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THE ROLE OF PHOSPHOGLYCERATE KINASE IN THE METABOLISM OF *PSEUDOMONAS PUTIDA*

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1. Introduction

The absence of a functional Embden-Meyerhof pathway due to the lack of phosphofructokinase has been shown in *Pseudomonas putida* [1] and other bacteria [2–4]. The Entner-Doudoroff enzymes [5] appear to be essential for glucose metabolism in these organisms. Glyceraldehyde-3-phosphate (G3P) and pyruvate are the products of the Entner-Doudoroff pathway and it is generally admitted that the former is directed to the tricarboxylic acid cycle through the corresponding steps of the Embden-Meyerhof pathway, including that catalyzed by phosphoglycerate kinase (PGK). The properties of a mutant of *P. putida* with impaired PGK activity are described in this report, which show that the mentioned enzyme has no role in the degradation of G3P by this bacterium. In contrast, PGK is necessary to perform gluconeogenesis in *P. putida*.

2. Methods

Pseudomonas putida strain A.3.12 (ATCC 12633) was used. Mutants unable to grow on a mixture of gluconeogenic substrates, were obtained after mutagenesis with *N*-methyl-*N*′-nitrosoguanidine [6]. Mutagenized cells were allowed to grow on glucose minimal medium for 12 hr and subsequently transferred into succinate plus lactate medium where penicillin-cycloserine selection was carried out [7].

The conditions of cultivation and the enzyme assays have been previously described [8]. In addition, phosphoenolpyruvate carboxykinase was determined according to Ruiz-Amil et al. [9].

3. Results and discussion

Among *P. putida* mutants unable to grow on a mixture of gluconeogenic substrates, strain 412-3 has been well characterized and its properties are somewhat puzzling. This strain fails to grow on succinate, lactate, acetate or glycerate but is able to grow on glucose, gluconate, fructose or glycerol, suggesting a kind of deficiency in the gluconeogenic process.

To determine the enzymic defect, cell free extracts were prepared from glucose grown cells and the gluconeogenic enzymes were assayed (table 1). Values for phosphoglycerate mutase are not indicated because this activity is not detectable in *P. putida* with the usual methods. All the enzymes assayed with the exception of G3P dehydrogenase, are constitutive in *P. putida*, including phosphoenolpyruvate carboxykinase and fructosediphosphatase. The synthesis of G3P dehydrogenase is highly enhanced during growth on glucose [8]. The only difference found between 412-3 and wild type cells was the absence of detectable PGK activity in the mutant. This result is actually due to the lack of an active protein and not to the presence of a product interfering with the enzyme or with the assay method, as shown by the fact that PGK activity measured in extracts from wild type cells was not affected by adding mutant extract to the assay mixture.

That the inability of strain 412-3 to grow on the various substrates was due to a single lesion was confirmed by experiments with spontaneous revertants obtained from succinate, lactate or acetate plates. Every type or revertant had regained the ability to

Table 1
Specific activities of gluconeogenic enzymes in extracts of strain 412-3 and the wild type of *Pseudomonas putida* grown on glucose.

Enzyme	412-3	Wild type
	nmoles/min/mg protein	
Phosphoenolpyruvate carboxykinase	62	61
Enolase	259	246
Phosphoglycerate kinase	< 0.1	966
Glyceraldehyde-3-phosphate dehydrogenase	233	308
Triosephosphate isomerase	1,050	605
Fructosediphosphate aldolase	81	97
Fructosediphosphatase	16	18
Glucosephosphate isomerase	26	28

grow on all the other carbon sources mentioned earlier. The revertants showed restored PGK activity from 60 to 85% of the original activity.

The ability of *P. putida* to grow on glucose or gluconate in the absence of PGK could be explained by considering that these substrates are catabolized into pyruvate and G3P through the Entner-Doudoroff pathway. The lesion does not interact with pyruvate utilization. However, the growth rate, or at least the final yield in cell mass, should be affected by the block in one of the steps to channel G3P into the tricarboxylic acid cycle. Contrary to this expectation, the growth characteristics of 412-3 on glucose or gluconate were identical to that of the wild type. The growth curves for both strains on glucose are compared in fig. 1. Even more difficult is to understand the ability of 412-3 to grow on glycerol. Fig. 1 also shows that the utilization of this substrate by the mutant is normal. The same results were obtained when fructose was used as the carbon source. Respirometric experiments equally showed that strain 412-3 is not affected at all in the rate of oxidation of hexoses, gluconate and glycerol.

These results suggest that PGK is essential for gluconeogenesis in *P. putida* and that both 3-phosphoglycerate and 1,3-diphosphoglycerate, the substrate and the product of the reaction catalyzed by this enzyme, are likely intermediates of the process. Additional support was provided by the fact that strain 412-3 grows normally on any of the gluconeogenic substrates when the culture media are supplemented with small amounts of glucose, fructose or glycerol.

The behaviour of mutant 412-3 raises the problem of the nature of the reactions involved in the assimilation of G3P by *P. putida.* The reported data clearly show that the mutation at the structural gene for PGK completely blocks the synthesis of G3P from intermediates of the tricarboxylic acid cycle, but has no effect on the utilization of this compound as source of carbon and energy (for instance during growth on glycerol or fructose). The possibility exists that two molecules of G3P are converted into hexose and thereafter into pyruvate through the Entner-Doudoroff pathway as suggested for *Entamoeba histolytica* [10]. This organism lacks G3P dehydrogenase. The operation of this cycle as a major route for the utilization of G3P by *P. putida* must be discarded because a mutant with impaired 6-phosphogluconate dehydrase, one of the Entner-Doudoroff enzymes, although unable to grow on glu-

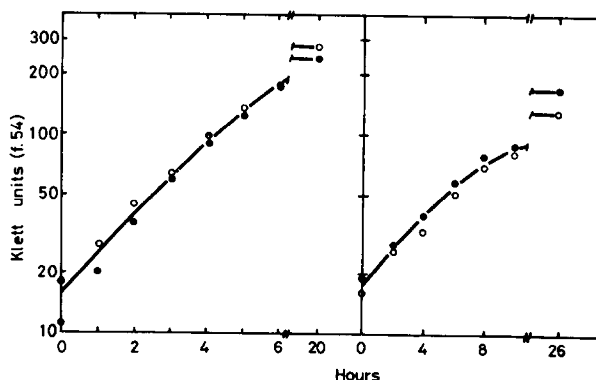

Fig. 1. Growth of strain 412-3 (•) and the wild type (o) of *Pseudomonas putida* on glucose (left) and glycerol (right).

cose or gluconate, grows readily on glycerol and fructose (unpublished results). One alternative would be that the conversion of 1,3-diphosphoglycerate into 3-phosphoglycerate to supply intermediates of the tricarboxylic acid cycle from G3P is mediated by a phosphatase, as suggested by Diederich and Grisolia [11]. Two different methods were used to assay a possible 1,3-diphosphoglycerate phosphatase activity: i) measurement of inorganic phosphate production from 3-phosphoglycerate plus ATP by extracts supplemented with PGK; ii) determination of the continuous reduction of NAD by G3P catalyzed by G3P dehydrogenase in the absence of arseniate ions or the PGK system. Unfortunately, our efforts to detect such a phosphatase activity in extracts prepared from the mutant or wild type cells were not successful. Evidently, further evaluation of other possible alternatives is required to understand the metabolism of G3P by *P. putida* according to the fact demonstrated in this paper that the enzyme PGK has no role in its conversion into intermediates of the tricarboxylic acid cycle.

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