The Methylglyoxal By-Pass of the Embden–Meyerhof Pathway

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Methylglyoxal was for many years considered to be a possible intermediate in normal glycolysis (Harden, 1932; Cori & Cori, 1933). One of the main arguments in support of this glycolytic role was its apparent formation from hexose diphosphate and the wide-spread occurrence, at high activity, of an enzyme system that converted methylglyoxal into lactate. However, no enzyme capable of forming methylglyoxal from likely glycolytic intermediates could be identified, and subsequent work showed that phosphorylated C₃ compounds were intermediates in the conversion of hexose phosphates into lactate. Methylglyoxal formation was then considered to be due to non-enzymic side reactions of the triose phosphates, and thus of little or no importance (Meyerhof, 1948).

During studies on bacterial gluconeogenesis we isolated Escherichia coli mutants defective in triose phosphate isomerase (Anderson & Cooper, 1969) and found that, although such mutants were, as expected, incapable of gluconeogenesis from lactate, they were still capable of gluconeogenesis from glycerol (Cooper & Anderson, 1970). A key step in this process was found to be the formation of methylglyoxal, which was subsequently converted into pyruvate via D-lactate (Scheme 1). The glyceraldehyde 3-phosphate required for fructose bisphosphate formation was thus produced indirectly from pyruvate via the gluconeogenic sequence rather than directly from dihydroxyacetone phosphate.

We have paid particular attention to the formation of methylglyoxal and shown that it is produced enzymically from dihydroxyacetone phosphate by a phospho-lyase that we have called methylglyoxal synthase. This enzyme has been found in all the Enterobacteriaceae tested (Hopper & Cooper, 1971) and in various Enterobacteriaceae-like organisms such as Aeromonas formicans and Obesumbacterium proteus. It is present in certain strict anaerobes such as Clostridium pasteurianum and Clostridium tetanomorphum, and has also been detected in the strict aerobe Pseudomonas saccharophila (Cooper, 1974). Another pseudomonad, Pseudomonas G6 (Rizza & Hu, 1973), appears to have methylglyoxal synthase, but in this case the enzyme seems to be different from the E. coli enzyme in some kinetic properties.

Methylglyoxal synthase has been purified extensively from E. coli (Hopper & Cooper, 1972) and appears to show regulatory properties, since it is strongly inhibited by low concentrations of P₃ and this inhibition is overcome in a co-operative manner by increasing dihydroxyacetone phosphate concentration. P₃ appears to cause changes in the enzyme structure since low concentrations (1 mm) increase the electrophoretic mobility of the enzyme.
of the enzyme during disc-gel electrophoresis and protect the enzyme against inactivation.

A strict regulation of methylglyoxal production, or an effective system for its utilization, is necessary because of the toxicity of the 2-oxo aldehyde. Concentrations greater than 0.5 mM are toxic to *E. coli*, but mutants that are resistant to such killing can be isolated (Freedberg et al., 1971). These mutants have a 5–8-fold increase in lactoylglutathione lyase activity, but the hydroxyacylglutathione hydrolase activity is unchanged.
Under certain physiological conditions *E. coli* produces methylglyoxal in excess of its rate of utilization, and it can be detected in the growth medium. Thus triose phosphate isomerase-negative mutants growing on gluconate, fructose bisphosphate aldolase-negative mutants growing on glycerol and wild-type strains growing on glucose 6-phosphate all produce an excess of methylglyoxal. Surprisingly the presence of increased lactoylglutathione lyase activity does not diminish the amount of methylglyoxal accumulated by such cells.

The amount of methylglyoxal produced by wild-type cells growing on glucose 6-phosphate is increased dramatically by the addition of cyclic AMP, and to such an extent that the cells are killed (Ackerman *et al.*, 1974). However, in this situation the presence of increased lactoylglutathione lyase activity prevents the lethal accumulation of methylglyoxal. In a similar way an *E. coli* strain that produced lethal concentrations of methylglyoxal from glucose 6-phosphate in the absence of added cyclic AMP is able to grow on glucose 6-phosphate if it has increased lactoylglutathione lyase activity.

That the effect of cyclic AMP may be mediated via an increase in the dihydroxyacetone phosphate concentration, thereby leading to increased methylglyoxal synthase activity, is suggested by the inability of a temperature-sensitive fructose bisphosphate aldolase mutant (in which the aldolase activity may be the rate-limiting step for growth at 30°C) to produce an excess of methylglyoxal when challenged with cyclic AMP during growth on glucose 6-phosphate at 30°C.

Although our experiments suggest that for *E. coli* the lactoylglutathione lyase–hydroxyacetylglutathione hydrolase system is important in the catabolism of methylglyoxal, another enzyme, 2-oxo aldehyde dehydrogenase (Monder, 1967), can convert methylglyoxal into pyruvate. This latter enzyme has also been reported in bacteria (Higgins & Turner, 1969), but its species distribution is unknown.

Since all the enzymes of the methylglyoxal glycolytic by-pass are formed constitutively by *E. coli*, the pathway may function under a variety of growth conditions, and a number of possible physiological roles may be proposed. The reactions by-pass both substrate-linked phosphorylation steps of the Embden–Meyerhof pathway, making the by-pass energetically unfavourable as a route for the catabolism of glucose. However, glucose catabolism without concomitant ATP synthesis does provide a means of uncoupling catabolism and anabolism, a phenomenon that has been recognized for many years (Gunsalus & Shuster, 1961). Alternatively, the sequence may be a route for the production of D-lactate, which appears to be involved in the transport of various sugars and amino acids into *E. coli* (Kaback, 1974.)

One approach that we are using in an attempt to understand the role of the by-pass is to obtain mutants that are defective in the appropriate enzymes. To date we have been unable to obtain mutants lacking methylglyoxal synthase or the methylglyoxal-utilizing system, but we have obtained mutants lacking D-lactate dehydrogenase. When such mutants are grown on 5 mM-glucose 6-phosphate about 1 mM-D-lactate is found in the medium at the end of growth. Since some of this lactate may have been formed by the reduction of pyruvate, it represents a maximum flow through the by-pass. However, no lactate was detected in the growth medium when the mutants grew on glucose under similar conditions, so it would seem that a significant proportion of the glucose 6-phosphate was catabolized via the methylglyoxal by-pass.

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The wall teichoic acid in Staphylococcus aureus is a polymer of ribitol phosphate in which each ribitol possesses an N-acetylglicosaminyl substituent in addition to D-alanyl ester residues; in a mutant of this organism the amino sugar substituents are absent. From a study of carefully prepared walls of the wild type and of the mutant it is found that a short oligomer of three or four glycerol phosphate residues is present in a molar ratio 1:1 with the ribitol teichoic acid. The same conclusion is reached from a study of soluble teichoic acid–glycan complexes obtained by dissolution of walls with an amidase. The number and nature of chain ends supports the view that the glycerol phosphate oligomer is interposed between the poly(ribitol phosphate) chain and the glycan chain, thus constituting a structurally distinct 'linkage oligomer'. The walls of several other organisms possessing ribitol teichoic acids or polymers of N-acetylglucosamine phosphate also contain small amounts of bound glycerol phosphate, and it is possible that such linkage oligomers are widespread.

The possibility that glycerol phosphate units occur in the linkage between ribitol teichoic acids and peptidoglycan raises a number of questions related to teichoic acid biosynthesis. These include ones concerning the origin of these glycerol phosphate residues and the connexion, if any, between the synthesis of wall teichoic acids and the membrane lipoteichoic acids, and also possible interrelationships between the synthesis of phospholipids and teichoic acids and the role of CDP-glycerol.

Lipids in the Synthesis of Lipopolysaccharides and Exopolysaccharides

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Since the identification of lipid intermediates in the biosynthesis of mucopeptides and lipopolysaccharides, such compounds have been recognized in numerous other polysaccharide-synthesizing systems (Hemming, 1974). The lipids involved in these systems are isoprenoid alcohols, and in bacteria the active compound is C_{25}-isoprenoid alcohol phosphate (undecaprenyl phosphate). In addition, several of the enzymic systems involved in aspects of polysaccharide formation demonstrate a requirement for lipid other than the isoprenoid alcohol (Rothfield & Horecker, 1964; Sandermann, 1974). In whole bacteria or in cell membrane preparations both types of lipid are present, but solvent extraction permits the study of lipid-depleted enzymes and an assessment of the lipid requirements.

In Gram-negative bacteria such as Escherichia coli, Salmonella typhimurium or