REGULATION OF THE SYNTHESIS OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN PSEUDOMONAS PUTIDA

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1. Introduction

The problem of hexose and pentose biosynthesis by members of the *Pseudomonas* group remains unclear. Studies on ribose formation by *Ps. saccharophila* during aerobic growth on glucose suggest that a modified pentose cycle must be involved in such a process [1]. One of the enzymes of the Embden-Meyerhof pathway, fructosediphosphate aldolase, it not detectable in some pseudomonads [2,3]. It must be stated that the absence of fructosediphosphate aldolase does not conflit with glucose dissimilation by *Pseudomonas spp.* because this enzyme plays no role in the Entner-Doudoroff pathway [4] which appears to be the predominant glycolytic route in these bacteria. Lessie and Neidhardt after considering the apparent absence of fructosediphosphate aldolase in *Ps. aeruginosa* suggest that the cells can form hexose and pentose phosphates during growth on succinate by an alternate route [3]. The operation of an alternate gluconeogenic route is rather probable in members of the *Enterobacteriaeceae. Escherichia coli* mutants with severely impaired fructosediphosphate aldolase activity seem to grow normally on many gluconeogenic substrates [5].

To gain information about gluconeogenic processes in the *Pseudomonadaceae* a survey of enzymes of the Embden-Meyerhof pathway has been carried out in *Ps. putida.* Our data show that the synthesis of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is highly regulated in these bacteria and in such a way that its participation in the "de novo" formation of carbohydrates appears improbable.

2. Experimental

Pseudomonas putida strain A.3.12 (ATCC 12633) was used. The culture media used for its maintenance and for experimental purposes were those described by Ornston and Stanier [6]. Cells were grown at 30° in agitated liquid cultures in synthetic media and harvested during exponential growth. The cells were suspended in a pH 7.5 buffer containing 20 mM Tris-HCl and 10 μM Na-EDTA, and were disrupted by exposure to the output of an ultrasonic oscillator. These sonnicated suspensions were clarified and particulate material also removed by centrifugation at 100,000 × g for 2 hr. The clear supernatant was decanted and used for enzymic assays. All the assays were performed by spectrophotometric methods; measurements were made in a Cary 15 recording spectrophotometer. Published procedures for phosphohexose isomerase (D-glucose-6-phosphate ketol-isomerase EC 5.3.1.9) [7], fructosediphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase EC 3.1.3.11) [8], fructosediphosphate aldolase (D-fructose-1,6-diphosphate D-glyceraldehyde-3-phosphatelyase EC 4.1.2.13) [9], triosephosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase EC 5.3.1.1) [10], phosphoglycerate kinase (ATP: 3-phospho-D-glycerate 1-phosphotransferase EC 2.7.2.3) [11], glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating) EC 1.2.1.12) [11], and enolase (2-phospho-D-glycerate hydrolyase EC 4.2.1.11) [12] were used, with minor changes to provide optimum pH and proper substrate

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and cofactor concentrations. Protein was determined by the method of Lowry et al. [13]. The chemicals and auxiliary enzymes employed were of reagent grade or highly purified preparations obtained from commercial sources.

3. Results and discussion

Pseudomonas putida grows at high rates on non sugar substrates such as succinate, lactate and acetate as sole carbon and energy sources. The growth of *Ps. putida* on succinate is even faster than its growth on glucose, indicating that an active route for hexose and pentose biosynthesis must operate in these bacteria. The conventional pathway involves the conversion of phosphoenolpyruvate to glyceraldehyde-3-phosphate (GAP), key metabolite for condensation reactions leading to hexose and pentose phosphate formation. Four enzymes are known to be involved in the synthesis of GAP from phosphoenolpyruvate. One of them, GAPDH, is barely detectable in succinate grown cells (table 1). The enzyme was assayed either with DPNH or TPNH as co-substrate. The significance of these results becomes apparent when compared with the activity of this enzyme in glucose grown cells, which is at least two orders of magnitude higher. The level of GAPDH is also high in cells grown on glycerol but its synthesis is not derepressed if the carbon source is lactate or acetate.

Data not reported here show that *Ps. putida* catabolizes glycerol through GAP, and glucose via an inducible Entner-Doudoroff pathway which yields GAP and pyruvate. The role of *Ps. putida* GAPDH in the degradation of GAP to intermediates of the TCA cycle appears clear. Accordingly the synthesis of the enzyme seems to be regulated through an inductive event elicited as a consequence of the accumulation of GAP. The extremely low values of GAPDH activity in cells grown on succinate, lactate or acetate might be regarded as a result of a regulatory mechanism involving repression rather than induction. This possibility must be discarded because the levels of the enzyme are only moderately reduced in cells grown on glucose plus succinate (table 1), presumably due to catabolic repression interfering with the inductive process.

The high regulatory control of GAPDH in *Ps. putida* is a striking feature if the bifunctional role in gluconeogenesis and GAP degradation, conventionally ascribed to this enzyme is considered. The levels of other enzymes of the Embden-Meyerhof pathway are not subjected to similar changes in *Ps. putida* (table 2). Even fructose-diphosphate aldolase, an enzyme undetectable in some pseudomonads [2,3], is clearly demonstrated in cells of this strain grown on succinate as well as glucose. To give a certain rationale to the strict

Table 1
Specific activities of glyceraldehyde-3-phosphate dehydrogenase in extracts of *Pseudomonas putida* grown on different carbon sources.

Substrates used for growth	Specific activities (μmoles/min/mg protein)	Relative activities
Glucose (20 mM)	0.240	100
Glucose (20 mM) + succinate (20 mM)	0.090	38
Glycerol (30 mM)	0.210	88
Glycerate (30 mM)	0.025	10
Succinate (20 mM)	0.002	1
Lactate (30 mM)	0.003	1
Acetate (40 mM)	0.002	1

The reaction mixture contained in a final volume of 3 ml: 200 μmoles of Tris-HCl (pH 8.5), 10 μmoles of $MgCl_2$, 6 μmoles of cysteine, 5 μmoles of ATP, 5 μmoles of 3-phosphoglycerate, 0.4 μmole of DPNH, 2.5 units of phosphoglycerate kinase and the proper amount of cell-free extract.

Table 2
Specific activities of enzymes of the Embden-Meyerhof pathway in extracts of *Pseudomonas putida* grown on glucose and succinate.

Enzyme	Substrate used for growth	
	Glucose (20 mM)	Succinate (20 mM)
	μmoles/min/mg protein	
Phosphohexose isomerase	0.033	0.042
Fructosediphosphatase	0.014	0.025
Fructosediphosphate aldolase	0.182	0.137
Triosephosphate isomerase	0.960	1.090
Phosphoglycerate kinase	0.347	0.079
Enolase	0.212	0.178

mode of regulation found in the synthesis of GAPDH, the gluconeogenic role of this enzyme in *Ps. putida* must be regarded as rather questionable. This is also adduced by theoretical considerations which show that the activity of this enzyme found in succinate grown cells, is far below the minimum estimated to supply raw material for hexose and pentose biosynthesis in accordance with the growth rate.

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