The pyridine nucleotide-dependent D-glucose dehydrogenase of *Nostoc* sp. strain Mac, a cyanobacterium, is subject to thioredoxin modulation

A. Juhász, V. Csizmadia, G. Borbély, J. Udvardy and G.L. Farkas*

Institute of Plant Physiology, Biological Research Center, PO Box 521, Szeged H-6701, Hungary

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A pyridine nucleotide-dependent D-glucose dehydrogenase (GDH) was isolated and purified about 1000-fold from *Nostoc* sp. strain Mac. The activity of this preparation with NADP as cofactor was 2.8-times that with NAD. This ratio did not change during purification. The enzyme both in crude extracts and after purification proved to be subject to redox modulation. Homologous and heterologous (*Anacystics nidulans*, *Anabaena* sp. strain PCC 7120, spinach) thioredoxins, in the presence of 0.5 mM DTT, deactivated the enzyme. The thioredoxin from *Nostoc* was active with heterologous enzymes: it activated the fructose-1,6-bisphosphatase of *Anacystis nidulans* and the NADP-dependent malate dehydrogenase of spinach. The thioredoxin-mediated reduction decreased the apparent V_{max} value for D-glucose by about 65% and that for NADP by about 51%. The apparent K_m value for NADP increased upon reduction by about 10-fold. The apparent K_m value for D-glucose was but slightly affected by the redox state of the enzyme.

Cyanobacterium *D-Glucose dehydrogenase* (Nostoc *sp. strain Mac*) *Redox modulation* *Thioredoxin*

1. INTRODUCTION

It is generally accepted that in cyanobacteria, a group of photoautotrophic prokaryotes, the only route of carbohydrate breakdown is the oxidative pentose phosphate pathway [1,2]. Most heterotrophic bacteria have alternative routes of carbohydrate breakdown, including the 'direct' oxidation of non-phosphorylated glucose by various types of glucose dehydrogenase [3]. Glucose oxidation by these enzymes is followed by the processing of D-gluconic acid, the reaction product of glucose oxidation [4,5]. Although a pyridine nucleotide (NAD(P)) dependent D-glucose dehydrogenase (GDH; EC 1.1.1.118(9)) has also been found in some cyanobacteria (mainly in those strains which have some heterotrophic capacities [6–8]), little attention has been paid and, in general, no functional role attributed to this enzyme [2,7]. One reason for this is that the general enzymological properties (pH optimum, K_m values, low affinity for D-glucose, substrate and coenzyme specificities, etc.) of the two cyanobacterial GDHs studied [7,8] have been found to be similar to those of the pyridine nucleotide-dependent GDHs isolated from heterotrophic bacteria and liver cells (for pertinent literature see [7,8]). We describe here the observation that the pyridine nucleotide-dependent GDH of *Nostoc* sp. strain Mac, a filamentous cyanobacterium, which is capable of growing both under photoautotrophic conditions and on glucose in the dark, is subject to thioredoxin modulation. This property of the enzyme may be of interest from a functional point of view. No thioredoxin sensitivity of any GDH from any source has so far been reported.

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* To whom correspondence should be addressed

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2. MATERIALS AND METHODS

Nostoc sp. strain Mac was grown axenically in Allen medium [9] illuminated with warm fluorescent light (14 W/m^2) and aerated with a sterile air-CO_2 mixture (95:5, v/v). The heterotrophic cultures were kept in the dark and grown in the same medium supplemented with 1% D-glucose (no CO_2 was given). The samples were centrifuged, the sediment washed with 50 mM Tris-HCl buffer (pH 7.5), and the resedimented sample suspended in a 3-fold volume of the same buffer. The algal suspension was sonicated under cooling for 2 min. The sonicates were centrifuged at 40000 × *g*. The supernatants contained 98% of the total GDH activity. These supernatants were used in the experiments with crude extracts.

The enzyme was purified by $(NH_4)_2SO_4$ fractionation followed by repeated column chromatography on hydroxyapatite and Sephacryl S-200 gels. An approx. 1000-fold purification was achieved.

GDH activity was measured in a reaction system containing 50 mM Tris-HCl buffer at pH 7.5, 5 mM $MgCl_2$, 0.5 mM NADP, 100 mM D-glucose and a suitable amount of the enzyme preparation. NADP was used as a coenzyme in all experiments because both in crude extracts and in the highly purified preparations the enzyme was about 3-times more active with NADP than with NAD. NADP-dependent malate dehydrogenase (MDH) from spinach leaves was purified and analysed as described by Jaquot et al. [10]. Fructose-1,6-bisphosphatase (FBPase) was purified from *Anacystis nidulans* and assayed as in [11].

Thioredoxin-rich protein fractions were isolated as described in [12] for pea chloroplasts and characterized according to the criteria discussed in [13].

Unless stated otherwise in the text, the experiments were carried out with photoautotrophically grown cultures.

3. RESULTS

3.1. *Detection of the redox properties of D-glucose oxidase in crude extracts*

Freshly prepared, highly concentrated crude extracts from cyanobacteria (10000–40000 × *g* supernatants) have proved to be useful in the detection of the redox nature of several cyanobacterial glucose-6-phosphate dehydrogenases (G6PDHs). In such extracts, the oxidized and reduced G6PDH molecules have been found to be in equilibrium [14,15]. Aeration of these preparations led to the formation of highly active, oxidized G6PDH forms, whereas flushing the supernatants with argon resulted in a dramatic decrease in G6PDH activity [14,15]. The redox activation $\rightleftharpoons$ deactivation process could be repeated several times. This has shown that the concentrated crude extracts contain an endogenous, reversible redox system which modulates G6PDH activity according to the electron pressure in the medium [14]. Similarly, the aeration of 10000 × *g* supernatants from *Nostoc* extracts increased their GDH activity. Although simple flushing of the *Nostoc* extracts with argon had little effect, the GDH in freshly prepared extracts from *Nostoc* could be deactivated upon the addition of low amounts of dithiothreitol (DTT). This deactivation proved to be reversible (table 1).

Since thioredoxins, low-M_r naturally occurring compounds, have been shown to be involved in the redox modulation of a number of cyanobacterial and chloroplast enzymes [15], we tested the *Nostoc* extracts for the presence of thioredoxin(s).

3.2. *Detection of thioredoxin in* Nostoc *sp. strain Mac cells and its action on* Nostoc *GDH*

Low-M_r (M_r ~12000) protein fractions (fractions 65–85 in fig.1) were obtained from *Nostoc* cultures as described in the legend to fig.1. These

Table 1

Redox modulation of D-glucose dehydrogenase in 10000 × *g*, freshly prepared supernatants from sonicates of *Nostoc* sp. strain Mac

Treatment	Enzyme activity (μmol · mg protein^{-1} · min^{-1})
None	30.5
Aeration for 60 min	59.0
Treatment with 0.5 mM DTT for 60 min	10.2
Aeration for 60 min followed by treatment with 0.5 mM DTT for 40 min	19.1

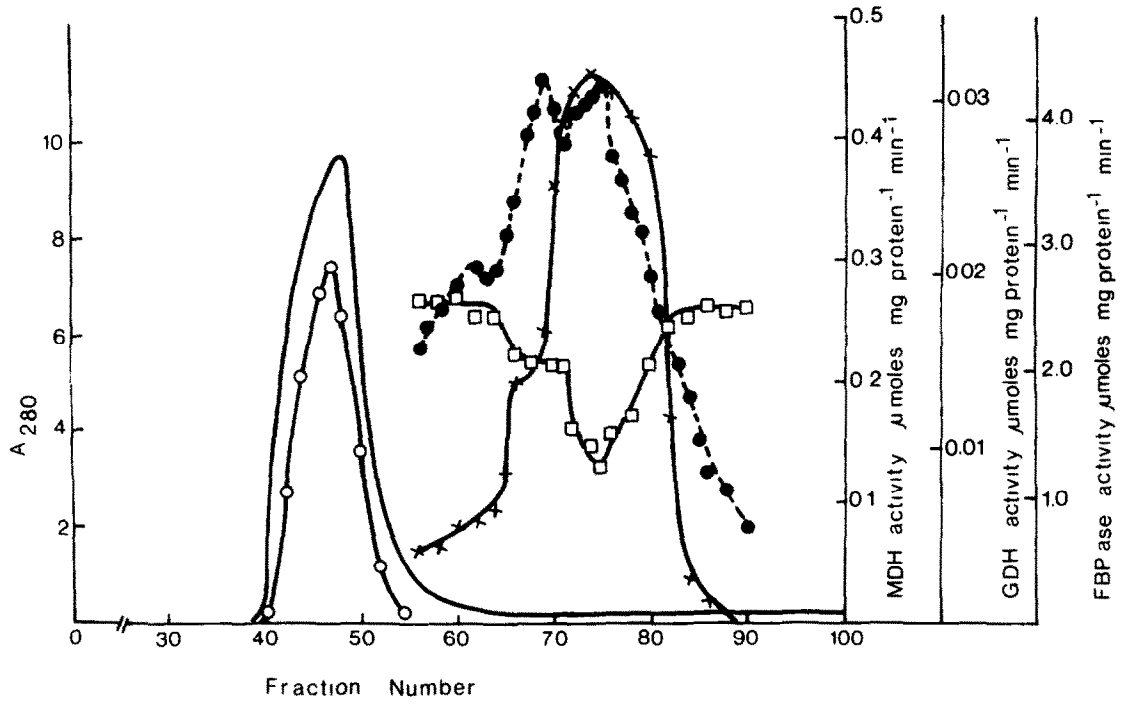

Fig.1. Separation by gel chromatography of high-M_r compounds (including GDH) from low-M_r compounds (which modulate GDH, FBPase and MDH) in extracts from cells of *Nostoc* sp. strain Mac. Supernatants of sonicated cells obtained at 40000 × *g* were fractionated by $(NH_4)_2SO_4$ precipitation and chromatographed on a Sephadex G-50 column as described by Jacquot et al. [10]. Thioredoxin activity in the fractions was tested by the deactivation of purified *Nostoc* GDH and activation of purified *Anacystis* FBPase as well as spinach NADP-dependent MDH added to the eluted fractions with 0.5 mM DTT, essentially as described for chloroplast G6PDH and thioredoxin by Ashton et al. [12]. In the absence of DTT, none of the eluted fractions affected the activity of these enzymes. A 0.5 mM amount of DTT had no direct effect on the purified GDH, FBPase or MDH. (——) Proteins; (○) GDH activity in the eluted, macromolecular fractions; (□) GDH, (●) FBPase, and (×) MDH activity, respectively, in the eluted low-M_r fractions in the presence of 0.5 mM DTT.

were tested for thioredoxin-content as reported in [13]. The pooled fractions deactivated the purified GDH enzyme preparation in the presence of 0.5 mM DTT but not by DTT alone at this low concentration, as expected from a thioredoxin. Fig.1 shows that the *Nostoc* thioredoxin was also active in other thioredoxin-dependent reactions. It activated a cyanobacterial FBPase isolated from *A. nidulans* and also activated the NADP-dependent malate dehydrogenase obtained from spinach. Alternatively, reduced, heterologous thioredoxins (isolated from *A. nidulans, Anabaena* sp. strain 7120 and spinach, respectively) deactivated the *Nostoc* GDH (table 2).

3.3. *The effect of reduced thioredoxin on the kinetic constants of the glucose dehydrogenase from* Nostoc *sp. strain Mac*

The K_m and V_{max} values of the enzyme for glucose and NADP were determined from Lineweaver-Burk plots in both the absence and presence of thioredoxin + 0.5 mM DTT. As shown in table 3, the reduction of the enzyme with thioredoxin resulted in a 10-fold increase in the K_m value for NADP. No significant change was observed in the K_m value for glucose. The V_{max} values were also affected (decreased by reduction of the enzyme), although to a lesser extent. It appears that thioredoxin may regulate GDH activity,

Table 2

Modulation of glucose dehydrogenase from *Nostoc* sp. strain Mac by homologous and heterologous thioredoxin preparations

Specific activity of enzyme from *Nostoc* sp. strain Mac	Additions to the reaction mixture					
	− DTT	+ DTT	+ DTT + thioredoxin preparation from			
			Nostoc sp. strain Mac	*A. nidulans*	*Anabaena* sp. strain 7120	Spinach
μmol · mg protein^{-1} · min^{-1}	105	95	39	32	38	31

Samples of purified GDH preparations (100 μl) were preincubated for 40 min in the absence or presence of 0.5 mM DTT as well as the same amount of DTT plus a suitable amount of protein fraction endowed with thioredoxin activity (50–100 μl, depending on the modulator capacity of the preparation) for 40 min in a final volume of 200 μl Tris-HCl buffer, pH 7.5. The concentration of DTT was chosen so that DTT did not affect the enzyme activity markedly without the addition of a low-M_r protein fraction. After preincubation, a suitable amount of the mixture was assayed for GDH activity. The figures given were calculated from values obtained after 1 min of measurement

Table 3

Kinetic constants of the oxidized and reduced forms of D-glucose dehydrogenase purified from *Nostoc* sp. strain Mac

Substrate	Apparent K_m value		V_{max} (μmol · mg protein^{-1} · min^{-1})	
	Oxidized[a]	Reduced[b]	Oxidized	Reduced
D-Glucose[c]	14 mM	15 mM	0.40	0.14
NADP[d]	24 μM	285 μM	0.78	0.40

[a] Untreated
[b] Treated with 0.5 mM DTT + *Nostoc* thioredoxin
[c] Measured in the presence of 0.5 mM NADP
[d] Measured in the presence of 100 mM glucose

at least in part, by a thioredoxin-induced shift of the K_m value for NADP.

4. DISCUSSION

Buchanan [16] has entertained the idea that the reductively activated enzymes of chloroplast carbohydrate metabolism are involved in synthetic reactions whereas the few reductively deactivated enzymes (e.g. G6PDH) participate in breakdown processes. The more limited observations on cyanobacteria are in line with this hypothesis. The behavior of GDH of *Nostoc* corroborates this view. The advantage for the chloroplast or a cyanobacterial cell of a greatly 'segregated' carbon flow in the light (reductive conditions) and in the dark (oxidizing conditions), respectively, is obvious from the point of view of carbon economy. The question arises: what is the advantage for a cyanobacterial cell capable of growing under heterotrophic conditions in the dark to be subject to redox modulation? No unequivocal answer can be given to this question as yet. One possibility is that, to be able to grow in the dark (not only to survive as the obligate photoautotrophs do), the cyanobacterial cells need more energy which may be supplied by the oxidation of NADPH produced and/or by the utilization of NADPH for reductive syntheses. The NADPH produced by GDH may supplement the NADPH supplied by the operation of the oxidative pentose phosphate pathway which functions in chloroplasts and cyanobacteria in the dark [1]. It is pertinent to emphasise that in agreement with Pulich and Van Baalen [6], we found in heterotrophically grown *Nostoc* cells a 3-fold higher GDH activity than in the autotrophically grown ones (not shown). It should be stressed that the specific GDH activity in *Nostoc* is not at all negligible. It is comparable to that of *Nostoc* G6PDH, the key enzyme of the oxidative pentose phosphate cycle.

The results presented well establish the modulatability of the *Nostoc* GDH by thioredoxins. More has to be learned about the exact mechanism of enzyme modulation. We have preliminary evidence that the apparent M_r of the enzyme (~230000) decreases upon reduction. This might indicate subunit interaction. Indeed, reversible dissociation of GDH into subunits has just been reported for the GDH of *Bacillus megatherium* [17].

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