Glycine Formation During Growth of Pseudomonas AM1 on Methanol and Succinate

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1. The mechanism of regeneration of glycine during the growth of Pseudomonas AM1 on C₁ compounds has been investigated by brief incubation of bacterial suspensions with [2,3-¹⁴C₂]succinate and observing the incorporation of radioactivity into various metabolites. 2. With the wild-type organism growing on methanol, radioactivity appeared rapidly in glycine and tricarboxylic acid-cycle intermediates, but there was a relatively slow labelling of serine and phosphorylated compounds. Serine became labelled predominantly in the C-2 position. 3. The proportion of radioactivity incorporated into glycine at earliest times was greatly diminished when succinate-grown cells were used. 4. Radioactivity was also incorporated from [2,3-¹⁴C₂]succinate into glycine and serine by methanol-grown mutant 20S, which lacks phosphoserine phosphohydrolase. Both the glycine and serine were labelled mainly in C-2. 5. The formation of predominantly [2-¹⁴C]serine from [2,3-¹⁴C₂]succinate in wild-type Pseudomonas AM1, and of [2-¹⁴C]-serine and [2-¹⁴C]glycine in the mutant lacking the phosphorylated pathway from succinate to serine, is taken as strong evidence for a mechanism of glycine regeneration involving cleavage of a C₄ skeleton between C-2 and C-3, rather than by a direct combination of two C₁ units derived from the growth substrate. 6. The cleavage mechanism is quantitatively more significant during growth on methanol than on succinate.

Several lines of evidence (see Harder & Quayle, 1971b) have led to the suggestion that glycolytic intermediates are formed during growth of Pseudomonas AM1 on C₁ compounds by a pathway (the serine pathway) involving the following steps:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁ unit + cofactors + H₄folate → 5,10-CH₂-H₄folate</td>
<td>(1)</td>
</tr>
<tr>
<td>5,10-CH₂-H₄folate + glycine → H₄folate + serine</td>
<td>(2)</td>
</tr>
<tr>
<td>Serine + glyoxylate → hydroxypyruvate + glycine</td>
<td>(3)</td>
</tr>
<tr>
<td>Hydroxypyruvate + NADH + H⁺ → d-glycerate + NAD⁺</td>
<td>(4)</td>
</tr>
<tr>
<td>d-Glycerate + ATP → phosphoglycerate + ADP</td>
<td>(5)</td>
</tr>
</tbody>
</table>

The serine pathway uses one molecule of glyoxylate to produce each molecule of phosphoglycerate. The mechanism of net synthesis of glyoxylate from C₁ units remains unknown. It is likely to happen by one of two general ways (Large et al., 1962a): (a) direct condensation of two C₁ units, which the radioisotopic data would suggest to be a reduced C₁ compound and carbon dioxide, or (b) extension of the carbon skeleton of phosphoglycerate by at least one carbon atom followed by cleavage to give glyoxylate (or a precursor) and a residual multicarbon molecule.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoglycerate + NAD⁺ → phosphohydroxypropyuvate + NADH + H⁺</td>
<td>(6)</td>
</tr>
<tr>
<td>Phosphohydroxypropyuvate + L-glutamate → O-L-phosphoserine + α-oxoglutarate</td>
<td>(7)</td>
</tr>
<tr>
<td>O-L-Phosphoserine + H₂O → serine + P₁</td>
<td>(8)</td>
</tr>
<tr>
<td>Serine + H₄folate → glycine + 5,10-CH₂-H₄folate</td>
<td>(9)</td>
</tr>
</tbody>
</table>
The multicarbon molecule would represent the net biosynthetic product of the overall cycle of C1 fixation. An obvious possibility for alternative (b) is carboxylation of phosphoenolpyruvate via phosphoenolpyruvate carboxylase (Large et al., 1962b) followed by cleavage of the resulting oxaloacetate, or a derivative, to two C2 fragments.

All attempts so far to demonstrate the generation of glyoxylate or its precursor in cell-free extracts by either mechanism have been unsuccessful and, similarly, the results obtained from work with whole cells, including mutants, have not enabled a choice to be made between the two general types of mechanism. This paper records an approach to the problem through a study of the metabolism of [14C]succinate by Pseudomonas AM1 and a mutant, 20S, lacking phosphoserine phosphohydrolase (reaction 8). The study was designed to test out three specific predictions, which may be made on the basis of regeneration of glyoxylate by cleavage of a C4 compound identical with or derived from succinate.

The predictions, based on the assumption that glyoxylate leads directly to glycine (reaction 3), are: (a) exposure of cells growing on methanol to [14C]succinate should result in early labelling of glycine, which should precede that of phosphorylated compounds and serine; (b) early labelling of glycine from [14C]succinate should be more pronounced in cells growing on methanol than in cells growing on succinate; (c) [2,3-14C2]succinate administered to cells growing on methanol should lead to glycine and serine labelled predominantly in C-2.

Materials and Methods

Maintenance and growth of the organism

Pseudomonas AM1 was maintained in slope culture on an inorganic salts medium (Jayasuriya, 1955) containing methymelamine hydrochloride (50mm) and agar (1.5%, w/v). The organism was subcultured monthly and stored at 4°C after incubation for 3-4 days at 30°C. Mutant 20S was maintained similarly on the above medium containing sodium succinate (50mm) and L-serine (1mm) in place of methymelamine hydrochloride.

The organisms were grown in liquid culture as described by Heptinstall & Quayle (1970) with methanol (0.5, v/v) as carbon source. The wild-type organism was also grown on sodium succinate (50mm). Growth was assessed by measuring the extinction at 650nm.

Incubation of cells with [14C]succinate

 Cultures of bacteria growing on methanol were harvested during exponential growth by centrifuging at room temperature and resuspended at a density of 6mg dry wt. of bacteria/ml in a solution containing 10mm-sodium phosphate buffer, pH 7.0, 10mm-NH4Cl and 100mm-methanol. For succinate-grown cells, methanol was replaced by sodium succinate (10mm) in the resuspension medium. The suspension was aerated vigorously in a cylindrical vessel (10cm x 4.9cm2) fitted with a sintered-glass base. After equilibration for 5 min at 30°C, 1 ml of a solution of sodium [2,3-14C2]succinate (73μmol; 100μCi) was added and samples (approx. 1ml) were rapidly removed and transferred to boiling aq. 95% (v/v) ethanol (3ml) by means of a hypodermic syringe. After cooling, cell debris and precipitated protein were removed by centrifugation (5000g; 10min) and washed twice with 0.5ml of cold aq. 20% (v/v) ethanol. The combined supernatant fluids were evaporated to dryness under reduced pressure at 40°C and residues redissolved in 0.2ml of aq. 20% (v/v) ethanol for chromatography.

For each experiment, a corresponding control incubation was performed at one-fifth of the scale, with cells that had been previously heated at 100°C for 5min.

Chromatography and identification of labelled compounds

Samples of the final ethanolic extracts were chromatographed in two dimensions on Whatman no. 4 paper (18½ in x 22in) in descending fashion, with, as solvents, phenol-formic acid (90%, w/v)-water (500:13:167, w/v/v) (Kornberg, 1958) and butan-1-ol-propionic acid-water (47:22:31, by vol.) (Benson et al., 1950). The radioactive compounds were located by radioautography (Large et al., 1961) and the radioactivity was measured directly with a mica end-window Geiger-Müller tube (type 2B2) coupled with a scaler (type D657; Panax Equipment Ltd., Redhill, Surrey, U.K.). The observed values were corrected for background radioactivity and for counts appearing in corresponding positions in chromatograms from the control incubations with boiled cell suspensions.

After elution from the paper with water, radioactive materials were identified by co-chromatography or co-electrophoresis with authentic samples of suspected compounds. Carboxylic acids were identified chromatographically on Whatman no. 1 paper either with the original solvents or with ethanol-aq. NH3 (sp. gr. 0.88)-water (80:4:16, by vol.; Long et al., 1951). Alternatively, satisfactory separation of carboxylic acids was achieved by t.l.c. on cellulose (average thickness 100μm) as described by Myers & Huang (1969). Carrier acids were detected by spraying with a solution of Bromphenol Blue in ethanol (0.1%, w/v). Identification of amino acids was achieved by one-dimensional co-electrophoresis on Whatman no. 3MM paper with authentic samples at 40 V/cm on a flat-plate apparatus (Savant Instruments 1972).
The ethanol-soluble extract after incubation of cell suspensions with [2,3,4-14C]succinate was evaporated to dryness at 40°C under reduced pressure. The extract was dissolved in 1 ml of reduced pressure water, and the solution applied to a column of Dowex 50 (X8; 100-200 mesh) in the (20:0.8:279, by vol.) form, pH 6.4. After washing with 3 ml of water, the column was eluted with 45 ml of formic acid-water (40:30:20:10, by vol.). The latter buffer system was described by Benson et al. (1959).

The formic acid-water (20:0.8:279, by vol.) was added to the crude extracts of Pseudomonas AM1 incubated with [2,3,4-14C]succinate. The radioactivity in the formic acid-water (20:0.8:279, by vol.) was added to the crude extracts of Pseudomonas AM1 incubated with [2,3,4-14C]succinate. The radioactivity in the formic acid-water (20:0.8:279, by vol.) was added to the crude extracts of Pseudomonas AM1 incubated with [2,3,4-14C]succinate.

Table 1. Distribution of 14C among the non-volatile labelled components of ethanol extracts of methanol-grown wild-type Pseudomonas AM1 incubated with [2,3,4-14C]succinate

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Malate</th>
<th>Fumarate</th>
<th>Glycine</th>
<th>Serine</th>
<th>Glutamate</th>
<th>Aspartate</th>
<th>Citrate</th>
<th>Trehalose</th>
<th>Alanine</th>
<th>Phosphorylated compounds</th>
<th>Other compounds</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1635</td>
<td>632</td>
<td>493</td>
<td>107</td>
<td>156</td>
<td>768</td>
<td>567</td>
<td>0</td>
<td>0</td>
<td>928</td>
<td>0</td>
<td>5286</td>
</tr>
<tr>
<td>20</td>
<td>3843</td>
<td>128</td>
<td>1162</td>
<td>481</td>
<td>460</td>
<td>1443</td>
<td>706</td>
<td>180</td>
<td>1220</td>
<td>1431</td>
<td>2355</td>
<td>13409</td>
</tr>
<tr>
<td>37</td>
<td>4732</td>
<td>0</td>
<td>1251</td>
<td>514</td>
<td>1232</td>
<td>2459</td>
<td>1583</td>
<td>690</td>
<td>2012</td>
<td>4001</td>
<td>3582</td>
<td>22056</td>
</tr>
<tr>
<td>61</td>
<td>7174</td>
<td>0</td>
<td>1060</td>
<td>468</td>
<td>2608</td>
<td>2495</td>
<td>1879</td>
<td>1588</td>
<td>3192</td>
<td>6030</td>
<td>4526</td>
<td>31020</td>
</tr>
<tr>
<td>85</td>
<td>5009</td>
<td>0</td>
<td>1038</td>
<td>419</td>
<td>4560</td>
<td>2377</td>
<td>1900</td>
<td>3389</td>
<td>3975</td>
<td>9349</td>
<td>5077</td>
<td>37093</td>
</tr>
<tr>
<td>126</td>
<td>5095</td>
<td>0</td>
<td>1352</td>
<td>497</td>
<td>7320</td>
<td>2821</td>
<td>2992</td>
<td>7910</td>
<td>4993</td>
<td>12807</td>
<td>7101</td>
<td>52888</td>
</tr>
<tr>
<td>207</td>
<td>4219</td>
<td>0</td>
<td>1180</td>
<td>743</td>
<td>15930</td>
<td>2296</td>
<td>3587</td>
<td>16231</td>
<td>8655</td>
<td>20010</td>
<td>9623</td>
<td>82474</td>
</tr>
</tbody>
</table>
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Fig. 1. Variation with time of the percentage distribution of $^{14}$C incorporated from [2,3-$^{14}$C$_2$]succinate into (a) amino acids and (b) other constituents of the ethanol-soluble fraction of wild-type Pseudomonas AM1 growing on methanol.

o, Glutamate; ▼, alanine; □, aspartate; ▼, glycine; ●, serine; □, malate; △, trehalose; ■, citrate; ◇, phosphorylated compounds.

Table 2. Distribution of $^{14}$C in serine derived from wild-type Pseudomonas AM1 growing on methanol in the presence of sodium [2,3-$^{14}$C$_2$]succinate

Sodium [2,3-$^{14}$C$_2$]succinate (73μmol; 100μCi) was added at zero time to an aerated suspension of bacteria (60mg dry wt.) in 9ml of 10mM-sodium phosphate buffer, pH7.0, containing 10mM-NH$_4$Cl and 100mM-methanol. After incubation for 90s, the suspension was poured into 40ml of boiling aq. 95% (v/v) ethanol. The isolation and degradation of radioactive serine are described in the Materials and Methods section.

<table>
<thead>
<tr>
<th>Carbon atom no.</th>
<th>Percentage yield of CO$_2$ obtained from oxidation of each C atom</th>
<th>Percentage of total radioactivity in molecule contributed by each C atom</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$H</td>
<td>94</td>
<td>17.6</td>
</tr>
<tr>
<td>CH$_2$NH$_2$</td>
<td>64</td>
<td>75.6</td>
</tr>
<tr>
<td>CH$_2$OH</td>
<td>69</td>
<td>6.8</td>
</tr>
</tbody>
</table>
precipitation with barium chloride (5%, w/v) and then plated in triplicate on filter-paper discs (2.69 cm²) as detailed by Sakami (1955). Weighed samples of barium [¹⁴C]carbonate were suspended in 7 ml of NE213 liquid scintillator containing Cab-O-Sil (4%, w/v) (both from Nuclear Enterprises Ltd., Sighthill, Edinburgh, U.K.) as described by Kemp & Quayle (1967) and radioactivity was measured with a liquid scintillation counter (Nuclear Chicago Corp., Des Plaines, Ill., U.S.A.). Quench corrections were applied by the channels-ratio method.

Serine and glycine were degraded as described by Large et al. (1962a).

Assay of phosphoserine phosphohydrolase (EC 3.1.3.3)

Measurement of this enzyme was used to confirm the phenotype of mutant 20S of Pseudomonas AM1. The enzyme was assayed in ultrasonic extracts, by measuring the Pi liberated from dl-O-phosphoserine, by the procedure of Heptinstall & Quayle (1970).

Chemicals

[2,3-¹⁴C₂]Succinic acid was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.) and neutralized with sodium hydroxide before use. dl-O-Phosphoserine was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.) and neutralized with sodium hydroxide before use.

Results

Incorporation of [2,3-¹⁴C₂]succinate by wild-type Pseudomonas AM1 growing on methanol or succinate

A culture of wild-type Pseudomonas AM1, containing 32 mg dry wt. of organisms growing exponentially on methanol, was centrifuged and suspended in 5.5 ml of resuspension medium containing 10 mM-sodium phosphate buffer, pH 7.0, 10 mM-NH₄Cl and 100 mM-methanol. The suspension was incubated at 30°C with sodium [2,3-¹⁴C₂]succinate (73 μmol; 100 μCi) as described in the Materials and Methods section. The distribution of radioactivity among non-volatile compounds formed during the first 2 min after addition of labelled succinate is recorded in Table 1. Curves showing the percentage of total radioactivity incorporated into individual metabolites expressed as a function of time (Fig. 1) suggest that malate, fumarate, aspartate, citrate and glycine were early-labelled compounds. The initial positive slopes for glutamate, phosphorylated compounds and serine indicate less-rapid incorporation of radioactivity from labelled succinate into these metabolites.

The finding of a negative slope to the curve for glycine, together with its high percentage of radioactivity (9%) in the earliest sample, implies a direct

Table 3. Distribution of ¹⁴C among the non-volatile labelled compounds of methanol-grown mutant 20S of Pseudomonas AM1 after addition of labelled [2,3-¹⁴C₂]succinate and treated as described in Table 1.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Malate</th>
<th>Glycine</th>
<th>Serine</th>
<th>Glutamate</th>
<th>Aspartate</th>
<th>Citrate</th>
<th>Trehalose</th>
<th>Alanine</th>
<th>Compound Phosphorylated</th>
<th>Radioactivity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4495</td>
<td>79</td>
<td>34</td>
<td>25</td>
<td>25</td>
<td>533</td>
<td>185</td>
<td>0</td>
<td>6</td>
<td>4439</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>2769</td>
<td>196</td>
<td>95</td>
<td>46</td>
<td>515</td>
<td>199</td>
<td>46</td>
<td>100</td>
<td>8500</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>3370</td>
<td>231</td>
<td>97</td>
<td>48</td>
<td>944</td>
<td>228</td>
<td>108</td>
<td>150</td>
<td>2482</td>
</tr>
<tr>
<td>6</td>
<td>30.5</td>
<td>2391</td>
<td>333</td>
<td>71</td>
<td>53</td>
<td>916</td>
<td>239</td>
<td>108</td>
<td>175</td>
<td>64</td>
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<tr>
<td>8</td>
<td>64</td>
<td>2799</td>
<td>278</td>
<td>106</td>
<td>71</td>
<td>749</td>
<td>248</td>
<td>106</td>
<td>187</td>
<td>114.5</td>
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<tr>
<td>10</td>
<td>114.5</td>
<td>4439</td>
<td>473</td>
<td>149</td>
<td>3251</td>
<td>938</td>
<td>4513</td>
<td>149</td>
<td>2046</td>
<td>938</td>
</tr>
</tbody>
</table>

A suspension of mutant 20S (32 mg dry wt. of organisms) grown on methanol (0.5%, w/v) was incubated with sodium [2,3-¹⁴C₂]succinate and treated as described in Table 1.
pathway for its formation from succinate, not involving the phosphorylated pathway. To determine whether these results were peculiar to the methanol-grown organism, the experiment was repeated with cells grown on 50 mM-sodium succinate, the methanol in the resuspension medium being replaced by 10 mM-sodium succinate. Under these conditions radioisotope appeared predominantly in tricarboxylic acid-cycle intermediates and derived amino acids such as aspartate and glutamate, and a much smaller percentage of radioactivity appeared in glycine (<2%) than with the methanol-grown organism. Furthermore, labelled serine did not appear in succinate-grown cells until very much later than with methanol-grown cells, and even then in negligible amounts.

It is inferred from these results that glycine can arise from succinate by a route not involving the phosphorylated pathway, and that this pathway is of greater quantitative importance during growth on methanol than on succinate.

Labelling pattern of serine formed from [2,3-14C2]succinate during growth of wild-type Pseudomonas AM1 on methanol

The labelling pattern of serine formed from [2,3-14C2]succinate during short-term incubation with methanol-grown cells was determined. If the serine were formed predominantly by hydroxymethylation of glycine derived from a C4-cleavage reaction, it should be labelled predominantly in C-2. If, however, serine arose from [2,3-14C2]succinate mainly by the pathway involving phosphorylated C3 intermediates, it would be expected to be equally labelled in the C-2 and C-3 positions.

The labelling pattern of serine obtained from growing cells incubated for 90s with sodium [2,3-14C2] succinate is shown in Table 2. That 75.6% of the total radioactivity in the serine molecule occurs in C-2, whereas C-3 contains only 6.8%, is in accordance with a cleavage of the succinate carbon skeleton giving rise to [2-14C] glycine and hydroxymethylation.

Fig. 2. Variation with time of the percentage distribution of 14C incorporated from [2,3-14C2] succinate into (a) amino acids and (b) other constituents of the ethanol-soluble fraction of mutant 20S of Pseudomonas AM1 growing on methanol

○, glutamate; ▼, alanine; □, aspartate; ▽, glycine; ●, serine; □, malate; △, trehalose; ■, citrate; △, phosphorylated compounds; ×, compound H.
of the latter by a C₃ unit obtained from unlabelled methanol.

The results might, however, be explained by the formation of [2,3-¹⁴C]serine from the labelled succinate via the phosphorylated pathway, followed by an exchange reaction of C-3 of serine with a C₃ unit derived from unlabelled methanol. The availability of a mutant, 20S, deficient in phosphoserine phosphohydrolase (Harder & Quayle, 1971a), and which therefore lacks a functional phosphorylated pathway from phosphoglycerate to serine, permitted this possibility to be tested as follows.

**Brief incubation of mutant 20S with [2,3-¹⁴C₂]succinate**

A culture of mutant 20S of *Pseudomonas AM1* growing exponentially on methanol was harvested and its phenotype confirmed by assay of phosphoserine phosphohydrolase: no detectable P₃ was liberated when ultrasonic extracts were incubated with either DL-O-phosphoserine or 3-phosphoglycerate. The mutant was resuspended in aerated buffered medium containing methanol and the incorporation of radioactivity into non-volatile metabolites immediately after the introduction of [2,3-¹⁴C₂]succinate was determined (Table 3). The pattern of curves tracing the incorporation of radioactivity into each metabolite (Fig. 2) showed differences from the corresponding results with the wild-type organism (Fig. 1). The most striking difference was the appearance of an unknown compound H, which contained 36% of the fixed radioactivity at the earliest time of sampling and whose percentage curve fell steeply with time. Since this compound was not observed with the wild-type organism, its appearance is presumably connected with the lack of a complete phosphorylated pathway from phosphoglycerate to serine in mutant 20S. Compound H has Rₜ values 0.48–0.50 and 0.52–0.57 in the aq. phenol-formic acid and butanol-propionic acid solvents respectively and it is distinguishable chromatographically from fumarate, glycollate and phosphorylated compounds. Preliminary tests indicate that compound H is neither an amino acid nor a carbonyl compound, since its chromatographic properties are not altered after heating with a solution of ninhydrin and attempts to form a 2,4-dinitrophenylhydrazone have been unsuccessful. Another difference between the results obtained with mutant 20S and the wild-type organism was that glycine and serine did not contain as large a percentage of the total radioactivity in the earliest samples. Possible causes of this are suggested in the Discussion section. Nevertheless, these amino acids did become sufficiently labelled to permit determination of the distribution of radioactivity between the carbon atoms of each compound (Table 4).

The results of the degradation clearly show that both the glycine and serine produced during incubation...
tion of methanol-grown mutant 20S with [2,3-14C2]-
succinate are labelled predominantly in C-2. Since
this mutant lacks phosphoserine phosphohydrolase,
glycine and serine must have been formed by a route
other than the known pathway involving phosphoryl-
ated C3 intermediates. Moreover, the labelling
patterns of glycine and serine are consistent with a
cleavage of the succinate carbon skeleton between
C-2 and C-3 to give C2 products, at least one of which
is convertible into glycine.

Discussion

The three specific predictions, which were based at
the outset on the assumption of regeneration of the
glycine skeleton by cleavage of the succinate carbon
skeleton into two C2 compounds, have been verified.
The possibility that the results could be explained
either by reversal of the serine pathway, or by
operation of the phosphorylated pathway, followed
by exchange of the C-3 atom of serine with C1 units
derived from methanol, is unlikely for the following
reasons.

(a) The labelling patterns of serine and malate
resulting from incorporation of [14C]methanol and
[14C]bicarbonate into methanol-grown Pseudomonas
AM1 are consistent with the formation of malate by
carboxylation of a C3 fragment derived from serine,
rather than the reverse (Large et al., 1962a).

(b) Reversal of the serine pathway would necessitate
conversion of phosphoglycerate into glyceraldehyde. It is
most unlikely that reaction (5) is appreciably re-
versible and no evidence could be found for release of
Pi from phosphoglycerate in ultrasonic extracts of
Pseudomonas AM1.

(c) The labelling patterns of serine and glycine
obtained from mutant 20S incubated with [2,3-14C2]-
succinate appear to eliminate a major involvement of the
phosphorylated pathway in supplying the carbon
skeletons of these amino acids. However, a role for the
phosphorylated pathway during growth on C3
compounds has been suggested previously by Harder
& Quayle (1971a). They observed that although
mutant 20S was able to grow on methanol alone, its
growth rate was lower than that of the wild-type
organism. The addition of serine (1 mM) increased the
growth rate of the mutant, whereas that of the wild-type
organism and of revertants of mutant 20S
to wild type was unaffected. The function of the
phosphorylated pathway during growth of wild-type
Pseudomonas AM1 on C3 compounds was suggested to be
net replenishment of amino groups necessary for
the cyclic formation of glycine and serine with
serine-glyoxylate aminotransferase, when these
amino acids are withdrawn for macromolecular
synthesis (Harder & Quayle, 1971b). A consequence of
the lesion in mutant 20S may therefore be a short-
age of amino groups normally provided by the
phosphorylated pathway for net synthesis of glycine
and serine, and decreased intracellular pools of these
amino acids may result. Indeed, this could be reflected in the early appearance of a considerable
proportion of radioactivity incorporated from
[2,3-14C2]succinate in compound H, especially if, for
example, compound H is itself a precursor or is in
equilibrium with a precursor of glyoxylate and
glycine. These factors would be expected to contribute
greatly to the observed differences in kinetic data on
the incorporation of radioactivity into serine and
glycine by mutant 20S (Fig. 2) when compared with
the wild-type organism (Fig. 1).

Although the results with whole cell suggest a
cleavage reaction as the source of the glycine skeleton,
no such reaction has yet been demonstrated in cell-
free extracts. Of the possible C3 products, glyoxylate,
glycollate and acetate have each been shown to
satisfy the requirement for C3 compounds by
mutants of Pseudomonas AM1 unable to grow on
methanol unless supplied with substrate quantities
of C2 compounds (Anthony et al., 1971b; Salem
& Quayle, 1971). If acetate, or a derivative, is a cleavage
product, then the postulated oxidation of acetate to
glycollate, which has been implicated in the growth
of Pseudomonas AM1 on ethanol (Anthony et al.,
1971a), would form an essential part of the pathway
of glycine regeneration during growth on C1
compounds.

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