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PURIFICATION AND PROPERTIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM A METHANOL-UTILIZING YEAST, *CANDIDA BOIDINII*

NOBUO KATO *, HERMANN SAHM **, HORST SCHÜTTE and FRITZ WAGNER

Gesellschaft für Biotechnologische Forschung mbH, D-3300 Braunschweig-Stöckheim (F.R.G.)

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Summary

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase, EC 1.1.1.44) were purified approx. 1700 fold and 330 fold, respectively, from *Candida boidinii* grown on methanol. The final enzyme preparations were homogeneous as judged by polyacrylamide gel electrophoresis. The molecular weights of the enzymes were estimated to be 118 000 and 110 000, respectively. Both enzymes are composed of two probably identical subunits and the molecular weights of the polypeptide chains were calculated to be 61 000 and 58 000, respectively.

From a consideration of enzyme activities and types of inhibition by different metabolites the role of these two enzymes in glucose- and methanol-metabolism is discussed.

Introduction

The catabolic pathway of methanol found in many methanol-utilizing bacteria and yeasts proceeds by a stepwise oxidation of methanol via formaldehyde to formate and finally to CO_2 [1—5]. Recently, however, in some obligate methylotrophic bacteria very low or negligible activities of formaldehyde and formate dehydrogenases have been found [6—8]. In these bacteria formaldehyde is mainly oxidized to CO_2 via the dissimilatory ribulose monophosphate cycle, since they contain high specific activities of glucose-6-phos-

* Present address: Department of Environmetal Chemistry and Technology, Tottori University, Tottori, 680 (Japan).
** Present address: Institut für Biotechnologie der KFA Jülich, D-5170 Jülich (F.R.G.).

phate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase, EC 1.1.1.44). Previous studies have indicated that methanol utilizing yeasts of *Candida* species assimilate methanol by a sugar phosphate pathway similar in concept to the ribulose monophosphate cycle. Experiments with whole yeast cells and cell-free extracts demonstrated that fructose-phosphate is one the primary intermediates in the assimilation of methanol in these organisms [4,9—11]. The presence of glucose-6-phosphate isomerase (EC 5.3.1.9), glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in *Candida boidinii* [4] indicates that formaldehyde could also be oxidized to CO_2 by a cyclic pathway similar to the dissimilatory ribulose monophosphate cycle in some methylotrophic bacteria. Since in this yeast formaldehyde and formate dehydrogenases are inducibly formed during growth on methanol [4] this means that there are two pathways for the catabolism of methanol to CO_2. Therefore, we were interested in the regulation of the cyclic pathway for the oxidation of formaldehyde to CO_2. It has been demonstrated in other organisms that glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase play an important role in the regulation of the oxidative pentose phosphate cycle. We have purified these enzymes from *Candida boidinii* and studied their kinetic and regulatory properties.

Materials and Methods

Chemicals

Blue Sepharose CL-6B and 2′,5′-ADP-Sepharose 4B were purchased from Pharmacia Fine Chemicals (Freiburg). For concentration and dialyzation of enzyme solutions, a Diaflo® ultrafilter-PM 10 and Minicon®-B 15, obtained from Amicon (Witten) were used. D-Ribulose 5-phosphate (Na-salt) was bought from Sigma-Chemical (Munich). All the other substrates, nucleosides phosphates and auxiliary enzymes were products of Boehringer Mannheim (Mannheim, G.F.R.).

Organism and cultivation

Candida boidinii (ATCC 32195) which is able to grow on methanol as a sole carbon and energy source [12] was used throughout this work. The cultivation on methanol (1% v/v) or glucose (1% w/v) was carried out in a 80 l fermenter and cells were harvested at the end of exponential growth phase by continuous flow centrifuge and stored at —25°C until required. In order to determine various enzyme activities during growth, *C. boidinii* was grown in 500-ml Erlenmeyer flasks containing 100 ml basal medium with 1% of carbon source as described previously [12]. Cell free extracts were prepared using an X-press® or a Dyno-Mill®.

Enzyme assays

Enzyme activities were assayed spectrophotometrically at 340 nm using a Zeiss PMQII photometer equipped with a lin-log converter and recorder. The assay mixture of the glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was composed of 33 mM glycylglycine buffer (pH 8.0), 2 mM $MgCl_2$, 4 mM glucose

6-phosphate, 0.1 mM NADP, and limiting amounts of enzyme. The 6-phosphogluconate dehydrogenase (EC 1.1.1.44) was measured in a mixture of 33 mM glycylglycine buffer (pH 8.0), 2 mM $MgCl_2$, 0.1 mM 6-phosphogluconate, 0.1 mM NADP and limiting amounts of enzyme. All mechanistic studies for both enzymes were performed in 33 mM glycylglycine buffer (pH 7.0) without $MgCl_2$ at 30°C, unless otherwise stated.

Alcohol oxidase (EC 1.1.3.13), formaldehyde dehydrogenase (EC 1.2.1.1) and formate dehydrogenase (EC 1.2.1.2) were assayed as described previously [13,14]. Hexokinase (EC 2.7.1.1) was assayed by the method of Bergmeyer et al. [15]. One enzyme unit is defined as the amount of enzyme producing 1 μmol of product per min.

The nomenclature and equations used for analysis of the data are those of Cleland [16—18].

Analysis

Protein was estimated by the method of Lowry et al. [19] using bovine serum albumin as a standard. Analytical gel electrophoresis was carried out according to Jovin et al. [20]. Activity staining for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase was done by the method of Lessie and Vander Wyk [21]. The molecular weight of the subunit(s) was determined by polyacrylamide gel electrophoresis in 1% sodium dodecyl sulfate (SDS) according to Shapiro et al. [22], using pepsin, ovalbumin and bovine serum albumin as marker proteins. The molecular weights of the native enzymes were determined by gel filtration on Sephadex G-200 according to the method of Andrews [23] using β-galactosidase, lactate dehydrogenase, alkaline phosphatase and ovalbumin as marker proteins.

Purification of the enzymes

All procedures were carried out at 2—6°C and with 0.05% mercaptoethanol and 10% glycerol in all buffers to maintain the enzyme activities.

Step 1. Crude extract. The frozen cells (600 g wet weight) were softened and suspended in 1500 ml 10 mM potassium phosphate buffer (pH 7.5) and then disrupted in a Dyno-Mill® disintegrator, as described previously [14]. Cell debris was removed by centrifugation at 25 000 × *g* for 30 min.

Step 2. Protamine sulfate. To the crude extract obtained by step 1, a 2% protamine sulfate solution was added to give a ratio of protein to protamine sulfate of 1 : 0.3. The resulting precipitate was removed by centrifugation at 25 000 × *g* for 20 min.

Step 3. Phosphocellulose. The clear supernatant of the previous step was applied to a phosphocellulose column (5 × 80 cm) equilibrated with 250 mM acetate/NaOH buffer (pH 6.0). The chromatography was carried out according to Domark and Chilla [24] and Rippa and Signorini [25]. 6-Phosphogluconate dehydrogenase was eluted with the equilibration buffer and glucose-6-phosphate dehydrogenase was then eluted with 200 mM potassium phosphate buffer (pH 6.8). The enzymes were located in the eluate and concentrated by ultrafiltration.

Purification of glucose-6-phosphate dehydrogenase

Step 4. DEAE-cellulose. The concentration glucose-6-phosphate dehydro-

genase solution obtained in step 3 was dialyzed against 50 mM potassium phosphate buffer (pH 7.5) and applied to a DEAE-cellulose column (5 × 30 cm) equilibrated with the same buffer. The column was washed with starting buffer and elution was carried out by a linear gradient between 5 l 50 mM potassium phosphate buffer, pH 7.5 and 5 l of the same buffer containing 100 mM NaCl. The eluate was collected in fractions of 15 ml and those exhibiting the highest enzyme activities were pooled and concentrated by ultrafiltration.

Step 5. Sephadex G-200. The concentrated enzyme from the previous step was applied to a Sephadex G-200 column (2.5 × 80 cm) and eluted with 50 mM potassium phosphate buffer (pH 7.5). The active fractions were combined, concentrated and dialyzed against 10 mM Tris-HCl buffer (pH 7.5).

Step 6. Blue Sepharose CL-6 B. A quarter of this enzyme solution was placed on a column (2.6 × 14 cm) packed with Blue-Sepharose CL-6 B which had been equilibrated with 10 mM Tris-HCl buffer (pH 7.5). After the column was washed with 700 ml of the same buffer, glucose-6-phosphate dehydrogenase was eluted with this buffer containing 50 mM NADP. The flow rate was 0.45 ml/min. The remaining enzyme solution was treated in the same manner. The active fractions were combined, concentrated and dialyzed against 100 mM Tris-HCl buffer (pH 7.5).

Step 7. 2',5'-ADP-Sepharose 4B. The dialyzed enzyme solution was placed on a 2',5'-ADP-Sepharose 4B column (2.6 × 14 cm) which had been equilibrated with 100 mM Tris-HCl buffer (pH 7.5) according to Brodelius et al. [26]. After the column was washed with 300 ml equilibration buffer containing 0.1 mM NADP, the enzyme was eluted with the same buffer +0.5 mM NADP. After being concentrated, the enzyme was dialyzed against 50 mM potassium phosphate buffer (pH 7.5) containing 20% glycerol and 0.05% 2-mercaptoethanol and stored at 5°C.

Purification of 6-phosphogluconate dehydrogenase

Step 4'. DEAE-cellulose. The concentrated 6-phosphogluconate dehydrogenase solution obtained from the phosphocellulose column chromatography (Step 3) was dialyzed against 50 mM potassium phosphate buffer (pH 7.5) and applied to a column (5 × 30 cm) equilibrated with the same buffer. The column was washed and elution was carried out by a linear gradient between 5 l of 50 mM potassium phosphate buffer (pH 7.5) and 5 l of the same buffer containing 100 mM NaCl. The fractions with high activity were combined, concentrated and dialyzed against 10 mM maleate/NaOH buffer (pH 6.0).

Step 5'. CM-Sephadex. The enzyme solution of the previous step was placed on a column (5 × 30 cm) packed with CM Sephadex C 50 equilibrated with 10 mM maleate/NaOH buffer (pH 6.0). The enzyme was eluted with a linear gradient from 2 l 10 mM maleate/NaOH buffer (pH 6.0) and 2 l of the same buffer containing 500 mM NaCl. The enzyme activity was located in the effluent and concentrated by ultrafiltration.

Step 6'. 2',5'-ADP-Sepharose 4B. 6-Phosphogluconate dehydrogenase obtained at step 5' was dialyzed against 100 mM Tris-HCl buffer (pH 7.5) and applied to a 2',5'-ADP-Sepharose column (2.6 × 14 cm) which had been equilibrated with the same buffer. After the column was washed with 100 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM NADP, the enzyme was eluted

with a linear gradient between 250 ml starting buffer with 0.1 mM NADP and 200 ml of the same buffer with 1 mM NADP. After the enzyme was concentrated by ultrafiltration and dialyzed against 50 mM potassium phosphate buffer (pH 7.5), 20% glycerol, 0.05% 2-mercaptoethanol; it was stored at 5°C.

Results

A. Glucose-6-phosphate dehydrogenase

Purification. Using the purification procedures described in Materials and Methods, the enzyme was purified 1714-fold from the cell-free extract of methanol-grown *C. boidinii* (Table I). The purified enzyme gave one single protein band on polyacrylamide gel electrophoresis that coincided with the glucose-6-phosphate dehydrogenase activity. Isoenzymes and multimolecular forms of the enzyme, which are common in mammalian systems [30] were not found as indicated by the fact that only one band of activity was found by polyacrylamide gel electrophoresis of crude or purified protein.

Molecular weight and subunit structure. The molecular weight of the native enzyme was determined by gel filtration with Sephadex G-200 to be 118 000. When the enzyme was subjected to gel electrophoresis in the presence of sodium dodecyl sulfate only one protein band was observed. The molecular weight was estimated to be 61 000, half the value found by gel filtration. These results demonstrate that the glucose-6-phosphate dehydrogenase from *C. boidinii* is composed of two subunits of equal size, which are probably identical.

General properties. The enzyme exhibits activity exclusively with $NADP^+$ as electron acceptor. NAD^+ could not replace $NADP^+$ in a concentration up to 3 mM. Different sugar phosphates were examined for their ability to replace glucose 6-phosphate in the standard assay system. However, only glucose 6-phosphate supported the dehydrogenase reaction. The maximal enzymatic activity for glucose-6-phosphate dehydrogenase occurred at pH 8.5—9.0 at 55°C. The enzyme was stable in the pH range of 6.0—10.5 at 5°C for 48 h. It was stimulated by the addition of up to 20 mM $MgCl_2$.

Initial velocity. Steady state initial velocity studies on the enzyme were

TABLE I

SUMMARY OF PURIFICATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM METHANOL-GROWN *CANDIDA BOIDINII*

	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Recovery (%)	Purification
Crude extract	9050	30 066	0.301	100	1
Protamine sulfate	8356	16 720	0.500	92.3	1.7
Phosphocellulose	6300	1 016	6.20	69.6	20.6
DEAE-cellulose	4720	134	35.2	52.1	117
Sephadex G-200	4348	24	181	48.0	601
Blue-Sepharose CL-6B	2548	5.2	490	28.1	1628
2′,5′-ADP-Sepharose 4B	2166	4.2	516	23.9	1714

performed with respect to $NADP^+$ at fixed concentrations of glucose 6-phosphate. As shown in Fig. 1, the results are consistent with a sequential mechanism. The Michaelis constants for $NADP^+$ and glucose 6-phosphate were 0.014 and 0.86 mM, respectively. The dissociation constant for $NADP^+$ was 0.025 mM.

Enzyme inhibition. Product inhibition studies indicated that NADPH is a competitive inhibitor with respect to $NADP^+$ and a noncompetitive inhibitor with respect to glucose 6-phosphate at non-saturating levels of $NADP^+$ (Table II). The results of initial velocity studies and the NADPH inhibition patterns are consistent with an ordered Bi-Bi mechanism in which $NADP^+$ is bound first to the enzyme and NADPH released last [31—34].

All of the nucleoside phosphates tested inhibited glucose-6-phosphate dehydrogenase (Table III). Each nucleoside triphosphate inhibited the enzyme more strongly than the corresponding nucleoside monophosphate. The inhibition by nucleoside triphosphates was influenced significantly by the pH of the assay system and was much more pronounced at neutral or slightly acidic pH values. Addition of $MgCl_2$ counteracted the ATP inhibition as described for the enzymes from some other yeast strains [30,35]. From the point of physiological conditions, i.e. neutral or slightly acidic and lower $MgCl_2$ concentration, these phenomena are thought to be of significance to the metabolic regulation in methanol-grown cells. ATP inhibition is noncompetitive with respect to both substrates (Table II). ATP may be assumed to combine with E and E· $NADP^+$ forms of the enzyme, in the same manner as reported for the pig liver enzyme [34].

The effects of various sugar phosphates, glucose 1-phosphate, fructose 1,6-biphosphate, galactose 6-phosphate, 3-deoxyglucose 6-phosphate, phospho*enol*-pyruvate and glyceraldehyde 3-phosphate on the enzyme activity were tested in concentrations up to 3 mM. Among the sugar phosphates, glyceraldehyde

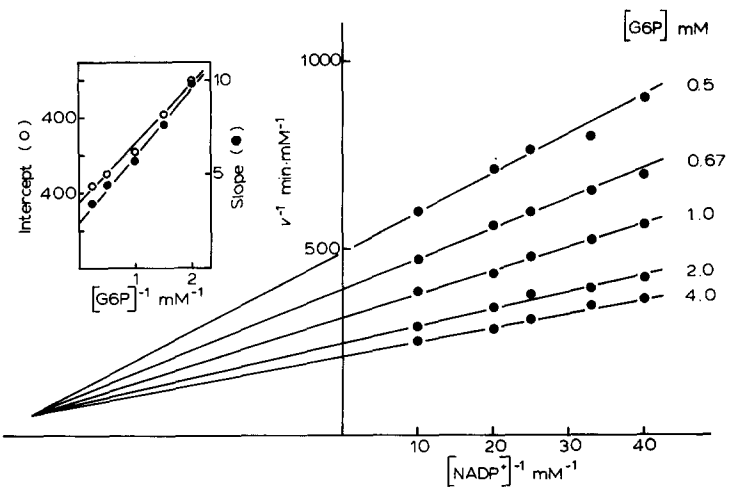

Fig. 1. Double reciprocal plots for the reaction of glucose-6-phosphate dehydrogenase. Initial velocity versus $NADP^+$ concentration at various constant levels of glucose 6-phosphate. Inset: replot of the slopes and intercepts versus the reciprocal concentration of glucose 6-phosphate.

TABLE II

INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Enzyme reaction was carried out at 30°C, using glycylglycine buffer, pH 7.0. K_i was measured by a replot of the slopes or intercepts obtained from the double reciprocal plots versus inhibitor concentrations.

Inhibitor	Substrate varied	Pattern	K_i (mM)	
			Slope	Intercept
NADPH	$NADP^+$	c *	0.018	—
NADPH	Glucose 6-phosphate	nc **	0.028	0.050
ATP	$NADP^+$	nc	1.7	2.6
ATP	Glucose 6-phosphate	nc	0.5	4.5
Glyceraldehyde 3-phosphate	$NADP^+$	nc	0.35	0.45
Glyceraldehyde 3-phosphate	Glucose 6-phosphate	nc	0.25	0.40

* c: competitive.
** nc: noncompetitive.

3-phosphate was the most potent inhibitor, it is a noncompetitive inhibitor with respect to both substrates $NADP^+$ and glucose 6-phosphate (Table II). No inhibition with NADH was observed in the concentrations up to 0.5 mM.

B. 6-Phosphogluconate dehydrogenase

Purification. By means of the purification procedure described in Materials and Methods, the enzyme was purified 331-fold from the cell-free extract of methanol-grown *C. boidinii* (Table IV). The purified enzyme exhibited a single band on polyacrylamide gel electrophoresis. Samples of preparations from different stages of purification, which were subjected to polyacrylamide gel electrophoresis and stained for enzyme activity, demonstrated that no iso-

TABLE III

EFFECT OF NUCLEOSIDE PHOSPHATES ON THE ACTIVITIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE

Glucose-6-phosphate dehydrogenase was measured in a mixture of 33 mM glycylglycine buffer (pH 7.0 or 8.0), 0.1 mM $NADP^+$, 0.5 mM glycose 6-phosphate, 3 mM nucleoside phosphate and 5 mU/ml enzyme. 6-Phosphogluconate dehydrogenase was measured in a mixture of 33 mM glycylglycine buffer (pH 7.0), 0.01 mM $NADP^+$, 0.2 mM 6-phosphogluconate, 1 mM nucleoside phosphate and 5 mU/ml enzyme.

Nucleoside phosphate	Inhibition (%)		
	Glucose-6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase pH 7.0
	pH 8.0	pH 7.0	
AMP	8.0	25.5	2.0
ADP	6.5	41.5	44.1
ATP	0	90.0	70.5
GMP	5.0	26.0	4.1
GTP	0	40.5	12.5
UMP	9.0	24.3	4.2
UTP	0	59.4	42.5
CMP	6.3	20.7	0
CTP	0	60.1	18.0

TABLE IV

SUMMARY OF PURIFICATION OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM METHANOL-GROWN *CANDIDA BOIDINII*

	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Recovery (%) (%)	Purification
Crude extract	1804	15 033	0.120	100	1
Protamine sulfate	1717	8 896	0.193	95.2	1.6
Phosphocellulose	1471	203.2	7.240	81.5	60.3
DEAE-cellulose	894	44.5	20.1	49.5	167
CM-Sephadex	511	14.8	34.5	28.3	288
2′-5′-ADP-Sepharose 4B	318	8.0	39.7	17.6	331

enzymes and multimolecular forms of the enzyme occurred.

Molecular weight. The molecular weight of the native enzyme was determined to be 110 000 by Sephadex G-200 gel filtration. Gel electrophoresis of 6-phosphogluconate dehydrogenase in the presence of sodium dodecyl sulfate revealed a single band with a relative mobility corresponding to a molecular weight of 58 000. This is approximately half of the value found under native conditions by gel filtration. Therefore it appears that the enzyme is a dimer, composed of two probably identical subunits.

General properties. The enzyme is specific for $NADP^+$, NAD^+ can not be used as an electron acceptor. The maximal enzymatic activity was observed at pH 8.0—8.5 and at 35—40°C, it was enhanced by the addition of $MgCl_2$ in concentrations up to 20 mM.

Initial velocity. Steady state initial velocity studies on the enzyme reaction were performed with respect to $NADP^+$ at fixed concentrations of 6-phosphogluconate. Double reciprocal plots indicated a sequential mechanism for the enzyme reaction. The Michaelis constants for $NADP^+$ and 6-phosphogluconate are 0.013 and 0.011 mM, respectively. The dissociation constant for $NADP^+$ is 0.011 mM.

Inhibition of enzyme. Inhibition patterns of the 6-phosphogluconate dehydrogenase are summarized in Table IV. Product inhibition studies were conducted with NADPH and ribulose 5-phosphate. NADPH was found to be a competitive inhibitor with respect to $NADP^+$ and a noncompetitive inhibitor with respect to 6-phosphogluconate at non-saturating levels of $NADP^+$. Ribulose 5-phosphate is an noncompetitive inhibitor with respect to both $NADP^+$ and 6-phosphogluconate. The results of the initial velocity and product inhibition studies described above agree well with the results reported for the pig liver enzyme [36], where the reaction mechanism is ordered Bi-Ter, with $NADP^+$ bound first, CO_2 released first and NADPH released last.

All of the nucleoside triphosphates tested and ADP inhibited 6-phosphogluconate dehydrogenase while nucleoside monophosphates inhibited very weakly (Table III). Among the nucleoside triphosphates, ATP is the most potent inhibitor. The extent of ATP inhibition is dependent on the pH of the assay system. The inhibition constant with respect to $NADP^+$ was found to be 0.56 mM at pH 7.0, 0.25 mM at pH 6.5 and 0.03 mM at pH 6.0. Furthermore $MgCl_2$ counteracts the ATP inhibition in the same way as in the case of the

TABLE V

INHIBITION OF 6-PHOSPHOGLUCONATE DEHYDROGENASE

Enzyme reaction was carried out at 30°C, using glycylglycine buffer (pH 7.0). K_i was measured by a replot of the slopes or intercepts obtained from the double reciprocal plots versus inhibitor concentrations.

Inhibitor	Substrate varied	Pattern	K_i (mM)	
			Slope	Intercept
NADPH	$NADP^+$	c *	0.012	—
NADPH	6-phosphogluconate	nc **	0.025	0.046
Ribulose 5-phosphate	$NADP^+$	un ***	—	2.6
Ribulose 5-phosphate	6-phosphogluconate	un	—	1.8
ATP	$NADP^+$	c	0.56	—
ATP	6-phosphogluconate	nc	0.70	0.44
Fructose 1,6-bis-phosphate	$NADP^+$	nc	0.60	0.90
Fructose 1,6-bis-phosphate	6-phosphogluconate	c	0.08	—

* c: competitive.
** nc: noncompetitive.
*** un: uncompetitive.

glucose-6-phosphate dehydrogenase. The kinetics of inhibition by nucleoside triphosphates have been studied on 6-phosphogluconate dehydrogenase from sheep liver [37], where nucleoside triphosphates are competitive inhibitors with respect to both substrates. In contrast to this result, ATP is a competitive inhibitor with respect to $NADP^+$ and a noncompetitive inhibitor with respect to 6-phosphogluconate for the enzyme from *C. boidinii*. In this yeast 6-phosphogluconate dehydrogenase is more susceptible to ATP inhibition than glucose-6-phosphate dehydrogenase.

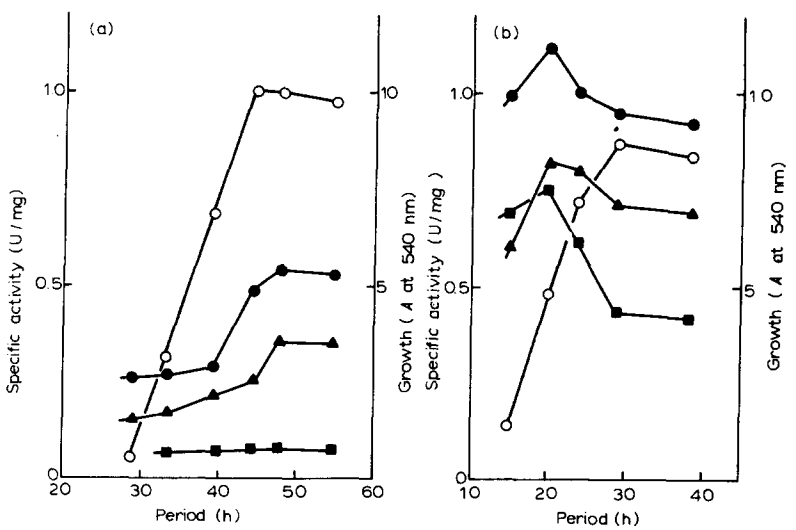

Fig. 2. Growth (○———○) and changes in specific activities of glucose-6-phosphate dehydrogenase (●———●), 6-phosphogluconate dehydrogenase (▲———▲), and hexokinase (■———■) in *Candida boidinii* during the growth on methanol (a) and glucose (b).

Among different sugar phosphates tested fructose 1,6-bisphosphate inhibited the enzyme. The inhibition is competitive with respect to 6-phosphogluconate and noncompetitive with respect to $NADP^+$. No inhibition was observed by NADH in concentration up to 0.5 mM.

Enzyme activities during growth on methanol and glucose

In the methanol-grown cells, activities of glucose-6-phosphate and 6-phosphogluconate dehydrogenases increased as the growth proceeded and reached their maximum in the stationary phase, while hexokinase activity, which is thought to participate only in the glucose metabolism, was very low during the whole growth phase (Fig. 2). On the other hand glucose-grown cells contained considerably higher activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and hexokinase in the exponential phase.

Discussion

Methanol-utilizing bacteria so far studied can be divided into three groups on the basis of their pathways for the oxidation of formaldehyde to CO_2 [7]. One group of bacteria which utilizes the serine pathway for the assimilation of C_1-compounds oxidizes formaldehyde via formate to CO_2. The second group assimilates methanol via the ribulose monophosphate cycle and oxidizes formaldehyde via the dissimilatory ribulose monophosphate cycle. The third group of methylotrophic bacteria assimilates C_1-compounds also via the ribulose monophosphate cycle, but oxidizes formaldehyde via formate and via the cyclic pathway. Published results [4] and experimental data presented in this work indicate that the methanol utilizing yeast *C. boidinii* contains the enzymes for oxidizing formaldehyde via both pathways and therefore belongs to the third group. However, in this yeast the specific activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are only half in methanol-grown cells in comparison with cells grown on glucose medium. During growth on methanol this substrate seems primarily catabolized by the inducibly formed enzymes, formaldehyde dehydrogenase and formate dehydrogenase. Thus the cyclic oxidation of the C_1-compound might serve mainly as a source of NADPH for biosynthetic purposes as in *Methylococcus capsulatus*, and *Pseudomonas methanica* [39]. Since in *C. boidinii* only a very low specific activity of 2-oxoglutarate dehydrogenase was found [4], glucose may be metabolized primarily via the oxidative pentose phosphate cycle as shown for the oleaginous yeast, *Candida* 107 [38]. Assuming that there is no compartmentalization of either substrates or these enzymes in *C. boidinii*, the levels of 6-phosphogluconate and $NADP^+$ appear to be high enough to allow maximum activity of 6-phosphogluconate dehydrogenase while the level of glucose 6-phosphate seems too low to allow maximal activity of glucose-6-phosphate dehydrogenase. The intracellular levels of NADPH and ATP are in the range of the K_i values for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Thus the activities of these enzymes may be regulated by the intracellular levels of these metabolites. Since both enzymes are not inhibited by NADH in constrast to the $NADP^+$-dependent glucose-6-phosphate dehydrogenase from *Escherichia coli* [40] there is no direct interaction between the

oxidation of formaldehyde via formate to CO_2 and the dissimilatory ribulose monophosphate cycle.

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