BBA 63279

The effect of some metabolites on glucose-6-phosphate dehydrogenase activity in loach embryos

It is well known that Glc-6-*P* dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49) is the key enzyme of the hexose monophosphate shunt. The excellent investigations of DICKENS, GLOCK AND MCLEAN[1] and GLOCK AND MCLEAN[2] have shown that the rate of Glc-6-*P* oxidation is determinated by the $NADP^+$:NADPH ratio in cytoplasm. Consequently, the rate of NADPH oxidation to a considerable degree controls Glc-6-*P* oxidation *via* the hexose monophosphate shunt. Recently, several authors have demonstrated that the activity of Glc-6-*P* dehydrogenase is inhibited by palmityl-CoA, stearyl-CoA[3,4] and by some steroids[5,6]. According to AVIGAD[7] ATP appeared to be the inhibitor of yeast Glc-6-*P* dehydrogenase.

In this report results are given on the control of Glc-6-*P* dehydrogenase activity by ATP, NADPH and by Fru-1,6-P_2. The Glc-6-*P* dehydrogenase from loach embryos was isolated by the following procedure. 40 g (fresh weight) of unfertilized loach (*Misgurnus fossilis*) eggs or 40 g of fertilized eggs at the early stages of cleavage were homogenized with 15 ml of 0.1 M $NaHCO_3$. The large granules were sedimented by centrifugation at 8000 × *g* for 15 min. 17 g of $(NH_4)_2SO_4$ were slowly added to the supernatant during 40 min. After centrifugation at 8000 × *g* for 20 min, the resulting residue was discarded. 2 g of $(NH_4)_2SO_4$ were added to the clear supernatant and the sediment which contained the main part of Glc-6-*P* dehydrogenase activity was collected after centrifuging at 18 000 × *g* for 30 min. The residue was redissolved in 50 ml of cold water, and 3.5 g of $(NH_4)_2SO_4$ were slowly added to the solution. After the centrifugation at 18 000 × *g* for 15 min, the sediment was collected and redissolved in 0.1 M Tris (pH 7.5).

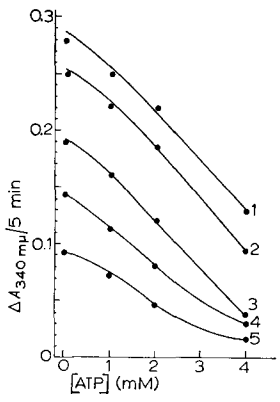
The resulting preparation of Glc-6-*P* dehydrogenase was free from traces of adenylate kinase (EC 2.7.4.3), ATPase (EC 3.6.1.3), fructosediphosphate aldolase (EC 4.1.2.13), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), fructosediphosphatase (EC 3.1.3.11) and glyceraldehydephosphate dehydrogenase (EC 1.2.1.12). Contamination with 6-phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44) did not exceed 2%.

The activity of Glc-6-*P* dehydrogenase was determined by the increase of absorbance at 340 mμ for 5 min in a 1-cm quartz cell. The reaction mixture contained 40 mM Tris (pH 7.3–6.2), 0.35 mM $NADP^+$ and 0.05–1.0 mM Glc-6-*P*.

Maximum activity was found in the pH range 8.0–8.5, but the maximum inhibiting effect of ATP was observed at relatively low pH (7.3–6.2). The effect of ATP is clearly dependent on the substrate concentration: at 1 mM of substrate the increase in ATP concentration from 0.5 to 4.0 mM reduced the activity of Glc-6-*P* dehydrogenase approx. 2-fold; the lowering of the substrate concentration to 0.05 mM resulted in a 5-fold decrease of enzyme activity (Fig. 1).

The double-reciprocal plot method gives a K_m value of about $9 \cdot 10^{-5}$ M; in the presence of ATP, this value is considerably increased (Fig. 2).

Citrate, ADP, 5-AMP and 3,5-cyclic AMP in concentrations between 1 and 3 mM did not change the activity of Glc-6-*P* dehydrogenase from loach embryos; in



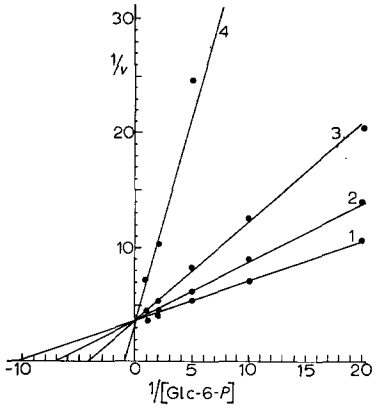


Fig. 1. Effect of ATP on Glc-6-*P* dehydrogenase activity at different substrate concentrations. The assay mixture contained 40 mM Tris (pH 7.3), 0.35 mM $NADP^+$, 0.05–1.0 mM Glc-6-*P*, 0–4 mM ATP and 0.03 unit of enzyme in a total volume of 3 ml. 1, 1.0 mM Glc-6-P; 2, 0.5 mM Glc-6-*P*; 3, 0.2 mM Glc-6-*P*; 4, 0.1 mM Glc-6-*P*; 5, 0.05 mM Glc-6-*P*. Activity is expressed in increase of absorbance at 340 mμ in 5 min.

Fig. 2. Plot of reciprocal of reaction velocity *versus* reciprocal of Glc-6-*P* concentration. The conditions for the enzyme assay are the same as those in the legend to Fig. 1. 1, without ATP; 2, with 1 mM ATP; 3, with 2 mM ATP; 4, with 4 mM ATP.

contrast, the addition of Fru-1,6-P_2 enhanced the activity. This effect could be observed only at pH 6.2 (Tris maleate) and at substrate concentrations not exceeding 1.5 mM (Fig. 3). The activating effect of Fru-1,6-P_2 is completely abolished by the addition of 3 mM $MgCl_2$. The presence of 1–3 mM EDTA caused no detectable effect. The Fru-1,6-P_2 used in the experiments was free from hexose monophosphates according to the enzymatic tests using an excess of yeast Glc-6-*P* dehydrogenase and $NADP^+$ (ref. 8).

Thus, it seems that ATP and Fru-1,6-P_2 are the factors controlling the activity of Glc-6-*P* dehydrogenase and the level of 6-phosphogluconate in the embryo cell under aerobic and anaerobic conditions. As was shown earlier[9] in anaerobiosis the ATP level decreases about 60% with respect to the control and the Fru-1,6-P_2 level

TABLE I

THE EFFECT OF NADH ON THE ACTIVITY OF Glc-6-*P* DEHYDROGENASE

The reaction mixture contained 40 mM Tris (pH 7.5), 0.35 mM NADH, 1 mM Glc-6-*P*, 0.02 unit of enzyme and variable amounts of NADPH (Boehringer)*. The activity of enzyme is expressed in increase of absorbance at 340 mμ in 5 min.

Source of enzyme	*Control*	*NADPH added (mM)*		
		0.017	*0.035*	*0.35*
Glc-6-*P* dehydrogenase from loach eggs	0.22	0.18	0.16	0.08
Glc-6-*P* dehydrogenase isolated from yeast according to KORNBERG AND HORRECKER[10]	0.21	0.16	0.14	0.04

* The results with NADPH preparation from Lawson were similar to those obtained with NADPH Boehringer.

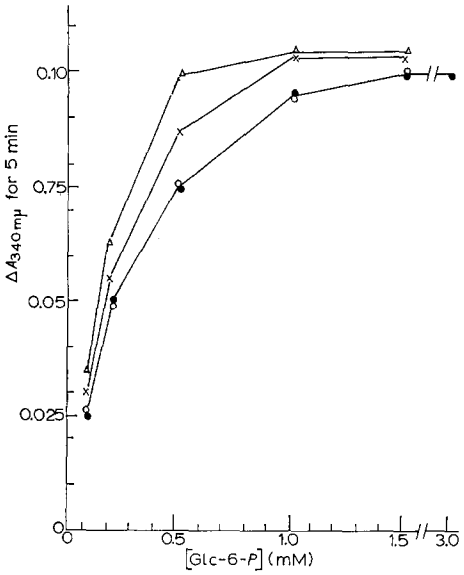


Fig. 3. Effect of Fru-1,6-P_2 on activity of Glc-6-*P* dehydrogenase. The assay mixture consisted of 40 mM Tris maleate (pH 6.2), 0.35 mM $NADP^+$ and 0.03 unit of enzyme in a total volume of 3.0 ml. △—△, 0.12–1.0 mM Fru-1,6-P_2; ×—×, 0.06 mM Fru-1,6-P_2; ●—●, without Fru-1,6-P_2; ○—○, 0.12 mM Fru-1,6-P_2 + 3 mM $MgCl_2$.

increases approx. 2-fold. This is followed by an increase in the intracellular activity of Glc-6-*P* dehydrogenase. The above conclusion is also supported by the fact that the level of 6-phosphogluconate in anaerobiosis considerably increased (from 7.7 to 13 mμmoles per 100 embryos). The increase in intracellular activity of Glc-6-*P* dehydrogenase in anaerobiosis, however, is limited by the inhibiting effect of NADPH—the product of this reaction. Excess of NADPH was found to inhibit the activity of Glc-6-*P* dehydrogenase from loach eggs and from yeast (Table I).

Institute of Developmental Biology,
Academy of Sciences of the U.S.S.R.,
Moscow (U.S.S.R.)

YU. G. YUROWITZKI
L. S. MILMAN

1 F. DICKENS, G. E. GLOCK AND P. MCLEAN, *Ciba Found. Symp. Regulation Cellular Metab.*, (1959) 206.
2 G. E. GLOCK AND P. MCLEAN, *Biochem. J.*, 55 (1953) 400.
3 I. EGER-NEUFELD, A. TEINZER, L. WEISS AND O. WIELAND, *Biochem. Biophys. Res. Commun.*, 19 (1965) 43.
4 K. TAKETA AND B. POGELL, *J. Biol. Chem.*, 241 (1966) 720.
5 P. A. MARKS AND J. BANKS, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 447.
6 HSIEN-GIEH SIE AND A. HABLALIAN, *Biochem. J.*, 97 (1965) 32.
7 G. AVIGAD, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 1543.
8 P. A. SRERE, J. COOPER AND E. RACKER, *Arch. Biochem. Biophys.*, 74 (1958) 295.
9 L. S. MILMAN AND YU. G. YUROWITZKI, *Biokhimiya*, 32 (1967) 417.
10 A. KORNBERG AND B. HORRECKER, *J. Biol. Chem.*, 182 (1950) 805.

Received August 14th, 1967
Revised manuscript received October 11th, 1967