Interaction between hysteretic regulation and redox modulation of glucose-6-phosphate dehydrogenase from *Anacystis nidulans*

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The glucose-6-phosphate dehydrogenase (G6PDH) of cyanobacteria is a hysteretic enzyme which is also subject to redox modulation [FEBS Lett. 126 (1981) 85–88]. We have found that the hysteretic and redox properties of G6PDH exhibit specific interactions: (1) The hysteretic forms of G6PDH ('hypoactive' $\rightleftharpoons$ 'hyperactive'), obtained at pH 7.5 and 6.5, respectively, differ in their redox properties. The 'hypoactive' form is easily activated by oxidation whereas the 'hyperactive' form is easily deactivated by reduction. (2) At low G6P concentrations (>1 mM) only the oxidized form of G6PDH has significant activity. An increase in G6P level diminishes the difference between the activity of oxidized and reduced G6PDH forms.

Anacystis nidulans *Glucose-6-phosphate dehydrogenase* *Hysteretic enzyme* *Oxidative pentose phosphate pathway* *Redox modulation*

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), the first committed enzyme of the oxidative pentose phosphate pathway, is known to play a role in the regulation of carbon flow in the cell [1]. The regulatory properties of different G6PDHs vary to a great extent [1]. Those of the cyanobacterial G6PDHs are of special importance because the oxidative pentose phosphate pathway is the only route of carbohydrate breakdown in cyanobacteria [2].

The regulatory function of the cyanobacterial G6PDHs is supposed to be based, at least in part, on their hysteretic properties (they exhibit slow responses in enzyme activity to rapid changes in ligand concentration [3,4]). (For a review on hysteretic enzymes see [5].)

The cyanobacterial G6PDHs are, however, also subject to redox modulation [6]. The hysteretic and redox properties of these enzymes can affect each other and this interaction may provide novel mechanisms for delicate metabolic control.

In spite of their relevance to the regulation of enzyme activity in vivo, little is known about hysteretic/redox interactions. No pertinent data are available on G6PDH and no role for such interactions in the regulation of the oxidative pentose phosphate pathway has been considered. Here we present evidence for the interaction of hysteretic and redox properties in a cyanobacterial G6PDH. The possible significance of these interactions in the regulation of carbon flow in the cyanobacterial cell will be discussed.

2. MATERIALS AND METHODS

Anacystis nidulans cells were grown as described [7] and extracted with as small amounts of 50 mM Tris–maleate buffer as possible (1:3 v/v) in order to keep the in vitro concentrations of proteins and small M_r substances close to the in vivo levels [4,6]. If not stated otherwise, freshly prepared, 10000 × *g*, membrane-containing supernatants were used for the assays the details of which are described in

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the legends to the tables 1 and 2, and fig.1. In some experiments a purified G6PDH preparation was used. The steps of purification were essentially the same as those recently described for a cyanobacterial fructose-1,6-bisphosphatase [8], except that the cells were extracted in 50 mM Tris–maleate buffer containing 1% β-mercaptoethanol and 50 mM $MgCl_2$ and that the fractions were assayed for G6PDH activity. The specific activity of the preparation was 12.8 μmol.mg protein^{-1}.min^{-1}. Partially purified thioredoxin preparations were obtained by the procedure described in [9], with slight modifications. Oxidative and reductive modulation of the enzyme preparations was carried out as described in [6].

2.1. *The G6PDH forms exhibit different redox properties*

An aggregated, high-activity ('hyperactive') molecular species of the cyanobacterial G6PDH is slowly formed upon incubation of the enzyme at pH 6.5 before assay [3,4]. Preincubation at pH 7.5 leads to deaggregation of the oligomeric enzyme and to the formation of a low-activity ('hypoactive') enzyme form (for terminology and more details see [3,4]). Since light induces shifts in the pH in photosynthetic prokaryotes [10,11] and also produces reducing power involved in the redox modulation of photosynthetic enzymes [12], it appeared to be of interest to find out whether or not the pH-induced hysteretic forms of G6PDH [3] have identical or different redox properties. To test this, 10000 × *g* supernatants were prepared from sonicated *Anacystis* cells at pH 6.5 and 7.5, respectively. The extracts were subjected to oxidation or reduction [6] and aliquots were assayed for G6PDH activity. As shown in table 1, the hyperactive form could be activated by oxidation only to a small extent but it was dramatically deactivated upon reduction. In contrast, the hypoactive enzyme could be highly activated upon aeration and was but moderately deactivated under reducing conditions. Thus, the two pH-induced forms of G6PDH were affected differently by the redox milieu.

Slow shifts in the equilibrium of the 'hypoactive' $\rightleftharpoons$ 'hyperactive' G6PDH enzyme forms can also be induced by changes in the substrate concentration. High substrate levels (~10 mM) tend to shift the equilibrium to the right and low

Table 1

Redox modulation of the pH-induced 'hyperactive' and 'hypoactive' forms of G6PDH in 10000 × *g* supernatants from *Anacystis*

Redox treatment before assay	G6PDH activity[a]			
	'Hyperactive' form[b] (pH 6.5)	% activity	'Hypoactive' form[c] (pH 7.5)	% activity
Fresh sample (control)	30.0	100	10.0	100
Oxidized after preincubation at the suitable pH[d]	35.0	117	41.0	410
Reduced after preincubation at the suitable pH[e]	6.0	20	4.0	40

[a] μmoles of NADP reduced during the first 5 min of the reaction per 10^8 cells. This comparatively long assay period was chosen to allow sufficient time for slow interactions to occur. The assay system contained 2 mM G6P and 0.12 μM NADP in 50 mM Tris–maleate buffer, pH 7.5 (the pH-optimum of the enzyme). Aliquots of the supernatant were preincubated at pH 6.5 and 7.5, respectively (b,c), then oxidized or reduced (d,e), and 5 μl of the supernatant were injected into the assay medium (1 ml). The reaction was followed spectrophotometrically at 340 nm

[b] Obtained by preincubation of aliquots of the supernatant at pH 6.5 for 20 min [3,4]

[c] Obtained as 'b', at pH 7.5 [3,4]

[d] Aerated for 20 min [6]

[e] Flushed with argon for 20 min [6]

substrate concentrations (<0.5 mM) to the left [3,4]. Since light/dark transitions induce changes in both the G6P level [13] and the redox milieu [6], the interaction of substrate-induced and redox effects on the enzyme may also have relevance for the in vivo conditions.

To study this problem, we measured the effect of different substrate levels on the activity of the oxidized and reduced forms of G6PDH, respectively. It may be seen in fig.1 that the activity versus substrate concentration curve of the oxidized enzyme was hyperbolic whereas that of the reduced en-

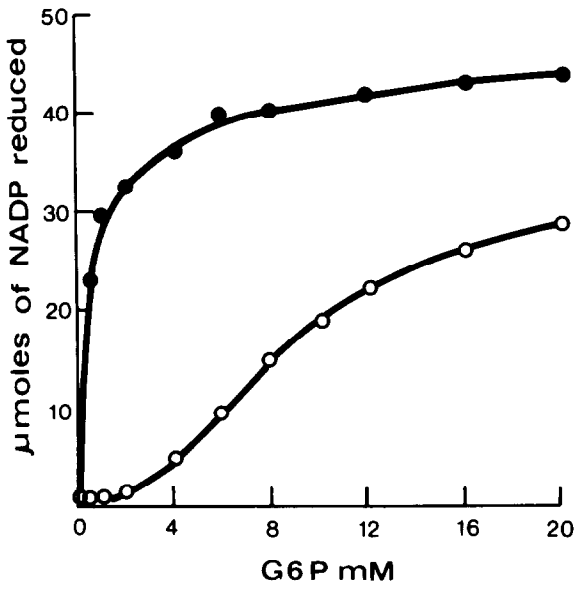

Fig.1. Activity of the oxidized (●) and reduced form (○) of *Anacystis* G6PDH as a function of G6P concentration. 10000 × *g* supernatants from *Anacystis* cells aerated for 20 min or flushed with argon for 30 min as in [6], yielded the oxidized and reduced enzyme forms, respectively. G6PDH activity was measured in aliquots as described in the legend to table 1, except for varying the G6P concentration.

zyme exhibited strong cooperativity. This observation is in line with the earlier finding [6] that the oxidized enzyme is hyperactive (aggregated). The hyperactive state is apparently not changed considerably by increasing the substrate concentration. Thus, a regular, Michaelis–Menten type substrate saturation curve is obtained. However, the reduced enzyme is in the hypoactive (deaggregated) form [6] and should, therefore, undergo a transition into a more active form upon incubation with high substrate concentrations [3]. This transition may explain the sigmoid character of the substrate saturation curve of the hypoactive enzyme.

Figure 1 shows that the redox modulation of G6PDH is efficient only at low substrate levels. In the µM substrate concentration range, the enzyme is up to 50-fold more active in its oxidized than in its reduced state. This property is quite important for the operation of a redox, regulatory enzyme in vivo.

2.2. *The substrate concentration affects the thioredoxin-mediated modulation of G6PDH*

Results similar to those presented in the previous paragraph can also be obtained on purified, reconstituted systems. Thioredoxin, coupled to the photosynthetic electron transport chain, or reduced chemically by dithiothreitol (DTT), affects the activity of most redox (light/dark modulated) enzymes in the photoautotrophic cell [12]. Thioredoxins are present in *Anacystis* [14] and may, therefore, be involved in hysteretic/redox interactions.

Table 2, indeed, shows that the purified *Anacystis* G6PDH could be deactivated by reduced thioredoxins to a high extent if a hyperactive G6PDH preparation, obtained by preincubation at pH 6.5, was used. Smaller thioredoxin-induced effects were obtained with G6PDH preparations preincubated at pH 7.5 (cf., the results with those presented in table 1).

Table 2

Modulation by thioredoxins of the activity of the pH-induced different molecular forms of a purified G6PDH preparation

Treatment	G6PDH activity			
	'Hyper-active' form (pH 6.5)	% activity	'Hypo-active' form (pH 7.5)	% activity
Fresh sample	25.0	100	12.0	100
Anacystis thioredoxin (30 µl purified fraction) + 5 mM DTT added	12.5	50	9.6	80
Spinach thioredoxin (30 µl purified fraction) + 5 mM DTT added	2.5	10	9.0	75
Fresh sample + 5 mM DTT	23.8	95	12.0	100

The two pH-dependent forms of G6PDH were produced as described in the legend to table 1 and treated with thioredoxin + DTT for 30 min or with DTT alone as control. For further details see the legend to table 1

3. DISCUSSION

Three different mechanisms have been proposed to play a role in the regulation (inhibition in the light) of the cyanobacterial G6PDH:

(i) Photosynthetically produced ATP and NADPH, negative effectors of G6PDH, inhibit the enzyme in the light (for a review see [2]).

(ii) G6PDH is a hysteretic enzyme which has interconvertible low- and high-activity forms. The two forms are in equilibrium which can be shifted, e.g., by the pH. Light increases the pH in the cell [10,11] and leads to a relative increase in the low activity form [3].

(iii) G6PDH is a redox protein which is reductively deactivated in the light via the photosynthetic electron transport chain [6] (cf., also the chloroplast G6PDH [15]).

These mechanisms may interact in the regulation of G6PDH. We have studied several of these interactions, especially those between (ii) and (iii). The following major conclusions are worth stressing:

(1) The redox modulation of G6PDH is extremely powerful, but only at low G6P concentrations. Any increase in G6P level tends to decrease the difference in activity between the oxidized and the reduced forms of G6PDH (fig.1).

(2) The high- and low-activity hysteretic forms of G6PDH have distinctly different redox properties. The hypoactive form, prevalent at the pH of the illuminated cell, is easily activated by oxidation, in contrast to the hyperactive form, prevalent at a lower pH, which is easily deactivated by reduction (table 1). A very powerful system, the photosynthetic electron transport chain, and compounds reduced by it, must keep the hypoactive form in an inactive, reduced state to inhibit the uneconomic flow of carbon via the oxidative pathway in the light. The fact that an easily oxidizable enzyme form is kept reduced and inactive in the light may have a physiological significance. The enzyme is triggered to change readily into its high-activity oxidized form as soon as the electron pressure stops.

REFERENCES

[1] Levy, R.H. (1979) Adv. Enzymol. 48, 97–192.
[2] Stanier, R.Y. and Cohen-Bazire, G. (1977) Ann. Rev. Microbiol. 31, 225–274.
[3] Schaeffer, F. and Stanier, R.J. (1978) Arch. Microbiol. 116, 9–19.
[4] Balogh, Á., Borbély, G., Cséke, Cs., Udvardy, J. and Farkas, G.L. (1979) FEBS Lett. 105, 158–162.
[5] Frieden, C. (1979) Ann. Rev. Biochem. 48, 471–489.
[6] Csékc, Cs., Balogh, Á. and Farkas, G.L. (1981) FEBS Lett. 126, 85–88.
[7] Udvardy, J., Sivók, B., Borbély, G. and Farkas, G.L. (1976) J. Bacteriol. 126, 630–633.
[8] Udvardy, J., Godeh, M. and Farkas, G.L. (1982) J. Bacteriol. 151, 203–208.
[9] Jacquot, J.P., Vidal, J., Gadal, P. and Schürmann, P. (1978) FEBS Lett. 96, 243–246.
[10] Falkner, G., Horner, F., Werdan, K. and Heldt, H.W. (1976) Plant Physiol. 58, 717–718.
[11] Kallas, T. and Dahlquist, F.W. (1981) Biochemistry 20, 5900–5907.
[12] Buchanan, B.B. (1980) Ann. Rev. Plant Physiol. 31, 341–374.
[13] Pelroy, R.A., Kirk, M.R., Levine, G.A. and Bassham, A. (1976) J. Bacteriol. 128, 623–632 and 633–643.
[14] Schmidt, A. (1981) Planta 152, 101–104.
[15] Scheibe, R. and Anderson, L.E. (1981) Biochim. Biophys. Acta 636, 58–64.