GLUCONEOGENESIS IN *ESCHERICHIA COLI*
The Role of Triose Phosphate Isomerase

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Studies on animal tissues have led to the belief that several glycolytic enzymes including fructose diphosphate aldolase and triose phosphate isomerase (TPI) are involved in both glycolysis and gluconeogenesis [1]. However, the isolation of mutants of *Escherichia coli* unable to grow on glucose due to the apparent absence of fructose diphosphate aldolase activity [2,3], but capable of almost normal growth rates on gluconeogenic substrates, along with the ability of other bacteria to carry out gluconeogenesis in the absence of glyceraldehyde 3-phosphate dehydrogenase [4] has raised the problem of the nature of the reactions involved in bacterial gluconeogenesis. In an attempt to resolve this question we are currently investigating the reactions likely to be common to glycolysis and gluconeogenesis in *E. coli.* It is the purpose of this communication to show that triose phosphate isomerase is involved in gluconeogenesis in *E. coli* and that both glyceraldehyde 3-phosphate (G 3-P) and dihydroxyacetone phosphate (DHAP) are likely intermediates in the process.

Mutants of *E. coli* K12 (strain K10), unable to grow on glucose as sole carbon source, were obtained after mutagenesis with ethyl methansulphonate [5] and subsequent penicillin selection [6]. Of these mutants, two were also unable to grow on lactate alone but were able to grow on gluconate or on lactate supplemented with a small amount of ribose. The glucose-negative lactate-negative mutants also failed to grow on acetate, succinate, glycerol, rhamnose, ribose, xylose, maltose, fructose or glucose 6-phosphate plates, suggesting that they were unable to catalyse a reaction common to both glycolysis and gluconeogenesis.

To determine the particular enzymic defect, cell-free extracts were prepared from gluconate-grown cells. The cells were suspended in 10 mM Tris-HCl-mM $MgCl_2$ buffer pH 7.3 and subjected to ultrasonication followed by centrifugation at 160 000 g for 90 min to remove NADH-oxidase activity. The appropriate glycolytic enzymes were assayed and the only difference between the wild-type cells and the mutants was in their content of TPI. In a coupled spectrophotometric assay containing 50 μmoles of Tris-HCl pH 8.0, 0.15 μmole of NADH, 0.75 μmole of G 3-P, 10 μg of crystalline α-glycerophosphate dehydrogenase and 5 μg of crude wild-type protein or 500 μg of crude mutant protein, the wild-type extract had an activity of 16.6 μmoles G 3-P isomerised min^{-1} mg $protein^{-1}$, whereas no activity could be detected with the mutant extract. Mixtures of mutant and wild-type extracts showed the amount of TPI expected from their content of wild-type extract, suggesting that there was no inhibitor of TPI present in the extract of the mutant.

That the inability of the mutants to grow on the various substrates was due to a single lesion was confirmed by experiments with spontaneous revertants obtained from glucose or lactate plates. Both types of revertants had regained the ability to grow on all the other carbon sources mentioned earlier. The revertants had regained TPI activity but all eight so far tested showed only 5% of the original activity, although their absolute specific activity, 0.8 μmoles min^{-1} mg $protein^{-1}$, is high. They all grow on lactate and glucose at the same rate as the wild-type strain suggesting either that their *in vivo* activity of TPI is appreciably higher than that observed *in vitro,*

Table 1
Glycogen synthesis from [1-^{14}C] lactate by a *tpi*$^-$-mutant and the parent strain.

E. coli strain	[1-^{14}C] lactate		[1-^{14}C] lactate *plus* glycerol	
	cpm/μg glycogen	Glycogen (μg)	cpm/μg glycogen	Glycogen (μg)
tpi$^-$-mutant	–	<2	524	248
K10	928	416	604	528

Glycogen synthesis was measured by the previously published method [8]. [1-^{14}C] lactate, 50 μmoles (1.4×10^5 cpm/μmole) and where appropriate, glycerol, 10 μmoles, were used along with 6 mg dry wt of lactate/ribose-grown cells. The figures in the table represent counts incorporated into glycogen by 6 mg dry wt of cells in 90 min at 30°.

or that normal growth can occur with only 5% of the wild-type amount of the enzyme. The lactate-positive recombinants obtained from a cross between a female strain devoid of TPI activity and strain K10 (HfrC), had regained the full amount of TPI activity.

One of the glucose-negative lactate-negative TPI-negative (*tpi*$^-$)-mutants has been used to investigate the nature of the reactions involved in gluconeogenesis. Although the *tpi*$^-$-mutant was able to oxidise glucose and lactate at the same rate as the wild-type cells and thereby obtain energy readily, it produced glycogen only from glucose. However, glycogen was produced in appreciable amounts if the lactate was supplemented with a small amount of glycerol (table 1).

By using [1-^{14}C] lactate more information was obtained on the reactions occurring in the *tpi*$^-$-mutant. As can be seen from table 1 the lactate was indeed utilised for glycogen synthesis when glycerol was included. Further, the specific radioactivity (cpm/μg glycogen) of the glycogen formed was almost 50% of that produced from [1-^{14}C] lactate alone by the wild-type cells. This implies that lactate and glycerol contribute equally to glycogen synthesis in cells which are unable to form DHAP from G 3-P, but which can form DHAP from glycerol.

These results suggest that TPI is essential for gluconeogenesis in *E. coli* and that both G 3-P and DHAP are likely intermediates in the process. Further, since no glycogen was formed from lactate alone, and the *tpi*$^-$-mutant is unable to grow on lactate in the absence of ribose, it seems that a condensation between G 3-P and hydroxypyruvate [7] to yield pentose (and thereby hexose) is unlikely to occur in *E. coli*.

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