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NAD(P)$^+$-glucose dehydrogenase from *Haloferax mediterranei*: kinetic mechanism and metal content

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Abstract

The kinetic mechanism and metal content of *Haloferax mediterranei* NAD(P)$^+$-glucose dehydrogenase have been investigated. The kinetic mechanism has been determined by initial rate and inhibition studies. Initial velocity studies were performed with D-glucose as well as with the alternative substrate D-xylose, with NADP$^+$ as coenzyme. The results show that the mechanism is sequential with respect to substrate addition. The product inhibition patterns agree with an ordered binding of NADP$^+$ and D-glucose, followed by an ordered release of gluconolactone and NADPH.

The activity of *Hf. mediterranei* glucose dehydrogenase was markedly dependent on the concentration of metal ions. Inactivation by metal chelators and reactivation by certain divalent ions indicated that glucose dehydrogenase from *Hf. mediterranei* contains tightly bound metal ions which are essential for activity. Metal analyses demonstrated that the enzyme binds 3.6 ± 0.3 mol of Zn(II)/mol of protein, which corresponds to the binding of two atoms of Zn(II) per subunit. Alignment of the N-terminal sequence of glucose dehydrogenase from *Hf. mediterranei* with medium chain zinc-containing dehydrogenases reveals a clear similarity between them, suggesting that glucose dehydrogenase from *Hf. mediterranei* belongs to this family.

Keywords: Glucose dehydrogenase; *Haloferax mediterranei*; Halophilic; Kinetic mechanism; Medium chain zinc-containing dehydrogenases

1. Introduction

Glucose dehydrogenase (EC 1.1.1.47) is the first enzyme of the modified Entner–Doudoroff pathway for the catabolism of glucose. The enzyme catalyses the oxidation of β-D-glucose to D-glucono-1,5-lactone using NAD$^+$ or NADP$^+$ as coenzyme. The enzyme is present in all three domains of life and there are a number of reports on its molecular and enzymatic properties [1–14]. Previous biochemical studies have shown the dependence of enzymatic activity on the presence of divalent metal ions, Mg^{2+}, Mn^{2+} or Ca^{2+} [1–3]. Gene sequences have been obtained for the *Bacillus subtilis, B. megaterium* and *Thermoplasma acidophilum* en-

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zymes [15–17]. However, there are data on the kinetic mechanism only for the *Schizosaccharomyces pombe* glucose dehydrogenase [18], and metal analysis has not been reported on any glucose dehydrogenase. In this report we investigate the kinetic mechanism of the *Haloferax mediterranei* glucose dehydrogenase, the dependence of enzymatic activity on divalent ions and the metal content of the enzyme.

Glucose dehydrogenase from *Hf. mediterranei* has been purified and characterised previously [19]. It is a homodimer of 39 ± 4 kDa/subunit, and has been shown to have broad substrate specificity. It can use NAD^+ and $NADP^+$ as coenzyme, the affinity for $NADP^+$ being higher than for NAD^+, and it requires a divalent metal ion for full enzymatic activity. With the substrate D-xylose the reaction rate is very high with both coenzymes. The D-xylose pyranose ring presents equatorial hydroxyl groups at C-2, C-3 and C-4, as in D-glucose. It seems that, like other archaeal glucose dehydrogenases, this enzyme shows sugar-specific stereoconfiguration at C-2, C-3, and C-4 [3,5].

The kinetic mechanism has been determined by initial velocity and product inhibition studies. Initial velocity studies have been carried out with the substrates D-glucose and D-xylose with $NADP^+$ as coenzyme in order to determine which is the best substrate of the enzyme, and metal ion studies have shown that *Hf. mediterranei* glucose dehydrogenase is a zinc metalloenzyme.

2. Experimental

2.1. Materials

β-D-glucose, D-xylose, D-glucono-1,5-lactone (simply referred to as gluconolactone) were from Sigma. $NADP^+$ and NADPH were obtained from Boehringer Mannheim. All other reagents were analytical reagent grade from Merck. Gluconolactone was used within 5 min after dissolution to prevent hydrolysis into gluconic acid and conversion into gluconic acid 1,4-lactone.

2.2. Enzyme preparation and protein determinations

A purified preparation of $NAD(P)^+$-glucose dehydrogenase from *Hf. mediterranei* was obtained as described previously [19]. Protein concentrations were determined by the method of Bradford [20], with at least three determinations of bovine serum albumin standards and samples.

The subunit molecular weight was determined on a Fisons VG Platform electrospray mass spectrometer (ES-MS). The sample was 20 μl of glucose dehydrogenase in water at a concentration of 88 μg/ml. The operating conditions for ES were as follows: the source was operated in the positive ion mode, the electrospray needle was held at 4.25 kV, and the heated capillary was operated at 90°C. Calibration was performed using a standard solution of horse heart myoglobin (Sigma). Full scan data acquisition was performed scanning from 670 to 2430 m/z in continuous mode, using a cycle time of 3.6 s and an inter-scan time of 0.13 s.

2.3. Kinetic assays

Initial velocity and inhibition studies were performed in 20 mM Tris–HCl buffer pH 8.8, containing 2 M NaCl and 25 mM $MgCl_2$. The concentration ranges of substrates and inhibitors are shown in Tables 1 and 2. The reaction was followed by measuring the appearance of NADPH at 340 nm with an Ultrospec 2000 spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol NADPH/min under the assay conditions (40°C).

The effect of several metal ions was assayed by varying the metal concentration in assays at 40°C in 20 mM Tris–HCl buffer, pH 8.8, containing 2 M NaCl, 0.22 mM $NADP^+$ and 25 mM D-glucose.

Table 1
Kinetic parameters from initial velocity studies with halophilic glucose dehydrogenase
Variable substrate D-glucose: 2–20 mM. Fixed substrate $NADP^+$: 0.04, 0.05, 0.067, 0.1, and 0.2 mM. Variable substrate $NADP^+$: 0.04–0.2 mM. Fixed substrate D-glucose: 2, 2.5, 3.3, 5, 10, and 20 mM. Variable substrate D-xylose: 1.25–25 mM. Fixed substrate $NADP^+$: 0.025, 0.04, 0.05, 0.067, and 0.2 mM. Variable substrate $NADP^+$: 0.025–0.2 mM. Fixed substrate D-xylose: 1.25, 1.67, 2.5, 5, 12.5, and 25 mM.

Substrate D-glucose		Substrate D-xylose	
V (U/mg)	93 ± 7	V (U/mg)	80 ± 3
$K_{m\ NADP^+}$ (mM)	0.039 ± 0.010	$K_{m\ NADP^+}$ (mM)	0.011 ± 0.003
$K_{m\ D\text{-glucose}}$ (mM)	4.7 ± 1.0	$K_{m\ D\text{-xylose}}$ (mM)	6.9 ± 0.6
$K_{i\ NADP^+}$ (mM)	0.09 ± 0.02	$K_{i\ NADP^+}$ (mM)	0.037 ± 0.006
$V/K_{D\text{-glucose}}$ (min^{-1})	0.46 ± 0.06	$V/K_{D\text{-xylose}}$ (min^{-1})	0.270 ± 0.017

2.4. Kinetic data processing

The nomenclature used in this paper is that of Cleland [21]. Reciprocal velocities were plotted against the reciprocals of substrate concentrations, and linear plots were obtained in all cases.

Primary plots of initial velocity vs. reciprocal substrate concentrations were based on, at least, triplicate velocity measurements. Reciprocal plots of initial velocity and product inhibition data were examined to determine the pattern, and the slopes and intercepts were plotted against either the reciprocal of the unchanged substrate concentration or the inhibitor concentration to determine the linearity of these replots.

Kinetic data were fitted by using the FORTRAN programs described by Cleland [22] to obtain values for kinetic constants. All kinetic constants are expressed with the standard error obtained in the computer fit. The best fit was selected by the criteria defined by Cleland.

Initial rate studies were done by varying the concentration of one substrate (A) at several fixed concentrations of a second substrate (B). Initial velocity data yielding intersecting patterns were fitted to Eqs. (1) and (2) in order to discriminate between a sequential mechanism and a rapid equilibrium ordered addition of substrates, where v is the experimentally determined velocity, V is the maximum velocity, A and B are the substrate concentrations, K_a and K_b are the respective Michaelis constants, and K_{ia} is the dissociation constant of A. Inhibition studies were done varying one substrate at several fixed concentrations of a single product (I), the other substrate concentration being kept constant at a saturated and non-saturated concentration. Linear competitive, non-competitive and uncompetitive inhibitions were fitted to Eqs.

Table 2
Product inhibition patterns for halophilic glucose dehydrogenase
Inhibitor concentration ranges: gluconolactone 0–18 mM; NADPH 0–0.4 mM. Substrate concentration ranges: glucose 2–20 mM; $NADP^+$ 0.025–0.5 mM.

Inhibitor	Variable substrate	Fixed substrate	Pattern	$K_{is} \pm$ S.E. (mM)	$K_{ii} \pm$ S.E. (mM)
NADPH	$NADP^+$	D-glucose 50 mM	C	0.137 ± 0.010	
NADPH	$NADP^+$	D-glucose 0.2 M	C	0.31 ± 0.04	
NADPH	D-glucose	$NADP^+$ 0.048 mM	NC	0.43 ± 0.19	0.42 ± 0.06
Gluconolactone	$NADP^+$	D-glucose 20 mM	NC	43 ± 12	8.5 ± 0.6
Gluconolactone	$NADP^+$	D-glucose 0.2 M	NC	44 ± 15	11.6 ± 1.3
Gluconolactone	D-glucose	$NADP^+$ 0.125 mM	NC	3.5 ± 0.3	15.5 ± 1.3
Gluconolactone	D-glucose	$NADP^+$ 1.25 mM	NC	2.3 ± 0.3	9.3 ± 1.0

(3)–(5), respectively. The results of metal activation were fitted to Eq. (6).

$$v = \frac{VAB}{K_{ia}K_b + K_aB + K_bA + AB} \quad (1)$$

$$v = \frac{VAB}{K_bA + AB + K_{ia}K_b} \quad (2)$$

$$v = \frac{VA}{K\left(1 + \frac{I}{K_{is}}\right) + A} \quad (3)$$

$$v = \frac{VA}{K\left(1 + \frac{I}{K_{is}}\right) + A\left(1 + \frac{I}{K_{ii}}\right)} \quad (4)$$

$$v = \frac{VA}{K + A\left(1 + \frac{I}{K_{ii}}\right)} \quad (5)$$

$$v = \frac{VA}{K_A + A} \quad (6)$$

The points drawn in figures showing double-reciprocal plots are the experimentally determined values. The lines drawn through these points are calculated from the fit of these data to the corresponding equations.

2.5. Metal analyses

Purified glucose dehydrogenase was dialysed against three changes of 20 mM Tris–HCl pH 7.4, containing 20% glycerol. The dialysis volume ratio was 1:100. To remove trace metals this buffer was treated with Chelex-100 chelating resin (Bio-Rad) using 5 g of resin for every 100 ml of buffer. Metal analyses were performed on a Perkin-Elmer Optima 3000 inductively coupled plasma emission spectrometer (ICP). Calibration curves were carried out with seven metal ion standards containing between 20 and 150 ppb, using the Merck 11355 ICP Multi Element Standard IV. The final dialysis buffer was used as a control. Three trials of each sample were taken and averaged. The metals analysed were Mg, Fe, Mn, Zn, Cu, Co and Ni.

3. Results

3.1. Kinetic mechanism

3.1.1. Initial velocity studies

Initial velocity studies were performed in the forward reaction with D-glucose as well as with the alternative substrate D-xylose, with $NADP^+$ as coenzyme. Intersecting lines were obtained with all combinations of substrates, confirming a sequential mechanism. The effect of variation in D-glucose concentration at different fixed $NADP^+$ concentrations is shown in Fig. 1. When D-xylose was plotted as the variable substrate, with different concentrations of $NADP^+$ as fixed

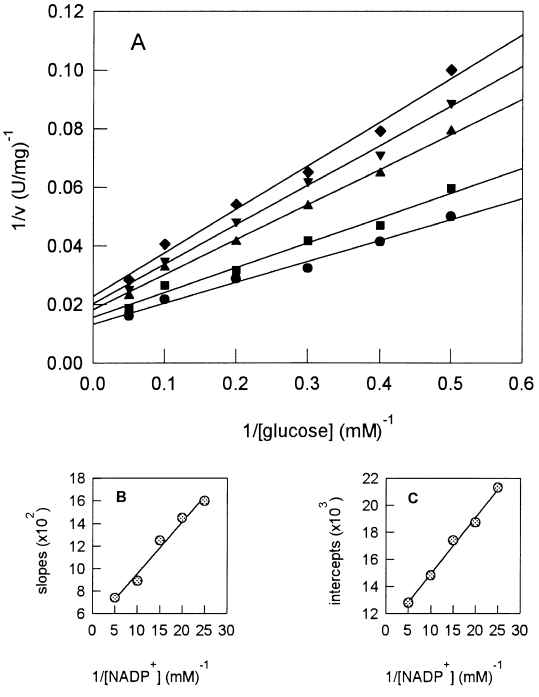


Fig. 1. (A) Initial velocity pattern with D-glucose as variable substrate (range 2–20 mM) and with $NADP^+$ as fixed substrate: ● 0.2, ■ 0.1, ▲ 0.067, ▼ 0.05, ◆ 0.04 mM. Re-plot of slopes (B) and intercepts (C) vs. $1/[NADP^+]$.

substrate, the intersecting point was close to the intercept axis, but the fit to Eqs. (1) and (2) showed that the lowest value of 'sigma' was obtained with Eq. (1). The results of initial velocity studies are shown in Table 1. The initial velocity studies ruled out a ping-pong mechanism and also a rapid equilibrium ordered addition of substrates to the enzyme. The value of V/K_m for the substrate D-glucose is greater for D-xylose indicating that D-glucose is the best substrate of the enzyme.

3.1.2. Product inhibition studies

Product inhibition studies were carried out to delineate clearly the order of substrate addition to and product release from the enzyme. All patterns gave linear inhibition. Product inhibition data are shown in Table 2. K_{is} and K_{ii} are the apparent slope and intercept inhibition constants, respectively, obtained from fits of data to Eqs. (3) and (4). Product inhibition by NADPH was competitive with respect to $NADP^+$ at the two D-glucose concentrations studied, and non-competitive with D-glucose at non-saturated $NADP^+$. The competitive pattern observed is shown in Fig. 2. This pattern indicates mutually exclusive binding of inhibitor (NADPH) and substrate ($NADP^+$) and thus suggests that they bind to the same enzyme form (free enzyme). Therefore, $NADP^+$ may be the first substrate added and NADPH the last product released in the reaction. Product inhibition by gluconolactone with respect to $NADP^+$ was non-competitive at 20 mM D-glucose, indicating that the inhibitor and the variable substrate bind to different forms of the enzyme and that they are separated in the reaction sequence by reversible steps. Saturating with D-glucose makes one of these steps irreversible, and the inhibition pattern should be uncompetitive. In this study, we observed non-competitive inhibition at 0.2 M D-glucose, which indicates saturation has not been reached at this concentration. Otherwise, substrate inhibition would be produced, since substrate inhibition becomes obvious at concentrations over 0.2 M (Fig. 3). Product inhibition by gluconolactone with respect to D-glucose was non-competitive at the two $NADP^+$ concentrations studied. This pattern is consistent with gluconolactone being the first product to be released in the reaction. Thus, the variable substrate cannot prevent combination of the inhibitor, and the points of combination of gluconolactone and $NADP^+$ are connected by a reversible sequence. Saturation with $NADP^+$ has no effect on D-glucose addition since this step is later in the reaction sequence.

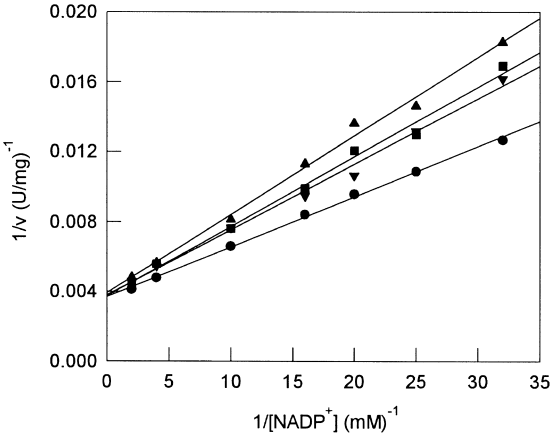


Fig. 2. Product (NADPH) inhibition pattern of glucose dehydrogenase with $NADP^+$ as the variable substrate (range 0.03–0.5 mM) at fixed concentration of D-glucose 0.2 M. NADPH concentrations are: ● 0 mM, ▼ 0.05 mM, ■ 0.1 mM, ▲ 0.2 mM.

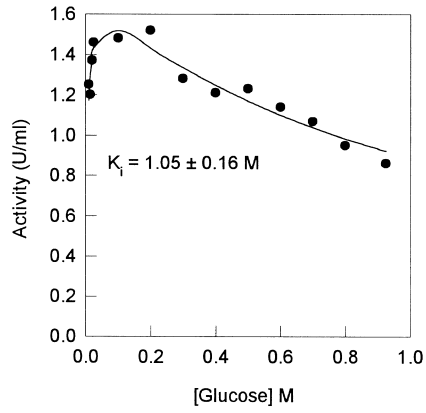


Fig. 3. Inhibition of glucose dehydrogenase from *Hf. mediterranei* by high concentrations of D-glucose.

3.2. Metal analyses on glucose dehydrogenase

The purified enzyme contains 10 mM Mg^{2+} since that is necessary in the final purification step. To eliminate this metal, the enzyme was extensively dialysed against 20 mM Tris–HCl pH 7.4 containing 20% glycerol.

The activity of *Hf. mediterranei* glucose dehydrogenase was markedly dependent on the concentration of metal ions. In the absence of such ions, glucose dehydrogenase preparations presented low enzymatic activity (the residual activity in the absence of metal ion in the assay mixture was always between 9% and 12% of the activity with 25 mM Mg^{2+}). The cations that produced activation were Mn^{2+}, Mg^{2+} and Ni^{2+}; activation was not observed with Zn^{2+}. The values of the kinetic constants obtained are shown in Table 3.

In order to investigate whether *Hf. mediterranei* glucose dehydrogenase was inhibited by chelating agents, the enzyme (53 μg/ml) was treated with EDTA at concentrations between 0.01 and 2.0 mM. The mixture was incubated for 5 min at room temperature and then the enzymatic activity was measured in the absence at any divalent metal in the assay medium. As show in Fig. 4, incubation with EDTA at concentrations higher than 1 mM results in the complete inactivation of the enzyme. This suggests that a tightly bound metal ion is essential for the expression of glucose dehydrogenase activity. The addition of Mn^{2+}, Mg^{2+}, Ni^{2+} and Zn^{2+} to the assay mixture results in the immediate recovery of the enzymatic activity (data not shown).

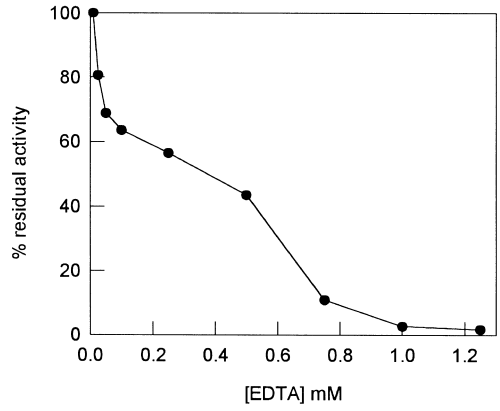


Fig. 4. Effect of chelating agent concentration on inactivation of glucose dehydrogenase from *Hf. mediterranei*. Metal-free purified enzyme was incubated for 5 min with the indicated concentrations of EDTA at 25°C, and then assayed for glucose dehydrogenase activity.

In order to investigate whether glucose dehydrogenase is a metalloenzyme, metal analyses on the purified enzyme were carried out. Prior to analysis the enzyme was dialysed against 3×100 vol. of 20 mM Tris–HCl, pH 7.4, containing 20% glycerol, and the concentration of the enzyme was determined. As shown in Table 4, the only metal ions detectable in significant amounts were Zn(II) and Mg(II). Three additional preparations of the enzyme yielded similar values for the metal content of glucose dehydrogenase. When the molecular weight of glucose dehydrogenase subunit was determined by ES-MS, a value of $39{,}248 \pm 4$ Da was obtained. This agrees with the mass determined previously by CTAB-PAGE (39 ± 4 kDa) [19]. The Zn(II) to enzyme stoichiometry is 3.6 ± 0.3

Table 3
Activation of glucose dehydrogenase by divalent metal ions
The kinetic parameters were obtained by fitting the data to Eq. (6), varying the metal concentration in the ranges: [Mg^{2+}]: 0–10 mM, [Mn^{2+}]: 0–0.1 mM, [Ni^{2+}]: 0–1 mM.

Metal ion	K_a (μM)	$V_{max.}$ (U/mg)
Mg^{2+}	290 ± 130	228 ± 14
Mn^{2+}	5.4 ± 1.5	360 ± 20
Ni^{2+}	3 ± 2	30 ± 4

Table 4
Metal content of glucose dehydrogenase from *Hf. mediterranei*

Metal ion	Moles metal/mole enzyme
Zn	3.6 ± 0.3
Mg	26.6 ± 0.9
Fe	0.02 ± 0.09
Mn	0.15 ± 0.03
Ni	1.0 ± 0.7
Co	0.30 ± 0.12
Cu	0

(for an enzyme Mr of 78,496 Da), which indicates the binding of two atoms of Zn per subunit of glucose dehydrogenase. Also a high Mg(II) content has been obtained.

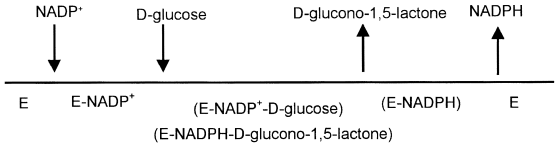
4. Discussion

4.1. Kinetic mechanism

Intersecting patterns were obtained when the reciprocal of initial velocity was plotted as a function of the reciprocal of one substrate concentration at several fixed levels of the other substrates. Thus, a ping-pong mechanism is ruled out, and the kinetic mechanism of *Hf. mediterranei* NAD(P)-glucose dehydrogenase should be of the sequential type. The product inhibition patterns agree with an ordered Bi–Bi mechanism, and allow the identification of the order of the addition of substrates. Thus we propose that the kinetic mechanism of halophilic glucose dehydrogenase involves an ordered binding of $NADP^+$ and D-glucose, followed by an ordered released of gluconolactone and NADPH, as shown in Scheme 1. The halophilic character of the enzyme does not involve any change in relation to the kinetic mechanism described for the enzyme from *S. pombe*, which also proceeds as a sequential order mechanism with the same order of substrate addition [18].

The mechanism suggested has been based in the following observations.

1. NADPH is a competitive inhibitor vs. $NADP^+$ and a non-competitive inhibitor vs. D-glucose, indicating that NADPH and $NADP^+$ both bind to free enzyme.



Scheme 1.

2. Gluconolactone is a non-competitive inhibitor vs. D-glucose and $NADP^+$, confirming that the order of substrate addition is $NADP^+$ followed by D-glucose.

This is the first archaeal glucose dehydrogenase mechanism described and provides information necessary for the further industrial application of the enzyme. This enzyme is very useful in recycling of NAD(P)H in a coupled-enzyme method, since the auxiliary substrate D-glucose is easily available and inexpensive, and the product, gluconic acid, has a great number of applications [23]. The enzyme from *Hf. mediterranei* is highly stable [19] and the enzyme is stable when salt is replaced by 20% glycerol, and it can act with relatively high concentrations of D-glucose. Therefore, it is an appropriate enzyme for use in industrial reactors. Also the enzyme can act with D-xylose as substrate.

4.2. Metal analyses

The inactivation by metal chelators and reactivation by certain divalent ions indicate that *Hf. mediterranei* glucose dehydrogenase contains tightly bound metal ions which are essential for activity. This fact led us to determine the metal content of the enzyme by atomic absorption analysis, which clearly revealed the binding of four atoms of Zn(II) per molecule of protein. The apparent high-Mg(II) content can be explained as non-dialysable Mg(II) binding to the enzyme surface and involved in the stabilisation of the protein.

Halophilic enzymes catalyse reactions identical to those mediated by their non-halophilic counterparts, although they require high salt concentration in the range of 1–4 M both for stability and for enzymatic activity. These enzymes also possess an excess of acidic over basic amino acids residues [24]. The structural features that stabilise halophilic malate dehydrogenase (hMDH) from *Haloarcula marismortui* have been extensively studied [25–27]. A stabil-

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GDH   Hf. mediterranei              MKAIAVKRGEDRPVVIEKPRPEPES.GEALVRTLRVGVD
GDH   T. acidophilum              TEQKAIVTDAPKGGVKYTTIDMPEPEHY.DAKLSPVYIGIC
GDH   S.solfataricus                MKAIIVKPPNAGVQVKDVDEKKLDSYGKIKIRTIYNGIC
GDH   T.tenax                       MRAVTVTPGVPESLRLRE.VPEPKP.GPGQV
ADH   S. cerevisiae         MPSQVIPEKQKAIVFYETDGKLEYKDVTVPEPKP.NEILVHVKYSGVC
ADH   A. eutrophus               MTAMMKA.AVFVEPGRIELADKPIPDIGP.NDALVRITTTTIC
ADH   S. solfataricus               MRAVRLVEIGKPLVLKDIDIPKPKG.AQVLIKVEAAGVC
XDH   Pichia stipitis            MTANPSLVLNKIDDISFETYDAPEISEP.TDVLVQVKKTGIC
SDH   B. subtilis              THTVPQNMKA.AVMHNTREIKIETLPVPDINH.DEVLIKVMAVGIC
SDH   H. sapiens                AAAAKPNNLSLVVHGPGDLRLENYPIPEPGP.NEVLLRMHSVGIC
BADH  Pseudomonas putida           MEIKAAIVRQKMGPFLLEHVALNEPAE.DQVLVRLVATGLC
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Fig. 5. Alignment of the N-terminal sequence of glucose dehydrogenase from *Hf. mediterranei* with glucose dehydrogenases from *Archaea* and other zinc-containing dehydrogenases. The amino acid sequences of the enzymes were derived from SWISSPROT database except glucose dehydrogenase from *T. tenax* (5) and glucose dehydrogenase from *Sulfolobus solfataricus* (EMBL acc. no. SSO 012093).

isation model for hMDH was proposed in terms of solvent interaction that depends on solution conditions and temperature. At molar concentrations of NaCl, KCl and $MgCl_2$, hMDH forms a particle that binds salt ions and water molecules. According to the model, in order to maintain its active structure the protein competes for water in the high-salt environment by co-ordinating a network of hydrated salt ions [25]. In the procedure for the purification of glucose dehydrogenase from *Hf. mediterranei*, we have replaced the salt by glycerol, which has a similar effect on protein stability to salts [28], but we have maintained $MgCl_2$ at a concentration of 10 mM. This Mg(II) must form part of the active structure of the protein, binding by ionic interactions to its surface.

The zinc content of glucose dehydrogenase is similar to that found in alcohol dehydrogenases. Currently three structurally and catalytically different types of alcohol dehydrogenases are known: medium-chain, zinc-containing alcohol dehydrogenases (350–375 residues), short-chain, non-zinc-containing alcohol dehydrogenases ($\sim$250 residues) and iron-containing alcohol dehydrogenases [29–31]. The glucosedehydrogenases from *B. megaterium* and *B.*
subtilis belong to the short-chain, non-zinc-containing dehydrogenases family, as do several ribitol dehydrogenases, several hydroxysteroid dehydrogenases and others [30], whereas glucose dehydrogenase from *T. acidophilum* belongs to the medium-chain, zinc-containing dehydrogenase family [32]. Zinc-containing alcohol dehydrogenases are dimeric or tetrameric enzymes that bind two atoms of zinc per subunit. One of the zinc atoms is essential for catalytic activity while the other has a structural function. The catalytic zinc is normally co-ordinated by two cysteines and one histidine, but variations occur. A number of other zinc-dependent dehydrogenases, including xylitol dehydrogenases and sorbitol dehydrogenases, are closely related to zinc-containing alcohol dehydrogenases.

The 17 first amino acids of the glucose dehydrogenase from *Hf. mediterranei* were determined [19], and recently we have obtained a longer N-terminal sequence of 38 amino acids. This sequence is shown in comparison with glucose dehydrogenase from different thermophilic *Archaea*, and with some other zinc-dependent dehydrogenases (Fig. 5). This comparison reveals a clear similarity of the *Hf. mediterranei* enzyme to zinc-containing dehydrogenases, suggesting that they belong to the same family. So we can postulate that the Zn ions present in the glucose dehydrogenase from *Hf. mediterranei* could have a similar role as that in the alcohol dehydrogenases of known structure.

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