THE RÔLE OF POLYGLUTAMYL PTERIDINE COENZYMES
IN SERINE METABOLISM

I. COFACTOR REQUIREMENTS IN THE CONVERSION OF
SERINE TO GLYCINE

BY BARBARA E. WRIGHT AND THRESSA C. STADTMAN

(From the Laboratory of Cellular Physiology, National Heart Institute, National
Institutes of Health, Department of Health, Education, and Welfare,
Bethesda, Maryland)

(Received for publication, July 12, 1955)

It has been previously reported that serine is converted to alanine in
Clostridium HF, and that formate is important in the metabolism of this
organism (1). When grown in the presence of formate-C$^{14}$, the major non-
volatile C$^{14}$-containing component in the culture medium is alanine.$^{1}$ Val-
ine is also similarly labeled, whereas aspartic and glutamic acids are not.
It was therefore of interest to observe that, when formate-C$^{14}$ and serine
were incubated with dried cells of Clostridium HF, C$^{14}$ was present in equal
amounts in the carboxyl and $\beta$-carbons of the alanine formed. A possible
mechanism for the incorporation of C$^{14}$ into the $\beta$ position could involve an
initial fixation of formate-C$^{14}$ into serine which would thereafter be con-
verted to alanine. In view of the activity of Clostridium HF with respect
to these reactions, the organism appeared to be good experimental material
for investigations of serine metabolism.

This paper deals with the formation of glycine from serine, as catalyzed
by cell-free extracts of Clostridium HF. Preliminary reports of the work
to be presented have been given elsewhere (2–4). It will be shown that
the conversion of serine to glycine is dependent upon DPN$^{2}$, Mn$^{++}$, pyri-
doxal phosphate, orthophosphate, and factors present in boiled extracts of
Clostridium cylindrosporum (5). The latter cofactors have been charac-
terized as a family of polyglutamyl pteridines (6). The nature and func-
tion of these folic acid derivatives will be the subject of subsequent publica-
tions.

Materials and Methods

Materials—Clostridium HF was used as the source of the enzyme. For
preparations of extracts, 20 liter cultures were grown anaerobically in a

$^{1}$ According to further degradation of the alanine, most of this label was in the
$\beta$- and the carboxyl carbons.

$^{2}$ The following abbreviations are used: trichloroacetic acid, TCA; diphosphopy-
ridine nucleotide, DPN; reduced diphosphopyridine nucleotide, DPNH; unidentified
cofactor obtained from Clostridium cylindrosporum boiled extracts, Co C.
medium containing 2.25 per cent Difco tryptone, 0.5 per cent Difco yeast extract, 0.15 per cent sodium formate, 0.174 per cent K$_2$HPO$_4$, and 0.03 per cent Na$_2$S·9H$_2$O (added after sterilization) in tap water. When the cultures had attained maximal turbidity (30 to 40 hours at 37°), the cells were collected by centrifugation in a Sharples supercentrifuge, washed two or three times in cold 0.5 per cent NaCl + 0.5 per cent KCl, and dried in vacuo over CaCl$_2$. The yield was about 350 to 500 mg. of dried cells per liter of culture medium.

The dried cells were ground with Alumina A-301 (Aluminum Corporation of America), according to the method of McIlwain (7), and extracted with 0.01 M potassium phosphate buffer, pH 7.4. The clear extracts obtained after centrifugation at 15,000 × g are stable when stored at -20°. Dowex-treated enzyme (8) was prepared by a 10 minutes exposure of the extract to 0.1 volume of wet Dowex 1 Cl⁻, with subsequent centrifugation and filtration to free the extract from the resin.

The DL-serine-3-C$^{14}$ was a gift from Professor H. Tarver, University of California, Berkeley; pyridoxal phosphate was synthesized by Dr. E. A. Peterson and Dr. H. A. Sober (9).

Methods—Protein was determined by a turbidimetric method with sulfosalicylic acid, standardized against crystalline bovine serum albumin (10).

Glycine was determined by the method of Alexander et al. (11) in which formaldehyde is produced and assayed colorimetrically. The experimental samples to be analyzed were deproteinized with TCA and added directly into a 25 ml. round bottomed flask containing the phosphate buffer-ninhydrin mixture (3.0 ml.) to which was added enough NaOH to neutralize the TCA present in each sample. Each flask was then attached to a small condenser, six of which were arranged in series on a Kjeldahl heating unit. About one-half of the liquid was distilled over into an ice-chilled receiver containing 1 ml. of water. 2 ml. of water were then added slowly through an inlet above the flask without interrupting the distillation. 2 ml. of the 5 ml. distillate were used to determine the formaldehyde colorimetrically in the presence of chromotropic acid and concentrated H$_2$SO$_4$.

Formaldehyde was determined by the procedure described above for glycine, except that ninhydrin was omitted from the mixture to be distilled.

Methanol-C$^{14}$ was determined by a modification of the method of Widmark (12). Part of a reaction mixture was adjusted to pH 9.0 and distilled. The distillate containing methanol was mixed in a closed vessel with an equal volume of 3.0 n potassium dichromate in 50 per cent H$_2$SO$_4$.
and heated at 55° overnight. CO₂ formed was trapped as BaCO₃ in a center well containing alkali.

Formic acid was determined by Friedemann's method (13).

**EXPERIMENTAL**

After extensive dialysis of cell-free extracts of Clostridium HF, it can be shown that the net production of glycine from serine is stimulated by DPN, Mn⁺⁺, and pyridoxal phosphate (Table I). Blakley has also shown a pyridoxal phosphate requirement for the interconversion of serine and glycine (14). Experimental samples were incubated in stoppered, small diameter test-tubes (10 mm.) under an atmosphere of hydrogen or helium, since aerobic conditions completely inhibit glycine formation. This is presumably partially due to the presence of a powerful DPNH oxidase in these extracts.

It was found that the activity of the system is completely dependent upon orthophosphate (Table II). The phosphate requirement can usually be replaced by arsenate, but for some enzyme preparations this substitution was not possible. Fig. 1 demonstrates the level of phosphate required under experimental conditions similar to those detailed in Table II. Succinate buffer was present in all samples at a concentration of 3 × 10⁻² M.

The pH optimum for the conversion of serine to glycine was found to be 6.5. Glycine production is directly proportional to enzyme and serine

### Table I

**Cofactor Requirements for Conversion of Serine to Glycine**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Omitted</th>
<th>Glycine</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment Ia</td>
<td>µmole</td>
<td>µmole</td>
</tr>
<tr>
<td>1</td>
<td>DPN</td>
<td>0.314</td>
<td>0.420</td>
</tr>
<tr>
<td>2</td>
<td>Mn⁺⁺</td>
<td>0.078</td>
<td>0.166</td>
</tr>
<tr>
<td>3</td>
<td>Pyridoxal phosphate</td>
<td>0.108</td>
<td>0.202</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.134</td>
<td>0.276</td>
</tr>
</tbody>
</table>

The assay mixture contains the following substances in a total volume of 0.6 ml.: 20.0 µmoles of DL-serine, 0.02 µmole of DPN, 2.0 µmoles of MnSO₄, 0.02 µmole of pyridoxal phosphate, and 0.04 ml. of M potassium phosphate buffer, pH 6.5. Experiment Ia had 2.8 mg. and Experiment Ib 3.5 mg. of enzyme protein which had been dialyzed 48 hours against 0.01 M phosphate buffer, pH 7.2. The samples were incubated anaerobically 2 hours at 38°. The control value in the absence of serine (=0.04 µmole) is subtracted from each experimental value given.
CONVERSION OF SERINE TO GLYCINE

ccentration over a fairly wide range (Fig. 2, A and B). It will be shown below that, as might be expected in a crude enzyme system, serine

### TABLE II

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Phosphate and Arsenate Effect</th>
<th>Glycine (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphate</td>
<td>0.394</td>
</tr>
<tr>
<td>2</td>
<td>&quot; + succinate</td>
<td>0.398</td>
</tr>
<tr>
<td>3</td>
<td>Succinate</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>Arsenate + succinate</td>
<td>0.248</td>
</tr>
<tr>
<td>5</td>
<td>&quot; + phosphate</td>
<td>0.316</td>
</tr>
</tbody>
</table>

The assay mixture contains the following in a final volume of 0.5 ml.: 10.0 μmoles of dl-serine, 0.02 μmole of DPN, 1.0 μmole of MnSO₄, 0.02 μmole of pyridoxal phosphate, and an appropriate buffer at pH 6.5. Each sample contained 1.8 mg. of enzyme which had been dialyzed 16 hours against 0.01 M tris(hydroxymethyl)aminomethane buffer, pH 7.4. Potassium salts of phosphate, succinate, and arsenate were all used at a concentration of $3 \times 10^{-3}$ M. Samples were incubated anaerobically 2 hours at 38°C.

**ORTOPHOSPHATE REQUIREMENT**

![Graph showing the dependence of glycine formation on orthophosphate under experimental conditions similar to those detailed in Table II.](http://www.jbc.org/)

The influence of serine concentration on glycine formation was deter-
mined, as a function of time, in the presence of excess serine (11 \mu M) and of limiting serine (4 \mu M). No serine remained in the mixture after 2 hours incubation with limiting serine, as could be shown by paper chromatography. The results are evident in Fig. 3. It can be seen that, when serine is limiting, the glycine level remains almost constant over a period of about 1 hour and then slowly decreases. It can therefore be concluded that glycine produced under these experimental conditions is not readily utilized in subsequent reactions. Experiments were routinely carried out in the presence of a slight excess of serine (upper curve, Fig. 3) and terminated after an incubation period of 2 hours at 38°C.

![GLYCINE FORMATION AS A FUNCTION OF:](image)

**Fig. 2.** The formation of glycine as a function of the concentration of enzyme and serine. The samples were incubated under the conditions described in Table I.

Table III demonstrates that, when the enzyme is treated with Dowex 1 Cl⁻ and subsequently dialyzed, another cofactor in addition to those de-

³ A solvent system developed by Dr. Seymour Korkes for the separation of serine, glycine, and alanine was used. It consists of acetone (80 parts) and triethylamine (5 parts), diluted to 100 with water.

⁴ Since the chemical determination of glycine is interfered with in the presence of high levels of serine, it is necessary, for each enzyme preparation, to determine the minimal level of serine required for a linear reaction rate over the incubation period used. Control experiments were carried out for samples which might contain inhibitory levels of serine. Such samples were prepared in duplicate, and a determined quantity of glycine was added to one sample after incubation and prior to the glycine determination. Thus the per cent inhibition of color yield was determined and the value for the experimental sample corrected accordingly. The only values reported in this paper for which such a control experiment was necessary were the first ones of each curve in Fig. 3. These values were found to have been suppressed about 15 per cent.
The conversion of serine to glycine described above is required (3). This cofactor (Co C) is present in boiled extracts of *C. cylindrosporum* and has been shown to be a folic acid derivative not identical to previously described pteridines (3, 6). The role of Co C in serine metabolism, its purification, and chemical nature will be the subject of future publications.

**Table III**

*Cofactor Requirements of Dowex-Treated Enzyme*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Boiled extract</th>
<th>Glycine (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Present</td>
<td>0.750</td>
</tr>
<tr>
<td>2</td>
<td>Absent</td>
<td>0.060</td>
</tr>
</tbody>
</table>

The assay mixture contained the components of Sample 1 in Table I, except that Dowex-treated enzyme was used and that boiled extract of *C. cylindrosporum* was present in Sample 1.

**Fate of Serine-3-C<sup>14</sup>**—Although the enzyme system has not yet been purified and many compounds other than glycine are formed from serine, preliminary experiments were carried out to determine the fate of the 1-carbon fragment necessarily arising in the course of glycine production from serine. Serine-3-C<sup>14</sup> was incubated with Dowex-treated enzyme for 2 hours at 38° in the presence of phosphate buffer, pH 6.5, enzyme, DPN, Mn<sup>++</sup>, pyridoxal phosphate, and Co C. Alkali was placed in a center well. After incubation, an aliquot was analyzed for glycine content, and the remainder

---

**Fig. 3.** The rate of glycine formation in the presence of limiting serine and excess serine. Experimental conditions are similar to those described in Table I.
was examined to determine the extent of labeling in CO₂, HCHO, CH₃OH, and HCOOH. The results indicated that no C¹⁴O₂ or formaldehyde was produced. 9 per cent of the β-carbon of serine could be accounted for as methanol and 50 per cent as formate (Table IV). The labeling in the acetate-C¹⁴ was determined according to the Schmidt reaction (15), and the other labeled compounds in Table IV were identified by paper chromatography.

**Table IV**

*Fate of β-Carbon of Serine in Dowex-Treated Dialyzed Enzyme System*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Specific activity</th>
<th>Glycine formed</th>
<th>C³H₂OH</th>
<th>HC¹⁴OOH</th>
<th>C³H₃OH</th>
<th>HC¹⁴OOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>serine-3-C¹⁴</td>
<td></td>
<td>c.p.m.</td>
<td>μm</td>
<td>c.p.m.</td>
<td>μm</td>
</tr>
<tr>
<td>1</td>
<td>36,700</td>
<td>0.62</td>
<td>2138</td>
<td>0.058</td>
<td>15,400</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>36,700</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No C¹⁴ was found in CO₂ or HCHO. Other labeled compounds formed from serine-3-C¹⁴ were C¹⁴H₂COOH, alanine C¹⁴, pyruvate C¹⁴, glycolate-C¹⁴, succinate-C¹⁴.

**DISCUSSION**

In the formation of glycine from serine-β-C¹⁴ about 50 per cent of the 1-carbon fragment can be accounted for as formate. Although the formation of serine from glycine and formate has not been studied, there is ample evidence in other systems (e.g. (16)) that formate can serve as a precursor to the β-carbon of serine. The observed formation of alanine-β-C¹⁴ from formate-C¹⁴ discussed in the introduction could be accounted for through the initial formation of serine, which is thereafter further reduced to alanine, or through the formation of an "active" 1-carbon intermediate which, either before or after combination with glycine, serves as a common precursor for alanine and serine.

It has been stated that reduced products, methanol and alanine, also arise from serine-β-C¹⁴. Such results indicate that future studies with more purified enzyme systems may uncover balanced dismutation reactions.⁵

⁵ In recent collaborative experiments carried out by one of the authors (T. C. S.) with Dr. J. Szulmajster, it has been found that an *Escherichia coli* enzyme fraction, capable of synthesizing methionine from serine and homocysteine in the presence of appropriate cofactors (17), also forms labeled formate from serine-3-C¹⁴. This oxidation of the serine 1-carbon fragment to formate occurs concomitantly with its reduction to a methyl group; i.e., during methionine synthesis when homocysteine is present. The close parallelism observed between the amounts of formate-C¹⁴ and...
The Mn\(^{++}\) requirement is interesting to consider in relation to Kisliuk and Sakami's observation that it is needed for formate but not for formaldehyde incorporation into serine (16). Although pyridoxal phosphate has long been implicated in the interconversion of serine and glycine (18), the bacterial system described here and the animal system investigated by Blakley (14) are the first in which direct evidence for this cofactor requirement has been obtained. By analogy with the chemical model system of Metzler, Longenecker, and Snell (19), wherein pyridoxal and a metal catalyze the cleavage of serine to glycine and formaldehyde, it is possible that a formaldehyde derivative may be formed initially in the bacterial system described here. This "active" 1-carbon compound is probably associated with a folate acid derivative (6), and could be either oxidized to formate or reduced to methanol, for example.

Although this paper deals primarily with the formation of glycine as an index of serine metabolism, it has been observed that alanine and at least two other compounds which react with ninhydrin are always detected with glycine on paper chromatograms. The nature of the interdependence of these amino acids in relationship to each other and to the cofactor requirements, particularly the dependence of glycine formation on orthophosphate, awaits clarification.

**SUMMARY**

In cell-free extracts of *Clostridium* HF, serine-\(\beta\)-C\(^{14}\) is converted to glycine and formate-C\(^{14}\). Glycine formation is proportional to the concentration of serine and of enzyme. This reaction is also dependent upon Mn\(^{++}\), DPN, pyridoxal phosphate, orthophosphate, and a factor obtained from boiled extracts of *Clostridium cylindrosporum*.

The authors wish to express their thanks to Miss Amelia Cherkes for her assistance in some phases of this work.

**BIBLIOGRAPHY**


methionine-C\(^{14}\) synthesized in a number of samples suggests that a dismutation reaction may be involved. No free formaldehyde-C\(^{14}\) or methanol-C\(^{14}\) is formed by the *E. coli* preparation in either the presence or the absence of the methyl group acceptor.
THE RÔLE OF POLYGLUTAMYL PTERIDINE COENZYMES IN SERINE METABOLISM: I. COFACTOR REQUIREMENTS IN THE CONVERSION OF SERINE TO GLYCINE
Barbara E. Wright and Thressa C. Stadtman


Access the most updated version of this article at http://www.jbc.org/content/219/2/863.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/219/2/863.citation.full.html#ref-list-1