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Glucose dehydrogenase from the thermoacidophilic archaebacterium *Sulfolobus solfataricus*

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Glucose dehydrogenase has been purified to homogeneity from cell extracts of the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus*. The enzyme utilizes both NAD^+ and $NADP^+$ as coenzyme and catalyses the oxidation of several monosaccharides to the corresponding glyconic acid. Substrate specificity and oxidation rate depend on the coenzyme present; when NAD^+ is used, the enzyme binds and oxidizes specifically sugars presenting equatorial orientation of hydroxy groups at C-2, C-3 and C-4. The M_r of the native enzyme is 124000 and decreases to about 60000 in the presence of 6 M-guanidinium chloride and to about 30000 in the presence of 5% (w/v) SDS. The enzyme shows maximal activity at pH 9, 77 °C and 20 mM-Mg^{2+}, -Mn^{2+} or -Ca^{2+} and is fairly stable in the presence of chaotropic agents and water-miscible organic solvents such as methanol or acetone.

INTRODUCTION

Sulfolobus solfataricus, previously named *Caldariella acidofila* (De Rosa *et al.*, 1975; Zillig *et al.*, 1980), is an extreme thermoacidophilic archaebacterium able to grow on simple media containing a mono- or a di-saccharide (glucose, galactose, allose, xylose, ribose, lactose, maltose, trehalose) as the sole source of carbon atoms (De Rosa *et al.*, 1984). In a series of studies on the metabolic conversions of carbohydrates occurring in *S. solfataricus*, we have isolated a β-galactosidase activity (Buonocore *et al.*, 1980) and investigated glucose and galactose metabolism. As both glycolysis and hexose monophosphate shunt are absent in this micro-organism, glucose degradation takes place through a modified Entner–Doudoroff pathway showing the unique feature of producing two C_3 fragments (pyruvate and glyceraldehyde) in the absence of any phosphorylation step (De Rosa *et al.*, 1984). Galactose also is converted into the two C_3 products by this route, and the same enzymic proteins appear to be involved in the breakdown of both hexoses (V. Buonocore, M. De Rosa, A. De Simone, A. Gambacorta & P. Giardina, unpublished work).

The first reaction of the degradative pattern is catalysed by a pyridine-dependent dehydrogenase that oxidizes D-glucose actively; however, by using either NAD^+ or $NADP^+$ as electron acceptor, this enzyme is able to oxidize, even though at lower rates, all the monosaccharides utilized by the micro-organism for its growth. Thus the glucose dehydrogenase may represent a key enzyme in the physiology of the micro-organism, enabling it to convert a number of carbohydrates into easily utilizable metabolic intermediates.

Nicotinamide-nucleotide-dependent glucose dehydrogenase (EC 1.1.1.47) has been isolated from a variety of sources, including mammalian liver (Campbell *et al.*, 1982), a blue–green alga (Pulich *et al.*, 1976), a number of bacteria such as *Pseudomonas*, *Klebsiella*, *Serratia* and other oxidative species (Matsushita *et al.*, 1980), an alkalophilic *Corynebacterium* (Kobayashi & Horikoshi, 1980) and many members of the family Bacillaceae, in which the dehydrogenase is produced in the early stage of sporogenesis (Vasantha *et al.*, 1983). The only report concerning the presence of glucose dehydrogenase in archaebacteria is by Tomlinson *et al.* (1974), who observed such an activity in homogenates of the extreme halophile *Halobacterium saccharovorum*.

In the present study we have purified glucose dehydrogenase from *S. solfataricus* to homogeneity and investigated some molecular and kinetic properties of the protein and its stability to a number of chemico-physical parameters.

EXPERIMENTAL

Materials

Sulfolobus solfataricus strain MT-4 was grown in standard medium as already described (De Rosa *et al.*, 1984). SP-Sephadex C-50 and phenyl–Sepharose CL-4B were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Affi-Gel Blue (100–200 mesh) and Protein Assay were the products of Bio-Rad Laboratories (Richmond, CA, U.S.A.). The TSK-G 3000 SW (Blue) column was obtained by LKB (Bromma, Sweden). Standard proteins used in the M_r studies were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All the other reagents were of analytical grade.

Enzyme purification

Unless otherwise stated, the purification process was carried out at 4 °C and the buffers used contained 20 mM-$MgCl_2$ and 20% (v/v) ethylene glycol. Wet cells (30 g) were ground with 50 g of glass beads and 50 ml of 10 mM-triethanolamine/HCl buffer, pH 7.0, in the stainless-steel chamber of a Sorvall Omni-Mixer for 5 min at

Abbreviations used: H_4furan, tetrahydrofuran; SP-Sephadex, sulphopropyl-Sephadex.

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half speed and for 10 min at full speed; the mixture was then centrifuged at 35000 *g* for 50 min. To the supernatant solution was added solid $(NH_4)_2SO_4$ up to 80% saturation to eliminate most of the lipid fraction; after centrifugation at 10000 *g* for 30 min, the precipitate was dissolved and extensively dialysed against the triethanolamine/HCl buffer. The homogenate (about 2 g of protein) was loaded on to a column (2.8 cm × 40 cm) of SP-Sephadex C-50 equilibrated with the triethanolamine/HCl buffer. After the column had been washed with the equilibration buffer to remove unadsorbed material, elution was carried out with a linear salt gradient of 1 litre of the triethanolamine/HCl buffer containing 0–200 mM-NaCl. Fractions (5 ml each) were collected at a flow rate of 20 ml/h. Those with glucose dehydrogenase activity, which were eluted at about 140 mM-NaCl, were pooled, concentrated by ultrafiltration on an Amicon PM-10 membrane and loaded on a phenyl–Sepharose column (1.5 cm × 30 cm) equilibrated with 50 mM-triethanolamine/HCl buffer, pH 7.0. After washing with the same buffer (containing 40% ethylene glycol) the enzyme was eluted with 50% ethylene glycol. Active fractions, collected at a flow rate of 12 ml/h, were pooled, concentrated by ultrafiltration and loaded on an Affi-Gel Blue column (1 cm × 20 cm) equilibrated with 20 mM-triethanolamine/HCl buffer, pH 7.0. Unadsorbed material was washed with the equilibration buffer, then the enzyme was eluted with the same buffer containing 50 mM-D-xylose and 0.5 mM-$NADP^+$ at a flow rate of 8 ml/h. Active fractions were pooled and washed with the triethanolamine/HCl buffer on an ultrafiltration membrane to remove the specific eluents.

Activity assay and protein determination

The dehydrogenase activity was measured at 70 °C by monitoring for 3 min the $NAD(P)^+$ reduction at 340 nm in a reaction mixture (1 ml) containing 100 mM-triethanolamine/HCl buffer, 20 mM-$MgCl_2$, either 5 mM-NAD^+ or 0.4 mM-$NADP^+$ and substrate; the pH value of the buffer was 9.0 as measured at room temperature; however, the true value at 70 °C was lower by about one pH unit. Optimal concentrations of each substrate in the assay were: glucose (NAD^+), 40 mM; glucose ($NADP^+$), 2 mM; galactose ($NADP^+$), 50 mM; xylose ($NADP^+$), 25 mM. One unit of dehydrogenase activity was the amount of the enzyme producing 1 μmol of NAD(P)H/min under the conditions of the assay. The effect of organic solvents or dissociating agents on the activity was studied by preincubating at room temperature for the reported time the enzyme and each compound, then assaying the residual activity at 70 °C, under the usual assay conditions, after a 1000-fold dilution.

Protein concentration was determined by using the Bio-Rad Protein Assay Kit, following the manufacturer's instructions and using bovine serum albumin as standard.

Gel electrophoresis

Polyacrylamide-gel electrophoresis was carried out in 50 mM-Tris/383 mM-glycine buffer, pH 8.5, with 5% (w/v) acrylamide as described by Davis (1964). Glucose dehydrogenase bands were specifically detected on the gels by incubating at 70 °C, at the end of the electrophoretic run, each gel with the assay mixture containing 0.1 mg of *p*-Nitrotetrazolium Blue and 0.01 mg of phenazine methosulphate/ml.

M_r studies

Gel filtration under native conditions was carried out by using a fast-protein-liquid-chromatography (Pharmacia Fine Chemicals) apparatus on a TSK gel 3000 SW column (7.5 mm × 600 mm) equilibrated and eluted with 50 mM-triethanolamine/HCl buffer (pH 7.0)/20 mM-$MgCl_2$; the flow rate was 6 ml/h. Reference proteins were aldolase, aspartate aminotransferase, ovalbumin and cytochrome *c* with M_r values 158000, 96000, 43000 and 11700 respectively. M_r studies under dissociating conditions were performed with the same apparatus by using as eluent 6 M-guanidinium chloride or 0.1% SDS in the triethanolamine/HCl buffer; reference proteins were bovine serum albumin (M_r 68000), ovalbumin, chymotrypsinogen A (M_r 25700) and cytochrome *c*. Before loading, protein samples were either made 6 M with respect to guanidinium chloride or incubated for 3 min at 100 °C in 1% or 5% (w/v) SDS. Sedimentation-equilibrium runs were performed in a Beckman L870 analytical ultracentrifuge equipped with a u.v. scanner at 3700 *g* and 10 °C in 50 mM-triethanolamine/HCl buffer, pH 7.0, containing 300 mM-KCl; the protein concentration was 0.24 mg/ml. The partial specific volume used for M_r calculation was 0.73 cm^3/g. Electrophoresis in 0.1% (w/v) SDS with and without 1% (v/v) 2-mercaptoethanol was performed as described by Laemmli (1970) on a polyacrylamide-gel slab. Protein samples were incubated for 3 min at 100 °C with 5% SDS and, in some cases, with 1% 2-mercaptoethanol.

Amino acid analysis

Protein samples (20 μg) were hydrolysed *in vacuo* at 110 °C for 24, 48 and 72 h in 6 M-HCl and analysed with a LKB 4400 amino acid analyser as described by Spackman *et al.* (1958). Appropriate corrections were made for the slow release of some amino acids.

Identification of the reaction product

Glucose dehydrogenase (1.7 units) was incubated for 2 h at 70 °C in a standard assay mixture. After deproteinization by ultrafiltration, the mixture was applied to a thin layer of silica gel; the plate was developed in butan-1-ol/acetic acid/water (3:1:1, by vol.), air-dried, sprayed with 10% (v/v) H_2SO_4 in methanol and 3% (w/v) phosphomolybdic acid in methanol and dried again in an oven at 100 °C. The product was identified by comparison with an authentic standard.

RESULTS

Glucose dehydrogenase purification

A typical purification procedure of glucose dehydrogenase from *Sulfolobus solfataricus* is summarized in Table 1. The last purification step involved specific adsorption of pyridine-dependent enzymes on Affi-Gel Blue matrix and specific desorption of the dehydrogenase with $NADP^+$ and xylose. $NADP^+$ was used at this step as it showed, compared with NAD^+, a higher affinity for the enzyme.

The presence of protein contaminants in enzyme preparations was determined by disc electrophoresis on polyacrylamide gel; purified fractions showed a single

Table 1. Purification of glucose dehydrogenase from *Sulfolobus solfataricus*

Step	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)	Purification (fold)
Extraction and $(NH_4)_2SO_4$ precipitation	3620	2540	0.7	100	1.0
SP-Sephadex	325	1752	5.4	70	7.8
Phenyl-Sepharose	81	1480	18.2	59	26.4
Affi-Gel Blue	1.6	699	437	28	623.0

diffuse protein band with an electrophoretic mobility, compared with Bromophenol Blue, ranging from 0.13 to 0.18. Specific staining with the tetrazolium salt gave a reddish band that was superimposed on the protein band.

Molecular properties

The M_r of purified glucose dehydrogenase in the native form was determined by both gel filtration and sedimentation equilibrium. The equilibrium-sedimentation pattern of the native enzyme was typical of a homogeneous protein; by using a partial specific volume of 0.730, an M_r of 130000 ± 4000 has been calculated. A very close value (124000) was obtained by filtering the glucose dehydrogenase on a TSK-gel column under non-dissociating conditions. Evidence that the enzyme has an oligomeric nature was obtained by chromatography and electrophoresis under dissociating conditions. Filtration of the guanidine-treated enzyme on a 6 M-guanidine-equilibrated TSK-gel column gave a single peak at an elution volume corresponding to an M_r of 64000. A different picture appeared when SDS was used as dissociating agent. Treatment at boiling temperature with high concentration of the surfactant (5%, w/v) caused complete dissociation of the enzymic protein into four subunits identical in size; the M_r of the monomer was furnished from both electrophoresis on a polyacrylamide-gel slab (M_r 33000) and chromatography on guanidine–TSK-gel column (M_r 30000). When treated with a lower surfactant concentration (1%, w/v) the protein dissociated mainly to dimers (M_r 60000), as observed by TSK-gel chromatography.

The amino acid composition of the purified glucose dehydrogenase is reported in Table 2. The number of each residue per mol of protein was calculated on the basis of M_r 124000. Half-cystine and tryptophan residues have not been determined.

Table 2. Amino acid composition of glucose dehydrogenase from *Sulfolobus solfataricus*

Amino acid	Composition (Residues/100 residues)	(Residues/mol)
Lys	7.0	80
His	1.4	16
Arg	4.9	56
Asp	12.9	148
Thr	4.6	53
Ser	6.3	72
Glu	9.6	110
Pro	4.3	49
Gly	9.3	107
Ala	5.8	67
Val	8.4	96
Met	1.9	22
Ile	7.8	90
Leu	9.3	107
Tyr	2.6	30
Phe	3.9	45

Parameters affecting enzyme activity and stability

Glucose dehydrogenase preparations extensively dialysed against Mg^{2+}-free 20 mM-triethanolamine/HCl buffer, pH 7.0, were poorly active in the NAD^+-dependent glucose oxidation; a significant increase of enzyme activity was caused by the addition of some bivalent cations. Maximum activation was observed at Mg^{2+}, Mn^{2+} or Ca^{2+} concentrations of 20 mM; Zn^{2+} was partially effective, whereas Ni^{2+}, Cd^{2+} and univalent cations were ineffective in promoting enzyme reactivation. The presence of 50 mM-EDTA in the standard reaction mixture decreased enzyme activity to about 5%; addition of Mg^{2+} in excess restored the initial activity. Glucose dehydrogenase preparations became inactivated irreversibly when stored in the absence of ethylene glycol and Mg^{2+}, at very low protein concentration, or during freezing and thawing. Conversely, the enzyme was stable for several months at 4 °C in the presence of 20 mM-$MgCl_2$ and 20% (v/v) ethylene glycol. The stability experiments described below were carried out in the presence of these two compounds.

The effect of temperature on glucose dehydrogenase stability was studied by incubating the enzyme at 37 and 70 °C in the absence of substrates and assaying the residual activity at various times. At a protein concentration of 0.2 mg/ml, 50% of the activity was lost after 40 days at 37 °C and after 45 h at 70 °C. The addition of a substrate to the incubation mixture did not affect further the thermostability. The enzyme stability did not vary significantly in the pH range 5–9.

The effect of a number of organic solvents and dissociating agents on the enzyme stability was also studied. Preincubation of enzyme solutions up to 24 h at room temperature with methanol or acetone (1:1, by vol.), 2 M-urea and 0.05% (w/v) SDS did not cause any appreciable loss of enzymic activity. A slow time-dependent decrease of activity was observed in the presence of ethanol, 4 M-urea and 0.1% SDS. Lastly, a rapid inactivation was obtained in the presence of H_4furan and 4 M-guanidinium chloride.

Glucose dehydrogenase was rapidly inactivated by incubation at room temperature and pH 9 with 1 mM-

Table 3. Compounds examined as possible substrates for glucose dehydrogenase from *Sulfolobus solfataricus*

All compounds were tested at 40 mM concentration; the NAD^+-dependent D-glucose oxidation rate was taken as 100. D-Glucose 6-phosphate, L-glucose, L-rhamnose, D-lyxose, D-arabinose, DL-glyceraldehyde, glycerol, *myo*-inositol, lactose and maltose were not oxidized by either coenzyme.

Substrate	Relative rate (%)	
Coenzyme ...	NAD^+	$NADP^+$
D-Glucose	100	9
D-Mannose	0	4
D-Allose	8	13
D-Galactose	0	15
D-Altrose	5	12
D-Gulose	8	12
D-Idose	65	12
2-Deoxy-D-glucose	12	25
2-Amino-2-deoxy-D-glucose	26	5
6-Deoxy-D-glucose	66	9
D-Xylose	26	28
D-Ribose	0	4

2-mercaptoethanol; conversely, only 50% inactivation was observed after a 2 h treatment under the same conditions with 3 mM-*N*-ethylmaleimide or 1 mM-5,5′-dithiobis-(2-nitrobenzoic acid).

Kinetic properties

Glucose dehydrogenase has been tested at 70 °C and different pH values with glucose and NAD^+ as substrates; the pH–activity profile consisted of a bell-shaped curve with a maximum at pH 8.0. Similar profiles were obtained by monitoring, at various pH values, galactose or xylose oxidation in the presence of $NADP^+$.

At pH 8, the enzyme exhibited maximal activity at about 77 °C. It has to be noted that, at 37 °C, a temperature value where most enzymes from extreme thermophiles are practically inactive, the *S. solfataricus* dehydrogenase showed about 20% activity as compared with the maximal value.

A large number of carbohydrates has been examined as possible substrates for the dehydrogenase (Table 3) and some of them as possible inhibitors for the NAD^+-dependent glucose oxidation. When NAD^+ served as electron acceptor, D-glucose was oxidized at the highest rate (330 μmol/min per mg) among the products tested; other sugars oxidized at high rates were D-idose, D-xylose and some glucose derivatives. By using $NADP^+$ as coenzyme, a larger number of carbohydrates was oxidized by the dehydrogenase (Table 3); among them, D-xylose and 2-deoxy-D-glucose were oxidized at the highest rate (about 90 μmol/min per mg). It has to be noted that the $NADP^+$-dependent glucose oxidation was inhibited by high substrate concentrations (> 10 mM); the activity at 40 mM-glucose was 50% lower than that observed at 2 mM-glucose. Some of the Michaelis constants calculated from double-reciprocal plots for carbohydrates and coenzymes are presented in Table 4.

Table 4. Apparent K_m values for substrates and coenzymes of glucose dehydrogenase from *Sulfolobus solfataricus*

Substrate pair	K_m value (mM)	
	Carbohydrate	Coenzyme
Glucose–NAD^+	8.0	1.2
Glucose–$NADP^+$	0.44	0.03
Galactose–$NADP^+$	22.0	0.03
Xylose–NAD^+	68.0	1.2
Xylose–$NADP^+$	2.2	0.03

Assay conditions and concentration of the fixed substrate were those of the standard assay.

NADPH was found to inhibit the NAD^+-dependent carbohydrate oxidations in a competitive manner with respect to NAD^+; when glucose was used as substrate, the K_i for NADPH was 75 μM. Galactose, mannose and ribose, in concentrations as high as 40 μM, did not inhibit glucose oxidation by NAD^+.

Reaction product

As observed by t.l.c. on silica-gel plates, gluconic acid accumulated in the NAD^+-dependent glucose oxidation by glucose dehydrogenase. In a previous study (De Rosa *et al.*, 1984), g.l.c. was used to identify gluconic acid among the products of the NAD^+-dependent reaction between *S. solfataricus* homogenate and glucose. It is likely that the first product of this reaction is δ-gluconolactone, which is readily converted into the acid; the lactone hydrolysis is facilitated by the high temperature and the slight alkaline pH value of the standard reaction conditions, thus explaining why we were unable to observe any reverse reaction under these conditions when δ-gluconolactone was used as substrate. In fact a very slow NADH-dependent reduction of δ-gluconolactone (2 μmol/min per mg) by glucose dehydrogenase *has* been observed at pH 7.0 and 37 °C. Conversely, no reverse reaction was evident, under various reaction conditions, when gluconic acid was used as substrate.

DISCUSSION

The purification of glucose dehydrogenase from crude extracts of *Sulfolobus solfataricus* was facilitated by the enzyme ability to bind to an Affi-Gel Blue matrix and to be specifically desorbed by elution with substrates. Purified enzyme preparations exhibited on polyacrylamide-gel electrophoresis one protein band, coincident with the activity staining, and oxidized a number of aldoses at different rates. The following evidence demonstrates that a single enzyme was capable of catalysing the observed carbohydrate oxidations: (i) the relative rates of glucose, galactose and xylose oxidation did not vary significantly throughout the purification procedure (Table 5); (ii) chromatography on SP-Sephadex and phenyl-Sepharose gave elution profiles in which the different activities were superimposable; (iii) specific elution of the glucose dehydrogenase from the Affi-Gel Blue column was accomplished by washing with $NADP^+$ and xylose; (iv) enzymic-activity bands detected on polyacrylamide gels by specific staining with different

Table 5. Relative rates of glucose, xylose and galactose oxidation throughout the enzyme purification

The oxidation of glucose was determined in the presence of NAD^+ as coenzyme, that of xylose and galactose in the presence of $NADP^+$

Step	Relative rate	
	Glucose/xylose	Glucose/galactose
$(NH_4)_2SO_4$ precipitation	3.2	6.4
SP-Sephadex	3.6	—
Phenyl-Sepharose	3.5	6.2
Affi-Gel Blue	3.7	6.6

carbohydrates were coincident; (v) the $NAD(P)^+$-reduction rate measured with saturating concentrations of either glucose or galactose did not increase by adding xylose to the assay mixture.

The glucose dehydrogenase from *S. solfataricus* is an oligomeric protein of M_r about 124000 consisting of four polypeptide chains similar or identical in size. The enzyme showed the ability to dissociate to dimers in the presence of guanidine or low concentrations of SDS and to monomers under stronger denaturing conditions. Direct evidence that monomeric or dimeric forms of the enzyme are active or that the dissociation process is effectively reversible are not yet available; however, at present we have not been able to restore activity of enzyme preparations dissociated by guanidine treatment.

When NAD^+ served as coenzyme, among a large number of sugars examined only D-glucose, D-idose and D-xylose were oxidized at a high rate by the *S. solfataricus* glucose dehydrogenase. It appears that any configuration change at C-2, C-3 and C-4 of the glucose molecule prevented the new product from binding the enzyme; the absence of the hydroxy group at C-2 or its substitution with an amino group were still compatible with oxidation by the enzyme, even though at a much lower rate. Consequently, it can be assumed that the enzyme is capable of binding a substrate sugar in the pyranose-ring form when it presents equatorial hydroxy groups at C-2, C-3 and C-4 (Fig. 1). Strong support for this hypothesis has come from the observation that D-idose was a good substrate for the dehydrogenase; in fact it is well documented that in the sugars of the idose series predominate in the reverse conformation, where the hydroxy groups at C-2, C-3 and C-4 are equatorial (Davidson, 1967). The specificity of the enzyme for sugars of the pentose series supported further the hypothesis, as the only substrate was D-xylose, which, in the pyranose-ring form, presents equatorial hydroxy groups at C-2, C-3 and C-4 (Fig. 1).

In contrast with normal glucose, idose presents, in the reverse conformation, an axial hydroxymethyl group at C-5 (Fig. 1). It appears that binding of the substrate to the enzyme did not depend on configuration at C-5 or even on the presence of an hydroxy group in this position; as a fact, 6-deoxy-D-glucose was as good a substrate for the dehydrogenase as was idose (Table 3). The fact that D-glucose 6-phosphate was not oxidized at all might indicate that steric factors or electrostatic repulsion prevent substrate attachment to the protein.

As in the case of other glucose dehydrogenases, one may expect that the anomeric carbon (C-1) of glucose is oxidized when in the β-configuration (equatorial hydroxy

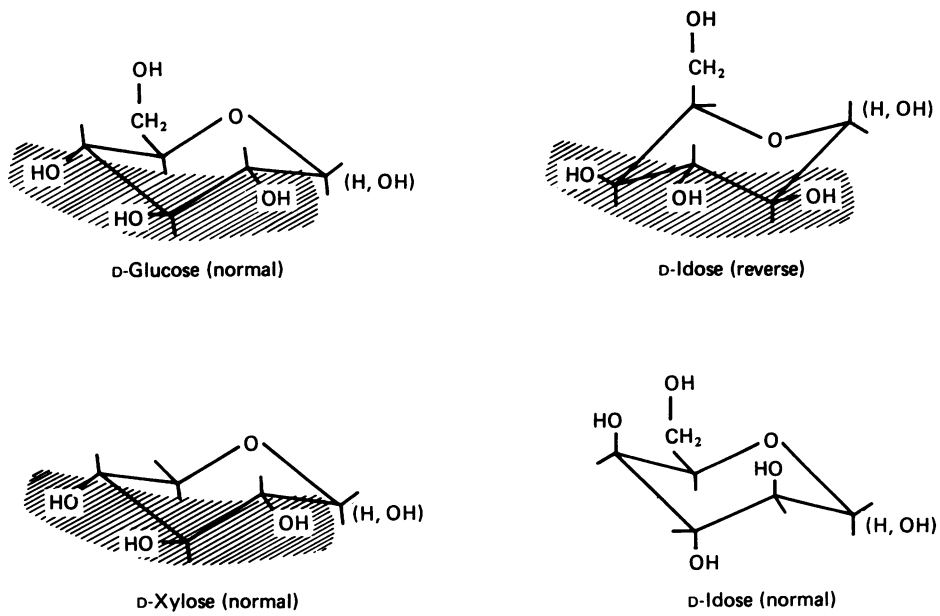

Fig. 1. Normal or reverse conformation of sugars oxidized by glucose dehydrogenase from *Sulfolobus solfataricus* in the presence of NAD^+ as electron acceptor

Minimal steric requirements of sugars for binding the enzyme are shaded (equatorial hydroxy groups at C-2, C-3 and C-4). D-Idose is a good substrate as its preferred conformer in aqueous solution has, in contrast with other sugars, a reverse ring form.

group in the normal conformation) and that the primary product of oxidation is the hexono-δ-lactone. However, direct evidence to support these assumptions have yet to be predicted.

A rather broad substrate specificity was showed by the enzyme when $NADP^+$ served as coenzyme, thus indicating that the extra negative charge on the adenosine moiety may influence the substrate-binding site. However, in the presence of this coenzyme, all the substrates were oxidized at a much lower rate with respect to that of glucose in the dehydrogenase–NAD^+ system (Table 3).

Glucose dehydrogenase purified from different microbial sources vary in a number of molecular and catalytic properties, such as the oligomeric nature, the ability to utilize either nicotinamide nucleotide (NAD^+, $NADP^+$), or both, as coenzyme, the substrate specificity. The enzyme from *Corynebacterium* species is a single polypeptide chain of M_r about 55000 and is strictly dependent on NAD^+ for its activity; it oxidizes glucose and xylose and is inactive towards other monosaccharides (Kobayashi & Horikoshi, 1980). Conversely, glucose dehydrogenase from *Gluconobacter* species is a $NADP^+$-dependent tetramer of M_r 150000 that oxidizes glucose and mannose and is inactive towards xylose and galactose (Avigad *et al.*, 1968; Adachi *et al.*, 1980). Glucose dehydrogenase from *S. solfataricus* differs significantly from the enzymes which, like those from coryneform and acetic acid bacteria, are produced during the vegetative growth, whereas it strongly resembles the developmental enzymes produced by sporulating bacilli. In fact, most of the glucose dehydrogenases produced by *Bacillus* species late during sporulation are tetramers of M_r about 120000, exhibiting monomer–oligomer equilibrium; these enzymes can use both NAD^+ and $NADP^+$ as coenzyme, have a pH optimum for activity in the range 8–9, show a good heat-resistance and are inactive towards mannose (Bach & Sadoff, 1962; Pauly & Pfleiderer, 1975; Fujita *et al.*, 1977). It is interesting to note that both heat-resistant eubacterial endospores and thermophilic archaebacterial cells have evolved a strikingly similar enzymic protein as part of an overall response to adverse external conditions.

The enzymic methods currently utilized for glucose determination involve multi-stage reactions that might affect the reproducibility and accuracy of the system. Therefore, in the last decade, efforts have been made to render suitable for analytical purposes the single-step reaction catalysed by nicotinamide-nucleotide-dependent glucose dehydrogenases (Price & Spencer, 1979; Sundaram *et al.*, 1979). The *S. solfataricus* enzyme presents a number of characteristics which make it a promising reagent, both in a free state and in immobilized forms, for direct enzymic determinations of glucose. This protein is fairly stable at temperatures below 40 °C and in the presence of several organic solvents and dissociating agents, valuable features in increasing half-life of the reactant and when mixed-solvent systems for bioreactor preparation and/or regeneration are required. Moreover, by using NAD^+ as electron acceptor, only xylose at high concentration may significantly interfere with glucose determination, as idose and 6-deoxyglucose are not usually present in biological fluids. Lastly, unlike most enzymes from highly thermophilic organisms, glucose dehydrogenase from *S. solfataricus* is still active at temperatures below 40 °C, being thus suitable for glucose determination by established manual and automatic procedures.

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REFERENCES

Adachi, O., Matsushita, K., Shinagawa, E. & Ameyama, M. (1980) Agric. Biol. Chem. **44**, 301–308
Avigad, G., Alroy, Y. & Englard, S. (1968) J. Biol. Chem. **243**, 1936–1941
Bach, J. A. & Sadoff, H. L. (1962) J. Bacteriol. **83**, 699–707
Buonocore, V., Sgambati, O., De Rosa, M., Esposito, R. & Gambacorta, A. (1980) J. Appl. Biochem. **2**, 390–397
Campbell, D. P., Carper, W. R. & Thompson, R. E. (1982) Arch. Biochem. Biophys. **215**, 289–301
Davidson, E. A. (1967) Carbohydrate Chemistry, pp. 126–130, Holt, Rinehart and Winston, New York
Davis, B. J. (1964) Ann. N.Y. Acad. Sci. **121**, 404–427
De Rosa, M., Gambacorta, A. & Bu'Lock, J. D. (1975) J. Gen. Microbiol. **86**, 156–164
De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio, E. & Buonocore, V. (1984) Biochem. J. **224**, 407–414
Fujita, Y., Ramaley, R. & Freese, E. (1977) J. Bacteriol. **132**, 282–293
Kobayashi, Y. & Horikoshi, K. (1980) Agric. Biol. Chem. **44**, 2261–2269
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Matsushita, K., Ohno, Y., Shinagawa, E., Adachi, O. & Ameyama, M. (1980) Agric. Biol. Chem. **44**, 1505–1512
Pauly, H. E. & Pfleiderer, G. (1975) Hoppe-Seyler's Z. Physiol. Chem. **356**, 1613–1623
Price, C. P. & Spencer, K. (1979) Ann. Clin. Biochem. **16**, 100–105
Pulich, W. M., Van Baalen, C., Gibson, J. L. & Tabita, F. R. (1976) Plant Physiol. **58**, 393–397
Spackman, D. H., Stein, W. H. & Moore, S. (1958) Anal. Chem. **30**, 1190–1206
Sundaram, P. V., Blumenberg, B. & Hinsch, W. (1979) Clin. Chem. **25**, 1436–1439
Tomlinson, G. A., Koch, T. K. & Hochstein, L. I. (1974) Can. J. Microbiol. **20**, 1085–1091
Vasantha, N., Uratani, B., Ramaley, R. F. & Freese, E. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 785–789
Zillig, W., Stetter, K. O., Wunderl, S., Schulz, W., Priers, H. & Scholz, I. (1980) Arch. Microbiol. **125**, 259–269

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