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Effect of Phosphoglycerate Mutase Deficiency on Heterotrophic and Autotrophic Carbon Metabolism of *Alcaligenes eutrophus*

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Mutants of *Alcaligenes eutrophus* were isolated on the basis of their inability to grow on succinate as the sole source of carbon and energy. The mutants also failed to grow on other gluconeogenic substrates, including pyruvate, acetate, and citrate. Simultaneously, they had lost their capability for autotrophic growth. The mutants grew, but slower than the wild type, on fructose or gluconate. Growth retardation on gluconate was more pronounced. The mutants lacked phosphoglycerate mutase activity, and spontaneous revertants of normal growth phenotype had regained the activity. The physiological characteristics of the mutants indicate the role of phosphoglycerate mutase in heterotrophic and autotrophic carbon metabolism of *A. eutrophus*. Although the enzyme is necessary for gluconeogenesis during heterotrophic growth on three- or four-carbon substrates, its glycolytic function is not essential for the catabolism of fructose or gluconate via the Entner-Doudoroff pathway. The enzyme is required during autotrophic growth as a catalyst in the biosynthetic route leading from glycerate 3-phosphate to pyruvate. It is suggested that the mutants accomplish the complete degradation of fructose and gluconate to pyruvate by a special metabolic process to bypass the phosphoglycerate mutase lesion. The catabolically produced triose phosphates are converted to fructose 6-phosphate which is rechanneled into the Entner-Doudoroff pathway. This carbon recycling mechanism operates less effectively in mutant cells growing on gluconate.

The facultatively chemolithoautotrophic hydrogen bacterium *Alcaligenes eutrophus* is able to grow autotrophically with a gas mixture of hydrogen, carbon dioxide, and oxygen as well as heterotrophically under air on a wide variety of organic substrates. Autotrophic carbon dioxide assimilation in this organism involves the reactions of the Calvin cycle (8). Fructose and gluconate are metabolized exclusively via the Entner-Doudoroff pathway since *A. eutrophus* lacks detectable phosphofructokinase (EC 2.7.1.11) and gluconate 6-phosphate dehydrogenase (EC 1.1.1.44) activities (4, 6, 14). The products of that pathway are pyruvate and glyceraldehyde 3-phosphate. The further conversion of the latter to pyruvate proceeds through reactions occurring also in the Embden-Meyerhof pathway. Pyruvate thus produced can be used for biosynthesis and for oxidation via the tricarboxylic acid cycle to yield additional energy (Fig. 1).

Depending on the carbon source supporting growth, the metabolic segment between glycerate 3-phosphate and pyruvate can serve different functions. During heterotrophic growth, it participates either in glycolysis or in the opposing process of gluconeogenesis. In autotrophic carbon metabolism, however, this reaction sequence has only an anabolic role in the synthesis of essential biosynthetic precursors from glycerate 3-phosphate. It was therefore of particular interest to investigate the importance of the pertaining enzymes for the heterotrophic and autotrophic carbon metabolism in *A. eutrophus*.

We succeeded in isolating mutants of *A. eutrophus* with deficient phosphoglycerate mutase (PGM; EC 2.7.5.3) activity. The properties of the mutants suggest that PGM is absolutely necessary for gluconeogenesis and for autotrophic metabolism in this organism. In contrast, the enzyme appears to be dispensable for the complete degradation of fructose and gluconate to pyruvate, although the PGM mutants grew slower than the wild type on these substrates. The latter finding can be explained by the operation of a special recycling process in the central carbon metabolism, which enables the mutants to bypass the PGM lesion during breakdown of fructose or gluconate.

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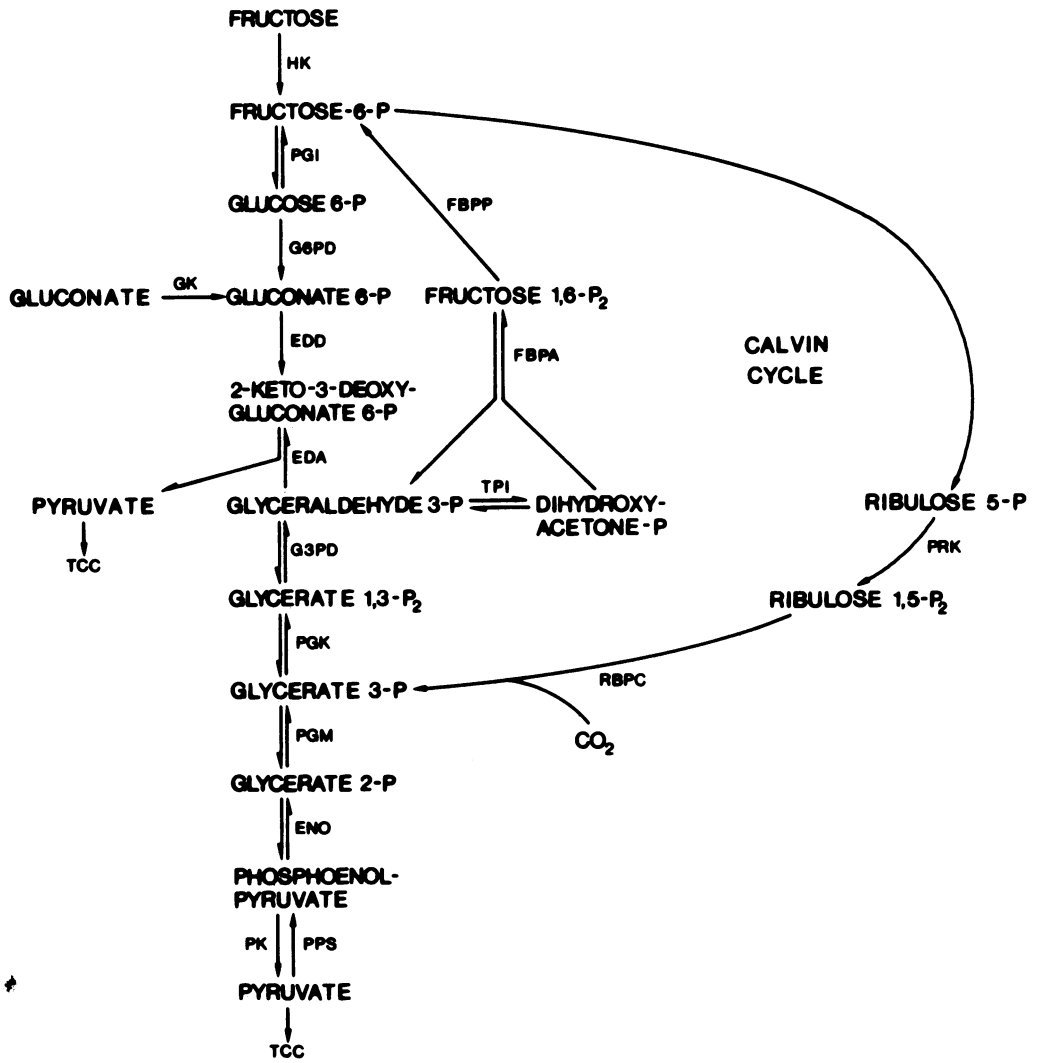

FIG. 1. Outline of the pathways in central carbon metabolism of *A. eutrophus* showing the interlocking of the Entner-Doudoroff pathway and the Calvin cycle. Abbreviations of enzyme names: HK, hexokinase; GK, gluconokinase; PGI, phosphoglucose isomerase; G6PD, glucose-6-phosphate dehydrogenase; EDD, gluconate-6-phosphate dehydratase; EDA, 2-keto-3-deoxygluconate-6-phosphate aldolase; TPI, triosephosphate isomerase; FBPA, fructosebisphosphate aldolase; FBPP, fructose bisphosphatase; G3PD, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase; PK, pyruvate kinase; PPS, phosphenolpyruvate synthetase; PRK, phosphoribulokinase; RBPC, ribulosebisphosphate carboxylase; TCC, tricarboxylic acid cycle.

MATERIALS AND METHODS

Bacterial strains. *A. eutrophus* H16 (DSM 428, ATCC 17699) was used in this study, and the mutant strains employed were derived from strain H16.

Culture media and growth conditions. The bacteria were grown in the mineral salts medium of Schlegel et al. (23) at 30°C. For heterotrophic growth and also for organoautotrophic growth on formate, carbon sources were added at a concentration of 0.2% (wt/vol) unless stated otherwise. The gas mixture supplied for lithoautotrophic cultivation consisted of hydrogen, carbon dioxide, and oxygen at a ratio of 8:1:1 (vol/vol). Solid media contained 1.5% (wt/vol) agar.

Growth experiments in liquid medium were performed with 30-ml cultures in 300-ml side-arm flasks incubated on a rotary shaker. Cell growth was followed turbidimetrically with a Klett-Summerson colorimeter equipped with no. 54 filter (520 to 580 nm). Klett unit readings corrected for deviation from linearity were converted into optical density values measured at 436 nm (OD_{436}) in a Zeiss PL4 spectrophotometer by means of a calibration curve.

Cells to be used for preparation of cell-free extracts

were grown in 300-ml cultures using baffled 2-liter Erlenmeyer flasks incubated on a rotary shaker. They were harvested in mid-exponential phase ($OD_{436}=3$), washed once with 10 volumes of 20 mM Tris-hydrochloride buffer (pH 7.8) containing 10 mM $MgCl_2$ and 1 mM EDTA, and stored at −20°C.

Isolation of mutants. Mutagenesis was effected following procedures described previously (7) using sodium nitrite at 20 mM or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 100 μg/ml as mutagens. Mutagenized cells were grown in mineral medium containing fructose to allow phenotypic expression and resuspended in mineral medium with pyruvate as sole carbon source. After treatment with colistine sulfate (final concentration, 75 μg/ml) for 12 h (21) to enrich the mutants of interest, survivors were plated onto mineral agar medium with fructose. Succinate-negative mutant clones were identified by replica plating on mineral agar medium plus succinate.

Isolation of spontaneous revertants of mutant strains. Cells of mutant strains were grown in fructose mineral medium, harvested in mid-exponential phase, washed twice in mineral medium without carbon source, and suspended in that medium to a density of 10^{10} cells/ml. Agar plates with mineral medium containing succinate as sole carbon source were seeded with 10^9, 10^8, 10^7, or 10^6 cells and incubated at 30°C for 120 h. Clones of succinate-positive revertants were isolated and verified by repetitive streaking on succinate mineral agar medium.

Preparation of cell extracts. All steps during the preparation of cell fractions were performed at 0 to 4°C. Thawed cells suspended in three volumes of 20 mM Tris-hydrochloride buffer (pH 7.8) containing 10 mM $MgCl_2$, 1 mM EDTA, and DNase I (10 μg/ml) were ruptured in a precooled French press at 140 MPa. Unbroken cells and cell debris were removed by centrifugation at 10,000 × *g* for 10 min. The supernatant was centrifuged at 140,000 × *g* for 60 min. The resulting supernatant served as cell-free extract for the assays of soluble enzymes. For stabilization of the soluble hydrogenase (EC 1.12.1.2.), ferricyanide was added at 0.5 mM to a portion of the extract (11). The membranes in the pellet fraction were homogenized in 1 ml of 50 mM potassium phosphate buffer (pH 7.0), recentrifuged at 140,000 × *g* for 60 min, and homogenized as before. This membrane suspension was used to assay the particulate hydrogenase.

Enzyme assays. The enzyme assays were carried out at 30°C. Assays involving NADH oxidation or $NADP^+$ reduction were monitored spectrophotometrically at 365 nm employing published methods for the following enzyme activities: phosphoglucose isomerase (EC 5.3.1.9), $NADP^+$-linked glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and combined activity of gluconate-6-phosphate dehydratase (EC 4.2.1.12) and 2-keto-3-deoxygluconate-6-phosphate aldolase (EC 4.1.2.14) (4); fructose-1,6-bisphosphatase (EC 3.1.3.11) (1); fructose-1,6-bisphosphate aldolase (EC 4.1.2.13), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), and 3-phosphoglycerate kinase (EC 2.2.2.3) (12); triosephosphate isomerase (EC 5.3.1.1) (3); phosphoglycerate mutase (glycerate-2,3-bisphosphate-dependent) and enolase (EC 4.2.2.11) (19); and pyruvate kinase (EC 2.7.1.40) (26). Phosphoenolpyruvate synthetase (EC 2.7.1.dd) activity was determined spectrophotometrically by coupling with excess phosphoenolpyruvate carboxylase (EC 4.1.1.31) and malate dehydrogenase (EC 1.1.1.37). The reaction mixture contained, in a total volume of 0.6 ml: 100 mM Tris-hydrochloride buffer (pH 8.8), 10 mM $MgCl_2$, 1.5 mM sodium pyruvate, 10 mM ATP, 20 mM $NaHCO_3$, 0.25 mM NADH, 0.5 U of phosphoenolpyruvate carboxylase, 5 U of malate dehydrogenase, and 0.2 to 0.5 mg of extract protein gel filtered on Sephadex G-25. The reaction was initiated by the final addition of ATP. All assay mixtures were incubated anaerobically by sparging the cuvettes with nitrogen in order to suppress the interfering NADH-oxidizing activity. The NAD^+-linked soluble hydrogenase was assayed by the method of Schneider et al. (24), and the particulate hydrogenase was assayed by following the reduction of methylene blue at 570 nm according to Schink and Schlegel (22). Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) activity was determined by ribulose 1,5-bisphosphate-dependent incorporation of $^{14}CO_2$ into acid-stable product as described previously (5). The two-stage radiometric assay of Siebert et al. (25) was used to measure phosphoribulokinase (EC 2.7.1.19) activity. Specific activities of enzymes were calculated as units per milligram of protein (micromoles of product formed per minute per milligram of protein).

Protein concentrations in soluble and particulate cell fractions were estimated by the method of Lowry et al. (18), using bovine serum albumin as standard.

Chemicals. Colistine sulfate was obtained from Chemie Grünenthal GmbH, Stolberg, Germany. Ribulose 1,5-bisphosphate was purchased from Sigma Chemical Co., St. Louis, Mo. Ribulose 5-phosphate was prepared according to Pontremoli and Mangiarotti (20). Sodium [^{14}C] bicarbonate was a product of The Radiochemical Centre, Amersham, England. Enzymes and further substrates employed in enzyme assays were purchased from C. F. Boehringer & Soehne, Mannheim, Germany. Other chemicals were of reagent-grade purity and were obtained from E. Merck AG, Darmstadt, Germany.

RESULTS

Isolation and enzyme defects of mutant strains. The mutants derived from *A. eutrophus* were isolated as clones unable to grow on succinate but retaining the ability to grow on fructose as sole source of carbon and energy. Thus, the selection procedure (see Materials and Methods) was designed to yield mutants deficient in enzymes of gluconeogenesis. Four mutant strains with very similar phenotypes have been isolated. Therefore, the characteristics of only one of the mutants obtained, strain BB4, will be described in detail. The specific activities of most gluconeogenic and glycolytic enzymes of the wild type and strain BB4 grown on fructose are presented in Table 1. All four mutants were found to contain insufficient PGM activities amounting to only 1 to 2% of the activity determined in cell-free extracts of the wild type. No other deficiencies in enzymes of the central carbohydrate metabolism, including those catalyzing interconversions of C_3- and C_4-metabolites (data not shown), were observed in mutant

TABLE 1. Activities of gluconeogenic and glycolytic enzymes in cell-free extracts of the wild-type strain *A. eutrophus* and of mutant BB4[a]

Enzyme	Sp act (U/mg of protein)	
	Wild type	BB4
Fructosebisphosphate aldolase	0.227	0.250
Fructose bisphosphatase	0.144	0.130
Triosephosphate isomerase	1.050	0.530
Glyceraldehyde-3-phosphate dehydrogenase	1.420	0.840
Phosphoglycerate kinase	1.260	1.050
Phosphoglycerate mutase	0.990	0.008
Enolase	0.180	0.110
Pyruvate kinase	0.052	0.102
Phosphoenolpyruvate synthetase	0.050	0.107

[a] The cells were grown in fructose mineral medium and harvested in mid-exponential phase (OD_{436} = 3).

BB4. Also, the activities of the key enzymes of lithotrophic energy metabolism, the soluble and particulate hydrogenases, as well as of autotrophic carbon dioxide assimilation, phosphoribulokinase, and ribulosebisphosphate carboxylase, were in the same range for both wild type and the mutant (Table 2). These latter enzymes are partially derepressed during heterotrophic growth of *A. eutrophus* on fructose (12).

Growth characteristics of mutant strain BB4. In Table 3 are listed the growth properties of mutant BB4 as determined on solid media supplied with various carbon sources. The heterotrophic growth of BB4 on fructose or gluconate was discernibly slower than that of the wild type. However, growth on gluconate seemed to be more strongly retarded. The mutant failed to grow on all gluconeogenic substrates tested, including pyruvate, succinate, malate, acetate, citrate, and glycerol. Supplementation of succinate- or pyruvate-containing media with fructose or glycerol did not result in growth, i.e., under these conditions fructose and glycerol could not satisfy the gluconeogenic carbon requirements of the mutant. Even nutrient broth did not support growth. Moreover, strain BB4 also lost the ability to grow autotrophically on either carbon dioxide plus hydrogen or on formate (11) as a consequence of the PGM deficiency.

Growth experiments in liquid media confirmed that the mutant grew slower on fructose (doubling time, 2.9 h) than the wild type (doubling time, 2.1 h; Fig. 2). Surprisingly, the mutant exhibited biphasic growth on gluconate if precultured on fructose. The cells grew exponentially with a doubling time of 3.1 h during the first three to four doublings after which they gradually entered a second, much slower exponential growth phase characterized by a doubling time of 9.3 h (Fig. 2). This slow growth phase was abolished by adding supplementary fructose to the gluconate medium (Fig. 2). Preculturing of the mutant on gluconate resulted in slow exponential growth on gluconate similar to that of the slow growth phase of fructose-precultured cells (data not shown). Despite the metabolic block, the growth yields of strain BB4 on fructose or gluconate were nearly identical to those of the wild type, indicating complete degradation of the substrates by the mutant.

To elucidate the metabolic basis for the strong growth retardation of mutant BB4 on gluconate, the activities of enzymes involved in fructose and gluconate catabolism and in gluconeogenesis were assayed (Table 4). It is evident that gluconate-grown wild type as well as mutant cells contained much lower levels of phospho-

TABLE 2. Activities of the key enzymes of lithoautotrophic metabolism in cell-free extracts of the wild-type strain *A. eutrophus* and of phosphoglycerate mutase mutant BB4[a]

Enzyme	Sp act (U/mg of protein)	
	Wild type	BB4
Phosphoribulokinase	0.036	0.057
Ribulosebisphosphate carboxylase	0.024	0.116
Soluble hydrogenase	0.160	0.380
Particulate hydrogenase	0.060	0.010

[a] The cells were grown in fructose mineral medium.

TABLE 3. Growth of wild-type strain *A. eutrophus* and of phosphoglycerate mutase mutant BB4 on various carbon sources[a]

Carbon source	Wild type	BB4
Fructose	+++	++
Gluconate	+++	+
Glycerol	+	−
Pyruvate	+++	−
Succinate	+++	−
Succinate + fructose[b]	+++	−
Nutrient broth[c]	+++	−
$CO_2(H_2)$[d]	++	−
Formate	+	−

[a] Bacteria were streaked on mineral agar medium containing the indicated carbon sources. Growth was scored after 72 h of incubation. Symbols: +++, luxuriant growth; ++, good growth; +, poor growth; −, no growth

[b] Fructose was supplemented at a concentration of 0.01% (wt/vol).

[c] Present at a concentration of 0.8% (wt/vol).

[d] Lithoautotrophic growth on mineral agar medium under a gas atmosphere containing hydrogen, carbon dioxide, and oxygen (8:1:1, vol/vol).

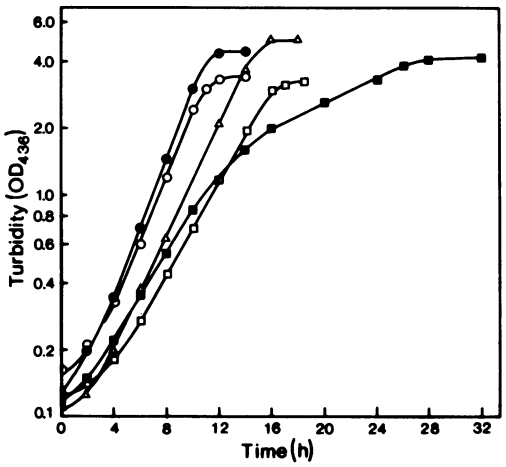

FIG. 2. Heterotrophic growth of *A. eutrophus* wild type and phosphoglycerate mutase-deficient mutant strain BB4. The liquid mineral medium contained 0.1% (wt/vol) fructose, 0.2% (wt/vol) gluconate, or 0.2% (wt/vol) gluconate plus 0.01% (wt/vol) fructose as sources of carbon and energy. Symbols: ○, wild type on fructose; ●, wild type on gluconate; □, BB4 on fructose; ■, BB4 on gluconate; △, BB4 on gluconate plus fructose. The strains were precultured on fructose.

glucose isomerase and glucose-6-phosphate dehydrogenase activities than fructose-grown cells. As expected, the key enzymes of the Entner-Doudoroff pathway, gluconate-6-phosphate dehydratase and 2-keto-3-deoxygluconate-6-phosphate aldolase, were present at high activities in both fructose- and gluconate-grown cells. The activity levels of the gluconeogenic enzymes fructosebisphosphate aldolase and fructose bisphosphatase were not significantly influenced by the two carbon sources.

The results of the growth experiments together with the patterns of enzyme activities suggest that, during breakdown of fructose or gluconate, mutant BB4 converts glyceraldehyde 3-phosphate into fructose 6-phosphate which is rechanneled into the Entner-Doudoroff pathway. However, this rechanneling process being essential for complete substrate utilization by the PGM mutant is very limited during gluconate degradation because of the low phosphoglucose isomerase and glucose-6-phosphate dehydrogenase activities. In mutant cells growing on gluconate in the presence of supplementary fructose, the latter enzymes were present at activity levels found in fructose-grown cells (data not shown). This is in accord with the faster monophasic growth of BB4 under these conditions (see Fig. 2).

Spontaneous revertants. Revertants were selected on succinate-containing mineral agar medium. Depending on the mutant strain examined, they occurred with frequencies of about 2×10^{-7} to 10^{-8}. All revertants exhibited the growth phenotype of the wild type, suggesting that the mutant phenotype was caused by a single mutation. Revertant strain BB4-R1, taken as representative, grew with the same rate as the wild type on fructose, succinate, or under autotrophic conditions (Fig. 3). The strain regained about 45% of the PGM activity found in the wild type.

DISCUSSION

Although the selection procedure used to isolate mutants of *A. eutrophus* with impaired gluconeogenic capacity was expected to yield different types of mutants, only strains deficient in PGM were obtained. Mutants with defects in glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, or enolase would possibly show similar phenotypes. Also, the isolation of fructosebisphosphate aldolase- or fructose bisphosphatase-deficient strains seemed to

TABLE 4. Activities of enzymes involved in fructose and gluconate catabolism and in gluconeogenesis in the wild-type strain *A. eutrophus* and in mutant BB4

Enzyme	Carbon source[a]	Sp act (U/mg of protein)	
		Wild type	BB4
Phosphoglucose isomerase	Fructose	0.183	0.189
	Gluconate	0.017	0.026
Glucose-6-phosphate dehydrogenase	Fructose	0.225	0.216
	Gluconate	0.016	0.023
Gluconate-6-phosphate dehydratase/2-keto-3-deoxygluconate-6-phosphate aldolase	Fructose	0.135	0.154
	Gluconate	0.142	0.125
Fructosebisphosphate aldolase	Fructose	0.210	0.236
	Gluconate	0.235	0.230
Fructose bisphosphatase	Fructose	0.115	0.122
	Gluconate	0.185	0.131

[a] Cells grown in 300 ml of fructose mineral medium were harvested in mid-exponential phase (OD_{436} = 3) and used to prepare cell-free extracts. Immediately before the harvest, 10 ml of the fructose cultures was withdrawn, and the cells were washed in mineral medium and used to inoculate a 300-ml culture of mineral medium containing gluconate. The wild-type cells of the gluconate culture were harvested in late exponential phase, and the mutant cells were harvested after entering the second, slower exponential growth phase (see Fig. 2) and also used for the preparation of cell-free extracts.

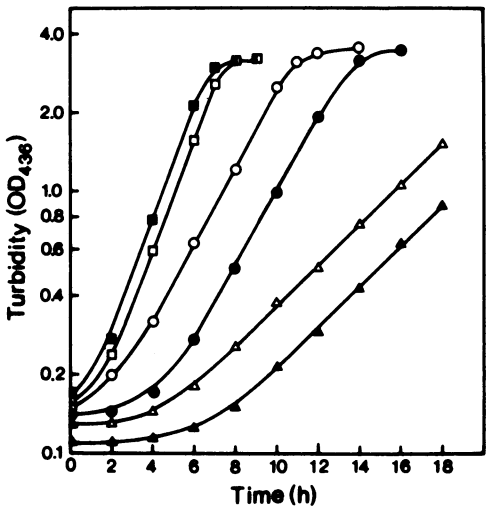

FIG. 3. Heterotrophic and autotrophic growth of *A. eutrophus* wild type (open symbols) and revertant strain BB4-R1 (closed symbols). The liquid mineral medium contained 0.1% (wt/vol) fructose (○, ●) or 0.2% (wt/vol) succinate (□, ■) for the heterotrophic cultures. A gas mixture consisting of hydrogen, carbon dioxide, and oxygen (8:1:1, vol/vol) was supplied to the autotrophic cultures (△, ▲). The strains were precultured on fructose.

be conceivable according to the rationale for the selection. The reason for the failure to isolate other than PGM mutants remains unclear. There may exist isozymic forms of the mentioned enzymes in *A. eutrophus*. This is unlikely in the case of PGM since the mutants lacked this activity almost completely. To our knowledge this is the first report on the isolation of a bacterial PGM mutant.

The characteristics of the PGM mutants indicate the role of the enzyme in heterotrophic and autotrophic carbon metabolism of *A. eutrophus*. The enzyme is necessary for the organism to perform gluconeogenesis during heterotrophic growth on four-carbon or three-carbon substrates except glycerol. Growth on glycerol probably requires the catabolic function of PGM because glycerol metabolites enter the glycolytic pathway presumably above this enzyme at the level of triose phosphate.

The glycolytic function of PGM is not essential for the growth of *A. eutrophus* on fructose or gluconate. It seems that the metabolic lesion in PGM did not prevent the complete degradation of fructose or gluconate to pyruvate. Analogous results with respect to the growth on glucose or gluconate were reported for a phosphoglycerate kinase-deficient mutant of *Pseudomonas putida* (2) which also catabolizes these substrates through the Entner-Doudoroff pathway.

The situation is different in *Escherichia coli*, which metabolizes hexoses primarily via the Embden-Meyerhof pathway but gluconate preferentially via the Entner-Doudoroff pathway (10). *E. coli* mutants defective in glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, or enolase were unable to grow not only on hexoses but also on gluconate (15, 16). Moreover, addition of hexoses or gluconate to a medium containing a permissive substrate mixture, e.g., succinate plus glycerol, inhibited the growth of these mutants possibly due to intracellular accumulation of inhibitory levels of fructose 1, 6-bisphosphate and triose phosphates produced from the nonmetabolizable part of the substrate carbon (16, 17).

Apparently, the PGM mutants of *A. eutrophus* are able to avoid such accumulation of metabolites during breakdown of fructose or gluconate, probably by converting glyceraldehyde 3-phosphate into fructose 6-phosphate which is rechanneled into the Entner-Doudoroff pathway. Thus, one half of the catabolically metabolized carbon from fructose or gluconate has to be recycled in the mutants by a process involving part of the gluconeogenic reaction sequence. The fact that the growth of the mutants on gluconate is considerably slower than that on fructose supports the operation of such a mechanism. In gluconate-grown wild-type and mutant cells, the activities of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase which are needed for the rechanneling of carbon into the Entner-Doudoroff pathway were at a basal repressed level. This confirms previous data (4, 6). However, the growth of the PGM mutants on fructose was also significantly slower than that of the wild type, indicating a limiting flow of carbon through the recycling sequence. Gluconate-6-phosphate dehydratase and 2-keto-3-deoxygluconate-6-phosphate aldolase are not induced in *A. eutrophus* during growth on glycerol (B. Bowien, unpublished data). Consequently, the PGM mutants did not grow on glycerol because of their inability to produce pyruvate.

There is obviously no further metabolic route in *A. eutrophus* to bypass the PGM lesion. One possible route, the methylglyoxal pathway (9) is unlikely to function since methylglyoxal synthase (EC 4.2.99.11) activity was not detected in the wild type and in mutant BB4 (data not shown). The failure of fructose or glycerol to satisfy the gluconeogenic carbon requirements of the mutants in the presence of succinate or pyruvate is presumably due to the catabolite repression of fructose and glycerol utilization by the organic acids (B. Bowien, unpublished data).

In *A. eutrophus* the synthesis of phosphoenolpyruvate from pyruvate does not involve carboxylation of the latter (13) but rather the action

of phosphoenolpyruvate synthetase (P. Schobert and B. Bowien, unpublished data). It is evident that the PGM mutants rely upon the activity of this enzyme during the growth on fructose or gluconate. Accordingly, fructose-grown mutant, but also wild-type cells, contained relatively high phosphenolpyruvate synthetase activities (see Table 1).

Finally, the data demonstrated that PGM activity is required in autotrophic carbon metabolism of *A. eutrophus* to accomplish the synthesis of phosphenolpyruvate and related metabolites. Bypassing of the metabolic block in the mutants is not possible because the Entner-Doudoroff enzymes are repressed in autotrophically growing cells (4, 6). The PGM mutants being deficient in an enzyme that converts the primary product of autotrophic carbon dioxide assimilation may lend themselves to studies on the regulatory mechanisms governing the expression of the key enzymes of the Calvin cycle in *A. eutrophus*.

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LITERATURE CITED

1. **Abdelal, A. T., and H. G. Schlegel.** 1974. Purification and regulatory properties of fructose 1,6-diphosphatase from *Hydrogenomonas eutropha*. J. Bacteriol. **120**:304–310.
2. **Aparicio, M. L., M. Ruiz-Amil, M. Vicente, and J. L. Canovas.** 1971. The role of phosphoglycerate kinase in the metabolism of *Pseudomonas putida*. FEBS Lett. **14**:326–328.
3. **Bergmeyer, H. U.** 1974. Triosephosphate-Isomerase, p. 550. *In* H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse, Band I. Verlag Chemie, Weinheim.
4. **Blackkolb, F., and H. G. Schlegel.** 1968. Katabolische Repression und Enzymhemmung durch molekularen Wasserstoff bei *Hydrogenomonas*. Arch. Mikrobiol. **62**:129–143.
5. **Bowien, B., F. Mayer, G. A. Codd, and H. G. Schlegel.** 1976. Purification, some properties and quaternary structures of the D-ribulose 1,5-diphosphate carboxylase of *Alcaligenes eutrophus*. Arch. Microbiol. **110**:157–166.
6. **Bowien, B., and H. G. Schlegel.** 1972. Der Biosyntheseweg der RNS-Ribose in *Hydrogenomonas eutropha* Stamm H16 und *Pseudomonas facilis*. Arch. Mikrobiol. **85**:95–112.
7. **Bowien, B., and H. G. Schlegel.** 1972. Isolierung und Charakterisierung katabolischer Defektmutanten von *Hydrogenomonas eutropha* Stamm H16. I. Fructose-negative Mutanten. Arch. Mikrobiol. **87**:203–219.
8. **Bowien, B., and H. G. Schlegel.** 1981. Physiology and biochemistry of aerobic hydrogen-oxidizing bacteria. Annu. Rev. Microbiol. **35**:405–452.
9. **Cooper, R. A., and A. Anderson.** 1970. The formation and catabolism of methylglyoxal during glycolysis in *Escherichia coli*. FEBS Lett. **11**:273–276.
10. **Fraenkel, D. G., and S. R. Levisohn.** 1967. Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase. J. Bacteriol. **93**:1571–1578.
11. **Friedrich, C. G., B. Bowien, and B. Friedrich.** 1979. Formate and oxalate metabolism in *Alcaligenes eutrophus*. J. Gen. Microbiol. **115**:185–192.
12. **Friedrich, C. G., B. Friedrich, and B. Bowien.** 1981. Formation of enzymes of autotrophic metabolism during heterotrophic growth of *Alcaligenes eutrophus*. J. Gen. Microbiol. **122**:69–78.
13. **Frings, W., and H. G. Schlegel.** 1971. Zur Synthese von C_4-Dicarbonsäuren aus Pyruvat durch *Hydrogenomonas eutropha* Stamm H16. Arch. Mikrobiol. **79**:204–219.
14. **Gottschalk, G., U. Eberhardt, and H. G. Schlegel.** 1964. Verwertung von Fructose durch *Hydrogenomonas* H16 (I.). Arch. Mikrobiol. **48**:95–108.
15. **Hillman, J. D., and D. G. Fraenkel.** 1975. Glyceraldehyde 3-phosphate dehydrogenase mutants of *Escherichia coli*. J. Bacteriol. **122**:1175–1179.
16. **Irani, M., and P. K. Maitra.** 1974. Isolation and characterization of *Escherichia coli* mutants defective in enzymes of glycolysis. Biochem. Biophys. Res. Commun. **56**:127–133.
17. **Irani, M. H., and P. K. Maitra.** 1977. Properties of *Escherichia coli* mutants deficient in enzymes of glycolysis. J. Bacteriol. **132**:398–410.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Maitra, P. K., and Z. Lobo.** 1971. A kinetic study of glycolytic enzyme synthesis in yeast. J. Biol. Chem. **246**:475–488.
20. **Pontremoli, S., and G. Manglarotti.** 1962. A simple method for the preparation of D-ribulose 5-phosphate. J. Biol. Chem. **237**:643–645.
21. **Reh, M., and H. G. Schlegel.** 1969. Anreicherung und Isolierung auxotropher Mutanten von *Hydrogenomonas* H16. Arch. Mikrobiol. **67**:99–109.
22. **Schink, B., and H. G. Schlegel.** 1979. The membrane-bound hydrogenase of *Alcaligenes eutrophus*. I. Solubilization, purification and biochemical properties. Biochim. Biophys. Acta **567**:315–324.
23. **Schlegel, H. G., H. Kaltwasser, and G. Gottschalk.** 1961. Ein Submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstumsphysiologische Untersuchungen. Arch. Mikrobiol. **39**:209–222.
24. **Schneider, K., R. Cammack, H. G. Schlegel, and D. O. Hall.** 1979. The iron-sulfur centres of soluble hydrogenase from *Alcaligenes eutrophus*. Biochim. Biophys. Acta **578**:445–461.
25. **Siebert, K., P. Schobert, and B. Bowien.** 1981. Purification, some catalytic and molecular properties of phosphoribulokinase from *Alcaligenes eutrophus*. Biochim. Biophys. Acta **658**:35–44.
26. **Wilke, D., and H. G. Schlegel.** 1975. Regulation of pyruvate kinase from *Alcaligenes eutrophus* H16 *in vitro* and *in vivo*. Arch. Microbiol. **105**:109–115.