The Assimilation of 2-C Compounds other than Acetate

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The tricarboxylic acid cycle is both the main terminal respiratory pathway in micro-organisms and a source of precursors for the net biosynthesis of cell constituents. Intermediates drained away from the cycle for these biosynthetic purposes must be replenished; a process accomplished by carboxylation reactions when suitable CO_2 -acceptor molecules (e.g. pyruvate and phosphopyruvate) are available. Any organism that utilizes a 2-C compound as sole source of carbon for growth must therefore be endowed with a means of forming from this substrate either the TCA cycle intermediates themselves or such 3-C CO_2 -acceptors.

The glyoxylate cycle (Kornberg & Krebs, 1957; Kornberg & Madsen, 1958) in which isocitrate lyase (Smith & Gunsalus, 1954; Olson, 1954) and malate synthase (Wong & Aji, 1956) operate in conjunction with enzymes of the TCA cycle to form 1 mol. unit of succinate from 2 mol. units of acetate, is the best documented of all such pathways. However, this biosynthetic route is not available to organisms utilizing 2-C compounds more highly oxidized than acetate. A number of pathways all involving glyoxylate or glycine or both have now been described whereby net synthesis of pyruvate from such compounds may be accomplished.

In micro-organisms such as *Escherichia coli* or *Pseudomonas* growing on glycollate, the necessary net formation of TCA cycle intermediates is effected via the 'glycerate pathway' (Kornberg, 1961). In this pathway glyoxylate (derived from the oxidation of the glycollate) undergoes a condensation reaction in which 2 mol. units of glyoxylate yield 1 mol. unit each of tartronic semialdehyde and carbon dioxide (Krakow & Barkulis, 1956; Krakow, Barkulis & Hayashi, 1961; Kornberg & Gotto, 1959, 1961): the enzyme catalysing this process has been named glyoxylic carboligase (Krakow, Barkulis & Hayashi, 1961). The tartronic semialdehyde thus formed is subsequently reduced to glyceric acid by tartronic semialdehyde reductase (Kornberg & Gotto, 1959, 1961; Krakow, Udaka & Vennesland, 1962). In the presence of ATP and *Pseudomonas* extracts, glyceric acid has been shown to yield pyruvate, presumably via the well-established Embden-Meyerhof sequence. The net effect of these reactions is to transform 2 mol. units of glycollate to 1 mol. unit of carbon dioxide and one of pyruvate which can either be carboxylated to oxaloacetate, or, after oxidation to acetylcoenzyme A, condensed with a third mol. unit of glyoxylate to yield malate (Kornberg & Sadler, 1961); in either case, the *de novo* formation of intermediates of the TCA cycle from glycollate has been effected.

The glycerate pathway operates not only during growth on glycollate but also on a number of 2-C compounds that first give rise to glyoxylate. Thus Callely & Dagley (1959) reported that glyoxylic acid carboligase and tartronic semialdehyde reductase were present in large amounts in extracts of a pseudomonad grown on glycine. *Pseudomonas oxalaticus* also formed these enzymes during growth on oxalate; this provided evidence for the initial reduction of oxalate to glyoxylate by this organism (Quayle & Keech, 1959). In this process oxalate is activated to oxalylcoenzyme A (Quayle, Keech & Taylor, 1961) which is reduced to glyoxylate by glyoxylic dehydrogenase and NADPH₂ (Quayle & Taylor, 1961). Thus pseudomonads growing on glycollate, glycine or oxalate are all in effect growing on glyoxylate and a source of 'reducing power'.

It is probable that in some organisms an alternative route for glycine utilization is available which does not require its preliminary deamination. Kornberg & Morris (unpublished) observed that serine, alanine and glutamate were early-labelled products during growth of *Arthrobacter globiformis* on [¹⁴C]-glycine. Extracts of the organism formed pyruvate, alanine and CO₂ from glycine in the presence of reduced pyridine nucleotides, pyridoxal phosphate and tetrahydrofolic acid. It would seem that in such coryneform organisms 2 mol. units of glycine might undergo a condensative decarboxylation in the presence of pyridoxal phosphate and a folic acid derivative to give serine, which would readily yield pyruvate through the action of serine dehydratase. This reaction sequence has been convincingly demonstrated in anaerobic organisms capable of fermenting glycine with acetate as end product, e.g. *Clostridium acidii-urici* (Radin & Barker, 1953; Sagers & Beck, 1956), *Diplococcus glycinophilus* (Barker, Volcani & Cardon, 1948; Sagers & Gunsalus, 1961). A similar route may be used for the metabolism of glycollate in plants (Rabson, Tolbert & Kearney, 1962).

Here, as in the glycerate pathway, 2 mol. units of the 2-C substrate undergo a condensation reaction wherein one 3-C product is formed with the loss of the carbon atom derived from one carboxyl group as carbon dioxide.

A novel metabolic route which avoids this loss of CO₂ in the initial formation of a compound containing more than 2 carbon atoms operates during growth of *Micrococcus denitrificans* on substrates catabolized to glyoxylate. Here both glycine and glyoxylate are required for the nett formation of TCA cycle intermediates. When *M. denitrificans* was grown on glycollate as sole carbon source, no glyoxylic carboligase activity was present despite initial oxidation of the substrate to form glyoxylate. Short-term incubation of growing cultures with [1-¹⁴C]-glycollate resulted in the appearance of isotope first in glycine, malate and aspartate. In contrast to the results obtained with *Pseudomonas* and *Escherichia coli*, no isotope was detected in glycerate or phosphoglycerate. Evidence was obtained that during growth on glycollate two hitherto unreported enzymes were inducibly formed by *M. denitri-*

ficans (a) an aldolase-type enzyme which catalysed the condensation of glycine and glyoxylate to form *erythro*- β -hydroxyaspartate, and (b) a β -hydroxyaspartate dehydratase capable of forming oxaloacetate from the *erythro* form of β -hydroxyaspartate (Kornberg & Morris, 1962*a*, *b*). Acting in conjunction with a mechanism for the production of glycine from glyoxylate these enzymes could account for the formation of 1 mol. unit of a 4-C compound (oxaloacetate) from 2 mol. units of glyoxylate without concomitant loss of CO₂ (though the subsequent utilization of oxaloacetate for biosyntheses would, of course, be accompanied by loss of CO₂ (Kornberg & Morris, 1963).

Other sources of glyoxylate, e.g. allantoin and ethyleneglycol, are also utilized by *Micrococcus denitrificans* via this β -hydroxyaspartate pathway.

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