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PURIFICATION AND PROPERTIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ($NADP^+/NAD^+$) AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ($NADP^+/NAD^+$) FROM METHANOL-GROWN *PSEUDOMONAS* C

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Summary

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADPH^+$ 1-oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ 2-oxidoreductase, EC 1.1.1.43) have been purified from methanol-grown *Pseudomonas* C.

Glucose-6-phosphate dehydrogenase exhibits activity with either $NADP^+$ or NAD^+ as coenzymes, V $NADP^+$ = 0.96 V NAD^+. K_m values of 22, 290, and 250 μM are obtained for $NADP^+$, NAD^+ and glucose 6-phosphate ($NADP^+$ as the coenzyme), respectively. ATP inhibits Glc-6*P* dehydrogenase activity with NAD^+ as coenzyme and to a less extent the activity with $NADP^+$. In the presence of $MgCl_2$, ATP inhibition of Glc-6*P* dehydrogenase activity is abolished.

6-Phosphogluconate dehydrogenase has a dual specificity for both $NADP^+$ or NAD^+ as coenzymes, V $NADP^+$ = 1.66 V NAD^+. K_m values of 20, 500 and 100 μM are obtained for $NADP^+$, NAD^+ and 6-phosphogluconate ($NADP^+$ as the coenzyme), respectively. With NAD^+ as the coenzyme ATP inhibits 6-phosphogluconate dehydrogenase activity, while with $NADP^+$ as the coenzyme, activity was less affected.

The possible role of these enzymes in the metabolism of one-carbon (C_1)-compounds in *Pseudomonas* C is discussed and compared with other methylotrphic microorganisms.

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Introduction

In a previous work [1] we have shown that *Pseudomonas* C oxidizes methanol to CO_2 both via formate and via a cyclic oxidation pathway [2]. *Pseudomonas* C assimilates C_1-compounds via the ribulose monophosphate pathway of formaldehyde fixation [3,4]. Thus, in C_1-utilizing bacteria such as *Pseudomonas* C, several enzymatic reactions are a part of both the cyclic oxidation pathway and the assimilation pathway of methanol. How the cell regulates the methanol carbon flux for energy production (NADH) and biosynthetic purposes (NADPH and C_3-, C_5- and C_6-building units) is unknown.

A possible site of regulation is the glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase, EC 1.1.1.49) step. This enzyme is known to be a regulatory enzyme in many microorganisms [5] and since in *Pseudomonas* C the enzyme exhibits activity with either $NADP^+$ or NAD^+ [1] it is a likely candidate for regulation of carbon flow in this bacterium. The same reasons are true for 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ 2-oxidoreductase, EC 1.1.1.43) [6—8], which in *Pseudomonas* C is found after the branch point of the oxidation and assimilation pathways [1].

Recently these enzymes were purified from *Candida boidinii*, a methanol-utilizing yeast, and their properties studies [9]. It was therefore of interest to compare the properties of the two enzymes from the yeast with those of *Pseudomonas* C, a bacterium which utilizes methanol via the similar biochemical pathways [1,9].

Materials and Methods

Growth of microorganisms. The growth of the bacterium used in this study, *Pseudomonas* C, has been described previously [10,11]. Cells from the late exponential phase of growth were harvest for purification of enzymes. Cells were washed once with 50 mM phosphate buffer (pH 7.0) containing 5 mM $MgCl_2$ and 1 mM dithiothreitol and stored at −20°C until used.

Enzyme assays. All assays involved $NADP^+$ or NAD^+ reduction which was followed at 340 nm using a Gilford recording spectrophotometer (model 240). Assays were performed at 30°C, and specific activities are expressed as international enzyme units (IU) per mg protein. Protein was measured as described by Lowry et al. [12], after precipitation in 10% trichloroacetic acid and resuspension in 0.2 N NaOH, using bovine serum albumin as standard.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) assay mixtures contained, in a total volume of 1.0 ml, 100 mM of Tris-HCl buffer (pH 8.4), 5 mM glucose 6-phosphate and 0.5 mM $NADP^+$ or 3 mM NAD^+.

6-Phosphogluconate dehydrogenase (EC 1.1.1.44) assay mixtures contained in a total volume of 1.0 ml, 100 mM of glycylglycine buffer (pH 8.4), 1 mM 6-6-phosphogluconate, 2 mM $MgCl_2$ and 0.2 mM $NADP^+$ or 5 mM NAD^+.

Purification of enzymes. All manipulations were carried out at 4°C unless stated otherwise.

(i) Glucose-6-phosphate dehydrogenase. Pseudomonas C cells (50 g cell wet weight) were suspended in about 100 ml of 5 mM Tris-HCl buffer (pH 7.5)

containing 5 mM $MgCl_2$ and 1 mM 2-mercaptoethanol (buffer A). The cell suspension was ruptured and deoxyribonuclease (5 μg/ml) was added to the suspension which was then incubated for 15 min at 25°C. This suspension was centrifuged at 25 000 × *g* for 20 min and the clear supernatant solution obtained is referred to as "crude extract" (Table I).

After fractionation of the crude extract with solid ammonium sulfate (Table I) the enzyme solution was applied onto a column (2.5 × 20 cm) of DEAE-cellulose pre-equilibrated with buffer A. Elution of the enzyme was carried out with a linear gradient of 0—0.5 M KCl (500 + 500 ml) in the same buffer. The fractions with enzyme activity (0.15—0.20 M KCl) were pooled and precipitated with solid ammonium sulfate (to 90% saturation).

The ammonium sulfate fraction was applied to a blue dextran-Sepharose 4B column (2.5 × 15 cm) [13] which was eluted by a gradient of 0—5 mM $NADP^+$ in buffer A. Fractions containing enzyme activity were collected between 3.3 and 4.6 mM $NADP^+$.

(ii) 6-Phosphogluconate dehydrogenase. Proamine sulfate (2% solution, pH 6.0) was added slowly to the crude extract of *Pseudomonas* C (to give a final concentration of 0.1%) and the resulting precipitate was removed by centrifugation. To the clear supernatant solution obtained, 180 g (wet wt.) of phosphocellulose (pre-equilibrated with 0.25 M sodium acetate buffer (pH 6.0) (buffer B)) were added and the mixture was stirred for 1 h. The enzyme was eluted from the phosphocellulose by 0.3 M sodium phosphate buffer (pH 6.3) and then applied onto a blue dextran-Sepharose 4B column pre-equilibrated with buffer A. The column was washed with buffer B which contained 5 mM NADH and then eluted with a linear gradient of 0—0.7 M KCl in buffer A (200 + 200 ml). Enzyme activity was eluted between 0.3 and 0.4 M KCl. Further purification of the enzyme was accomplished with a phosphocellulose column (2 × 5 cm), washed with buffer B (50 ml) and then eluted with a solution of 6-phosphogluconate (0.05%) in the same buffer.

Results

A. Purification of enzymes

The results of purifying glucose-6-phosphate dehydrogenase are given in Table I. A 220-fold purification of the enzyme was achieved, the yield obtained was 58% and a single band of protein was seen on polyacrylamide gel electrophoresis.

Table II summarizes the purification of 6-phosphogluconate dehydrogenase from *Pseudomonas* C. A 245-fold purification of the enzyme was achieved, the yield obtained was 50% and a single band of protein was evident on polyacrylamide gel electrophoresis.

B. Properties of glucose-6-phosphate dehydrogenase

General properties. The molecular weight of Glc-6*P* dehydrogenase was determined by gel filration on a Sepharose 4B column and a value of 150 000 was found.

Glucose-6-phosphate dehydrogenase exhibited activith with either $NADP^+$ or NAD^+ as coenzymes, V $NADP^+ = 0.96$ V NAD^+. Maximum activity of the

TABLE I

PURIFICATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM *PSEUDOMONAS* C

Enzyme activity was measured with $NADP^+$ as coenzyme.

Purification step	Volume (ml)	Total protein (mg)	Specific activity (IU/mg protein)	Recovery (%)	Purification (-fold)
1. Crude extract	125	4375	0.69	100	1.0
2. 40-80% $(NH_4)_2SO_4$ fraction	52	2028	1.33	89	1.9
3. DEAE-Cellulose chromatography (pooled fraction)	91	177	13.9	81	20.1
4. Blue dextran Sepharose 4B chromatography (pooled fractions)	125	12	152	58	220

enzyme with both coenzymes was obtained at pH values between 8.5 and 9.5 with 100 mM Tris-HCl, glycylglycine, or glycine/NaOH buffer.

The enzyme was specific for glucose 6-phosphate. Glucose 1-phosphate, glucose, 6-phosphogluconate and fructose 6-phosphate could not replace glucose 6-phosphate as a substrate in the reaction either with $NADP^+$ or with NAD^+. Enzyme activity did not require the presence of Mg^{2+} and at a concentration higher than 0.5 mM, Mg^{2+} had an inhibitory effect on the activity of the enzyme with NAD^+. Glucose-6-phosphate dehydrogenase activity with $NADP^+$ was less sensitive and the inhibition by Mg^{2+} was noted only above a Mg^{2+} concentration of 35 mM. Similar effects were obtained with $MnCl_2$.

Kinetic properties and inhibition studies of Glc-6P dehydrogenase. The saturation curves of G6PDH for glucose-6-phosphate, $NADP^+$ and NAD^+ were of hyperbolic type, and from the Lineweaver-Burk curves the K_m value of Glc-6*P* dehydrogenase with $NADP^+$ as coenzyme was calculated to be 0.25 mM and that for NAD^+ was 0.16 mM. With respect to the coenzymes the K_m value for $NADP^+$ was 0.022 mM, and that for NAD^+ was 0.29 mM.

NADH was found to be a competitive inhibitor of the enzyme with respect to either $NADP^+$ (Fig. 1A) or NAD^+ (Fig. 1B). Also, NADPH was a competitive

TABLE II

PURIFICATION OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM *PSEUDOMONAS* C

Enzyme activity was measured with $NADP^+$ as coenzyme.

Purification step	Volume (ml)	Total protein (mg)	Specific activity (IU/mg protein)	Recovery (%)	Purification (-fold)
1. Crude extract	140	4060	0.23	100	1
2. Phosphocellulose (pooled fractions)	72	98	7.30	77	32
3. Blue dextran-Sepharose 4B (pooled fractions)	66	17	30.4	55	132
4. Phosphocellulose (pooled fractions)	23	8.3	56.4	50	245

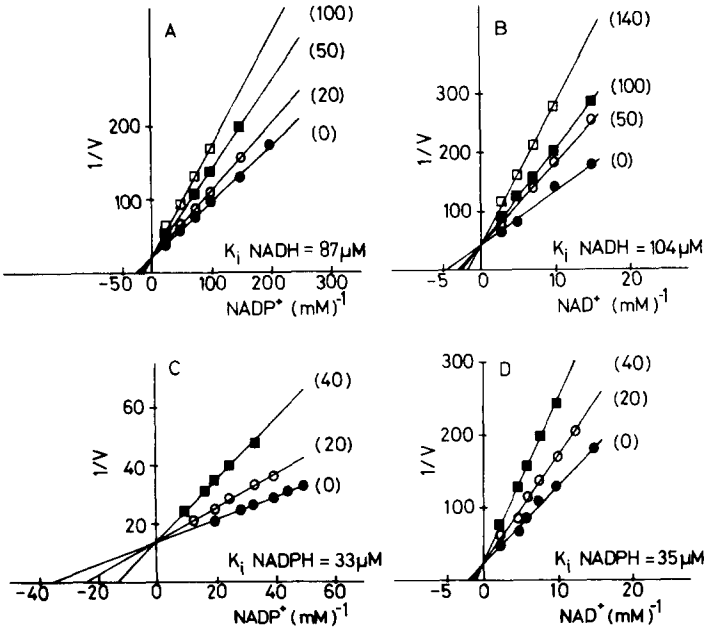

Fig. 1. Inhibition of glucose-6-phosphate dehydrogenase: A) by NADH at different concentrations of $NADP^+$; (B) by NADH at different concentrations of NAD^+; (C) by NADPH at different concentrations of $NADP^+$, and (D) by NADPH at different concentrations of NAD^+. Reaction mixtures containing in a final volume of 1.0 ml, 100 mM Tris-HCl buffer, pH 7.6; 0.5 mM glucose 6-phosphate and various concentrations of NAD^+ or $NADPH^+$. Reactions were started by the addition of 0.4 μg purified enzyme. Concentrations of inhibitors (mM) are given in parentheses in each figure.

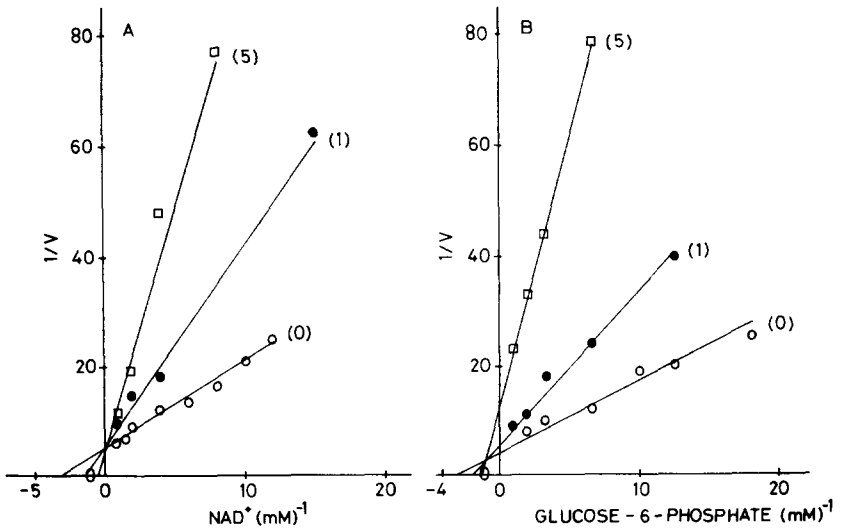

Fig. 2. Inhibition of glucose-6-phosphate dehydrogenase: (A) by ATP with different concentrations of NAD^+. (B) by ATP with various concentrations of glucose 6-phosphate. Standard reaction mixtures were started by the addition of 0.4 μg of purified enzyme. Concentrations of inhibitor (mM) are given in parenthesis.

inhibitor of the enzyme with respect to either $NADP^+$ (Fig. 1C) or NAD^+ (Fig. 1D). The K_i values for NADH and NADPH are given in Fig. 1.

Glucose, glucose 1-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, glucosamine, 2-deoxyglucose 6-phosphate, 6-phosphogluconate, ribose 5-phosphate, ribulose 5-phosphate, ribulose 1,5-diphosphate, erythrose 5-phosphate, xylulose 5-phosphate, sedoheptulose 7-phosphate, coenzyme A, acetylphosphate, pyruvate, phospho*enol*pyruvate, glycerol 3-phosphate and 3′,5′-cyclic AMP all at 1—2 mM level had almost no effect on the activity of Glc-6*P* dehydrogenase when measured at low concentration (2 mM) of $NADP^+$, NAD^+ and glucose 6-phosphate.

From the adenine nucleosides only ATP was found to be a significant inhibitor. The inhibitory effect of ATP on Glc-6*P* dehydrogenase activity was more pronounced with NAD^+ than with $NADP^+$ as coenzyme. Further studies showed that ATP was a competitive inhibitor with respect to NAD^+ (K_i = 1.1 mM, Fig. 2A) and a mixed-type inhibitor for glucose 6-phosphate (Fig. 2B). Addition of 5 mM $MgCl_2$ counteracted the ATP inhibition.

C. Properties of 6-phosphogluconate dehydrogenase

General properties. The molecular weight of 6-phosphogluconate dehydrogenase was determined by gel filtration on a Sepharose 4B column and a value of 220 000 was found. Upon electrophoresis in acrylamide gel containing urea, sodium dodecyl sulfate, and 2-mercaptoethanol the subunit's molecular weight of about 55 000 was evident.

6-Phosphogluconate dehydrogenase was specific for $NADP^+$ and NAD^+, V $NADP^+$ = 1.66 V NAD^+. Maximum activity of 6-phosphogluconate dehydrogenase with $NADP^+$ and NAD^+ was obtained at pH values between 8 and 9 in 100 mM of glycylglycine, Tris-HCl or glycine/NaOH buffer. The enzyme required the presence of Mg^{2+} for maximum activity and saturation was achieved at a concentration of 2 mM Mg^{2+}.

Kinetic properties and inhibition studies of 6-phosphogluconate dehydrogenase. The saturation curves of 6-phosphogluconate dehydrogenase for 6-phosphogluconate, $NADP^+$ and NAD^+ were of hyperbolic type and from the Lineweaver-Burk curves the K_m value of 6-phosphogluconate with $NADP^+$ was calculated to be 0.1 mM and with NAD^+ 0.04 mM. With respect to the coenzymes, the K_m value for $NADP^+$ was 0.02 mM and that for NAD^+ was 0.5 mM.

NADH and NADPH inhibited the activity of 6-phosphogluconate dehydrogenase both with $NADP^+$ and NAD^+. Using saturated concentrations of oxidized coenzymes, 50% inhibition of 6-phosphogluconate dehydrogenase activity with NAD^+ was obtained with 75 μM of NADH and 70 μM of NADPH. Enzyme activity with $NADP^+$ was inhibited by 50% with 48 μM of NADH and 28 μM of NADPH.

A survey for possible effectors of 6PGDH activity revealed that while ADP and AMP had almost no effect on enzyme activity, ATP was found to be a potent inhibitor of this enzyme. Fig. 3 shows that the inhibition is more pronounced on 6-phosphogluconate dehydrogenase activity with NAD^+ as coenzyme than with $NADP^+$. The addition of 5 mn $MgCl_2$ to the reaction mixture caused a decrease in the extent of ATP inhibition but did not abolish

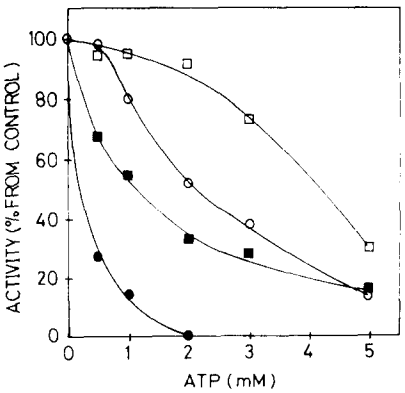

Fig. 3. Effect of ATP on the activity of 6-phosphogluconate dehydrogenase. Standard reaction mixtures containing different concentrations of ATP plus or minus $MgCl_2$ (5 mM final concentration) were started by the addition of 1 µg of purified enzyme. Percent activity was calculated from a control system without ATP. □——□, $NADP^+$ plus $MgCl_2$; ■——■, $NADP^+$ minus $MgCl_2$; ○——○, NAD^+ plus $MgCl_2$; ●——●, NAD^+ minus $MgCl_2$.

it. ATP was found to be a competitive inhibitor of 6-phosphogluconate dehydrogenase activity with respect to NAD^+ (K_i = 0.6 mM) and a non-competitive inhibitor with respect to 6-phosphogluconate.

Discussion

A partial purification of Glc-6*P* dehydrogenase has been reported for *Pseudomonas* W6, an obligate methylotroph able to grow on methane, methanol and methylated amines, but not on non C_1-compounds [14]. Preliminary results on the purification of Glc-6*P* dehydrogenase have been reported for *Methylomonas* M15 grown on methanol as a sole carbon source [15]. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were purified from a methanol utilizing yeast, *Candida boidinii* [9].

All these microorganisms have similar pathways as compared to *Pseudomonas* C for the oxidation and assimilation of C_1-compounds [9—10, 14—16].

Table III shows the comparison between the properties of Glc-6*P* dehydrogenase and 6-phosphogluconate dehydrogenase isolated from *Pseudomonas* C and *C. boidinii*. It appears that the major difference in the properties of these enzymes between the bacterium and the yeast is in their specificity toward the coenzymes.

In *Pseudomonas* C Glc-6*P* dehydrogenase and 6-phosphogluconate dehydrogenase react both with $NADP^+$ and NAD^+ at comparable rates. Similar coenzyme dual specificity is found in Glc-6*P* dehydrogenase of the methylotrophs *Pseudomonas* W6 [14], and *Methylomonas* M15 [15] and in other non-methylotrophs *Pseudomonas aeruginosa* [17], *Hydrogenomonas* H16 [18], *Leuconostoc mesenteroides* [19] and *Paracoccus denitrificans* [20]. 6-Phosphogluconate dehydrogenase with dual coenzyme specificity is found in the methylotroph *Pseudomonas* W5 [14], and in other non-methylotrophs

TABLE III

COMPARISON BETWEEN THE PROPERTIES OF G6PDH AND 6PGDH FROM *PSEUDOMONAS* C AND *CANDIDA BOIDINII*

Property	Glucose-6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase	
	Pseudomonas C	*C. boidinii* *	*Pseudomonas* C	*C. boidinii*
Purification (fold)	220	1 714	245	331
Final specific activity (HI/mg)	152	516	56.4	39.7
Molecular weight	105 000	118 000	220 000	110 000
Optimal pH	8.9—9.5	8.5—9.0	8.0—9.0	8.0—8.5
Requirement for $MgCl_2$	—	—	2 mM	20 mM
Activity with NAD^+	yes	no	yes	no
K_m values: (μM) $NADP^+$	22	14	20	13
G6P ** ($NADP^+$)	250	860	—	—
6PG *** ($NADP^+$)	—	—	—	—

* Results are taken from Kato et al. [9].

** Glucose 6-phosphate.

*** 6-Phosphogluconate.

Pseudomonas multivorans (Type I) [8] and *Streptococcus faecalis* (Type I) [7]. In all these cases the enzymes have higher affinities for $NADP^+$ than for NAD^+, but as the in vivo concentration of NAD^+ is in the range of 2—6 mM [21—25] as compared to $NADP^+$ concentrations of 0.3—1.8 mM [22—23,25] it can compensate for the lower affinities of both enzymes for NAD^+. It appears that the intracellular levels of substrates and coenzymes concentrations are high enough to allow maximum activities of these enzymes, which in *Pseudomonas* C might furnish both NADH and NADPH for cell metabolism, while in *C. boidinii* only NADPH might be formed [9].

The production of NADPH by these enzymes might be of importance under physiological conditions as the C_1-utilizing bacteria and *C. boidinii* do not possess a complete trichloroacetic acid cycle (α-oxoglutarate dehydrogenase activity cannot be detected) and the growth substrate carbon is oxidized to CO_2 by the cyclic oxidation pathway [1,9,14—15].

Inhibition studies with reduced coenzymes have shown that in *Pseudomonas* C NADH and NADPH inhibited Glc-6*P* dehydrogenase and 6-phosphogluconate dehydrogenase activities both with $NADP^+$ and NAD^+ as coenzymes. The inhibition caused by NADH in *Pseudomonas* C is in contrast with the results obtained in *C. boidinii* [9], where no inhibition by NADH on both enzyme activities was observed. As was pointed out by Kato et al. [9], the lack of inhibition by NADH on both enzyme activities in the methanol-grown yeast might indicate that there is no direct interaction between the oxidation pathway of formaldehyde via formate to CO_2 (which produces NADH) and the dissimilatory ribulose monophosphate cycle (the cyclic oxidation pathway), unlike the case of *Pseudomonas* C. In other methylotrophic bacteria (*Pseudomonas* W6 and *Methyomonas* M15) NADH inhibits the activity of Glc-6*P* dehydrogenase with $NADP^+$ and NAD^+ as coenzymes [9,15], while data for 6-phosphogluconate dehydrogenase have not yet been reported.

ATP was found to be an inhibitor of the activities of both enzymes in

Pseudomonas C; the activity of 6-phosphogluconate dehydrogenase was more sensitive than that of Glc-6*P* dehydrogenase to ATP inhibition. ATP inhibited more profoundly the activities of these enzymes with NAD^+ than with $NADP^+$, similar to the results obtained for Glc-6*P* dehydrogenase of *Pseudomonas* W6 when grown on methanol [14], or of other bacteria such as *Bacillus licheniformis* [22] and *P. denitrificans* [20]. In the yeast *C. boidinii* [9] the addition of $MgCl_2$ counteracted the ATP inhibition of both enzymes while in *Pseudomonas* C the inhibition of 6-phosphogluconate dehydrogenase activity caused by ATP was only partially reversed by the addition of $MgCl_2$ (Fig. 3).

The inhibitory effect of ATP on these enzymes might be of physiological importance in *Pseudomonas* C. It can be considered that as Glc-6*P* dehydrogenase and 6-phosphogluconate dehydrogenase are consecutive enzymes [1], small variations in ATP concentrations can result in profound changes in the flux of carbon via the different biochemical pathways. When the concentration of ATP increases the NAD^+-dependent activity of these enzymes is inhibited, and thus the continued flux of carbon through the cyclic oxidation pathway will result in a higher production of NADPH (relative to NADH). In *C. boidinii*, where these enzymes are specific only for $NADP^+$ [9], an increase in the intracellular concentration of ATP might lower the flux of carbon via the dissimilatory ribulose monophosphate cycle, and increase it via the direct oxidation pathway (via formic acid) causing a higher production of NADH (relative to NADPH).

For the elucidation of the in vivo regulation of Glc-6*P* dehydrogenase and 6-phosphogluconate dehydrogenase activities in *Pseudomonas* C the intracellular levels of the metabolites involved during growth need to be known.

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References

1 Ben-Bassat, A. and Goldberg, I. (1977) Biochim. Biophys. Acta 497, 586—597
2 Colby, J. and Zatman, L.J. (1975) Biochem. J. 148, 513—520
3 Stieglitz, B. and Mateles, R.I. (1973) J. Bacteriol. 114, 390—398
4 Strøm, T., Ferenci, T. and Quayle, J.R. (1974) Biochem. J. 144, 456—476
5 Bonsingnore, A. and DeFlora, A. (1972) in Current Topics in Cellular Regulation (Horecker, B.L. and Stadtman, E.R., eds.), Vol. 6, pp. 21—26, Academic Press, Inc. New York, NY
6 Bridges, R.B. and Wittenberger, C.L. (1975) Methods Enzymol. 41, 232—237
7 Brown, T.A. and Wittenberger, C.L. (1972) J. Bacteriol. 109, 106—115
8 Lessie, T.G. and Van der Wyk, J.C. (1972) J. Bacteriol. 110, 1107—1117
9 Kato, N., Sahm, H., Schutte, H. and Wagner, F. (1979) Biochim. Biophys. Acta 566, 1—11
10 Chalfan, Y. and Mateles, R.I. (1972) Appl. Microbiol. 23, 135—140
11 Mateles, R.I. and Battat, E. (1974) Appl. Microbiol. 28, 901—905
12 Lowry, O.H., Resenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
13 Ryan, L.D. and Vestling, C.S. (1974) Arch. Biochem. Biophys. 160, 279—284

14 Miethe, D. and Babel, N. (1976) Z. Allg. Mikrobiol. 16, 289—299
15 Sahm, H. and Steinbach, R. (1977) Proceedings of the International Symposium on Microbial Growth on C_1-compounds, Pushchino, pp. 50—51
16 Rock, J.S., Goldberg, I., Ben-Bassat, A. and Mateles, R.I. (1976) Agr. Biol. Chem. 40, 2129—2135
17 Lessie, T.G. and Neidhardt, F.C. (1967) J. Bacteriol. 93, 1337—1345
18 Blackkolb, F. and Schlegel, H.G. (1968) Arch. Mikrobiol. 63, 177—196
19 Olive, C., Geroch, M.E. and Levy, H.R. (1971) J. Biol. Chem. 246, 2047—2057
20 Slabas, A.R. and Whatley, F.R. (1977) Arch. Mikrobiol. 112, 225—227
21 Bowien, B. Cook, A.M. and Schlegel, H.G. (1974) Arch. Mikrobiol. 97, 273—281
22 Opheim, D. and Bernlohr, R.W. (1973) J. Bacteriol. 116, 1150—1159
23 Setlow, B. and Setlow, P. (1977) J. Bacteriol. 129, 857—865
24 Wimpenny, I.W.T. and Firth, A. (1972) J. Bacteriol. 111, 24—32
25 Andersen, K.B. and Meyenburg, K. (1977) J. Biol. Chem. 252, 4151—4156