by glycine and the absence of the enzymatic capacity to interconvert L-serine and glycine. Serine synthesis from 3-phosphoglycerate was observed in the same cell-free extracts which could not convert serine to glycine. The above results show that serine is the precursor of glycine under normal growth conditions. The C-2 of glycine provided "one-carbon" fragments when the C-3 of serine was not available as the source of "one-carbon" fragments. This condition occurred when a mutation produced a loss of serine aldolase activity or when a serine-glycine auxotroph was grown with glycine. Under these growth conditions, 30 to 40% of the "one-carbon" fragments used for cellular synthesis were derived from glycine.

On the basis of nutritional and metabolic studies with mutant strains of *Escherichia coli*, it has been concluded that this microorganism can interconvert serine and glycine (Roepke, Libby, and Small, 1944; Davis and Maas, 1949; Roberts et al., 1955; Meinhart and Simmonds, 1955). The question of which is precursor and which is product has not been answered, although the bulk of the available evidence indicates that serine gives rise to glycine and a one-carbon fragment at the oxidation level of a hydroxymethyl group.

The evidence consists of competition experiments (Roberts et al., 1955) which showed that exogenous serine inhibits the endogenous synthesis of both serine and glycine, whereas exogenous glycine fails to inhibit serine synthesis. Additional evidence that serine is the precursor of glycine comes from the demonstration by Umbarger, Umbarger, and Siu (1963) and Pizer (1963) that mutations producing enzymatic deficiencies in the biosynthetic pathway to serine are expressed phenotypically by a nutritional requirement satisfied by serine or glycine. An alternative pathway consists of a transamination of glyoxylate to give glycine, which gives rise to serine (Feldman and Gunsalus, 1950).

This paper presents direct evidence that serine is the precursor of glycine, since a mutation that causes the loss of the serine aldolase (glycine hydroxymethylase) has been shown to produce a growth requirement for glycine which is not satisfied by serine.

## MATERIALS AND METHODS

**Bacteria and growth conditions.** The mutant strains of *E. coli* K-12 were obtained from A. L. Taylor of the National Institutes of Health. *E. coli* strain AT-2046 (designated strain *gly*<sup>-</sup>) grows in a glucose salts medium supplemented with glycine and thiamine, at 370 and 1 μg/ml, respectively. *E. coli* strain AB-342 (designated strain *ser*<sup>-</sup>) requires that the glucose salts medium be supplemented with L-threonine, L-leucine, L-serine, and thiamine. The multiple requirements are the result of independent mutations. The final concentrations of nutrients in the medium were 200 μg/ml of L-threonine, 270 μg/ml of L-leucine, 300 μg/ml of L-serine, and 1 μg/ml of thiamine. The modified M-9 medium and the methods used to obtain and measure bacterial growth have previously been described (Pizer and Potochny, 1964). Glucose, at an initial concentration of 1 mg/ml, was the carbon and energy source in all the experiments reported in this paper.

To prepare cell-free extracts, the bacterial cells were harvested from the growth medium by centrifugation and disrupted by grinding with alumina. The disrupted cells were extracted with 0.05 M tris(hydroxymethyl)aminomethane (Tris)-Cl buffer, pH 7.5 [10 ml of buffer per g (wet weight) of cells] and the extract was clarified by centrifugation at 15,000 × *g* for 15 min.

**Chemical compounds and procedures.** Inorganic phosphate was determined by the method of Bartlett (1959). Protein was measured by the procedure of Lowry et al. (1951). C<sup>14</sup>-3-phosphoglycerate was a gift from N. Pon. C<sup>14</sup>-2-glycine was purchased from Calbiochem. C<sup>14</sup>-formaldehyde was purchased from Isotope Specialities

Co., Burbank, Calif. Radioactivity measurements were made by counting samples plated in stainless-steel planchets in a windowless flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.). All samples were counted to 2,000 counts. Radioactive areas on paper chromatograms were detected and measured with an Atomic Accessories Actograph model RSC-SA. L-Serine-phosphate was synthesized by the method of Neuhaus and Korke (1958). All other materials were purchased from Calbiochem.

For the fractionation of cell components and the isolation of amino acids and nucleic acid bases, a modified procedure of Roberts et al. (1955) was used (Pizer and Potochny, 1964). After growth, the cells were washed successively with 0.15 M NaCl and absolute ethyl alcohol. The lipids were twice extracted with an ethyl alcohol, diethyl ether, water mixture (40:50:10). This extraction was for 5 min at 58 C. The nucleic acids were extracted with 5% trichloroacetic acid at 90 C for 15 min. After the trichloroacetic acid was removed by ether extraction, the nucleic acid solution was evaporated to dryness. The extracted nucleic acid and the protein residue were hydrolyzed at 110 C in 6 N HCl for 3 and 18 hr, respectively. The nucleic acid bases were separated by two-dimensional paper chromatography, eluted from the paper, and their specific radioactivity was determined. The amino acids in the protein were converted to their dinitrophenyl derivatives, which were then separated by two-dimensional paper chromatography. Dinitrophenyl-glycine was eluted from the paper, and the radioactivity of the eluted material was determined.

**Enzyme assays.** The capacity to synthesize serine-phosphate was measured by incubating  $C^{14}$ -labeled 3-phosphoglycerate with bacterial extract in the presence of the required cofactors. The radioactive products of the incubation were separated by ion-exchange chromatography, and the amount of isotopically labeled serine-phosphate was determined (Pizer, 1963). L-Serine-phosphate phosphatase was assayed by measuring the release of inorganic phosphate from L-serine P as described previously (Pizer, 1963).

Serine aldolase was assayed in the direction of serine synthesis by determining the amount of  $C^{14}$ -formaldehyde added to glycine. The assay was a modification of that reported by Kisliuk and Sakami (1955). The incubation mixture contained the following: 0.1 M potassium phosphate buffer (pH 7.5), 0.01 M glycine, 0.002 M DL-tetrahydrofolic acid, 0.005 M  $C^{14}$ - $CH_2O$  (specific activity of 42,000 count/min per  $\mu$ mole), and enzyme, in a final volume of 0.5 ml. After addition of enzyme, the mixture was incubated at 37 C for 20 min and then chilled in an ice bath; 100  $\mu$ moles of  $C^{12}$ - $CH_2O$  and 5  $\mu$ moles of L-serine were added, and the protein was precipitated by the addition of 0.5 ml of 15% trichloroacetic acid. The precipitated material was removed by centrifugation and the supernatant fluid was placed on a column (1 by 3

cm) of Dowex 50  $H^+$  (100 to 200 mesh) previously washed with 0.01 N HCl. The column was washed with 20 ml of 0.01 N HCl, 5 ml of water, and 2.5 ml of 1.5 M  $NH_4OH$ . An additional 5 ml of 1.5 M  $NH_4OH$  eluted the serine, and the radioactivity in this fraction was determined as a measure of enzyme activity.

## RESULTS

**Growth studies.** Growth studies were designed to provide information on the L-serine and glycine requirements of these mutant organisms. The auxotrophic strains were grown overnight on the required nutrients and limiting glucose. The culture was diluted in the morning to a concentration of  $5 \times 10^7$  cells per milliliter and allowed to grow until a cell concentration of  $2 \times 10^8$  cells per milliliter was reached. The culture was chilled, centrifuged, and the cells were washed twice by suspension in cold M-9 medium lacking glucose. After washing, the cells were suspended in medium lacking only serine and glycine to give a concentration of about  $1 \times 10^8$  cells per milliliter. L-Serine and glycine were added according to the design of the experiment, and growth followed with time. The nutritional requirements of

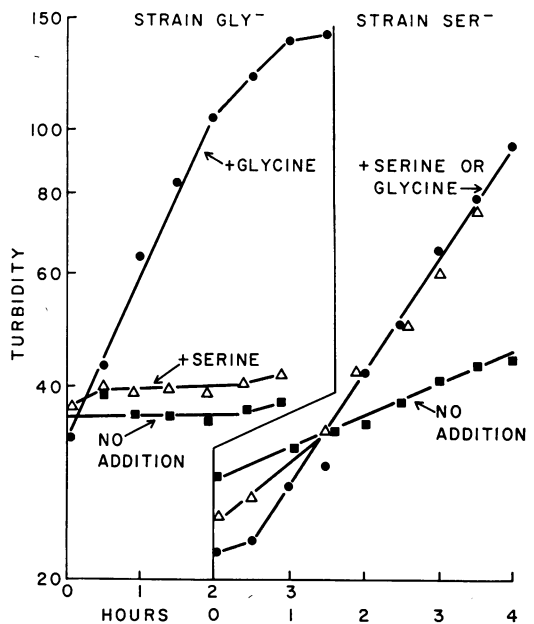


FIG. 1. Growth response of *Escherichia coli* strains *gly*<sup>-</sup> and *ser*<sup>-</sup> to the nutrients glycine and L-serine. The strain *ser*<sup>-</sup> had been grown on L-serine prior to washing and placement into the growth flasks as described in the text. The same growth response was obtained with cells grown on glycine. Glycine and L-serine when present were at an initial concentration of 500  $\mu$ g/ml.

strains *gly*<sup>-</sup> and *ser*<sup>-</sup> for L-serine and glycine are shown in Fig. 1. Strain *gly*<sup>-</sup> grew with a division time of 72 min when glycine was provided, but did not grow when glycine was omitted from the medium or replaced by L-serine. Strain *ser*<sup>-</sup> utilized either L-serine or glycine to satisfy its growth requirement and grew with a division time of approximately 100 min with either amino acid as a supplement. In the absence of L-serine or glycine, slow growth was observed with strain *ser*<sup>-</sup>, presumably due to the formation of glycine from exogenous L-threonine provided.

In contrast to other mutant strains of *E. coli* (Roepke et al., 1944; Davis and Maas, 1949) that have a growth requirement for glycine, strain *gly*<sup>-</sup> (AT-2046) cannot use L-serine to satisfy its growth requirement. The quantitative relationship between exogenous glycine and growth (Fig. 2) shows that 10<sup>10</sup> cells utilize for growth approximately 10 μmoles of glycine. It appears that the exogenous glycine supplied was used efficiently by the cell and was not converted to other cell components. A requirement for larger amounts of glycine (30 μmoles per 10<sup>10</sup> cells) would be expected if glycine were being utilized for the synthesis of L-serine (Pizer and Potochny, 1964).

*Enzyme studies.* The enzymes responsible for

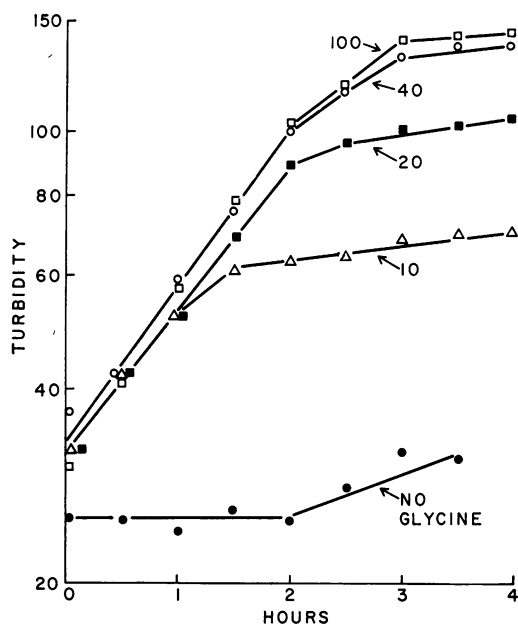


FIG. 2. Growth response of *Escherichia coli* strain *gly*<sup>-</sup> to limiting glycine. The change in turbidity with time is shown for cultures supplied with different amounts of glycine. The numbers on the curves give the initial glycine concentration in μg/ml.

L-serine biosynthesis and its cleavage to glycine were assayed in cell-free extracts of *E. coli* strain *gly*<sup>-</sup> and strain *ser*<sup>-</sup>. The ability to convert C<sup>14</sup>-3-phosphoglycerate to C<sup>14</sup>-serine-P was present in extracts from strain *gly*<sup>-</sup> but absent in comparable extracts from strain *ser*<sup>-</sup> (Table 1). Extracts from both strains contained the specific phosphatase which hydrolyzes L-serine-P to L-serine and phosphate. Since extracts of *E. coli* strain *gly*<sup>-</sup> have the enzymatic capacity to convert 3-phosphoglycerate to L-serine, their nutritional requirement for glycine is not due to an inability to synthesize serine or obtain serine from the medium.

Extracts of strain *gly*<sup>-</sup> did not possess the ability to synthesize L-serine from glycine and formaldehyde, but this reaction was catalyzed by comparable extracts of strain *ser*<sup>-</sup> (Table 2). The reaction was dependent on the presence of glycine, tetrahydrofolic acid, and unheated extract from bacterial cells. The product of the enzymatic reaction was characterized as serine by paper chromatography with phenol saturated with water as solvent. The detectable radioactivity possessed the same mobility in this solvent as the L-serine added as carrier at the end of the enzymatic incubation (Fig. 3A). Appreciable radioactivity was eluted by ammonia from the Dowex 50 H<sup>+</sup> column under all the incubation conditions used (Table 2). The radioactive compounds in the control incubations that were eluted from the Dowex 50 H<sup>+</sup> columns by ammonia did not chromatograph with serine in the phenol-water solvent system. This result was obtained with the incubations that lacked glycine or tetrahydrofolic acid and those that contained either the extract of strain *gly*<sup>-</sup> or the boiled extract from strain

TABLE 1. *Enzymes of serine synthesis\**

Incubation conditions	Serine phosphate formation (μmoles/30 min)	Serine phosphate hydrolyzed (μmoles/30 min)
Extract from strain <i>gly</i> <sup>-</sup>		
125 μg of protein	0.23	
250 μg of protein	0.44	
500 μg of protein		0.20
Extract from strain <i>ser</i> <sup>-</sup>		
100 μg of protein	0	
200 μg of protein	0	
400 μg of protein		0.12

\* Extracts were prepared from cells in the middle of the exponential growth phase. Glycine was the nutrient for both cultures. The C<sup>14</sup>-3-phosphoglycerate used as substrate had a specific activity of 20,000 counts per min per μmole.

TABLE 2. Enzymatic condensation of  $C^{14}$ -formaldehyde and glycine\*

Incubation conditions	Radio-activity in serine fraction
	count/min
Extract from strain <i>ser</i> <sup>-</sup>	
0.57 mg of protein and complete incubation mixture.....	24,260
1.14 mg of protein and complete incubation mixture.....	33,700
1.14 mg of protein and complete incubation mixture minus glycine.....	4,300
1.14 mg of protein and complete incubation mixture minus tetrahydrofolic acid.....	5,140
Boiled extract from strain <i>ser</i> <sup>-</sup> and complete incubation mixture.....	4,610
Extract from strain <i>gly</i> <sup>-</sup>	
0.97 μg of protein and complete incubation mixture.....	3,030
1.94 μg of protein and complete incubation mixture.....	4,660

\* Extracts were prepared from cells in the stationary phase of growth. Glycine was the nutrient in both cultures. The complete incubation mixture contained: 0.1 M potassium phosphate buffer (pH 7.5), 0.01 M glycine, 0.002 M DL-tetrahydrofolic acid, 0.005 M  $C^{14}$ -CH<sub>2</sub>O, and bacterial extract.

*ser*<sup>-</sup> (Fig. 3B). It is concluded that the radioactivity in these fractions was not due to enzymatically synthesized serine but to a nonenzymatic binding of  $C^{14}$ -formaldehyde to components in the incubation mixture.

*Utilization of  $C^{14}$ -2-glycine to provide "C<sub>1</sub>" units.*

Cells that split serine to produce glycine at the same time generate C<sub>1</sub> units for the synthesis of methionine, thymine, and the purines. When this reaction can occur, it provides essentially all of the C<sub>1</sub> units used in cellular synthesis (Pizer and Potochny, 1964) even though the glycine and formate can give rise to C<sub>1</sub> units (Roberts et al., 1955). The relative quantities of C<sub>1</sub> units coming from the C-2 of glycine and other sources have been measured in strains *gly*<sup>-</sup> and *ser*<sup>-</sup> under conditions in which the splitting of serine does not occur. In strain *gly*<sup>-</sup>, the supply of C<sub>1</sub> units from serine has been blocked because of the absence of serine aldolase, and in strain *ser*<sup>-</sup> it has been eliminated by growing the cells with glycine as nutrient rather than serine.

Cells (100-ml culture) were grown as described above except that  $C^{14}$ -2-glycine (11,500 count/min per μmole) was added as nutrient. Strain *gly*<sup>-</sup> was grown with an initial concentration of 1.35 mM glycine, and growth was allowed to proceed from approximately  $1.5 \times 10^8$  to approxi-

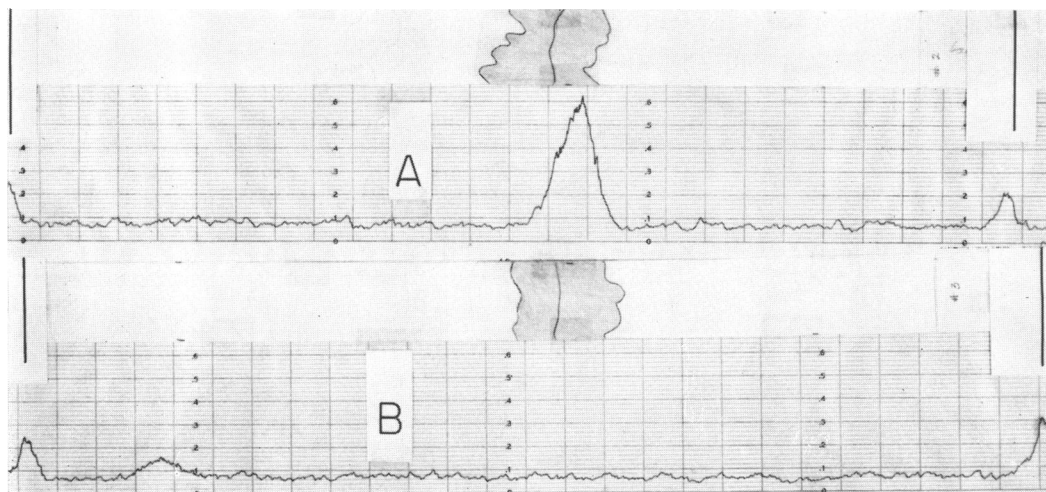


FIG. 3. Chromatography of the products of the serine aldolase reaction. The compounds eluted from the Dowex 50 H<sup>+</sup> column were evaporated to dryness and chromatographed on Whatman no. 1 paper with phenol saturated with water as the solvent system. After development the paper was cut into strips and the radioactive areas measured with an atomic accessories paper chromatogram scanner model RSC-SA. The paper was then sprayed with ninhydrin and the ninhydrin-positive areas were marked. A double spot due to the substrate, glycine, and serine added as carrier was detected. (A) A radioactive area corresponding to serine was found on the chromatographs of the compounds from the incubations that contained the complete incubation mixture and extract from strain *ser*<sup>-</sup>. (B) shows the absence of radioactivity in serine when the bacterial extract was prepared from strain *gly*<sup>-</sup>. Complete scale deflection corresponds to 1,000 count/min and the rate of scanning was 0.75 cm/min.

TABLE 3. *Specific activities of glycine and nucleic acid bases\**

Organism	Glycine	Adenine	Thymine	Uracil
Gly <sup>-</sup> .....	10,570	15,400	2,840	77
Ser <sup>-</sup> .....	6,350	10,700	2,380	27

\* Activities = counts per min per  $\mu$ mole.

mately  $6.5 \times 10^8$  cells per ml. Strain *ser<sup>-</sup>* was grown with an initial concentration of 6.75 mM glycine, and growth was allowed to proceed from approximately  $1.2 \times 10^8$  to  $6 \times 10^8$  cells per milliliter. Both cultures were chilled when the desired amount of growth had been attained, and the cells were collected by centrifugation. The cells from both cultures were then subjected to identical fractionation procedures as described in the Materials and Methods section. The specific activities of the isolated glycine and nucleic acid bases are presented in Table 3. Uracil was essentially unlabeled. Therefore, the radioactivity present in thymine resides in the methyl group rather than the pyrimidine ring and can be used as an indicator of C<sub>1</sub> units in the cell. In strain *gly<sup>-</sup>* approximately 26% of the one-carbon fragments are derived from C-2 of glycine as indicated by the specific radioactivity of the thymine methyl group. In strain *ser<sup>-</sup>* the specific activity of the intracellular glycine was reduced, presumably since exogenous threonine can be converted to glycine by the cell. When the reduction in the specific activity of glycine is taken into account, it can be calculated that approximately 40% of the thymine methyl group carbon comes from the C-2 of glycine.

The specific activities of adenine isolated from both strains indicate that the C<sub>1</sub> units at the level of formaldehyde and formate have approximately the same specific activities. The difference in the amount of glycine used to provide C<sub>1</sub> units by the two strains could be accounted for by the different amounts of glycine supplied to the cultures initially. The general conclusion appears warranted that both strains utilize sources other than the C-2 of glycine to provide the majority of the cellular C<sub>1</sub> units when the preferred source, the C-3 of serine, is unavailable because of enzymatic deficiencies or nutritional factors.

#### DISCUSSION

A mutation causing a loss of the enzyme responsible for the interconversion of serine and glycine produces a nutritional requirement for glycine. These data provide unequivocal evidence that under normal physiological conditions serine is the metabolic precursor to glycine. Glycine may arise from the cleavage of L-threo-

nine or the amination of glyoxylate, but the supply of glycine from these sources is inadequate to allow growth at the rate observed when either endogenous synthesis of glycine from serine is possible or an exogenous glycine supply is provided.

The long-standing observations that exogenous serine relieves sulfonamide inhibition of cell growth (Kohn and Harris, 1943) should be examined in connection with the experiments reported in this paper. Sulfonamides reduce the capacity of the cell to carry out C<sub>1</sub> transfer reactions, and, when the products of these reactions are provided, the inhibition of growth is relieved. Since a tetrahydrofolic acid-mediated C<sub>1</sub> transfer is not involved in serine biosynthesis, this pathway should not be sensitive to sulfonamide inhibition, and the effect serine has on the growth of sulfonamide-inhibited cells cannot be explained by a sparing of the cells' limited capacity for C<sub>1</sub> transfer reactions. A more likely explanation is that an increased supply of serine contributes to the efficiency of C<sub>1</sub> transfer by keeping the serine aldolase saturated with substrate and assuring maximal utilization of the available folic acid coenzymes.

Under conditions in which the C-3 of serine is not available as a source of C<sub>1</sub> units, C-2 of glycine is utilized to a limited extent. In the fermentation of glycine by *Diplococcus glycinophilus* (*Pediococcus glycinophilus* has recently been accepted as the name of this organism), the conversion of two molecules of glycine to serine and CO<sub>2</sub> is of major importance to the cell's economy (Sagers and Gunsalus, 1961). With these amino acids, as with other metabolic intermediates, the pathway that is involved in energy-producing reactions differs from that involved in the biosynthesis of the compound. With the bacterial strains described in this paper, the source of the C<sub>1</sub> units not arising from glycine has not been determined. Formate can give rise to C<sub>1</sub> units, but quantitative studies with this compound were not attempted in view of potential complications arising from the endogenous formate produced from pyruvate.

The possibility of C<sub>1</sub> units arising from at least three sources raises the question of whether the origin of C<sub>1</sub> units is regulated by mechanisms other than the simple supply of precursor compounds and their affinities for the enzymes involved. Newman and Magasanik (1963) have reported studies with serine-glycine auxotrophs of *E. coli* in which nutritional conditions, designed to spare the C<sub>1</sub> units from serine, produced an inability to utilize glycine to satisfy the growth requirement. It was concluded that a surplus of C<sub>1</sub> units represses the synthesis of the enzymes responsible for C<sub>1</sub> unit formation and serine

synthesis. Additional experiments should provide evidence for the nature of these controls and whether they differ in different strains of *E. coli*.

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