

Microbial Metabolism of C₁ and C₂ Compounds

THE ROLE OF GLYOXYLATE, GLYCOLLATE AND ACETATE IN THE GROWTH OF *PSEUDOMONAS* AM1 ON ETHANOL AND ON C₁ COMPOUNDS

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Succinate (or a product of succinate metabolism) is a catabolite repressor of some enzymes of the serine pathway (hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase) but not of methanol dehydrogenase nor methylamine dehydrogenase. A mutant (PCT64) of *Pseudomonas* AM1, which is unable to grow on C₁ compounds, lacks glycerate kinase, showing that this enzyme is essential for the operation of the serine pathway. Mutant PCT48, unable to convert acetate into glycollate, has lost the ability to grow both on C₁ compounds and on ethanol. The properties of a third mutant (PCT57) show that *Pseudomonas* AM1 contains enzymes catalysing the conversion of acetate into glyoxylate. Evidence is presented that hydroxypyruvate reductase is involved in the oxidation of glycollate to glyoxylate during growth on ethanol. A scheme is proposed for the conversion of ethanol and of C₁ compounds into glyoxylate in which acetate (or a derivative) and glycollate are intermediates.

Pseudomonas AM1 is a pink bacterium capable of growth on a variety of carbon sources including succinate, ethanol, oxalate and C₁ compounds. During the assimilation of C₁ compounds a C₁ unit condenses with glycine to give serine, which is then converted into glycolytic intermediates (Large *et al.*, 1961; Large & Quayle, 1963). To maintain the operation of this serine pathway, glyoxylate, the precursor of glycine (Harder & Quayle, 1971*b*), must be synthesized from C₁ units. The intermediates involved in this part of the pathway for the assimilation of C₁ compounds are unknown. In *Pseudomonas* AM1 ethanol is not assimilated by way of the glyoxylate bypass but probably by a novel route involving acetate, glycollate and glyoxylate as intermediates (Dunstan *et al.*, 1972). Results of work with mutants of *Pseudomonas* AM1 described in the present paper indicated that, during growth on C₁ compounds, glyoxylate is formed from C₁ units by way of a similar route involving acetate and glycollate as intermediates. Preliminary reports of this work have been published (Anthony *et al.*, 1971*a,b*).

Experimental

Chemicals

All chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., except the following. [U-¹⁴C]Acetate (sodium salt) (specific radioactivity, 56mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K.; ethyl methane-

sulphonate, DL-glyceric acid and glyoxylic acid monohydrate were from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; lactate dehydrogenase (crystalline suspension from pig heart, type IV), phosphoenolpyruvate (tricyclohexylamine salt), pyridoxal 5-phosphate and pyruvate kinase (crystalline suspension from rabbit skeletal muscle, type I) were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Other chemicals were obtained from sources given in the preceding paper (Dunstan *et al.*, 1972).

Bacterial strains

Pseudomonas AM1 (N.C.I.B. 9133) was obtained from the National Collection of Industrial Bacteria, Torrey Research Station, Aberdeen, U.K.

Methods

Maintenance and growth of the organism. The maintenance and growth of cultures of *Pseudomonas* AM1 have been described (Dunstan *et al.*, 1972). The basal growth medium, without any added carbon source, is referred to as 'salts medium'. Carbon sources included in growth media were used at a concentration of 0.2% except for methanol (0.4%), methylamine (0.4%) and sodium glyoxylate (5mM).

Isolation of mutants and revertants. Mutants and streptomycin-resistant revertants were isolated as described by Dunstan *et al.* (1972). For the isolation

of revertants of mutants PCT64 and PCT57, streptomycin-resistant organisms were plated on to methanol-agar plates containing 1 mg of streptomycin/ml. For the isolation of revertants of mutant PCT48, streptomycin-resistant mutant cells were plated on to agar plates containing streptomycin (1 mg/ml) and either methanol or ethanol as carbon source.

Determination of the growth responses of mutant strains. Initially the growth characteristics of mutant strains were determined by observation of growth in liquid media. When growth occurred the mutant was subcultured at least twice into the same medium (5 ml), by using a wire loop (2 mm diam.). After the second subculture the growth properties of the mutant were confirmed on solid media to ensure that revertants had not arisen during the subcultures.

Sometimes the growth of mutants on liquid media was very poor, and to determine whether or not the carbon source could be used as a growth substrate, alternative methods of testing the growth response were employed.

(a) Replica plating; a master plate with about 100 mutant colonies was replicated on to the solid medium to be tested, and the growth obtained was compared with the growth of wild-type organisms on similar replica plates and with any growth occurring on medium with no added carbon source.

(b) Single colony counts; a suspension of mutant cells in salts medium was spread on to a plate containing the substrate to be tested such that there were about 100 colonies per plate. The growth of single colonies on different media was compared with the growth of single colonies of wild-type cells under the same conditions.

When cultures of mutant cells were used for experiments, the growth responses of these cultures to succinate, methanol, methylamine and ethanol were tested to ensure that wild-type contaminants were not present.

Induction of the enzymes involved in the metabolism of C₁ compounds, measurement of oxygen uptake by whole cell suspensions, and bacterial incorporation of [¹⁴C]acetate. These methods have been described (Dunstan *et al.*, 1972).

Enzyme assays. Sonic extracts were prepared as described by Dunstan *et al.* (1972). Enzymes were assayed spectrophotometrically in a Unicam SP.500 spectrophotometer at room temperature; 10 mm light-path silica cells were used. The reference cell contained water.

Hydroxypyruvate reductase (EC 1.1.1.29). This enzyme was assayed by the method described by Dunstan *et al.* (1972).

Glyoxylate reductase (EC 1.1.1.26). This enzyme was assayed by the same method as hydroxypyruvate reductase, except that 6 μ mol of sodium glyoxylate was substituted for lithium hydroxypyruvate.

Serine-glyoxylate aminotransferase. This enzyme was measured by the method of Blackmore & Quayle (1970).

Glycerate kinase (EC 2.7.1.31). This enzyme was assayed by the method of Heptinstall & Quayle (1970).

Methylamine dehydrogenase. This enzyme was assayed by the method of Eady & Large (1968).

Methanol dehydrogenase. This enzyme was assayed by the method of Dunstan *et al.* (1972).

Glycollate oxidase (EC 1.1.3.1). This enzyme was assayed by the methods described by Kornberg &

Table 1. *Growth responses of mutants of Pseudomonas AM1*

Growth responses were determined in liquid media as described in the Experimental section. The relative amounts of growth observed after three serial subcultures in liquid medium are represented by values 0-5 (based on visual estimates).

Growth substrate	Strain ...	Growth response				
		Wild-type	PCT64	PCT57	PCT48	20BL
Succinate		4	4	4	4	4
Methanol		4	0	0	0	0
Methylamine		4	0	0	0	0
Formate		2	0	0	0	0
Oxalate		2	0	2	2	—
Ethanol		2	2	2	0	0
Methanol+glyoxylate		5	0	2	3	0
Methanol+glycollate		5	0	2	3	0
Methanol+acetate		4	—	3	0	—
Ethanol+glyoxylate		3	3	3	2	2
Ethanol+glycollate		3	3	3	2	0
Ethanol+acetate		3	3	3	0	0

Table 2. Effect of growth substrate(s) on the oxidative capabilities of whole cells of *Pseudomonas* AM1, and on the activities of the enzymes of the serine pathway in these organisms

Experimental details are given in the Experimental section. Rates of oxidation have been corrected for the O₂ uptake occurring in the absence of substrate.

Substrate for oxidation	Growth substrate(s)	...	Oxygen uptake (μl of O ₂ /h per mg dry wt.)				Methanol	Methylamine
			Ethanol	Succinate	Methanol	Methylamine	+ succinate	+ succinate
None			30	35	35	24	30	25
Methanol			131	27	156	—	129	—
Ethanol			130	26	140	—	125	—
Methylamine			—	0	—	127	—	110
			Specific activity (μmol of substrate used/h per mg of protein)					
Enzyme								
Hydroxypyruvate reductase			28.2	16.0	92.0	91.4	19.0	19.2
Serine-glyoxylate amino-transferase			0.2	0.0	5.0	5.17	0.52	0.95
Glycerate kinase			0.8	0.96	3.1	4.21	1.65	1.37
Methanol dehydrogenase			30.0	3.8	32.5	—	29.2	—
Methylamine dehydrogenase			—	0.2	—	7.2	—	6.8

Gotto (1961). A range of pH values and ion concentrations was used.

Results

A number of mutants of *Pseudomonas* AM1 that were unable to grow on C₁ compounds have been isolated. All the growth responses of these mutants are given for convenience in Table 1 but only the responses to single carbon sources are discussed in this first section.

Mutant PCT64 grows on ethanol but not on C₁ compounds nor on oxalate. Mutant PCT48 has lost the ability to grow on C₁ compounds and on ethanol, but growth on oxalate is unimpaired. Mutant PCT57 grows on ethanol and on oxalate, but not on C₁ compounds.

Mutant 20BL was isolated and characterized by Heptinstall & Quayle (1970); it differs from wild-type in its failure to synthesize hydroxypyruvate reductase, a key enzyme of the serine pathway. Results in Table 1 show that loss of this enzyme leads to loss of ability to use both C₁ compounds and ethanol as the sole carbon sources.

To characterize these mutants, the oxidative capabilities of whole cells were determined and the enzymes known to be involved in the assimilation of C₁ compounds were assayed. Because some of these enzymes are inducible (Large & Quayle, 1963) the conditions for induction were first determined.

Regulation of enzymes involved in the metabolism of C₁ compounds

Wild-type succinate-grown *Pseudomonas* AM1 contained low activities of some of the enzymes of the serine pathway (hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase), and methanol and methylamine were oxidized at low rates (Table 2). It was shown that the low oxidation rates for methanol and methylamine were a result of low activities of methanol dehydrogenase and methylamine dehydrogenase. These dehydrogenases were induced when their respective substrates were included in the growth medium together with succinate. By contrast, the enzymes of the serine pathway were not induced by C₁ compounds when succinate was present in the growth medium.

These results suggest that succinate, or a product of succinate metabolism, acts as a catabolite repressor of the serine-pathway enzymes but not of methanol dehydrogenase and methylamine dehydrogenase. For determination of oxidative capabilities, therefore, mutant organisms were grown on mixtures of succinate plus a C₁ compound. For assay of enzymes of the serine pathway, however, mutants were grown to exponential phase on succinate, harvested, and re-incubated overnight in medium containing methanol as the sole carbon source.

During these experiments it was observed that the specific activities of the serine-pathway enzymes bore

a well-defined relationship to one another. When the specific activity of hydroxypyruvate reductase was plotted against that for either serine-glyoxylate aminotransferase or glycerate kinase, a straight line was obtained (Fig. 1). No such well-defined relationship was observed between the specific activities of alcohol dehydrogenase and methylamine dehydrogenase nor between the specific activities of these dehydrogenases and of the serine-pathway enzymes. Statistical analysis of the results given in Fig. 1 shows that the three enzymes of the serine pathway (serine-glyoxylate aminotransferase, hydroxypyruvate reductase and glycerate kinase) may be regulated in a co-ordinate manner (Jacob & Monod, 1961). However, sophisticated genetic experiments, which are not possible at the present time with this organism, would be required to determine whether or not the genes coding for these enzymes are situated in an operon.

Characterization of mutants of Pseudomonas AM1

Oxidative properties of whole cells of mutants of Pseudomonas AM1. The rates of oxidation of methanol, ethanol, formaldehyde, formate, methylamine and succinate were measured with mutant PCT64, mutant PCT48 and mutant PCT57 grown in media containing succinate plus methanol plus methylamine. In no case were the rates with mutants significantly different from those measured in wild-type organisms.

Mutant PCT64: a mutant with no glycerate kinase. The results given in Table 3 show that this mutant differs from wild-type organisms in its lack of glycerate kinase. This mutant grows well on all substrates tested except for C_1 compounds and oxalate, confirming that glycerate kinase is an essential enzyme of the serine pathway as proposed by Large & Quayle (1963). A streptomycin-resistant revertant of this mutant was isolated; this revertant was

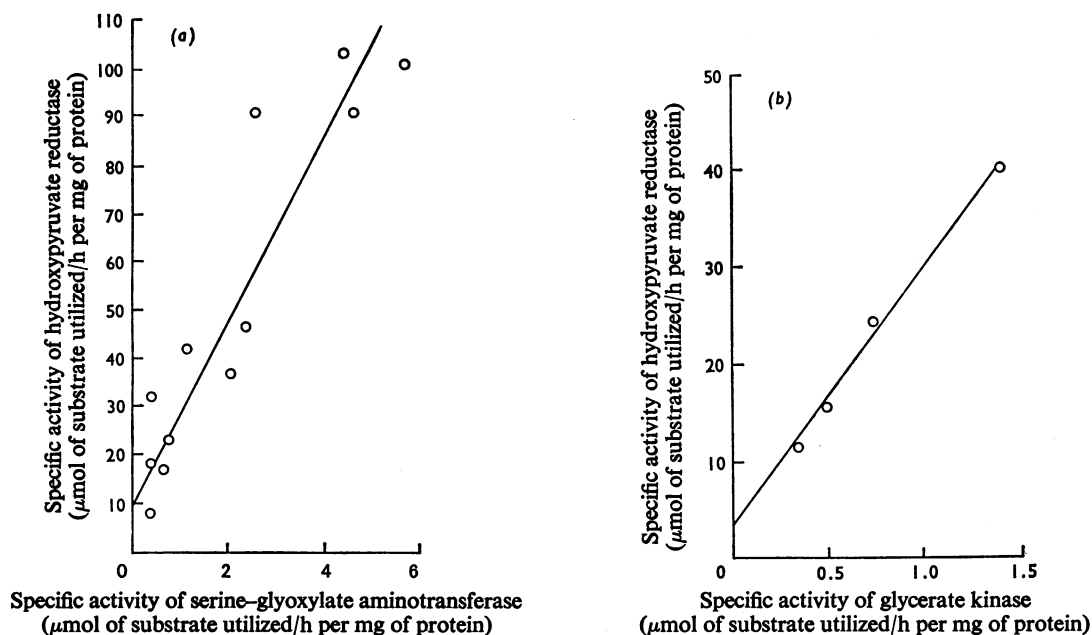


Fig. 1. Relationship between the specific activities of enzymes of the serine pathway in *Pseudomonas AM1*

Each point represents specific activities measured in extracts of cells grown on methanol, oxalate, succinate or ethanol or of cells grown on succinate and then induced with methanol for various periods of time. (a) Statistical analysis of the results shows that the best straight line has an intercept of 10.0 on the ordinate ($P < 0.001$). An analysis of variance made by using this regression curve and the regression curve for the best straight line passing through the origin shows that the intercept of 10.0 for the best straight line is not significantly different from zero ($F = 1.604$) (see Cooper, 1969, for statistical methods). (b) The best straight line has an intercept of 4.3 on the ordinate ($P < 0.001$) and an analysis of variance shows that this intercept is not significantly different from zero ($F = 5.524$).

Table 3. Specific activities of enzymes of the serine pathway in extracts of mutants of *Pseudomonas AM1*

Assay methods are described in the Experimental section. Cells were grown either on succinate or on methanol plus glycollate; some succinate-grown cells were then incubated overnight in salts medium in the presence of methanol (0.4%); this procedure was designed to induce the enzymes of the serine pathway.

Enzyme	Growth substrate(s) ...	Specific activity (μmol of substrate used/h per mg of protein)													
		Succinate			Succinate: incubated overnight with methanol			Succinate: incubated overnight in salts medium			Methanol plus glycollate				
		Wild type	PCT64	PCT48	Wild type	PCT64	PCT57	PCT48	Wild type	PCT64	PCT57	PCT48	Wild type	PCT57	PCT48
Hydroxypyruvate reductase	Strain ...	26.0	29.0	13.8	92.0	104.0	39.0	61.0	23.8	24.8	26.8	10.0	102.0	81.6	—
		0.96	0.0	0.35	1.4	0.0	0.9	3.5	0.75	0.0	1.9	0.96	—	1.8	—
Glycerate kinase	Strain ...	0.0	—	—	2.6	4.4	2.7	3.5	0.0	0.0	0.5	0.8	5.7	8.9	—
		—	—	—	—	—	—	—	—	—	—	—	—	—	—

able to grow on C₁ compounds and had regained glycerate kinase activity. The growth of this mutant on ethanol shows that glycerate kinase (and therefore probably the serine pathway) is not involved in the assimilation of ethanol by *Pseudomonas AM1*. The low activities of serine-glyoxylate aminotransferase, hydroxypyruvate reductase and glycerate kinase in ethanol-grown organisms (Table 2) support this conclusion.

Mutant PCT48. A consideration of growth responses (Table 1) suggests that mutant PCT48 is unable to form glyoxylate when a C₁ compound is provided as the potential carbon source. Growth on oxalate can occur because glyoxylate is formed from oxalate by reduction (Blackmore & Quayle, 1970). Ability to grow on oxalate indicates the presence of the serine-pathway enzymes and this was confirmed (for hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase) by using extracts of mutant PCT48 (Table 3). The failure of this mutant to grow on ethanol indicates that an enzyme involved in the pathway for glyoxylate formation from C₁ compounds is also essential for the assimilation of ethanol. It has been proposed (Dunstan *et al.*, 1972) that this pathway for ethanol assimilation involves the formation of glyoxylate from acetate (or a derivative) by way of glycollate. The observation that growth of mutant PCT48 on methanol or ethanol occurred when glyoxylate or glycollate, but not acetate, was also included in the growth medium (Table 1) suggests that this mutant is able to form glyoxylate from glycollate but cannot metabolize acetate to glycollate.

Revertants were not obtained from mutant PCT48 even after treatment with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine or ethyl methanesulphonate. The possibility that mutant PCT48 is a double mutant cannot therefore be completely excluded. If mutant PCT48 does have two lesions, one lesion may prevent metabolism of acetate to glycollate (thus precluding growth on ethanol), and the second lesion may prevent metabolism of C₁ compounds to glyoxylate (thus precluding growth on C₁ compounds). If mutant PCT48 is a double mutant of this sort, however, it should have been possible to obtain revertants able to grow with either ethanol or methanol as sole carbon source; such revertants were not obtained. Multiple penicillin screenings, as used in the isolation of mutant PCT48, are likely to lead to selection of stable (non-reverting) mutants such as deletion mutants and mutant PCT48 probably has a single lesion of this type.

Mutant PCT57. Extracts of this mutant contained the enzymes of the serine pathway (Table 3). Mutant PCT57 differs from mutant PCT48 in being able to grow on ethanol. Further, by contrast with mutant PCT48, acetate could replace glyoxylate or glycollate for growth with methanol. This result confirms the

Table 4. Variation with time of the percentage distribution of radioactivity incorporated from [$U\text{-}^{14}\text{C}$]acetate into non-volatile components of the ethanol-soluble fractions of mutant PCT48 and wild-type *Pseudomonas* AM1

Wild-type cells were grown on ethanol; mutant cells were grown on succinate and were induced overnight in the presence of ethanol (0.2%) and acetate (0.1%). This procedure was designed to allow for the synthesis of inducible enzymes and permeases for C_2 compounds. Washed organisms (6mg dry wt.) were then preincubated aerobically in 2ml of salts medium with 1 μmol of acetate at 30°C for 10min. [$U\text{-}^{14}\text{C}$]Acetate (1.7 μmol ; 20 μCi of ^{14}C) was added at zero time and 1ml samples were removed at suitable time-intervals into 3ml of boiling ethanol. The precipitates were extracted with 1ml of aq. 20% (v/v) ethanol and the combined extracts were analysed by two-dimensional chromatography. The radioactive areas were located by radioautography and the radioactive compounds were eluted, counted and identified. Full details of procedures are given by Dunstan *et al.* (1972). Correction was made for quenching and for the background radioactivity of vials (about 45 c.p.m.). The chromatogram background radioactivity was 60–150 c.p.m.; it is given for reference but was not used for correction purposes. A blank sample containing boiled cells and radioactive substrate was treated in an identical manner with the experimental samples; no radioactive compounds were detected in this sample after chromatography and radioautography.

Compound	Strain ... Time ...	Radioactivity incorporated into compound (% of total)			
		Wild-type cells		Mutant PCT48	
		16s	60s	15s	60s
Glutamate		32.6	65.0	45.0	73.0
Compound 'B'		12.2	8.0	55.0	7.2
Aspartate		8.0	7.1	<4.0*	<0.4*
Malate		2.8	0.9	<4.0*	<0.4*
Glycollate		2.8	1.3	<4.0*	<0.4*
'Succinate plus fumarate'		3.6	1.0	<4.0*	2.0
Others		38.0	17.0	<4.0*	17.8
Total radioactivity (c.p.m.)		76674	455548	7380	76980

* These compounds were not detected on the radioautogram; the lowest detectable limit under the conditions used was approx. 300 c.p.m. and this value is expressed as a percentage of the total radioactivity incorporated.

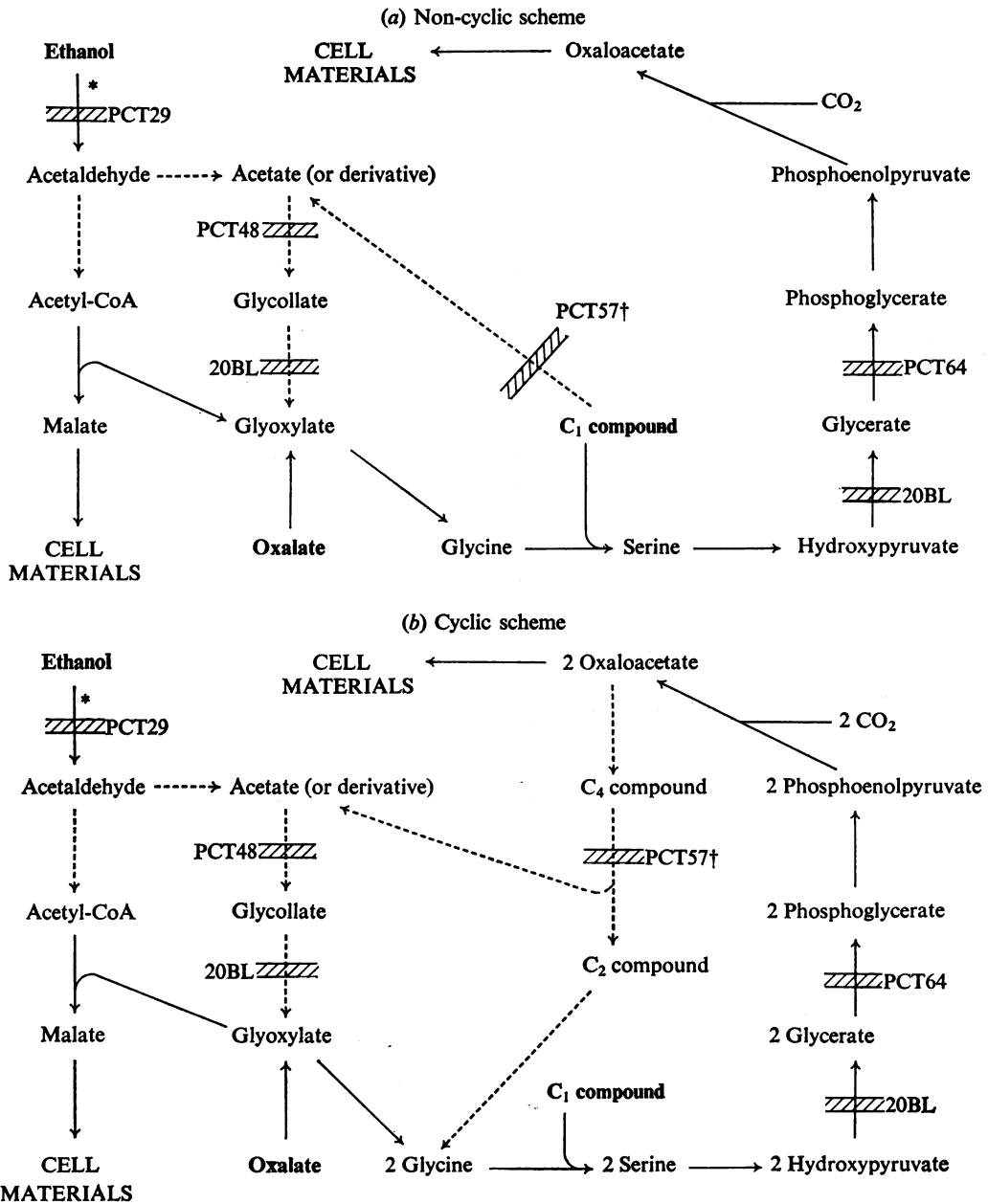
suggestion (Dunstan *et al.*, 1972) that enzymes capable of converting acetate into glyoxylate (probably by way of glycollate) are present in *Pseudomonas* AM1.

It is not known whether glyoxylate is formed from a product of the condensation of two C_1 units or by cleavage of a C_2 compound (Large *et al.*, 1962; Harder & Quayle, 1971b). Mutant PCT57 is unable to form glyoxylate from C_1 units, but its properties do not enable a distinction to be made between these two possible origins of glyoxylate during growth of *Pseudomonas* AM1 on C_1 compounds.

Mutant 20BL. The failure of this mutant to grow on ethanol indicates that hydroxypyruvate reductase has a role in the metabolism of ethanol as well as in the pathway for the assimilation of oxalate and of C_1 compounds. The results in Table 1 show that this mutant is able to grow on ethanol, at similar rates to wild-type organisms, when glyoxylate (but not glycollate) is included in the growth medium. This growth response indicates that the function of hydroxypyruvate reductase during growth on ethanol (and perhaps a second function during growth on methanol) is the oxidation of glycollate to glyoxylate.

Large & Quayle (1963) have shown that the glyoxylate reductase and the hydroxypyruvate reductase of *Pseudomonas* AM1 are the same enzyme; both activities are present in small amounts in succinate-grown organisms and are induced during growth on C_1 compounds. The specific activity of glyoxylate reductase in extracts of ethanol-grown *Pseudomonas* AM1 was 2–4 μmol of NADH used/h per mg of protein; this is sufficient to account for the growth rate on ethanol. Although the equilibrium of the reaction catalysed by this enzyme lies towards the formation of glycollate, net conversion of glycollate into glyoxylate might occur *in vivo* if the product (glyoxylate) is rapidly removed by metabolism to malate (or to glycine during metabolism of C_1 compounds). No flavoprotein glycollate oxidase could be detected in extracts of *Pseudomonas* AM1 (see the Experimental section).

Metabolism of [^{14}C]acetate by mutant PCT48. When ethanol-grown wild-type *Pseudomonas* AM1 is incubated with [^{14}C]acetate, radioactivity is rapidly incorporated into glutamate, aspartate, malate, glycollate, 'succinate plus fumarate' (which are not



* This enzyme also catalyses the oxidation of methanol to formaldehyde.

† Mutant PCT57 is unable to catalyse the condensation (Scheme a) or cleavage (Scheme b) reaction to give acetate, glycollate or glyoxylate from C₁ compounds. If mutant PCT48 has a single mutation then the product of the reaction is acetate (or a derivative), as shown above. If PCT48 is a double mutant then the product of the condensation or cleavage reaction could be glycollate or glyoxylate instead of acetate.

Scheme 1. Postulated relationships between pathways for the metabolism of C₁ and C₂ compounds by *Pseudomonas AM1*

(a) Non-cyclic scheme involving direct generation of glycine during growth on C₁ compounds; (b) alternative cyclic scheme in which glycine is regenerated by cleavage of a C₄ compound. Broken lines represent proposed reactions or series of reactions. Metabolic lesions in the designated mutants are indicated by hatched bars.

distinguished) and an unidentified compound (compound 'B') (Dunstan *et al.*, 1972). The properties of mutant PCT48, described above, indicate that it is unable to form glycollate from acetate. If this is the case, then radioactive glycollate should not accumulate when mutant cells are incubated with [^{14}C]acetate. Further, because the pool of glyoxylate should be negligible in this mutant, radioactive malate should not be formed by the condensation of glyoxylate with acetyl-CoA. The metabolism of radioactive acetate by mutant PCT48 was therefore investigated; the results were compared with those obtained with ethanol-grown wild-type organisms (Table 4). At 15 s after addition of [^{14}C]acetate to the mutant cells, radioactivity was detected in only glutamate and compound 'B'. After 60 s, radioactivity was also found in 'succinate plus fumarate' but not in glycollate, malate or aspartate. This pattern of incorporation (at 60 s) contrasts with that observed in wild-type organisms where similar amounts of radioactivity were incorporated from [^{14}C]acetate into glycollate, malate, 'succinate plus fumarate' and aspartate. Although the total amount of radioactivity incorporated into the mutant cells was less than that incorporated into wild-type cells, some label should have been found in glycollate, malate and aspartate if the metabolism of [^{14}C]acetate in the mutant is similar to that in wild-type organisms. These results confirm the suggestion that mutant PCT48 is unable to metabolize acetate to glycollate. The radioactive labelling found in compound 'B' indicates that glycollate formation is not required for synthesis of this unidentified compound.

Discussion

The results and conclusions presented in this paper are summarized in the two schemes shown in Scheme 1. Both schemes postulate a relationship between the pathways for the metabolism of C_1 and C_2 compounds in *Pseudomonas* AM1; they differ only in the route by which glycine is formed during growth on C_1 compounds. One of the schemes (Scheme 1a) involves non-cyclic formation of glycine from C_1 compounds. In the alternative cyclic scheme for glycine regeneration (Scheme 1b; modified from Large *et al.*, 1962) a C_4 compound is cleaved to a C_2 compound plus acetate (or a derivative of acetate). The metabolic lesions in the mutants of *Pseudomonas* AM1 described in the present paper are the same in the two schemes except for that in mutant PCT57. The growth properties of mutants PCT48, PCT57 and 20BL do not enable a distinction to be made between these two schemes. The properties of mutant PCT64 favour the cyclic scheme (Scheme 1b), because glycerate kinase is essential for conversion of C_1 compounds into glyoxylate by this route. If the alternative, non-cyclic, pathway (Scheme 1a)

operates it might be expected that mutant PCT64 would grow slowly on C_1 compounds by condensing glyoxylate with acetyl-CoA to give malate. However, it is possible that the non-cyclic scheme does operate in *Pseudomonas* AM1 and that mutant PCT64 is unable to grow on C_1 compounds because acetyl-CoA is not readily formed from these growth substrates.

When the results obtained with mutants PCT48 and PCT57 are considered together it can be concluded that acetate (or a derivative) and glycollate are intermediates in the formation of glyoxylate from C_1 compounds. If glycollate is an intermediate in this pathway then it is not obvious why radioactive glycollate was absent after incubation of methanol-grown *Pseudomonas* AM1 with [^{14}C]methanol (Large *et al.*, 1961). It should be noted, however, that radioactive glycollate was detected at early times when *Hyphomicrobium vulgare* was incubated with [^{14}C]methanol (Large *et al.*, 1961). Further, [^{14}C]glycollate accumulated when mutant 20BL was incubated with [^{14}C]methanol (Heptinstall, 1968). Such accumulation of glycollate would be expected if hydroxypyruvate reductase is required for the oxidation of glycollate to glyoxylate during the assimilation of C_1 compounds, as suggested in Scheme 1.

The failure to demonstrate conversion of [^{14}C]acetate into glycollate in mutant PCT48 confirms the proposal that metabolism of acetate to glycollate is essential for ethanol assimilation in *Pseudomonas* AM1 (Dunstan *et al.*, 1972). The proposed pathway for ethanol assimilation is further supported by the growth responses of mutant PCT57, which demonstrate that enzymes capable of catalysing the conversion of acetate into glyoxylate are present in this organism. Preliminary attempts to demonstrate conversion of acetate into glycollate in extracts of *Pseudomonas* AM1 have not been successful.

During growth on succinate, serine is synthesized by a pathway involving phosphoserine phosphatase (Heptinstall & Quayle, 1970). A mutant (20S) lacking this enzyme does not grow on succinate, lactate or ethanol (Harder & Quayle, 1971a). Consideration of Scheme 1 might suggest that serine could be synthesized by way of glyoxylate and glycine during growth on ethanol. In this case C_1 units for serine synthesis could be provided by the oxidative decarboxylation of glyoxylate (this reaction has been demonstrated in *Pseudomonas* AM1 by Harder & Quayle, 1971b). However, serine-glyoxylate aminotransferase is not induced during growth on ethanol (Table 2) and thus the phosphorylated pathway is the only possible route for serine synthesis.

It should be noted that most of the reactions in the proposed pathway for the assimilation of ethanol are also involved in the proposed pathway for methanol assimilation. The abundance of pink methanol-utilizing bacteria in Nature is probably related to

their relatively high growth rate on C₁ compounds (an unusual characteristic in bacteria). These bacteria grow slowly on ethanol compared with many other soil organisms. It is therefore suggested that growth of these bacteria on ethanol is not important in Nature and that when growth on this compound does occur this is a consequence of the reactions common to C₁ and C₂ metabolism as postulated in Scheme 1.

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