The Degradation of Glycine by *Pseudomonas aeruginosa*

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The breakdown of glycine by washed cells of *Proteus* and *Pseudomonas aeruginosa* has been described by Bernheim, Bernheim & Webster (1935). Janke & Tayenthal (1937) found that glyoxylic acid was an intermediate in the oxidation of glycine. It has been established that formaldehyde is an intermediate in the oxidation of glycine by a species of *Achromobacter* (Paretsky & Werkman, 1950). Campbell (1955) studied the degradation of glycine by cell-free preparations of a pseudomonas isolated from mud. He found that this amino acid was completely oxidized via glyoxylic and formic acids.

The present work deals with the mechanism of glycine degradation by washed and dried cells of *Ps. aeruginosa*.

MATERIALS AND METHODS

Organism. A laboratory strain of *Ps. aeruginosa* strain 18 was grown in the following medium: glycine 5 g., brain–heart infusion (Difco) 37 g., Bacto agar 15 g., water 1 l.; final pH 7·4. After incubation at 37° for 20 hr., the cells were harvested, washed three times with 0·85% NaCl solution and kept at −5° until used.

Reagents. Glycine, methylamine hydrochloride and potassium hypophosphite were products of British Drug Houses Ltd. (Poole, England). Glyoxylic acid was obtained from L. Light and Co. Ltd. (Colnbrook, Bucks, England). Sarcosine hydrochloride, betaine hydrochloride, hippuric acid and pyridoxal hydrochloride were products of Nutritional Biochemical Corp. (Cleveland 28, Ohio, U.S.A.). α-Oxoglutaric acid was obtained from Hopkin and Williams (Chadwell Heath, Essex, England). Glutathione and adenosine triphosphate (ATP) were from Roche (Basle, Switzerland).

Analytical methods. Glycine and other amino acids were identified by paper chromatography with ethanol–ammonia (95 ml. of 95% ethanol: 5 ml. of concn. aq. NH_3) as solvent. Paper chromatography was also used for the quantitative estimation of amino acids (Giri, Radhakrishnan & Vaidyanathan, 1952). Glyoxylate was identified by conversion into the 2:4-dinitrophenylhydrazone, which was then separated by paper chromatography as described by Block, Durrum & Zweig (1955). Glyoxylate was quantitatively estimated by the colorimetric method of Franke, Taha & Krieg (1952). Citric acid was estimated by the colorimetric method of Taylor (1953). Oxygen uptake and carbon dioxide evolution were determined by the conventional Warburg manometric technique. Ammonia was determined by the method described by Markham (1942).

Enzyme preparation. Dried cell preparations were obtained by drying the cells over phosphorus pentoxide at 0·3 mm. Hg for approx. 5 hr. Cell-free extracts were prepared by subjecting cell suspensions to the action of a 9 kc. Raytheon sonic oscillator for 20 min., and centrifuging for 20 min. at 11 000 rev./min. in a High Speed Angle Centrifuge (Measuring and Scientific Equipment Ltd. London).

RESULTS

Degradation of glycine by washed cell suspensions

Glycine was oxidized by washed cells of *Ps. aeruginosa* 18, the optimum pH being 7·8 (Fig. 1). The oxidation of glycine was complete, and 2·0 moles of carbon dioxide were produced and 1·5 moles of oxygen taken up for each mole of glycine which disappeared. Ammonia (1 mole) was liberated at the same time (Table 1). Glyoxylic acid was also completely oxidized by cells grown on glycine. Betaine and sarcosine, which are methylated glycine derivatives, were oxidized after a lag period by cells grown on glycine, but methylamine and hippuric acid were not oxidized (Table 1).

Degradation of glycine by dried cells and cell-free preparations

Formation of glyoxylate. Cell-free preparations failed to oxidize glycine, and dried cells oxidized it to a small extent only. The oxidation of glutamate

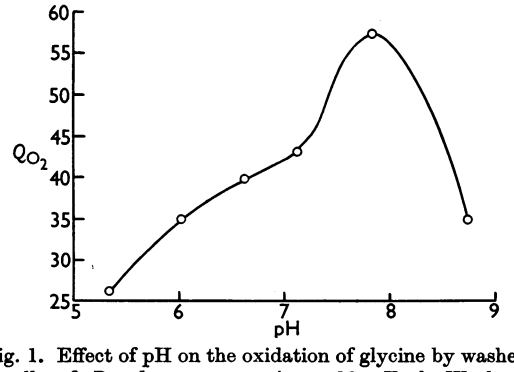

Fig. 1. Effect of pH on the oxidation of glycine by washed cells of *Pseudomonas aeruginosa* 18. Each Warburg vessel contained 5 μmoles of glycine, 1·0 ml. of cell suspension (approx. 10 mg. dry wt.) and 1·0 ml. of tris buffer. In the centre well was 0·2 ml. of 10% (w/v) KOH; total vol. 3·2 ml. Incubation was carried out in air at 30°.

Table 1. *Oxidation of glycine and related compounds by washed cells of* Pseudomonas aeruginosa 18

Each Warburg vessel contained 0·5 ml. of substrate, 1·0 ml. of cell suspension (approx. 10 mg. dry wt.) and 1·0 ml. of 0·2 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·9. In O_2 uptake experiments, 0·2 ml. of 10% (w/v) KOH was in the centre well; in CO_2 evolution experiments, 0·2 ml. of 2 N-HCl was tipped in from the side arm, and 0·2 ml. of water was in the centre well; total vol., 3·2 ml. Incubation was carried out in air at 30° for 90 min. with glycine and glyoxylate and for 240 min. with other substrates. Values have been corrected for endogenous activity. All results are expressed as μmoles.

Amount of substrate	O_2 uptake	CO_2 evolution	NH_3 formation
Glycine 2·8	4·25	5·6	2·85
Glyoxylic acid 4·0	3·85	8·1	0
Betaine hydrochloride 5·0	4·65	—	—
Sarcosine hydrochloride 5·0	3·30	—	—
Hippuric acid 5·0	0	0	0
Methylamine hydrochloride 5·0	0	0	0

Table 2. *Transamination of glycine by preparations of* Pseudomonas aeruginosa 18

Each Warburg vessel contained 20 μmoles of glycine, 20 μmoles of α-oxoglutarate, 20 μg. of pyridoxal hydrochloride, 1·0 μmole of ATP, 1·0 ml. of 0·067 M-phosphate buffer, pH 7·8, and 1·0 ml. of suspension of dried cells (40 mg.) or 1·0 ml. of cell-free preparation. Total vol., 2·6 ml. Incubated for 60 min. in H_2 at 30°. Recovery is 100 × total amount of amino acids (μmoles) at the end of experiment/initial amount of glycine (20 μmoles).

	Glycine left (μmoles)	Glutamic acid formed (μmoles)	Recovery (%)
Dried cells	10·8	9·8	103
Cell-free preparation	16·2	4·0	101

Table 3. *Disappearance of glyoxylic acid incubated with dried cells of* Pseudomonas aeruginosa 18

Each flask contained 10 μmoles of glyoxylic acid, 50 mg. of dried cells, 1·0 ml. of tris buffer, pH 7·9, and 50 μmoles of succinic acid when present. The cofactors glutathione (10 μmoles) and $MgSO_4$ (10 μmoles) were added as indicated. Total vol., 4·9 ml. Atmosphere, air, temp. 30°; values are expressed as the percentage of glyoxylic acid disappearing on incubation with glyoxylic acid alone or with the mixtures indicated.

Time of incubation (min.)	Glyoxylic acid only	Glyoxylic and succinic acids	Glyoxylic and succinic acids and cofactors
60	39	54	70
150	59	76	90
180	65	83	94

by dried cells was faster. The addition of α-oxoglutarate, pyridoxal hydrochloride and ATP enhanced the disappearance of glycine; glutamate was formed even under anaerobic conditions (Table 2). The glyoxylate formed was identified by paper chromatography. The reverse reaction, i.e. the formation of glycine from glyoxylate and glutamate by dried cells or cell-free preparations of *Ps. aeruginosa* 18, was also demonstrated.

Oxidation of glyoxylate. As shown in Table 1, glyoxylate was completely oxidized by washed cells of *Ps. aeruginosa* 18. Dried cells oxidized glyoxylate slowly. The oxidation of glyoxylate by cell-free extracts was very slow, whereas succinate and citrate were oxidized at a faster rate. Glyoxylate, when incubated with dried or washed cells under anaerobic conditions or in the presence of cyanide, was partly metabolized. This observation suggested the presence of a mechanism other than direct oxidation for the metabolism of glyoxylate.

It may be seen from Table 3 that the disappearance of glyoxylic acid was markedly enhanced by the addition of succinate, Mg^{2+} and glutathione. Citrate was formed when the experiment was carried out under anaerobic conditions. The reverse reaction, i.e. the formation of glyoxylate from citrate, was also demonstrated.

The oxidation of glycine by dried cells was also enhanced in the presence of Mg^{2+} ions and glutathione.

Effect of various factors on the oxidation of glycine by washed cells

*iso*Nicotinic acid hydrazide (isoniazid) reacts with pyridoxal phosphate and may therefore be used to detect the participation of this coenzyme in biological systems (Davison, 1956). Table 4 shows that isoniazid added to washed cells of *Ps. aeruginosa* 18 inhibited the degradation of glycine as measured by oxygen uptake.

The use of 'aged' cells presents another possibility for demonstrating the participation of pyridoxal in the oxidation of glycine. Table 4 shows that the activity of old cells was low. Some reactivation was obtained by the addition of ATP and pyridoxal hydrochloride.

Iodoacetate inhibited the oxidation of glycine (Table 4). The addition of potassium hypophosphite had no effect on the oxidation of glycine or glyoxylate by washed cells of *Ps. aeruginosa* 18.

Pre-incubation of the cells with potassium hypophosphite had no effect. On the other hand, the oxidation of formate was inhibited under similar conditions.

Table 4. *Effect of various factors on the oxidation of glycine by washed cells of* Pseudomonas aeruginosa 18

Each Warburg vessel contained 5 μmoles of glycine; cells for 'aging' were kept at 0° for 15 days; other conditions as given in Table 1.

	O_2 uptake (μl./90 min.)	Inhibition or activation (%)
Glycine	103	—
Glycine and isoniazid (300 μg./ml.)	42	−59
Glycine	93	—
Glycine and iodoacetate (5 mM)	54	−42
Glycine	93	—
Glycine and potassium hypophosphite (5 mM)	90	−3
Glycine ('aged' cells)	27	—
Glycine and ATP (2 μmoles) and pyridoxal hydrochloride (60 μg.) ('aged' cells)	43	+60

DISCUSSION

Glycine is oxidized to glyoxylate in animal tissues by glycine oxidase (Ratner, Nocito & Green, 1944). This enzyme has a low activity and its function in biological systems has been doubted (cf. Greenberg, 1954). The conversion of glycine into glyoxylate by bacteria has been demonstrated by Janke & Tayenthal (1937). Stumpf & Green (1944) found that cells of *Proteus vulgaris* lost their ability to oxidize glycine after being kept at 0° for 14 days; they could not obtain a cell-free preparation capable of oxidizing glycine.

The results presented here show that the formation of glyoxylate from glycine by dried cells and by cell-free preparations is due to a transamination process. It is suggested that transamination, a process which requires pyridoxal phosphate, is the only mechanism for the degradation of glycine by *Ps. aeruginosa* 18. This view is supported by the fact that the oxidation of glycine by washed cells was inhibited by isoniazid, which is known to inactivate pyridoxal phosphate (Davison, 1956). 'Aged' cells which had lost most of their activity were somewhat reactivated by the addition of pyridoxal hydrochloride and ATP, which presumably formed the coenzyme pyridoxal phosphate (Gunsalus, Bellamy & Umbreit, 1944).

According to Campbell (1955) glyoxylate is oxidized to formate. The mechanism proposed by him did not operate with *Ps. aeruginosa* 18, since potassium hypophosphite, which is known to inhibit the oxidation of formate, had no effect on the oxidation of glycine, and iodoacetate was inhibitory. In our experiments, when glyoxylate was metabolized under anaerobic conditions, citrate and not formate was produced. It seems therefore that the *iso*citratase pathway which is known to occur with *Ps. aeruginosa* was involved. The enhancing effect of Mg^{2+} ions, glutathione and succinate corroborates this suggestion. Under aerobic conditions citrate was further oxidized.

The methylated derivatives of glycine were metabolized by washed cells of *Ps. aeruginosa* 18, whereas methylamine and hippuric acid were not oxidized.

SUMMARY

1. Glycine and glyoxylic acid were completely oxidized by washed cells of *Pseudomonas aeruginosa* strain 18.

2. Isoniazid inhibited the oxidation of glycine. The low activity of 'aged' cells was increased by the addition of adenosine triphosphate and pyridoxal.

3. Glycine was metabolized very slowly by dried cells and not at all by cell-free preparations. In the presence of α-oxoglutaric acid, adenosine triphosphate and pyridoxal, glycine was readily converted into glyoxylic acid.

4. Glyoxylic acid was metabolized by cell-free preparations or by dried cells in the presence of succinic acid, Mg^{2+} ions and glutathione.

5. It is suggested that glycine is converted into glyoxylic acid by transamination, and that the glyoxylic acid formed is further metabolized by the *iso*citratase pathway.

REFERENCES

Bernheim, F., Bernheim, M. L. C. & Webster, M. D. (1935). *J. biol. Chem.* **110**, 165.

Block, R. J., Durrum, E. L. & Zweig, G. (1955). In *A Manual of Paper Chromatography and Paper Electrophoresis*, p. 170. New York: Academic Press.

Campbell, L. L. jun. (1955). *J. biol. Chem.* **217**, 669.

Davison, A. N. (1956). *Biochim. biophys. Acta*, **19**, 131.

Franke, W., Taha, E. E. M. & Krieg, L. (1952). *Arch. Mikrobiol.* **17**, 255.

Giri, K. V., Radhakrishnan, A. N. & Vaidyanathan, C. S. (1952). *Nature, Lond.*, **170**, 1025.

Greenberg, D. M. (1954). In *Chemical Pathways of Metabolism*, vol. 2, p. 53. New York: Academic Press.

Gunsalus, I. C., Bellamy, W. D. & Umbreit, W. W. (1944). *J. biol. Chem.* **155**, 685.

Janke, A. & Tayenthal, W. (1937). *Biochem. Z.* **286**, 76.

Markham, R. (1942). *Biochem. J.* **36**, 790.

Paretsky, D. & Werkman, C. H. (1950). *Arch. Biochem.* **25**, 288.

Ratner, S., Nocito, V. & Green, D. E. (1944). *J. biol. Chem.* **152**, 119.

Stumpf, P. K. & Green. D. E. (1944). *J. biol. Chem.* **153**, 387.

Taylor, T. G. (1953). *Biochem. J.* **54**, 48.