THE GLUCOSE DEHYDROGENASE ACTIVITY OF THE NAD-LINKED GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM *ACETOBACTER XYLINUM*

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1. Introduction

The pentose cycle is the major pathway for the oxidative dissimilation of carbohydrates in *Acetobacter xylinum* [1–3]. Entry into the cycle takes place by means of specific hexokinases and in several other ways in which the primary conversion of glucose to gluconate plays a part [2, 4]. *A. xylinum* was found to possess two distinct glucose 6-P dehydrogenases: an NAD-specific enzyme, which is optimally active at pH 5.6 and is inhibited by ATP, and an NADP-specific enzyme which is optimally active at pH 8.2 and is insensitive to ATP [5]. We subsequently observed that preparations of the NAD-linked enzyme also catalyze the NAD-dependent dehydrogenation of glucose. Evidence for the involvement of a single enzyme in the catalysis of these two reactions is presented in this communication.

2. Materials and methods

Cells of *A. xylinum* were grown on succinate, fructose or glucose and harvested after 24 hr as previously described [4, 6].

2.1. *Purification of the enzyme*

Crude extracts of succinate-grown cells were prepared, centrifuged and chromatographed on DEAE-cellulose as previously described [5]. Fractions rich in NAD-linked glucose dehydrogenase (GDH) and glucose 6-phosphate dehydrogenase (G6PDH) activities were pooled and further fractionated by the addition of solid $(NH_4)_2SO_4$. The protein which precipitated between 25–50% saturation was dissolved in 5 mM Tris–H_2SO_4 (pH 7.4), and then reprecipitated with $(NH_4)_2SO_4$. The fraction precipitating between 30–50% saturation was dissolved in a small volume of 0.05 M Tris–HCl (pH 7.4) buffer and applied to a Sephadex G-200 column (1.2 × 70 cm) which had been pre-equilibrated and was then eluted with the same buffer. Fractions of 1 ml were collected. Fractions 16–18 of the single activity peak contained about 20% of the GDH and G6PDH activities of the crude extract, and possessed a constant specific activity corresponding to a 315-fold overall purification. They were free from NADP-linked G6PDH, 6-P-gluconate dehydrogenase, glucokinase or phosphoglucomutase activities. The purification procedure is summarized in table 1.

G6PDH activiy was measured as previously described [5]. GDH activiy was measured similarly but with 15 mM glucose. In most experiments an equilibrated D-glucose solution (specific rotation +52°) was used. Polyacrylamide gel electrophoresis was carried out by the method of Davis [7]. Protein was stained with Amido Black and destained with acetic acid. In order to stain for G6PDH and GDH activity, gels were sliced lengthwise and then incubated for 30 min at 30°C in standard assay mixtures containing 0.2 mg phenazine methosulfate and 0.1 mg nitro blue tetrazolium per ml. Products and reactants of the two dehydrogenase reactions were determined as previously described [5, 4].

3. Results

Cell-free extracts of *A. xylinum* catalyzed the reduction of NAD in the presence of glucose 6-P or

Table 1
Purification pf glucose-6-P dehydrogenase.

Fraction	Volume (ml)	Total protein (mg)	Total activity (units)*	Specific activity units per mg protein	Recovery %	Activity ratio (G6PDH:GDH)
Crude extract	55	1750	37	0.02	100	0.90
High speed supernatant	55	835	35	0.04	95	0.88
DEAE-cellulose combined fractions	135	67	28	0.32	76	0.91
1st $(NH_4)_2SO_4$ (25–50%)	135	67	23	0.34	62	0.92
2nd $(NH_4)_2SO_4$ (30–50%)	2	7	18	2.58	51	0.87
Sephadex G-200 combined fractions	3	1	7.6	7.60	20	0.92

* Micromoles of NADH produced per min from glucose-6-P under standard assay conditions.

glucose. The specific activities of such extracts with glucose and glucose 6-P as substrates, were approximately equal and were virtually the same whether the cells were grown on glucose, fructose or succinate. During enzyme purification, the activity ratio G6PDH:GDH (table 1) remained essentially constant at 0.9. Polyacrylamide gel electrophoresis of purified preparations did not separate the two activities. Although several bands were noted upon protein staining, GDH and G6PDH activities always migrated as a single band. Thermal inactivation at 62°C also failed to distinguish between the two activities, as the rate of inactivation of GDH activiy paralleled that of G6PDH activity.

The depdendence of GDH activity on pH was identical with that of G6PDH activity [5], with a sharp activity maximum at pH 5.6 and little or no activity above pH 6.2 or below pH 4.5. To test the effect of the concentration of each substrate on enzyme activity, the appropriate assay methods were modified so that the non-variable component of the reaction was present at a saturation concentration and the concentration of the substrate under examination was varied. Kinetic curves for GDH activity are identical in type with those previously obtained for G6PDH [5] and are therefore not presented in this communication. As calculated from Lineweaver–Burk plots, the K_m values for NAD in both dehydrogenase reactions were identical (0.08 mM), and the values for the two substrates were similar (2.5 mM for glucose-6-P and 2.3 mM for glucose). It was also found that the K_m for NAD was not affected by the concentration of glucose or glucose-6-P and that, similarly, the K_m for either of the two sugar substrates was independent of NAD concentration.

The final products of the G6PDH and GDH reactions were identified as stoichiometric quantities of NADH and respectively, 6-P-gluconate and gluconate. No oxidation of the reduced coenzyme was demonstrable in the presence of gluconate or 6-P-gluconate. NADH oxidation occurred, however, with δ-gluconolactone as substrate. The K_m values for the substates of this reaction were calculated as 0.8 mM for the lactone and 0.05 mM for NADH. The rate of NAD reduction in the presence of a saturating concentration of glucose-6-P was not increased by the addition of glucose. On the other hand, the rate of 6-P-gluconate formation decreased in the presence of glucose. As NADH inhibits both dehydrogenase activities, these assays were carried out in the presence of an NAD-regenerating system composed of lactic dehydrogenase and pyruvate. The inhibitory effect of glucose was competitive with respect to glucose-6-P and a K_I value of 2.3 mM was calculated from Dixon and Lineweaver–Burk plots. This value is the same as K_m for glucose as substrate. ATP inhibited both dehydrogenase activities. The inhibition, which followed classical kinetic patterns was competitive with respect to glucose-6-P and glucose with an identical K_I value of

1.3 mM, and non-competitive with respect to NAD. Both enzymic activities were competitively inhibited by NADH with respect to NAD (K_I = 0.05 mM and 0.035 mM for the GDH and G6PDH reactions, respectively) and non-competitive with respect to glucose-6-P and glucose. The reaction with both substrates was similarly inhibited by *p*-hydroxymercuribenzoate, 50% inhibition being obtained at 0.04 mM inhibitor concentration.

At concentrations up to 2 mM, NADP was not reduced in the presence of either glucose or glucose-6-P. The initial rate of NAD reduction was about 10 times higher with β-D-glucose than with the α-anomer (the reaction was started by addition of 2 μmoles of each isomer, freshly dissolved in 50 mM Tris–maleate buffer, pH 5.6). The rates obtained using sugar solutions previously incubated for 30 min at 30°C, were not significantly different for the two sugar samples. D-glucose-6-sulfate also served as substrate for NAD reduction with a V_{max} equal to that obtained with glucose and a K_m value of 2.5 mM. Other sugars and sugar derivatives, at concentrations up to 10 mM, did not serve as substrates for NAD reduction and did not affect activity with either glucose or glucose-6-P (when present at K_m concentrations). These compounds are as follows: D-fructose; D(+)mannose; D(−)ribose; D(+)galactose; D(+)xylose; L(−)xylose; D(−)arabinose; L(+)rhamnose; D(+)glucosamine; 2-deoxyD(+)glucose; D-gluconate; D-glucoronate; D-glucose-1-P; D-fructose-6-P; D-fructose-1.6 di-P; D-ribose-5-P; D-gluconate-6-P and D-mannose-6-P. Both dehydrogenase activities were not significantly affected by the presence of bicarbonate, sulfate or phosphate anions at concentrations up to 50 mM. These measurements were made in 50 mM Tris–maleate pH 5.6 at K_m concentrations of glucose or glucose-6-P [cf. ref. 8].

4. Discussion

A single enzyme from *A. xylinum* appears to be responsible for the NAD-dependent dehydrogenation of both glucoe-6-P and glucose. The ratio of activities with the two substrates remains constant throughout an extensive purification. Both catalytic activities are similarly affected by various reagents (ATP, NADH, *p*-chloromercuribenzoate) and conditions (pH, heat). On gel electrophoresis, both activities always migrated as a single band. Finally the dehydrogenation of the two substrates was competitive rather than additive, K_I for inhibition by glucose being equal to its K_m as substrate.

Glucose dehydrogenase activity has been observed with purified glucose-6-P dehydrogenase preparations from various sources [8–11]. The enzyme from *A. xylinum* appears to be unique in its high GDH/G6PDH activity ratio (0.9) and relatively low K_m for glucose [cf. ref. 8–10, 12]. This makes both glucose and glucose-6-P potential physiological substrates for the enzyme. In this connection it should be noted that glucose oxidation by the enzyme is small, compared to that catalyzed by the particulate pyridine nucleotide-independent glucose dehydrogenase present in these cells [2], and at best could account for no more than 10% of the *in vivo* rate of glucose oxidation to gluconate [13]. On the other hand, the activity of the enzyme towards glucose-6-P is essential for the operation of the pentose cycle, especially in the utilization of sugars such as fructose, the metabolism of which does not involve gluconate formation [2, 13].

The inhibitory effect of glucose on the G6PDH reaction could account for the accumulation in the medium of significant amounts of glucose-6-P during the utilization of glucose by resting cells *A. xylinum* [our unpublished experiments]. It could also explain the greater suppression by glucose of the conversion of fructose carbon to CO_2 than to cellulose, reported previously [4].

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