Biochimica et Biophysica Acta, 358 (1974) 33–43

BBA 67247

GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM *CAULOBACTER CRESCENTUS*

JOSEPH G. SHEDLARSKI, Jr

Department of Biological Sciences, Louisiana State University in New Orleans, New Orleans, La.70122 (U.S.A.)

(Received January 15th, 1974)

SUMMARY

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) has been partially purified from *Caulobacter crescentus*. The enzyme is induced 10-fold when the organism is grown in complex or minimal salts medium containing glucose. Three molecular forms of the enzyme can be demonstrated in crude extracts or in purified preparations using polyacrylamide gel electrophoresis. Three peaks of enzymatic activity can be separated from each other by DEAE-Sephadex chromatography. However, only one sedimenting peak of enzymatic activity with an apparent molecular weight of 190 000 can be detected on a 5–20% linear sucrose density gradient. Substrate saturation curves for glucose 6-phosphate and NADP or NAD as electron acceptors are of the Michaelis–Menten type. Of the possible effector compounds tested, only phosphoenolpyruvate has been shown to exert a negative allosteric effect.

INTRODUCTION

In 1964, Poindexter presented the first review of the genus *Caulobacter* [1]. These are Gram-negative bacteria that possess a stalk at one pole of the cell and exhibit a distinctly dimorphic life cycle. Many of the physiological properties of caulobacters determined by Poindexter are also possessed by the *Pseudomonas–Vibrio* group The apparent absence of fructose-bisphosphate aldolase (fructose-1,6-bisphosphate: D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.13) and the presence of 2-keto-3-deoxy-6-phosphogluconate aldolase (6-phospho-2-keto-3-deoxy-D-gluconate:D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.14). in crude extracts of bacteria grown in the presence of glucose implicated the presence of the Entner–Doudoroff pathway and provided another criterion of similarity between this genus and the pseudomonads [1].

This report presents evidence that glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49), one of the enzymes associated with the degradation of glucose in the organism, occurs in more than one molecular form and can be induced when glucose is used as a carbon source. Although isozymes and multimolecular forms of glucose-6-phosphate dehydrogenase are common in mammalian systems [2], the occurrence of such forms in bacteria have been detected so far only in partially purified extracts of *Pseudomonas multivorans* [3].

MATERIALS AND METHODS

Organisms

Caulobacter crescentus CB2 (ATCC 15252) was used throughout.

Growth of bacteria

Bacteria were grown in either complex media with or without glucose or in minimal salts media containing a 0.2% carbon source. Both media were prepared according to Schmidt and Stanier [4]. Cultures were shaken in side-armed nephelometric flasks and contained a volume one-third that of their normal capacity. Incubation was at 30 °C in a gyratory shaker (New Brunswick Co., N.J.). Growth was monitored by following increase in turbidity using a No. 66 filter in a Klett–Summerson colorimeter.

Preparation of cell-free extracts for enzyme assay

Cultures were harvested at mid-log stage of growth and washed with 0.02 M Tris–Cl buffer, pH 7.8. The bacteria were suspended in the same buffer and an equal volume of acid-washed glass beads (0.1 mm diameter) were added. This suspension was sheared at the maximum speed in a Sorvall Omnimix cup cooled in an ice-water bath; shearing was done in three 2-min intervals interspersed with a 2-min cooling period. The suspension was then filtered through a course sintered glass disc or, in instances of volumes less than 5 ml, the supernatant was removed after the beads were allowed to settle. The crude extract was then centrifuged at 29 000 $\times$ *g* for 20 min at 10 °C.

Enzyme assay

Glucose-6-phosphate dehydrogenase assay mixture (3 ml) contained: $3 \cdot 10^{-2}$ M Tris–Cl buffer, pH 7.6; $4 \cdot 10^{-4}$ M NADP; $2 \cdot 10^{-3}$ M glucose 6-phosphate, and 20–100 μg of protein. The reactions were started by addition of enzyme. The assay involved the appearance of NADPH which was measured by monitoring changes in absorbance of the assay mixtures at 340 nm with a Cary 15 recording spectrophotometer. The values were converted to nmoles of NADPH formed per min per mg of protein, assuming a molar extinction coefficient of $6.2 \cdot 10^3$ for the reduced pyridine nucleotide. 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.

Sucrose gradient centrifugation

Rate-zonal centrifugation was performed essentially as described by Martin and Ames [5] using a 5–20% linear sucrose gradient in 0.05 M Tris–Cl, pH 7.6.

Polyacrylamide gel electrophoresis

A vertical slab gel apparatus was used (E. C. Apparatus, Co.). Gels were prepared by mixing 8.0 g Cyanogum-41 (E. C. Apparatus, Co.) in 160 ml 0.01 M Tris–Cl, pH 7.6. 1/1000 vol. of N,N,N′,N′-tetramethylethylenediamine was added as catalyst and the reaction initiated with addition of 0.16 g ammonium persulfate. Samples were applied in a 10% sucrose solution. Experimental runs were done at a constant current of 150 mA for 3 h during which the gel was cooled by circulating ice-water

through the cooling coils. Zones of glucose-6-phosphate dehydrogenase activity were visualized by incubating the gels for 6 h at 25 °C in 10 ml of a standard assay mixture containing 50 μg of phenazine methosulfate and 200 μg of nitro blue tetrazolium. The reaction was stopped by adding glacial acetic acid (3 ml), and the gels were stored in 7% (v/v) acetic acid. 6-Phosphogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase, EC 1.1.1.43) activity was visualized by substituting 6-phosphogluconate for glucose 6-phosphate in the incubation mixture.

Purification of glucose-6-phosphate dehydrogenase

A total of 2 l of bacteria were grown in complex media, harvested at late-log stage of growth, and the crude extract was prepared as stated above. All subsequent procedures were performed at 0–4 °C and all centrifugations were at 10 °C in an SS-34 rotor in a Sorvall refrigerated centrifuge.

The crude extract was diluted with glass-distilled water to give a protein concentration of 25 mg/ml. 1/60 vol. of 1.0 M $MnCl_2$ was added dropwise and the mixture was then equilibrated, with stirring, for 10 min. Precipitated material was removed by centrifugation at 23 000 $\times$ g for 20 min. To the supernatant was added sufficient crystalline $(NH_4)_2SO_4$ to bring the solution to 25% saturation. After equilibration for 30 min, the mixture was centrifuged at 29 000 $\times$ g for 20 min. To the clear supernatant was added crystalline $(NH_4)_2SO_4$ to bring the solution to 50% saturation (0.31 g $(NH_4)_2SO_4$ per ml of original volume). After equilibration for 20 min, the mixture was centrifuged at 29 000 $\times$ g for 20 min, supernatant discarded and the pellet drained. The pellet was resuspended in 0.05 M potassium phosphate buffer, pH 7.8, 1.0 mM dithiothreitol and dialyzed overnight against 1000 vol. of the resuspending buffer.

The dialyzed solution was diluted 5-fold with distilled water and admitted to a DEAE-cellulose column (2.5 cm $\times$ 30 cm) equilibrated with 0.01 M Tris–Cl buffer, pH 7.8. The column was then eluted with an exponential gradient of 100 ml of equilibrating buffer in the mixing chamber and the same buffer containing 0.5 M NaCl in the reservoir. The flow rate was 5 ml/h. The elution profile is given in Fig. 1.

The fractions of peak activity from the column chromatography were pooled, and crystalline $(NH_4)_2SO_4$ was added to bring the solution to 30% saturation. After the solution was cleared by centrifugation at 29 000 $\times$ g for 20 min, the supernatant was taken to 50% saturation. The solution was again cleared in the same way and the pellet was resuspended in 0.05 M potassium phosphate buffer, pH 7.8, 1.0 mM dithiothreitol and dialyzed overnight against the same buffer. The enzyme is recovered in a 37% yield and the specific activity at this stage is 48-fold above that in crude extract; it will remain stable over a period of months if kept frozen at -20 °C.

Protein determination

Protein was determined using the Folin phenol reagent by the method of Lowry et al. [6].

RESULTS

Induction of glucose-6-phosphate dehydrogenase

Batch cultures of bacteria grown in complex media exhibit low levels of glucose-6-phosphate dehydrogenase compared to those grown in minimal salts media contain-

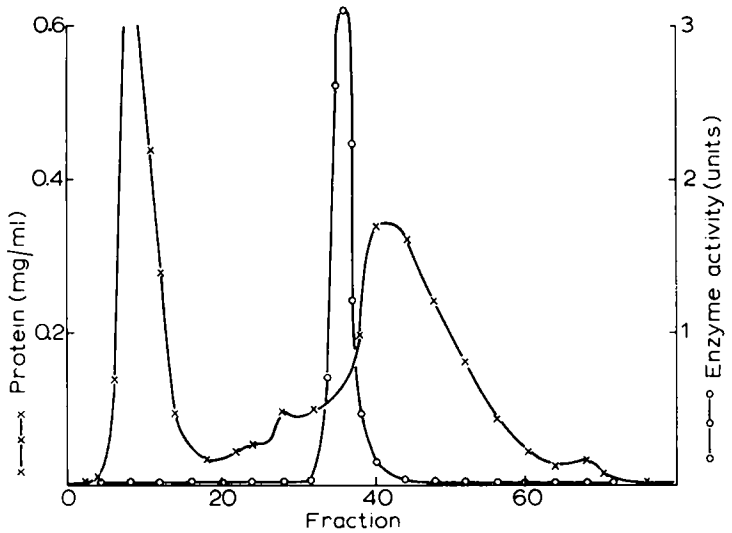

Fig. 1. Chromatographic profile of the partially purified enzyme on DEAE-cellulose. For elution conditions see Materials and Methods.

ing glucose or galactose (Table I). If the complex media contains glucose, however, a level of glucose-6-phosphate dehydrogenase is reached that is equal to that in the minimal salts medium containing the same carbon source. The presence of xylose or glycerol as a carbon source results in low levels of activity. When mixtures of extracts were prepared from bacteria grown on the pentose- or glycerol-containing media with that from the glucose-containing media, an average value of glucose-6-phosphate dehydrogenase activity was obtained, suggesting the absence of any agents interfering with the enzyme assay that may have been present in the extracts prepared with xylose or glycerol as sources of carbon and energy.

The growth rate constants in complex medium or minimal salts medium containing glycerol are equal and approximately half as great as that on the minimal media containing xylose, galactose, or glucose (Table I), yet the level of glucose-6-phosphate dehydrogenase activity in the xylose- or glycerol-containing media is the same. When

TABLE I

FORMATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE UNDER VARIED GROWTH CONDITIONS

Medium*	Growth rate constant** (h^{-1})	Specific activity (units/mg protein)
Complex	0.13	10.1
Complex + glucose	0.23	80.2
Minimal salt + glucose	0.23	79.5
Minimal salt + galactose	0.23	80.1
Minimal salt + xylose	0.22	5.5
Minimal salt + glycerol	0.13	7.0

* All carbon sources present at 0.2%.

** Growth rate as expressed in terms of the specific growth rate constant, k, calculated as ln 2/mass doubling time in h.

glucose is added to the complex medium, however, the growth rate increases to that on minimal medium containing glucose or xylose, but the level of glucose-6-phosphate dehydrogenase is now equivalent to that in bacteria grown in the minimal medium containing glucose. Consequently, caulobacters do not adjust the level of glucose-6-phosphate dehydrogenase based solely upon growth rate but upon carbon source as well.

Initial velocity studies

When the concentration of NADP was varied between $1.3 \cdot 10^{-5}$ M and $3 \cdot 10^{-4}$ M at a constant concentration of glucose 6-phosphate (3.9 mM), the rate–substrate concentration response curve was of the classical Michaelis–Menten type. A Lineweaver–Burk plot of these results is linear and extrapolates to an apparent K_m of $2.9 \cdot 10^{-5}$ M for NADP.

There are many reports in the literature concerning the dual response of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* [7] and *Pseudomonas aeruginosa* [8] to NADP and NAD. When a rate–substrate concentration curve for the enzyme was obtained using NAD as cofactor, the response was again hyperbolic; the same maximum velocity is obtained with either cofactor and a double reciprocal plot indicates an apparent K_m of $2.9 \cdot 10^{-4}$ M for NAD. The substrate saturation curve was the same in the presence of both cofactors as in the presence of NADP alone and did not reach a higher V.

Initial velocity studies indicate a classical hyperbolic response when glucose 6-phosphate is varied at saturating NADP ($2 \cdot 10^{-4}$ M). The double reciprocal plot is linear and extrapolates to an apparent K_m of $3.4 \cdot 10^{-4}$ M; no evidence of a homotropic effect of this substrate upon the *Caulobacter* enzyme could be discerned as the concentration of glucose 6-phosphate was varied between $20 \cdot 10^{-6}$ M and $2 \cdot 10^{-3}$ M.

Inhibition studies

ATP inhibits glucose-6-phosphate dehydrogenase, and the extent of inhibition depends upon the concentration of glucose 6-phosphate alone (Fig. 2). The inhibition is classically competitive and the apparent K_I for ATP calculated by a Dixon plot [9] was $9.4 \cdot 10^{-3}$ M. There is no indication that ATP exerts a heterotropic effect at these concentrations.

Phosphoenolpyruvate exerts a negative heterotropic effect, however (Fig. 3). The presence of pyruvate or P_i at a concentration of 0.01 M produces no inhibition whatsoever. The inhibitory effect of phosphoenolpyruvate has been reported previously with glucose-6-phosphate dehydrogenase from *Arthrobacter 7C* [10] and *Bacillus licheniformis* [11].

A number of enzymes involved in initial or terminal pathways of carbohydrate metabolism have been shown to be specifically regulated by NADH in a characteristic allosteric manner [12]. When initial velocity studies of glucose-6-phosphate dehydrogenase of *Caulobacter* were examined in the presence of NADH at $4.6 \cdot 10^{-5}$ M, no significant inhibition of the NADP-linked reaction occurred, nor was there any evidence of a sigmoidal response. The presence of NADPH at a concentration of $2 \cdot 10^{-6}$ M, on the other hand, gave a typical product inhibition response as the concentration of NADP was varied between $2 \cdot 10^{-6}$ M and $2 \cdot 10^{-4}$ M; the inhibition was specific to the NADP site only.

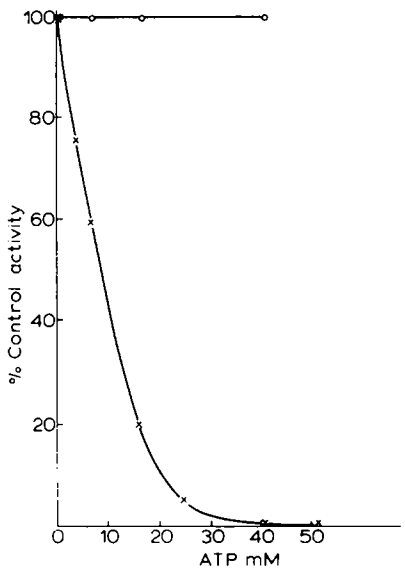

Fig. 2. Inhibition of the enzyme by ATP. Reaction mixtures (3.0 ml) contained 20 μg enzyme (750 nmoles/min per mg), 0.05 M Tris–Cl buffer, pH 7.6, and either $5 \cdot 10^{-5}$ M glucose 6-phosphate plus $2 \cdot 10^{-4}$ M NADP (×···×) or 3.9 mM glucose 6-phosphate plus $0.3 \cdot 10^{-5}$ M NADP (○—○). The data are expressed as a percentage of the activity measured in the absence of ATP.

pH optimum

Glucose-6-phosphate dehydrogenase exhibits a well-defined pH profile. An optimum range of 7.8–8.2 is indicated when either phosphate or Tris buffers at a concentration of 0.05 M are used.

Physical properties of glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase was characterized by comparing its sedimentation through sucrose gradients with that of rabbit muscle lactate dehydrogenase (L-lactic acid: NAD oxidoreductase, EC 1.1.1.27). On the basis of its sedimentation, an approximate molecular weight of 190 000 was assigned to the enzyme from *Caulo-*

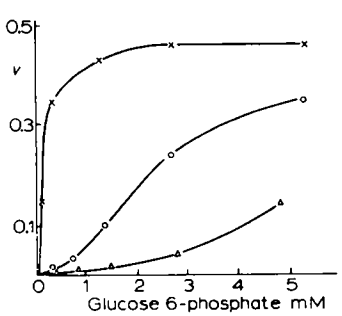

Fig. 3. Inhibition of the enzyme by phosphoenolpyruvate. For assay conditions see Materials and Methods. ×—×, control; ○- -○, 0.33 mM phosphoenolpyruvate; △—△, 3.3 mM phosphoenolpyruvate.

bacter. This value corresponds to the molecular weight determined for human erythrocyte [13] and *P. aeruginosa* [8] glucose-6-phosphate dehydrogenase. Calculations were made with the assumptions that the molecular weight of rabbit muscle lactate dehydrogenase was 142 000 and that the distances sedimented by both proteins were related to their molecular weights in the manner described by Martin and Ames [5]. Only one sedimenting peak of activity, comprising 70% of the total enzymatic activity put on the gradient, is detected after centrifuging for 12 or 42 h.

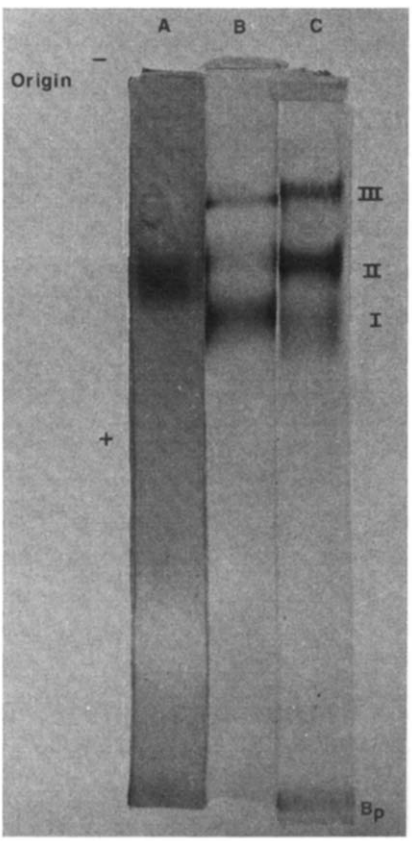

Fig. 4. Separation of three forms of glucose-6-phosphate dehydrogenase by polyacrylamide gel electrophoresis. Samples (0.025 ml) containing 80 μg protein from each preparation were layered in standard buffer containing 10% glucose onto a 5% acrylamide gel. The samples were run and activity detected as described in Materials and Methods. (A) Crude extract from glucose-grown bacteria replacing glucose 6-phosphate by 6-phosphogluconic acid in gel assay mixture. Bromphenol blue (Bp) used as tracking dye. (B) 50-fold purified enzyme. (C) Crude extract.

Electrophoresis of samples of crude extract or $(NH_4)_2SO_4$ fractions on vertical slab gels demonstrates the presence of three bands of enzymatic activity (Fig. 4). Zones of activity were detected by incubating the gel in assay mixtures containing nitro blue tetrazolium and a coupling agent (phenazine methosulfate) to promote the NADPH-dependent deposition of an insoluble tetrazolium pigment. A fast-migrating band (I) appears first, followed by a slower (II) and then the slowest-migrating (III)

bands. The same order of appearance occurs whether NADP or NAD are used as cofactors in the gel assay mixture. The presence of glucose 6-phosphate or NADP at 25 to 50% of their respective K_m or preincubation of the enzyme in the presence of the same range of glucose 6-phosphate concentrations does not alter the mobility and/or time of appearance of any single band. Control gels incubated in a staining solution lacking glucose 6-phosphate showed no enzymatic activity over the usual 6-h incubation period.

Replacement of glucose 6-phosphate by 6-phosphogluconate in the assay mixture containing the gel on which a sample of the $(NH_4)_2SO_4$ fraction was run produced no detectable bands of activity even after 48 h incubation, demonstrating that the partial purification has eliminated 6-phosphogluconate dehydrogenase activity. However, a single band of activity for this enzyme did appear when crude extract of bacteria grown in minimal salts medium containing 0.2% glucose was run and incubated for 48 h (Fig. 4); the band displayed a mobility intermediate between that of Bands I and II of glucose-6-phosphate dehydrogenase but does not begin to appear until 12 h of incubation. The simultaneous presence of glucose 6-phosphate and 6-phosphogluconate in the assay mixture results in the appearance of only the usual glucose-6-phosphate dehydrogenase bands within a 6-h incubation period. These observations demonstrate that 6-phosphogluconate dehydrogenase is present in crude extracts of bacteria grown in the presence of glucose, is absent from the purified preparations, and does not pose any ambiguity to the electrophoretic assay of glucose-6-phosphate dehydrogenase in crude extract under these conditions.

Although the enzyme is eluted as a single symmetrical peak from a DEAE-cellulose column (Fig. 1), the three forms of the enzyme exhibited on gel electrophoresis can also be shown to separate upon chromatography of the partially purified enzyme on DEAE-Sephadex (Fig. 5). This differential behavior in the presence of a

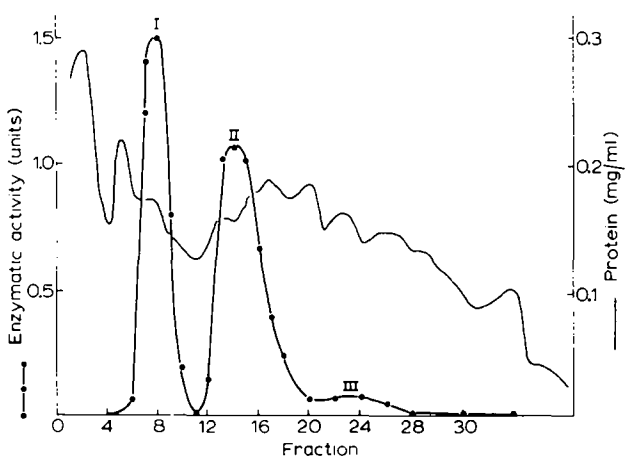

Fig. 5. Chromatography of 5-fold purified enzyme on DEAE-Sephadex column. A 0.5-ml aliquot of 5-fold purified enzyme was loaded onto a 0.9 cm × 30 cm column of DEAE-Sephadex A-25 (Pharmacia, Co.) equilibrated with 0.05 M Tris–Cl, pH 7.8. The column was eluted with an exponential gradient (0.4 M Tris–Cl, pH 7.8 in reservoir and 50 ml 0.05 M Tris–Cl, pH 7.8 in mixing chamber) at a flow rate of 5 ml per h. A 0.2-ml aliquot of each fraction (5.0 ml) was removed and assayed for enzyme activity. An aliquot of each fraction was assayed for protein content by the Lowry procedure using crystalline bovine serum albumin as a standard.

sieving matrix suggests that the three forms have closely similar or identical net charges at pH 7.8 but are different in molecular size. It was possible to recover 50% of the total activity put on the DEAE-Sephadex column in two major peaks and one minor peak. Peak I included 58% and Peak II 40% of the total activity recovered from the column. It was not possible to confirm or correlate the identity of each peak eluting from the column with those on the acrylamide gel; when each peak was concentrated by collodion membrane dialysis, applied to the gel, and run under the same conditions as the crude extract, all three bands of activity were generated and migrated with the same relative mobility to each other as the native preparation (data not shown). These results demonstrate that three molecular forms of glucose-6-phosphate dehydrogenase exhibit enzymatic activity, establish an equilibrium mixture in crude extracts, and that all three forms are present in these partially purified preparations.

DISCUSSION

The Entner–Doudoroff pathway of glucose dissimilation, which involves the formation of triose phosphate from gluconic acid phosphate rather than from glucose phosphate, is generally recognized as a major glucose-inducible system in Pseudomonads [14]. Poindexter observed that extracts of five strains of *Caulobacter* (including CB2) exhibit the presence of hexosephosphate dehydrogenase and 2-keto-3-deoxy-6-phosphogluconate aldolase [1]. These initial observations have been confirmed and extended in this report. When grown in the presence of glucose, glucose-6-phosphate dehydrogenase is induced to higher specific activity levels compared to growth on complex media lacking glucose or in minimal medium containing xylose or glycerol as the only source of carbon.

Glucose-6-phosphate dehydrogenase has been highly purified from a variety of microorganisms and mammalian tissues and has been extensively studied with respect to the enzymatic mechanism and control [2, 13, 15, 16]. Enzymes from bacterial sources can be distinguished on the basis of their pyridine nucleotide specificity. The enzyme from *Escherichia coli* reacts exclusively with NADP [12] whereas that from *L. mesenteroides* [7], *P. aeruginosa* [8] and *Hydrogenomonas eutropha* H16 [17] reacts equally well with NAD and NADP. The observation that the same maximum velocity is achieved at saturating concentrations of either pyridine nucleotide cofactor in the present case allows *C. crescentus* glucose-6-phosphate dehydrognasee to be included in the latter class.

In contrast to cooperative inhibition of glucose-6-phosphate dehydrogenase of *E. coli* by NADH [12], the activity of the *Caulobacter* enzyme is unaffected by this cofactor. Although this enzyme in *Caulobacter* is inhibited by ATP, the adenine nuleotide only decreases the apparent affinity of the enzyme for glucose 6-phosphate without significantly affecting the activity of the enzyme once it is saturated with substrate. Glucose 6-phosphate itself does not exhibit any cooperative binding, which is in contrast to the same enzyme from *Pseudomonas fluorescens*, *P. aeruginosa*, and *Hydrogenomonas* H16, where sigmoidal saturation curves for glucose 6-phosphate are observed or sigmoidicity is induced by ATP [8, 18]. No sigmoidicity has been observed when the enzyme from *Pseudomonas facilis* [18], *Thiobacillus ferrooxidans* [19], or *Nocardia opaca* 1b [10] was investigated, even in the presence of unphysiologically high concentrations of ATP. Evidently, ATP inhibition is focused only at the glucose

6-phosphate binding site in the *Caulobacter* enzyme, and neither ATP nor NADH are allosteric ligands.

Phosphoenolpyruvate is a key intermediate in the degradation of hexose via the Embden–Meyerhoff–Parnas as well as the Entner–Doudoroff pathway. The potent negative allosteric effect of this compound has been recently shown with glucose-6-phosphate dehydrogenase from two hydrogen-oxidizing bacteria, *Arthrobacter 7C* and *Hydrogenomonas H16* [10]. The presence of enzymes unique to the Entner–Doudoroff catabolic sequence in *Caulobacter*, coupled with the apparent absence of those enzymes characteristic of the glycolytic pathway [1], may suggest that phosphoenolpyruvate controls the rate of carbon flow in this organism by acting directly on glucose-6-phosphate dehydrogenase, which is at a strategic branch-point in hexose/pentose metabolism.

Three zones of glucose-6-phosphate dehydrogenase activity can be detected after polyacrylamide gel electrophoresis; all three bands respond to either NAD or NADP at concentrations of glucose 6-phosphate in the range of 25–50% of its K_m value. No single band of activity is inactive under these concentrations of glucose 6-phosphate, but the effect of this substrate upon the distribution of the three forms in the mixture is unknown. When eluted with an exponential salt gradient from DEAE-Sephadex, two major peaks of activity account for 98% of the total recoverable activity applied to the column; these may represent the two prominent bands (I and II) seen on the gel since they are the most prominent bands to appear in the gel assay early in the incubation period.

Lessie and Vander Wyk [3] recently reported finding two multiple forms of glucose-6-phosphate dehydrogenase in partially purified extracts of *P. multivorans*, which differed in pyridine nucleotide specificity at non-saturating values of glucose 6-phosphate; the two forms are clearly separated by centrifugation on a linear sucrose density gradient as well as upon gel electrophoresis. Clearly, this is not the case with glucose-6-phosphate dehydrogenase from *C. crescentus* since all three bands of activity appear in the presence of either cofactor at non-saturating values of glucose 6-phosphate. It is not immediately apparent why only one sedimenting peak of activity is recovered from a linear sucrose gradient centrifugation of glucose-6-phosphate dehydrogenase from *Caulobacter*. However, the sequental (as opposed to simultaneous) appearance over a 6-h interval in the gel assay suggests that the forms have different kinetic constants and/or occur in different concentrations in an equilibrium mixture. The short-term spectrophotometric assay used to detect the enzyme in the sucrose gradient may be sensitive to the form occurring in highest concentration only.

REFERENCES

1 Poindexter, J. S. (1964) Bacteriol. Rev. 28, 231–295
2 Bonsignore, A. and DeFlora, A. (1972) Current Topics in Cellular Regulation (Stadtman, E. and Horecker, B., eds), Vol. 6, pp. 21–62, Academic Press, New York
3 Lessie, T. and Vander Wyk, J. C. (1972) J. Bacteriol. 110, 1107–1117
4 Schmidt, J. M. and Stanier, R. Y. (1966) J. Cell Biol. 28, 423–436
5 Martin, R. G. and Ames, B. N. (1961) J. Biol. Chem. 236, 1372–1379
6 Lowry, O. H., Rosebrough, N. M., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
7 DeMoss, R. D., Gunsalus, I. C. and Bard, R. C. (1953) J. Bacteriol. 66, 10–16

8 Lessie, T. and Neidhardt, F. C. (1967) J. Bacteriol. 93, 1337–1345
9 Dixon, J. and Webb, E. C. (1964) Enzymes, 2nd edn, pp. 329–330, Longmans Green, London
10 Tunall, N. and Schlegel, H. G. (1972) Biochem. Biophys. Res. Commun. 49, 1554–1560
11 Opheim, D. and Bernlohr, R. W. (1973) J. Bacteriol. 116, 1150–1159
12 Sanwal, B. D. (1970) J. Biol. Chem. 245, 1626–1631
13 Chung, A. E. and Langdon, R. G. (1963) J. Biol. Chem. 238, 2309–2316
14 Kersters, K. and DeLay, J. (1968) Antonie van Leeuwenhoek J. Microbiol. Serol. 34, 393–408
15 Afloyan, A. (1972) Biochemistry 11, 4172–4178
16 Glaser, L. and Brown, D. H. (1955) J. Biol. Chem. 216, 67–79
17 Blckkolb, F. and Schlegel, H. G. (1968) Arch. Mikrobiol. 63, 177–196
18 Schindler, J. and Schlegel, H. G. (1969) Arch. Mikrobiol. 66, 69–78
19 Tabita, R. and Lundgren, D. G. (1971) J. Bacteriol. 108, 343–352