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Adenosine Triphosphate-Linked Control of *Pseudomonas aeruginosa* Glucose-6-Phosphate Dehydrogenase

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Extracts of *Pseudomonas aeruginosa* (ATCC 7700) cells grown on glucose, gluconate, or glycerol had enzyme activities related to the Entner-Doudoroff pathway. These activities were present in no more than trace amounts when the bacteria were grown on succinate. Fructose-1,6-diphosphate aldolase could not be detected in extracts of the bacteria grown on any of the above carbon sources. Therefore, it appears that *P. aeruginosa* degrades glucose via an inducible Entner-Doudoroff pathway. The apparent absence of fructose-1,6-diphosphate aldolase in cells growing on succinate suggests that the bacteria can form hexose and pentose phosphates from succinate by an alternate route. D-Glucose-6-phosphate dehydrogenase, a branch-point enzyme of the Entner-Doudoroff pathway, was purified 50-fold from glucose-grown cells. Its molecular weight, estimated by sucrose density gradient centrifugation, was found to be approximately 190,000. The enzyme was strongly inhibited by adenosine triphosphate, guanosine triphosphate, and deoxyguanosine triphosphate, which decreased the apparent binding of glucose-6-phosphate to the enzyme. It is suggested that adenine nucleotide-linked control of glucose-6-phosphate dehydrogenase may regulate the overall catabolism of hexose phosphates and prevent their wasteful degradation under certain conditions requiring gluconeogenesis.

Members of the *Pseudomonodaceae* group of bacteria are notable for their ability to respire at the expense of a large number of carbon compounds (15). Generally, they lack fermentative mechanisms for obtaining energy. In particular their catabolism of glucose differs from that of the more widely studied *Enterobacteriaceae*. The Entner-Doudoroff pathway of glucose dissimilation (7), which involves formation of triose from hexonic acid phosphate rather than from hexose phosphate, appears to be the dominant route of glucose degradation.

It has long been established that the levels of many bacterial catabolic enzymes are subject to regulation by induction and repression (*see* 12). More recently, adenine nucleotides have been found to promote changes in the activities of certain catabolic enzymes (*see* 1); for example, adenosine monophosphate (AMP) or adenosine triphosphate (ATP) modify the activity of *Escherichia coli* phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase; EC 2.7.1.11) by respectively increasing or decreasing the apparent strength of binding of fructose-6-phosphate to the enzyme (4). Similarly, the catabolic threonine deaminase (L-threonine hydro-lyase; EC 4.2.1.16) of *Clostridium tetanomorphum* (10) is activated by adenosine diphosphate (ADP), which increases the affinity of the enzyme for threonine. The in vitro pattern of regulation by adenine nucleotides indicates that the in vivo flow of metabolites through catabolic routes may be restricted under conditions of energy excess (high ATP concentration) and increased under conditions of energy deprivation (high AMP or ADP concentration).

In view of their capacity to catabolize a wide array of carbon compounds, the pseudomonads seemed an ideal group in which to study catabolic regulatory mechanisms. To gain information about control of catabolism in the *Pseudomonodaceae*, we studied several enzymes related to glucose dissimilation in *Pseudomonas aeruginosa*. In particular, our study centered upon regulation of glucose-6-phosphate dehydrogenase [D-glucose-6-phosphate:nicotinamide adenine di-

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nucleotidephosphate (NADP) oxidoreductase, EC 1.1.1.49], a branch-point enzyme of the Entner-Doudoroff pathway, which we found to be inhibited by ATP.

MATERIALS AND METHODS

Organisms. P. aeruginosa ATCC 7700 was used throughout. *Aerobacter aerogenes* strain XXXV was used in one of the experiments.

Growth of bacteria. Bacteria were grown in media containing salts solution P (8) supplemented with 0.2% ammonium sulfate and 0.3% of each indicated carbon source. Cultures were shaken in Erlenmeyer flasks filled to less than one-fifth their nominal capacity and incubated at 37 C. Growth was monitored by following the increase in optical density of the cultures at 420 mμ. An optical density of 1 was equivalent to 80 μg of protein per ml.

Chemical determinations. Protein was measured by the Folin phenol method (11). Whole cells were first digested for 2 hr at 40 C in 1 N NaOH. Glucose was determined by the Glucostat method as described previously (5).

Bacterial consumption of glucose. Portions of culture were sampled at intervals during growth and chilled. The samples were filtered through Schleicher and Schuell membranes of 0.4 μ pore size (Schleicher and Schuell Co., Keene, N.H.). Glucose remaining in the filtrates was plotted as a function of bacterial protein, and the milligrams of glucose consumed to form 1 mg of protein was calculated. This value was multiplied by the growth rate constant, k, expressed in minutes^{-1}, to give the milligrams of glucose consumed per minute per milligram of protein.

Preparation of cell-free extracts for enzyme assays. Portions of culture containing 3 to 10 mg of protein were centrifuged, and the bacteria were washed with 5×10^{-2} M tris(hydroxymethyl)aminomethane (Tris) chloride (*p*H 7.4). The bacteria were suspended in 2 ml of the same buffer, and were disrupted by sonic treatment for 2 min with a Branson Sonifier (Branson Instruments, Inc., Stamford, Conn.). The sonically treated material was centrifuged for 20 min at 12,000 $\times$ g, and the supernatant liquids were assayed for enzyme activity.

Enzyme assays. All assays involved appearance or disappearance of reduced nicotinamide adenine dinucleotide ($NADH_2$) or reduced NADP ($NADPH_2$), which was measured by monitoring changes in optical density of the assay mixtures at 340 mμ with a Gilford recording spectrophotometer (Gilford Instruments, Oberlin, Ohio). The values were converted to millimicromoles of $NADPH_2$ or $NADH_2$ formed or consumed per minute per milligram of protein, assuming a molar extinction of 6.2×10^3 for $NADPH_2$ and $NADH_2$. The assays were carried out at 25 C unless indicated otherwise, and in each case the rate of the reaction was proportional to enzyme concentration.

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49) assay mixtures (3 ml) contained: 5×10^{-2} M Tris chloride buffer (*p*H 8.2), 10^{-2} M $MgCl_2$, 4×10^{-4} M NADP; 2×10^{-3} M glucose-6-phosphate, and 90 to 300 μg of crude extract protein. The reactions were started by addition of glucose-6-phosphate.

6-Phosphogluconate dehydrogenase (6-phospho-D-gluconate:NADP oxidoreductase, EC 1.1.1.43) reaction mixtures (3 ml) were the same as for glucose-6-phosphate dehydrogenase, except that 6-phosphogluconate was substituted for glucose-6-phosphate.

Glucokinase (ATP:D - glucose - 6 - phosphotransferase, EC 2.7.1.12) activity was measured by coupling formation of glucose-6-phosphate to NADP reduction in the presence of excess rabbit muscle glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.). The reaction mixtures (3 ml) were the same as in the assay for glucose-6-phosphate dehydrogenase, except that glucose-6-phosphate was omitted, and the mixtures were supplemented with 10^{-2} M glucose, 4×10^{-3} M ATP, and 3 μg of glucose-6-phosphate dehydrogenase.

6-Phosphogluconate dehydratase (6-phospho-D-gluconate hydro-lyase, EC 4.2.1.12) and KDPG aldolase (6-phospho-2-oxo-3-deoxy-D-gluconate D-glyceraldehyde-3-phosphate-lyase) were assayed jointly by following the formation of pyruvate from 6-phosphogluconate. The pyruvate formed was converted to lactate by excess rabbit muscle lactate dehydrogenase (Sigma Chemical Co.), and $NADH_2$ consumption was measured. The rates of $NADH_2$ disappearance were corrected for nonspecific $NADH_2$ oxidation by subtracting the rate in assay mixtures containing no 6-phosphogluconate. Except in the case of succinate-grown bacteria, the crude extracts themselves had only low levels of $NADH_2$ oxidase activity (less than 10 mμmoles of $NADH_2$ oxidized per min per mg of protein). The $NADH_2$ oxidase activity in extracts of succinate-grown bacteria was about threefold higher. The reaction mixtures (3 ml) contained: 5×10^{-2} M Tris chloride buffer (*p*H 8.2), 10^{-2} M $MgCl_2$, lactate dehydrogenase (300 μg), 3×10^{-4} M $NADH_2$, 2×10^{-3} M 6-phosphogluconate, and between 150 and 600 μg of crude extract protein. The reaction was started by addition of 6-phosphogluconate.

Fructose-1,6-diphosphate aldolase (fructose-1,6-diphosphate-D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.b) was assayed as described previously (5). The reaction mixtures (3 ml) contained between 150 and 450 μg of protein. Rabbit muscle aldolase (Sigma Chemical Co.) was added to otherwise identical reaction mixtures as a control.

Purification of glucose-6-phosphate dehydrogenase. Glucose-grown bacteria from 8 liters of culture containing approximately 400 μg of protein per ml were washed with 5×10^{-2} M tris-HCl buffer (*p*H 7.4) containing 10^{-2} M $MgCl_2$, and were suspended in 75 ml of the same buffer to which deoxyribonuclease (Worthington Biochemical Co., Freehold, N.J.) had been added (final concentration, 2 μg/ml). The cell suspension was passed through a French pressure cell under 6,000 to 9,000 psi of pressure. The treated material was centrifuged for 20 min at 12,000 $\times$ g. The supernatant liquid was centrifuged for 2 hr at 40,000 rev/min in the 50 rotor of a Spinco model L preparative ultracentrifuge to remove ribosomes. Streptomycin

sulfate was added to the supernatant liquid (final concentration, 2%) to precipitate low-molecular-weight ribonucleic acid (RNA) remaining after the high-speed centrifugation. The precipitate was removed by centrifugation. An equal volume of 40% (w/v) ammonium sulfate was added to the supernatant liquid, and this preparation was incubated for 8 hr at 0 C. The precipitate was collected by centrifugation, and was dissolved in 5 ml of 5×10^{-2} M tris chloride buffer (*p*H 7.4). The enzyme solution was dialyzed overnight at 4 C against 2 liters of the same buffer, and 2 ml of the dialyzed material was applied to a column (25 cm by 1 cm^2) of diethylaminoethyl cellulose (Whatman DE 52; H. Reeve Angel and Co., Clifton, N.J.). A linear gradient (300 ml) of 0 to 0.5 M NaCl in 0.01 M Tris buffer (*p*H 7.4) was passed through the column. Fractions of 5-ml volume were collected. Glucose-6-phosphate dehydrogenase eluted between 0.22 and 0.28 M NaCl. Fractions containing enzyme activity were pooled, and dialyzed twice against 2-liter volumes of 5×10^{-2} M Tris chloride buffer (*p*H 7.4). The overall purification was about 50-fold, and 29% of the original enzyme activity was recovered. The individual steps in the purification are outlined in Table 1.

Sucrose gradient centrifugation. Zone centrifugation of glucose-6-phosphate dehydrogenase was performed essentially as described by Martin and Ames (13).

Sephadex gel filtration. Samples (1 ml) of 50-fold purified glucose-6-phosphate dehydrogenase containing 1 mg of hemoglobin (1 mg/ml) were loaded onto columns (20 cm by 1 cm^2) of Sephadex G-100 or G-200 gel (Pharmacia Fine Chemicals, Inc., New Market, N.J.), and the enzyme was eluted with 5×10^{-2} M Tris chloride buffer (*p*H 7.4). Fractions (0.5 ml) were collected and assayed for glucose-6-phosphate dehydrogenase and hemoglobin.

Chemicals. 6-Phosphogluconate in its sodium form was obtained from Calbiochem, Los Angeles, Calif. Glucose-6-phosphate, NAD, $NADH_2$, $NADPH_2$, and ATP were obtained as sodium salts from Sigma Chemical Co. All other chemicals were of reagent grade.

RESULTS

Induction of enzymes involved in glucose catabolism. The catabolic reactions I through IV comprise the Entner-Doudoroff pathway of glucose degradation (Fig. 1). Extracts of *P. aeruginosa* were examined for this pathway. Enzymes I and II were assayed singly; enzymes III and IV were assayed jointly by measuring the formation of pyruvate from 6-phosphogluconate. All of these enzymes were found to be present in extracts of glucose-grown bacteria (Table 2). The activity of glucose-6-phosphate dehydrogenase (enzyme II) was approximately twice each of the other two activities. When assayed at 37 C, the activity of glucose-6-phosphate dehydrogenase was 1.8 times greater than at 25 C, and was about 70% the in vivo rate of glucose consumption (420 mμmoles per min per mg of protein) in cultures growing aerobically with glucose as sole carbon and energy source. 6-Phosphogluconate dehydrogenase activity (enzyme V), which diverts 6-phosphogluconate from the Entner-Doudoroff pathway to pentose formation, was about one-tenth that of glucose-6-phosphate dehydrogenase (Table 2). Fructose-1,6-diphosphate aldolase was not detected in the extracts. Comparable extracts of *A. aerogenes*, which presumably degrade glucose primarily via the glycolytic pathway, had at least 50 times more aldolase activity (47 mμmoles of $NADH_2$ formed per min per mg of protein). When extracts of *P. aeruginosa* and *A. aerogenes* were supplemented with rabbit muscle aldolase, similar increases in aldolase activity were observed, indicating that the absence of aldolase activity in the *Pseudomonas aeruginosa* extracts was not due to the presence of agents interfering with the enzyme assay.

No more than trace amounts of the above

TABLE 1. *Purification of glucose-6-phosphate dehydrogenase*

Fraction	Volume	Protein	Enzyme[a]		Recovery	Relatve purification
			Units/ml	Total units		
	ml	*mg/ml*			%	
1. 12,000 × *g*	57	42	39	2,223	100	—
2. 40,000 rev/min	43	26	39	1,677	75	1.6
3. Streptomycin	45	—	42	1,890	85	—
4. $(NH_4)_2SO_4$	6	20	120	720	32	6.5
5. DEAE cellulose	24	0.18	9	216	29[b]	54

[a] One unit of enzyme is the amount of glucose-6-phosphate dehydrogenase which catalyzes an optical density increase of 1.0 per min in the standard assay system.

[b] Only one-third (2 ml) of the enzyme preparation from step 4 was placed on the diethylaminoethyl cellulose column.

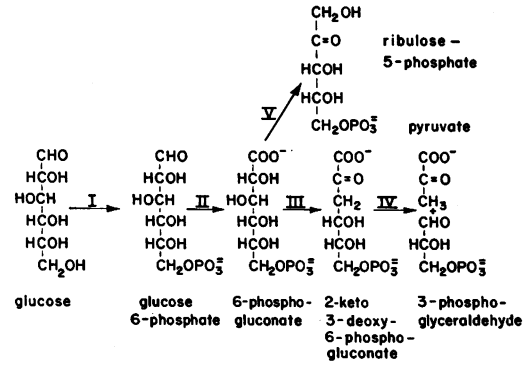

FIG. 1. *Enzymes related to the Entner-Doudoroff pathway.*

TABLE 2. *Effect of carbon source on levels of enzymes related to the Entner-Doudoroff pathway*

Carbon source	Growth rate constant[a]	Enzyme activity[b] (units/mg of protein)				
		I	II	III-IV	V	Aldolase
	hr^{-1}					
Glucose....	0.85	91	160	84	17	<1
Succinate...	0.92	8	8	<1	<1	<1
Pyruvate...	0.90	32	24	<1	<1	—
Pyruvate + glucose...	0.85	—	180	—	—	—
Gluconate..	0.85	85	184	80	10	—
Glycerol...	0.25	143	280	96	6	—
Fructose....	0.14	—	200	—	—	—

[a] Growth rate is expressed in terms of k, the specific growth rate constant, calculated as $ln2/$ mass doubling time in hours.

[b] For each enzyme, one unit is that amount which catalyzes the formation or oxidation of 1.0 mμmole of $NADH_2$ or $NADPH_2$ per min under the conditions of the assays.

activities were detected in extracts of bacteria which had been cultivated on succinate (Table 2).

Bacteria transferred from succinate medium to a medium containing only glucose as a carbon and energy source formed glucose-6-phosphate dehydrogenase preferentially before they resumed rapid growth (Fig. 2). Enzyme synthesis commenced immediately, and the resumption of growth paralleled the attainment of fully induced levels of the enzyme.

The above data suggest that *P. aeruginosa* catabolizes glucose primarily via an inducible Entner-Doudoroff pathway.

Glucose-6-phosphate dehydrogenase and other enzymes related to the Entner-Doudoroff pathway were induced to different levels in cultures growing upon different carbohydrates (Table 2). The highest and lowest levels of the enzymes were induced by growth on glycerol and pyruvate, respectively. The rapid growth on pyruvate in comparison with that on glycerol (see growth rate constants listed in Table 2) suggested that the low levels formed in the presence of pyruvate might be a result of catabolite repression. Two observations tend to rule out this notion. First, as indicated in Table 2, the level of glucose-6-phosphate dehydrogenase in cultures grown upon a mixture of glucose and pyruvate was not decreased below the level on glucose alone. Second, the results in Table 3 show that, in a nitrogen-limited chemostat culture grown on glucose at less than 15% the unrestricted growth rate,

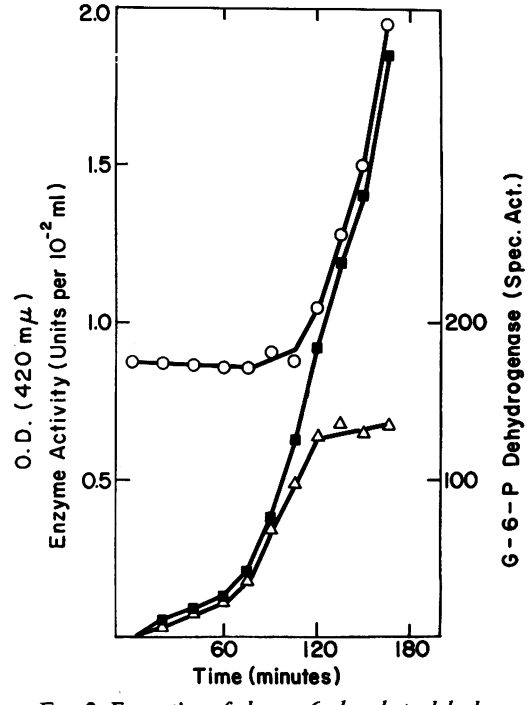

FIG. 2. *Formation of glucose-6-phosphate dehydrogenase during adaptation of succinate-grown bacteria to grow on glucose. Pseudomonas aeruginosa was grown in succinate medium until the culture had an optical density of 0.9 at 420 mμ. The bacteria were centrifuged, washed twice with medium containing no substrate, and then suspended in medium containing glucose as the sole source of carbon. The culture was incubated at 37 C, and at specified times samples were removed for determination of glucose-6-phosphate dehydrogenase. Units of enzyme per milliliter of culture* (○), *units of enzyme per mg of protein* (△), *and culture optical density at 420 mμ* (■) *are plotted as functions of time on linear coordinates; growth was accelerating between 90 and 170 min. Enzyme units are defined in Materials and Methods.*

TABLE 3. *Formation of glucose-6-phosphate dehydrogenase during growth under biosynthetic restriction*

Growth rate constant[a]	Glucose-6-phosphate dehydrogenase (units/mg of protein)
hr^{-1}	
0.85[b]	160
0.40[c]	172
0.19[c]	122
0.10[c]	118

[a] See Table 2, footnote *a*.

[b] Unrestricted (batch) growth in glucose medium.

[c] A culture (245 ml) was grown in a chemostat in basal salts medium containing a limiting supply of nitrogen as $(NH_4)_2SO_4$ (94 μg/ml), 0.2% Na_2SO_4, and 0.3% glucose. The culture was saturated with oxygen by bubbling air through the medium.

the level of glucose-6-phosphate dehydrogenase was 80% of that found in batch cultures.

Inhibition of glucose-6-phosphate dehydrogenase activity by ATP. The fact that biosynthetically restricted cultures did not repress glucose-6-phosphate dehydrogenase to match their growth rate suggested that formation of this enzyme was not tightly regulated by catabolite repression. Since this enzyme seemed a likely control point, regulating the entry of hexose phosphate into the Entner-Doudoroff pathway, we investigated the possibility that control of its activity governed operation of the pathway. Accordingly, we tested several products of the pathway, as well as certain metabolites of the tricarboxylic acid cycle, for their ability to inhibit glucose-6-phosphate dehydrogenase. Of the following compounds, only ATP was found to cause significant inhibition of the enzyme: glyceraldehyde phosphate, pyruvate, phosphoenolpyruvate, acetyl-coenzyme A, acetate, oxalacetate, isocitrate, α-ketoglutarate, succinate, glutamate, fumarate, and ATP all at 10^{-3}M).

ATP inhibited glucose-6-phosphate dehydrogenase activity in crude bacterial extracts prepared in buffers from which magnesium was omitted. The extent of inhibition depended upon the concentration of glucose-6-phosphate. The data of Fig. 3 show results with 50-fold purified enzyme. ATP decreased the apparent affinity of the enzyme for glucose-6-phosphate without significantly affecting the activity of the enzyme once it was saturated with substrate.

Even in the absence of ATP, the apparent binding of glucose-6-phosphate to the enzyme depended upon the concentration of glucose-6-phosphate. This is seen clearly in the reciprocal plot of enzyme activity versus glucose-6-phosphate concentration presented in Fig. 3B. To ascertain whether ATP modified the cooperative binding effects between substrate molecules, Hill plots were used to compare shapes of substrate saturation curves in the presence of different concentrations of ATP. The results are shown in Fig. 3C. The slopes of such curves at half-maximal velocity have been interpreted as a measure of cooperation between the ligands involved (*see* 3). Plots of the present data show that ATP causes no significant change in the parameter, indicating that it increases the concentration of glucose-6-phosphate required for full enzyme activity without altering the degree of cooperative binding between substrate molecules. Similar results have been observed for yeast phosphofructokinase and isocitrate dehydrogenase (2). A Hill plot relating enzyme activity and ATP concentration is presented in Fig. 3D. The slope of the line obtained was 2, indicating that ATP binds to at least two sites on the enzyme.

We have used reciprocal plots of enzyme activity versus substrate concentration to detect small changes in apparent affinity of glucose-6-phosphate dehydrogenase for substrate under different conditions. Figure 4 shows that such changes are induced by citrate, magnesium, and high *p*H. At low substrate concentrations, an increase in *p*H or the addition of citrate markedly decreased enzyme activity. Addition of magnesium at elevated *p*H (*p*H 9) caused a significant increase in enzyme activity at low substrate concentrations. These interactions with the enzyme were weaker than those of ATP, which are detectable even at high substrate concentrations. High concentrations of magnesium abolished both the weak inhibition by citrate and the stronger effects of ATP. Presumably, this was a result of direct interaction between magnesium and the inhibitors. Magnesium did not appear to be necessary for enzyme activity because the latter was not modified by the presence of 2×10^{-3} M ethylenediaminetetraacetate.

Inhibition of glucose-6-phosphate dehydrogenase by other nucleotides. Guanosine triphosphate (GTP) also inhibited the activity of glucose-6-phosphate dehydrogenase. In fact, GTP was slightly more effective than ATP. Inhibition by deoxyguanosine triphosphate (dGTP) was less than that by GTP. Table 4 shows the inhibitions promoted by a number of different nucleotides. In general, only purine di- and trinucleotides gave significant inhibition. In all cases, inhibition was overcome by increasing the substrate concentration.

Determination of K_m values for NAD and NADP. The apparent affinity of glucose-6-

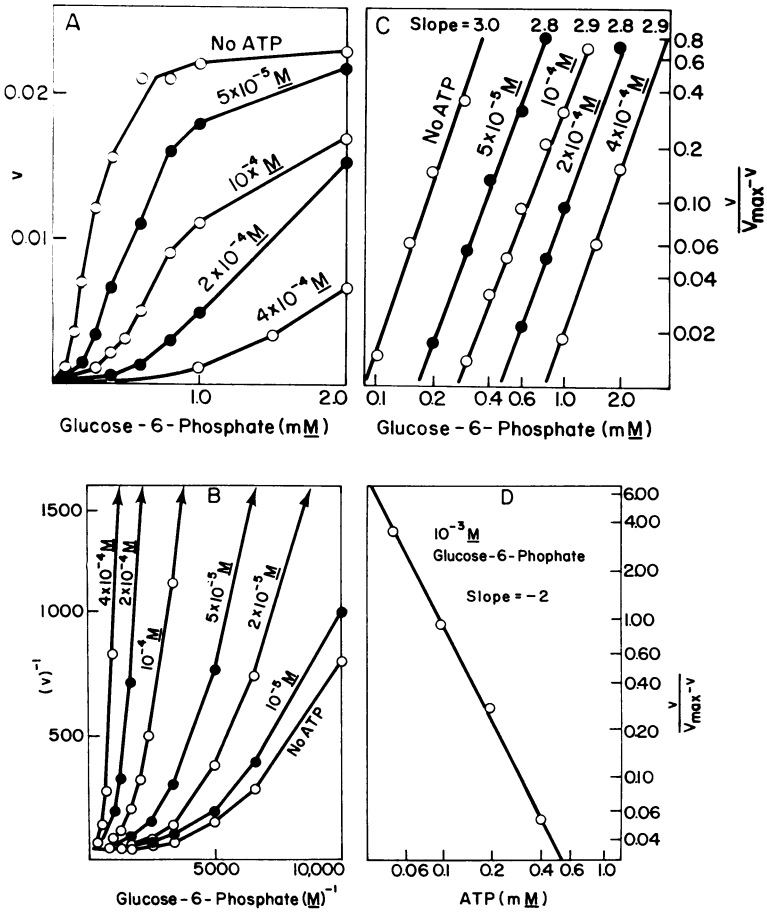

FIG. 3. *Activity of glucose-6-phosphate dehydrogenase in the presence of different concentrations of glucose-6-phosphate and ATP. The reaction mixtures contained about 1.5 μg of 50-fold purified glucose-6-phosphate dehydrogenase and no magnesium. Enzyme activity (v) is expressed as change in optical density at 340 mμ per minute. (A) Enzyme activity as a function of glucose-6-phosphate concentration with different concentrations of ATP. (B) Reciprocal plots of enzyme activity versus glucose-6-phosphate concentration with different ATP concentrations. (C) Hill plots (3) for estimating apparent binding of glucose-6-phosphate to the enzyme with different ATP concentrations. (D) Hill plot for estimating apparent binding of ATP to the enzyme.*

phosphate dehydrogenase for NADP was found to be approximately 10 times greater than that for NAD (Table 5). The presence of ATP did not significantly alter these values. There was no difference in enzyme activity with NAD or NADP when saturating concentrations of these cofactors were present. Sigmoid saturation curves for glucose-6-phosphate were observed with NAD as well as with NADP.

Physical properties of glucose-6-phosphate dehydrogenase. Glucose - 6 - phosphate dehydrogenase was characterized by comparing its filtration through Sephadex gels, and its sedimentation through sucrose gradients, with that of hemoglobin. The enzyme was completely excluded from Sephadex G-100 gels and eluted from columns of G-200 gels before hemoglobin. On the basis of its sedimentation (Fig. 5), an approximate molecular weight of 190,000 was assigned to the enzyme. This value corresponds to the molecular weight (190,000) determined for human erythrocyte glucose-6-phosphate dehydrogenase by Chung and Langdon (6). Our calculation was made with the assumptions that the molecular weight of hemoglobin was 68,000 and that the distances both proteins migrated during centrifugation were related to their molecular weights in the manner described by Martin and Ames (13).

There was no significant change in sedimenta-

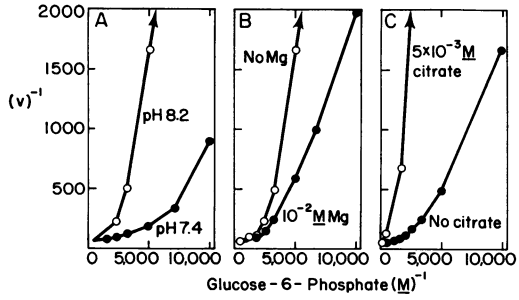

FIG. 4. *Modification of glucose-6-phosphate dehydrogenase activity by magnesium, citrate, and change in pH. The reaction mixtures contained about 1.5 μg of 50-fold purified glucose-6-phosphate dehydrogenase and no magnesium unless indicated. Enzyme activity (v) is expressed as change in optical density at 340 mμ per min. (A) With and without magnesium, pH 8.2. (B) With and without citrate, pH 8.2. (C) With magnesium at pH 7.4 and 8.2.*

TABLE 4. *Effect of various nucleotides on the activity of glucose-6-phosphate dehydrogenase*

Nucleotide[a]	Glucose-6-phosphate dehydrogenase activity[b]
	%
None	100
Adenosine monophosphate	100
Adenosine diphosphate	50
Adenosine triphosphate	15
Cytidine triphosphate	100
Guanosine monophosphate	80
Guanosine diphosphate	25
Guanosine triphosphate	5
Deoxyguanosine triphosphate	5
Uridine triphosphate	70

[a] Each at 10^{-3} M.

[b] The assay mixture (3 ml) contained 3 μg of 50-fold purified enzyme and, in the absence of added nucleotides, gave an optical-density change of 0.06 unit/min at 340 mμ.

tion behavior of the enzyme when gradients were prepared with solutions containing 5×10^{-3} M ATP.

DISCUSSION

It is possible that *P. aeruginosa* catabolizes glucose via more than one pathway. Radiorespirometric data, however, indicate that the Entner-Doudoroff pathway plays a predominant role in catabolism of glucose by this organism (9, 18). Hamilton and Dawes (9) reported that the enzymes of this route were present in bacteria grown upon glucose but not upon tricarboxylic acid cycle intermediates. On the other hand, Stokes and Campbell (16) found that this organism can oxidize glucose directly to ketogluconate without intermediate formation of hexose phosphate. We measured glucose oxidation in extracts of *P. aeruginosa* by coupling this activity to ferricyanide reduction (*unpublished data*) and found the same low activities in bacteria irrespective of whether they were grown on glucose or succinate. The values at 25 C were

TABLE 5. *Apparent K_m values for NAD and NADP of purified glucose-6-phosphate dehydrogenase in the presence and absence of ATP*

Assay condition	K_m	
	NAD	NADP
	M	M
No ATP	8×10^{-4}	6×10^{-5}
ATP (10^{-3} M)	6×10^{-4}	5×10^{-5}

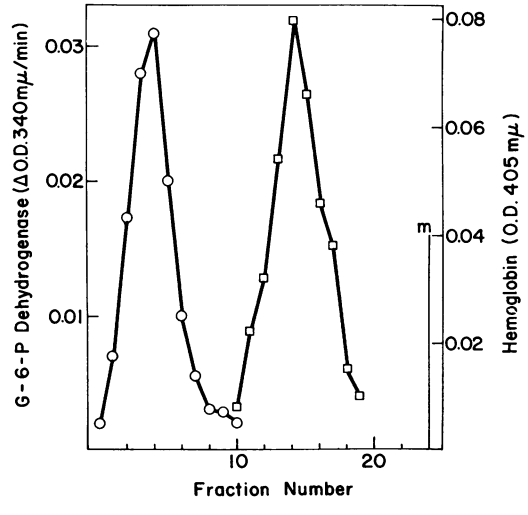

FIG. 5. *Zone centrifugation of a mixture of glucose-6-phosphate dehydrogenase and hemoglobin. Samples (0.2 ml) containing about 35 μg of 50-fold purified glucose-6-phosphate dehdyrogenase and 200 μg of hemoglobin were layered with 0.2 ml of 4% sucrose as an inverse gradient over 4.8 ml of 5 to 20% linear sucrose gradients containing 5×10^{-2} M Tris chloride buffer. The gradients were centrifuged for 20 hr at 35,000 rev/min in the SW-39 rotor of a Spinco preparative ultracentrifuge. The bottoms of the tubes were punctured, and seven-drop fractions were collected. Portions (0.05 ml) of each fraction were assayed for glucose-6-phosphate dehydrogenase (○). The remainder of each fraction was diluted with water, and hemoglobin was determined by measuring the optical density at 405 mμ (□). The meniscus is indicated by m.*

about 40 mμmoles of glucose oxidized per min per mg of protein (compared with about 150 mμmoles of succinate per min per mg of protein for succinate dehydrogenase measured similarly), and were insufficient to account for the observed in vivo rates of glucose utilization (420 mμmoles per min per mg of protein). Since in our experiments the ability to grow upon glucose closely followed the induction of glucose-6-phosphate dehydrogenase, it seems reasonable that this enzyme must be present for the bacteria to grow upon glucose.

Our finding that *P. aeruginosa* glucose-6-phosphate dehydrogenase is inhibited by ATP raises the question of the possible in vivo significance of such regulation. J. Passaneau (Federation Proc. **25:** 219, 1966) observed a similar inhibition of mammalian glucose-6-phosphate dehydrogenase by ATP, indicating that adenine nucleotide-linked control of glucose-6-phosphate dehydrogenase activity may be of general importance.

The simplest hypothesis is that regulation of glucose-6-phosphate dehydrogenase activity is necessary to attain the most efficient pattern of hexose phosphate consumption. In *P. aeruginosa*, the problem is exaggerated by the presence of large amounts of the enzyme, presumably reflecting its major role in the catabolism of glucose. In the absence of any control over glucose-6-phosphate dehydrogenase, sudden depletion of exogenous glucose might be expected to lead to rapid exhaustion of hexose phosphate, and thereby delay or prevent adaptation to growth on other substrates by wastefully consuming hexose phosphate required for biosynthesis. Two properties of *P. aeruginosa* glucose-6-phosphate dehydrogenase might function to avoid such a situation. First, its saturation curves suggest that a threshold level of glucose-6-phosphate must be reached before appreciable enzyme activity is observed. Second, generation of ATP by degradation of alternative substrates or of endogenous materials might be sufficient to inactivate the enzyme at low levels of glucose-6-phosphate.

The highest levels of glucose-6-phosphate dehydrogenase observed in our experiments occurred in bacteria growing upon glycerol, where growth was poor. If excess glucose-6-phosphate dehydrogenase does in fact tend to restrict the supply of hexose phosphate, then the poor growth on glycerol may be a consequence of the high enzyme level. To test for such a correlation, we are attempting to obtain mutants which have either gained the ability to grow rapidly upon glycerol or have lost the ability to form large amounts of glucose-6-phosphate dehydrogenase.

Although *P. aeruginosa* glucose-6-phosphate dehydrogenase appears to be primarily an NADP-linked enzyme, our results do not exclude the possibility that it has a dual function of generating both $NADH_2$ and $NADPH_2$ in vivo. It was attractive to speculate that these functions might be mutually exclusive and depend upon the supply of ATP; however, our results do not support such an idea.

The apparent absence of both fructose-1,6-phosphate aldolase and 6-phosphate gluconate dehydrogenase in extracts of succinate-grown bacteria poses the question of how hexose and pentose phosphates are formed during growth on this substrate. It is generally considered that pentose phosphate arises either from 6-phosphogluconate via its respective dehydrogenase or by transketolase-mediated transfer of active glycoaldehyde from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate. Both of these reactions depend upon a constant supply of hexose phosphate, whose origin is not obvious in the present case. Sable (14) suggested on the basis of labeling experiments that *P. saccharophila* forms pentose phosphate by transketolase-promoted transfer of active glycoaldehyde from hydroxypyruvate to glyceraldehyde-3-phosphate. Since *P. aeruginosa* can presumably form triose phosphates from tricarboxylic acid intermediates, such a mechanism may be of importance to this bacterium.

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