INHIBITION OF α-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY IN HUMAN PLACENTAL MITOCHONDRIA BY PHOSPHOENOLPYRUVATE

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Received 5 March 1976

1. Introduction

In previous paper we have shown that mitochondria isolated from human term placenta possess high activity of α-glycerophosphate dehydrogenase (EC 1.1.99.5) [1]. This paper reports the effect of phosphoenolpyruvate on the α-glycerophosphate dehydrogenase activity in human placental mitochondria. Phosphoenolpyruvate has been shown to inhibit competitively this enzyme; the K_i value being about 0.5 mM. 3-Phosphoglyceric acid also inhibits α-glycerophosphate dehydrogenase; however this inhibitory effect is smaller than the effect caused by phosphoenolpyruvate. Other glycolytic intermediates as glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and 2,3-diphosphoglyceric acid were found to be ineffective. It is proposed that phosphoenolpyruvate may be a regulatory factor of α-glycerophosphate dehydrogenase in human placental mitochondria.

2. Materials and methods

DL-α-glycerophosphate (disodium salt), phosphoenolpyruvate (trisodium and tricyclohexylamine salts), phenazine methosulfate (PMS), fructose 1,6-diphosphate and fructose 6-phosphate were obtained from Sigma Chemical Co.; carbonylcyanide *m*-chlorophenylhydrazone (CCCP), and 2,3-diphosphoglyceric acid were from Calbiochem, glucose 6-phosphate and 3-phosphoglyceric acid were from Koch-Light. All other compounds were of the highest purity available commercially.

Human placental mitochondria obtained as described previously [1] were suspended (about 50 mg of mitochondrial protein per 30 ml of solution) in 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, + 0.5 mM EGTA (ethyleneglycol-bis-(β-aminoethylether)*N,N'*-tetraacetic acid). The suspension was centrifuged at 16 000 *g* for 4 min. The pellet obtained was suspended in about 30 ml of 125 mM KCl + 10 mM Tris-HCl, pH 7.4, and centrifuged at 16 000 *g* for 4 min. The last step of the procedure was repeated twice. The final pellet was suspended in 125 mM KCl + 10 mM Tris-HCl pH 7.4 to obtain about 20–30 mg of mitochondrial protein per ml. Protein was estimated as described previously [2].

Respiration was measured with a Clark oxygen electrode at 25°C in 2.5 ml medium containing: 120 mM KCl, 20 mM Tris-HCl pH 7.35, 1 μM CCCP, 10 μM cytochrome *c* and 2–3 mg of mitochondrial protein.

α-Glycerophosphate dehydrogenase activity was assayed as described previously [1] by measuring the rate of oxygen uptake in the reaction coupled with PMS reduction and its subsequent reoxidation in the medium containing: 120 mM KCl, 20 mM Tris-HCl, pH 7.35, 2 mM KCN, 0.35 mM PMS and about 2 mg of mitochondrial protein. Assay temperature was 25°C.

3. Results and discussion

The effect of PEP on α-glycerophosphate oxidation by human placental mitochondria under uncoupled state is shown in fig.1. The addition of 1.2 mM PEP (trisodium or tricyclohexylamine salts) caused an immediate inhibition of α-glycerophosphate oxidation by human placental mitochondria if the substrate was

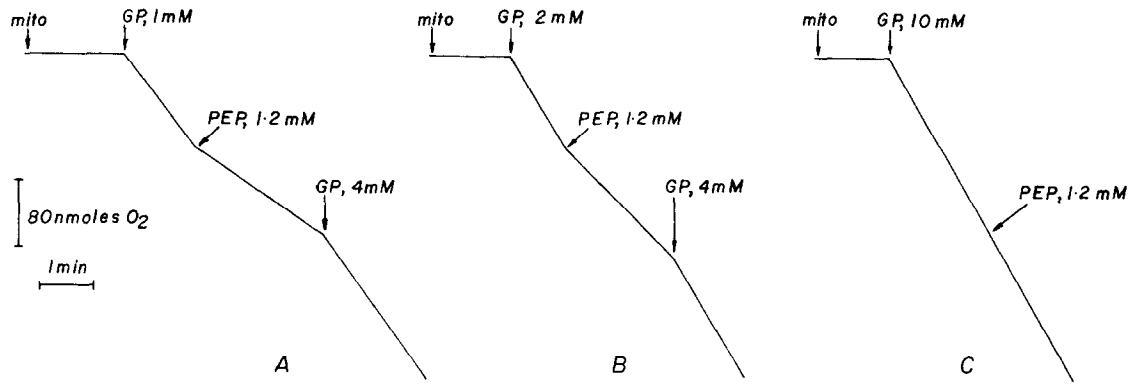

Fig.1. Effect of PEP on α-glycerophosphate oxidation by human placental mitochondria. Experimental conditions as described under materials and methods. Other additions as indicated on the figure. Mitochondria (mito), α-glycerophosphate (GP).

used at 1 or 2 mM concentrations. The extent of the inhibition was dependent on substrate concentration reaching about 65% or 40% at 1 mM and 2 mM α-glycerophosphate concentrations, respectively. This inhibition was almost completely reversed by addition of 4 mM α-glycerophosphate. No inhibitory effect was observed if 1.2 mM PEP and 10 mM α-glycerophosphate were added. The identical inhibition was obtained if oxygen uptake was determined under similar conditions as in fig.1 but in the presence of PMS and KCN. This suggests that PEP acts at the level of the α-glycerophosphate dehydrogenase.

Fig.2 exhibits the effect of increasing concentrations of PEP on α-glycerophosphate dehydrogenase activity. The results are expressed as per cent of the control activity in the absence of the inhibitor. A significant inhibition of α-glycerophosphate dehydrogenase activity was observed when as little as 0.2 mM PEP was present in the incubation medium. The activity of α-glycerophosphate dehydrogenase was considerably depressed by PEP at its concentration range 0.2–1 mM. The degree of inhibition increased as the concentration of PEP was raised, reaching about 50% at 1 mM PEP. 3-Phosphoglyceric acid inhibits also α-glycerophosphate dehydrogenase however considerably less than PEP. Other glycolytic intermediates as glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, pyruvate and 2,3-diphosphoglyceric acid were found to be ineffective at the concentration range 0.5–5 mM.

It is known that α-glycerophosphate dehydrogenase of flight muscle mitochondria [3], lung mitochondria [4] and brown adipose tissue mitocondria [5] are markedly stimulated by very low Ca^{2+} concentrations. In this respect mitochondria from human placenta resemble mitochondria of flight muscle, lung and brown adipose tissue. Therefore, the effect of PEP on α-glycerophosphate dehydrogenase activity was studied in the presence of an excess of calcium ions

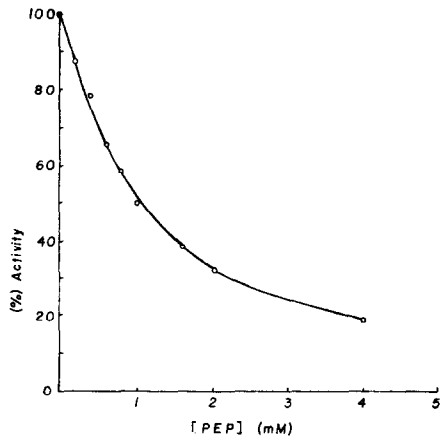

Fig.2. Effect of increasing concentrations of PEP on the activity of α-glycerophosphate dehydrogenase in human placental mitochondria. Mitochondria had been preincubated for 2 min in the presence of PEP at concentrations indicated on the figure in the medium described under materials and methods prior to the addition of 1 mM α-glycerophosphate. Oxygen uptake in the control was 60 nmol/mg of mitochondrial protein per 1 min.

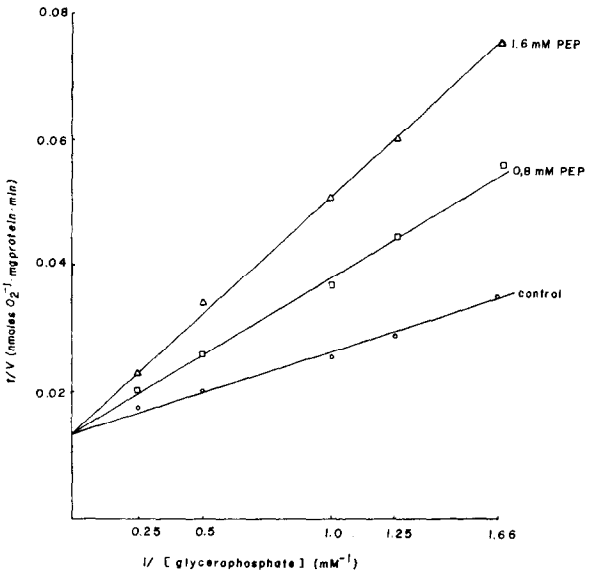

Fig.3. Inhibition by PEP of α-glycerophosphate dehydrogenase activity presented as double reciprocal plot. Mitochondria were preincubated in the medium as described under materials and methods with varied concentrations of PEP for 2 min. Then α-glycerophosphate was added at concentrations indicated on the figure.

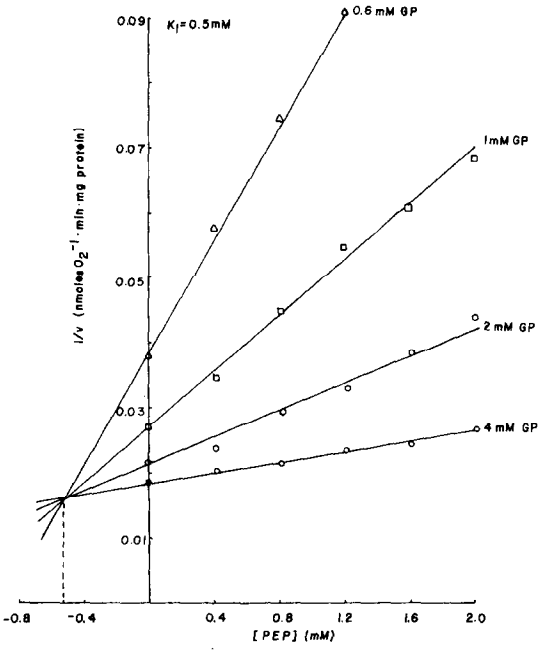

Fig.4. Inhibition by PEP of α-glycerophosphate dehydrogenase activity presented as a Dixon plot. Experimental conditions as described in the legend to fig.3.

under conditions described in the legend to fig.2. The extent of inhibition both in the presence and in the absence of calcium ions was identical (not shown here).

Data presented in fig.1 suggest that PEP is a competitive inhibitor of α-glycerophosphate dehydrogenase with respect to the α-glycerophosphate. Kinetic studies of the effect of PEP on α-glycerophosphate dehydrogenase activity are presented as Lineweaver–Burk plots (fig.3), and reveal an apparent competition between α-glycerophosphate and PEP. The results presented in the form of a Dixon plot (fig.4) showed that under these conditions the K_i value for PEP is about 0.5 mM.

The results of experiments presented above suggest that α-glycerophosphate dehydrogenase activity in human placental mitochondria may be controlled by cytosolic changes in PEP concentration. Taking into consideration that this enzyme catalyses the conversion of α-glycerophosphate to dihydroxyacetone phosphate, which can be metabolised to PEP by glycolytic pathway, a feed-back regulation of α-glycerophosphate dehydrogenase by PEP may be proposed. Our results, as well as the observations that phosphoenolpyruvate inhibits mitochondrial protein synthesis [6] and the net uptake of Ca^{2+} by rat heart and liver mitochondria [7] indicate that this glycolytic intermediate influences several mitochondrial processes.

Acknowledgements

The authors wish to thank Professor L. Zelewski for his interest. The technical assistance of Miss U. Bernatek is greatly acknowledged. This work was supported by a grant from Ministry of Higher Education, Science and Technology within the project I-22, 02, 02.

References

[1] Świerczyński, J., Ścislowski, P. and Aleksandrowicz, Z. (1976) Biochim. Biophys. Acta, in the press.
[2] Świerczyński, J. and Aleksandrowicz, Z. (1974) Biochim. Biophys. Acta 376, 66–75.
[3] Hansford, R. G. and Chappell, J. B. (1967) Biochem. Biophys. Res. Commun. 27, 686–692.
[4] Fisher, A. B., Huber, G. A. and Bassett, D. J. B. (1975) Comp. Biochem. Physiol. 50B, 5–8.
[5] Bukowiecki, L. J. and Lindberg, O. (1974) Biochim. Biophys. Acta 348, 115–125.
[6] McCoy, E. D. and Doeg, K. A. (1972) Biochem. Biophys. Res. Commun. 46, 1411–1417.
[7] Chudapongse, P. and Haugaard, N. (1973) Biochim. Biophys. Acta 307, 599–606.