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REGULATION OF α-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY IN HUMAN TERM PLACENTAL MITOCHONDRIA

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Summary

1. α-Glycerophosphate dehydrogenase (*sn*-glycerol-3-phosphate:(acceptor) oxidoreductase, EC 1.1.99.5) activity in mitochondria isolated from human term placenta was found to be inhibited by ethyleneglycolbis(β-aminoethyl ether)-*N*,*N'*-tetraacetic acid (EGTA). Addition of an excess of calcium ions to the incubation medium completely restored the original activity. The concentration of free calcium ion required to activate the α-glycerophosphate dehydrogenase was found to vary between 10 and 100 nM.

2. The pH optimum for α-glycerophosphate dehydrogenase activity varied with substrate concentration. The pH optima were 7.4 and 8.0 in the presence of 2 or 8 mM α-glycerophosphate, respectively. The apparent K_m for α-glycerophosphate also varied with pH; the values being 0.4 mM at pH 7.05, 1.5 mM at pH 7.8, and 3.5 mM at pH 8.5.

3. α-Glycerophosphate dehydrogenase activity was inhibited by palmitoyl-CoA in a competitive manner with an apparent K_i value of about 10 μM. This inhibition was less pronounced in the presence of calcium or magnesium ions.

4. The activity of α-glycerophosphate dehydrogenase was inhibited by phospho*enol*pyruvate, D- and DL-glyceraldehyde 3-phosphate and 3-phosphoglyceric acid, in a competitive manner, the apparent K_i values being 0.5, 0.95, 0.12 and 1.5 mM, respectively.

5. α-Glycerophosphate dehydrogenase activity in human placental mitochondria was found to be more sensitive to phospho*enol*pyruvate, than the activity of the same enzyme in rat skeletal muscle mitochondria. α-Glycerophosphate dehydrogenase activity in rat brown adipose tissue mitochondria was only slightly affected by phosph*enol*pyruvate under the same conditions.

6. The data obtained suggest that the activity of α-glycerophosphate dehydrogenase in human placental mitochondria may be controlled by changes of the cytosolic level of palmitoyl-CoA, some glycolytic intermediates, and pH.

Introduction

It has been reported that mitochondria isolated from some tissues posses high α-glycerophosphate dehydrogenase (*sn*-glycerol-3-phosphate:(acceptor) oxidoreductase, EC 1.1.99.5) activity [1—10]. Klingenberg and Buchholz [11] and Donnellan et al. [12] have provided evidence indicating that this enzyme is located on the external surface of the inner membrane. The location of α-glycerophosphate dehydrogenase on the part of mitochondria accessible from outside might facilitate the action of cytosolic metabolites on the enzyme activity. DL-glyceraldehyde 3-phosphate was found by Dawson and Thorne [13] to be a competitive inhibitor of partialy purified α-glycerophosphate dehydrogenase from pig brain mitochondria. Inhibition by DL-glyceraldehyde 3-phosphate of α-glycerophosphate dehydrogenase activity in flight muscle mitochondria was also demonstrated by Donnellan et al. [12]. α-Glycerophosphate dehydrogenase from brown adipose tissue is inhibited by long-chain fatty acyl-CoA esters and by long-chain free fatty acids [14,15].

Estabrook and Sacktor [1] have shown that oxidation of α-glycerophosphate by isolated flight muscle mitochondria is inhibited by addition of EDTA and that this inhibition is reversed by Mg^{2+}, Ca^{2+}, and other divalent metal ions. They concluded that EDTA inhibits α-glycerophosphate dehydrogenase activity. Hansford and Chappell [16] showed that α-glycerophosphate oxidation by isolated flight muscle mitochondria was significantly stimulated by very low concentrations of Ca^{2+} ions and that this cation lowered the K_m for α-glycerophosphate. They suggested that α-glycerophosphate dehydrogenase exhibits allosteric kinetics with regard to substrate. Mitochondrial dehydrogenase from lungs [17] and brown adipose tissue [14] possess similar properties.

Recently we have shown [18] that mitochondria isolated from human term placenta oxidize α-glycerophosphate at a relatively high rate. In the experiments presented here we have been looking for the factors which might regulate the activity of α-glycerophosphate dehydrogenase in placental mitochondria. It was found that the enzyme activity can be regulated by divalent cations, palmitoyl-CoA and some glycolytic metabolites. A part of this work has been already reported [19].

Materials and Methods

Human term placental and skeletal muscle mitochondria were prepared as described previously [18—20]. Brown adipose tissue mitochondria were prepared in 250 mM sucrose + 10 mM Tris · HCl (pH 7.4), from pooled interscapular tissue excised from rats who had lived for at least 10 days at about 0° C. Centrifugation procedure described by Houštěk and Drahota [21] was applied. Both rat skeletal and brown adipose tissue mitochondria were washed in 250 mM sucrose + 10 mM Tris · HCl (pH 7.4) + 0.5 mM ethyleneglycolbis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA) and then in KCl + Tris · HCl (pH 7.4), similarly as human placental mitochondria [19]. Mitochondrial protein was estimated as described previously [22].

α-Glycerophosphate dehydrogenase activity was assayed by measuring the

rate of oxygen uptake in the reaction coupled with phenazine methosulfate reduction and its subsequent reoxidation in the medium containing: 120 mM KCl, 20 mM Tris · HCl (pH 7.4), 2 mM KCN, 0.35 mM phenazine methosulfate and 1.5—3.0 mg mitochondrial protein. Assay temperature was 25° C. Other additions were as indicated in the figures.

DL-α-glycerophosphate (disodium salt), phospho*enol*pyruvate (trisodium and tricyclohexylamine salts), phenazine methosulfate, fructose 1,6-diphosphate, fructose 6-phosphate, palmitoyl-CoA, palmitoyl-carnitine, CoA and palmitic acid were obtained from Sigma Chemical Co. Glucose 6-phosphate, DL-glyceraldehyde 3-phosphate and 3-phosphoglyceric acid were from Koch-Light. 2-phosphoglyceric acid and D-glyceraldehyde 3-phosphate were generous gift from Dr. J. Kwiatkowska (Dept. Biochemistry, Medical School, Wrocław, Poland).

Results

It has been shown in previous paper [18] that the rate of α-glycerophosphate oxidation by human term placental mitochondria was strongly inhibited by EDTA or EGTA. This inhibition was reversed by the subsequent addition of calcium ions. Magnesium ion reversed the inhibition caused by EDTA, but was ineffective in the presence of EGTA. The stimulation of α-glycerophosphate dehydrogenase activity in human term placental mitochondria by calcium ion is shown in Fig. 1. The actual calcium ion concentration required for the enzyme stimulation was determined by using EGTA/Ca^{2+} buffer in which the free calcium ion could have been calculated according to Portzehl et al. [23]. Fig. 1 shows an increase of α-glycerophosphate dehydrogenase ac-

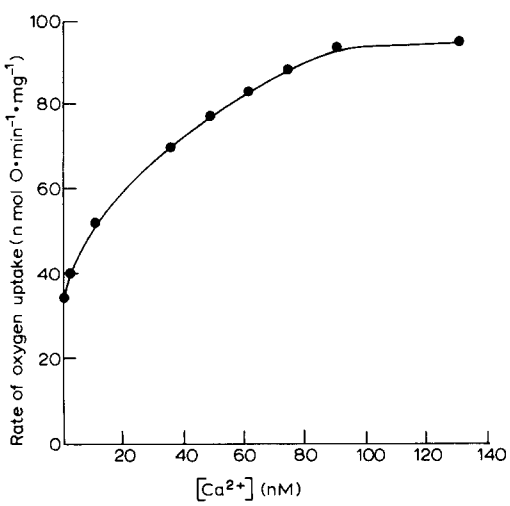


Fig. 1. The effect of stabilized low concentration of Ca^{2+} ion on α-glycerophosphate dehydrogenase activity in human placental mitochondria. Incubation was carried out in the medium described under Materials and Methods supplemented with 1 mM α-glycerophosphate, 2 mM EGTA, to which $CaCl_2$ had been added to give the free Ca^{2+} concentration indicated on the figure.

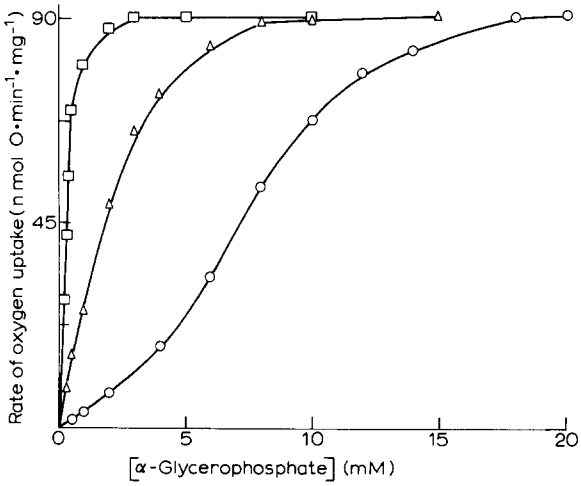


Fig. 2. α-Glycerophosphate dehydrogenase activity vs. α-glycerophosphate concentration in human placental mitochondria in the presence of added 0.2 mM (△) and 4 mM (○) EGTA, and 0.2 mM or 4 mM EGTA + 0.2 μM free Ca^{2+} ions (□). Experimental conditions were as described under Materials and Methods. Concentration of free Ca^{2+} was calculated according to the method Portzehl et al. [23].

tivity as a function of calcium ion concentration. Maximal stimulation was achieved at about 0.1 μM concentration of free calcium ion. Maximal rate of α-glycerophosphate oxidation under these conditions was identical with those obtained while mitochondria isolated and incubated in the absence of any cation-chelators were used. The concentration of free Ca^{2+} required for maximal stimulation of α-glycerophosphate dehydrogenase activity in human placental mitochondria was similar to that reported for mitochondria isolated from other tissues [9,14,16]. Fig. 2 shows α-glycerophosphate dehydrogenase activity versus α-glycerophosphate concentration in the presence of 0.2 or 4 mM EGTA, and in the presence of 0.2 or 4 mM + 0.2 μM free calcium ions (concentration of free Ca^{2+} was calculated according to Portzehl et al. [23]). In the absence of added calcium ion, EGTA caused an increase of the apparent K_m for α-glycerophosphate. At 0.2 mM EGTA the plot was hyperbolic; however, it changed for sigmoidal in the presence of 4 mM EGTA. Thus, calcium ion acted as a positive modifier by lowering the apparent K_m for the substrate. The allosteric kinetics in the presence of 4 mM EGTA were also revealed by calculating the Hill coefficient which was about 2.4 in a series of three different experiments (not shown).

Data presented in Fig. 3 demonstrate that the apparent Michaelis constant for α-glycerophosphate was increasing with the raising of pH, the values determined from Lineweaver-Burk plots being 0.4 mM at pH 7.05, 1.5 mM at pH 7.8 and 3.5 mM at pH 8.5. The response of α-glycerophosphate dehydrogenase activity to changes in pH varied with α-glycerophosphate concentration (not shown). In the presence of α-glycerophosphate at concentration of 2 and 8 mM the pH optima were about 7.4 and 8.0, respectively.

Fig. 4 shows the effect of increasing concentrations of palmitoyl-CoA on α-glycerophosphate dehydrogenase activity in human placental mitochondria.

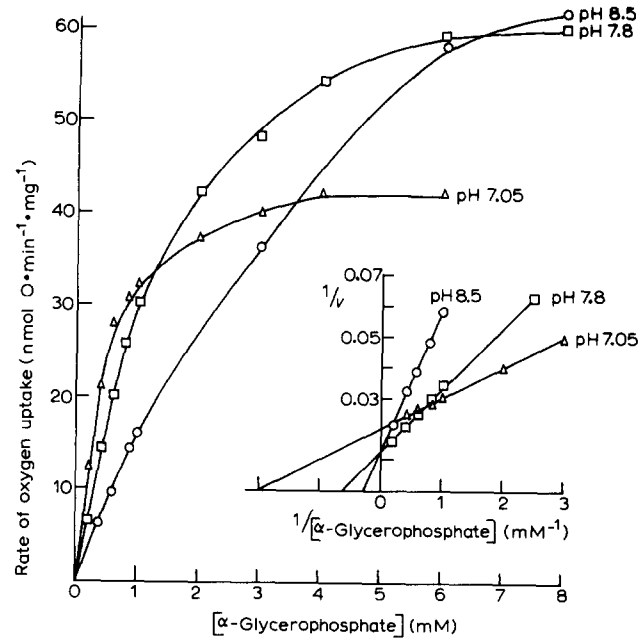


Fig. 3. The influence of pH on K_m values of α-glycerophosphate dehydrogenase in human placental mitochondria for α-glycerophosphate. Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration and pH were as indicated on the figure.

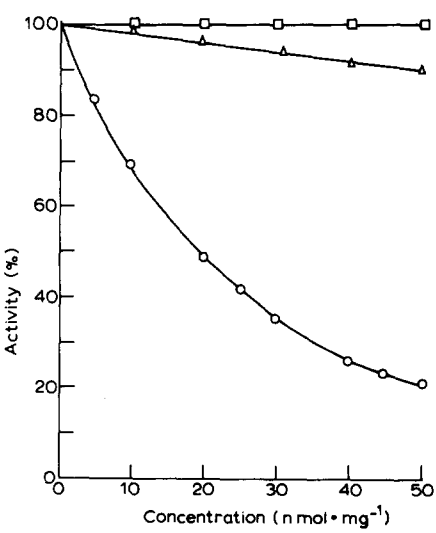


Fig. 4. The effect of palmitoyl-CoA (○), palmitoylcarnitine (△), palmitic acid, CoA, and carnitine (□) on α-glycerophosphate dehydrogenase activity in human placental mitochondria. Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration was 1 mM.

The results are expressed as per cent of the control activity in the absence of an inhibitor. The activity of this enzyme was considerably depressed by palmitoyl-CoA at its concentration range 6—50 nmol/mg^{-1} protein. The degree of inhibition increased as the concentration of palmitoyl-CoA was raised, reaching about 50% at 20 nmol · mg^{-1} protein. Palmitic acid, CoA and carnitine used at concentration range 10—100 nmol · mg^{-1} of mitochondrial protein were ineffective under these conditions. Palmitoylcarnitine affected the enzyme activity only slightly. Kinetic studies of the effect of palmitoyl-CoA on α-glycerophosphate dehydrogenase activity revealed that palmitoyl-CoA acted as a competitive inhibitor with respect to the substrate exhibiting apparent K_i value of about 10 μM. Data presented in Figs. 5 and 6 indicate that palmitoyl-CoA in the presence of added Ca^{2+} or Mg^{2+} ions exerted considerably less inhibitory effect on α-glycerophosphate dehydrogenase activity than in the absence of these cations.

Recently we have shown [19] that phospho*enol*pyruvate strongly inhibits the activity of α-glycerophosphate dehydrogenase from human term placental mitochondria. It is evident from the results presented in Fig. 7 that α-glycerophosphate dehydrogenase activity in human placental mitochondria was more sensitive to phospho*enol*pyruvate than the dehydrogenase in rat skeletal muscle mitochondria. In the same conditions, α-glycerophosphate dehydrogenase in mitochondria isolated from rat brown adipose tissue was only slightly affected

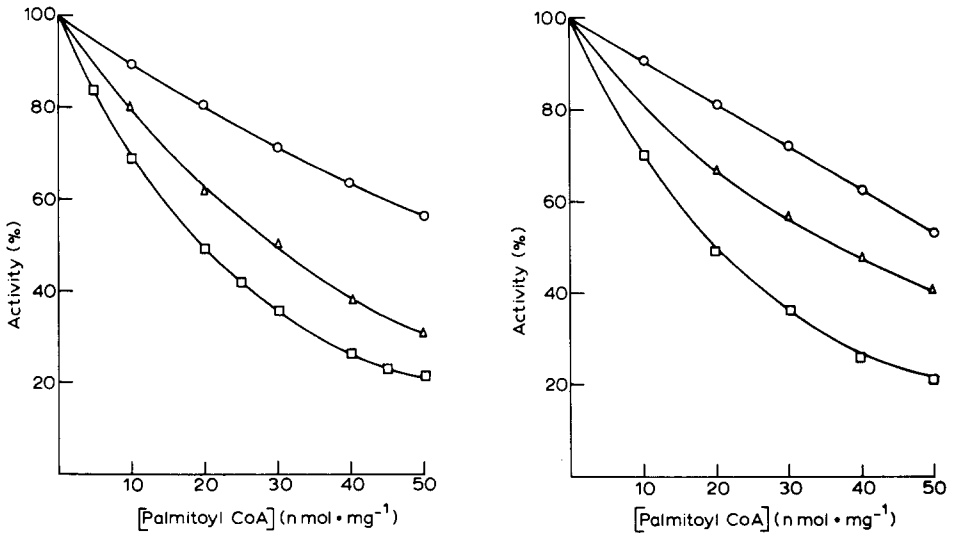
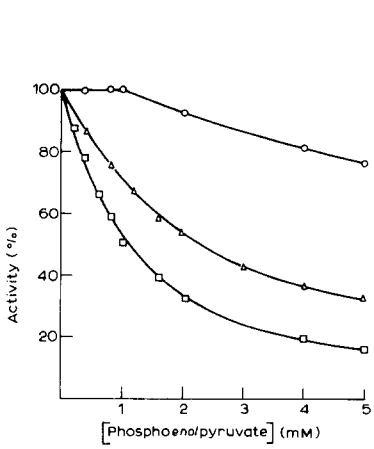


Fig. 5. Effect of added Ca^{2+} ions on α-glycerophosphate dehydrogenase activity in human placental mitochondria inhibited by palmitoyl-CoA. Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration was 1 mM. Palmitoyl-CoA (□), palmitoyl-CoA + 0.4 mM $CaCl_2$ (△), palmitoyl-CoA + 2 mM $CaCl_2$ (○).

Fig. 6. Effect of added Mg^{2+} ions on α-glycerophosphate dehydrogenase activity in human placental mitochondria inhibited by palmitoyl-CoA. Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration was 1 mM. Palmitoyl-CoA (□), palmitoyl-CoA + 1 mM $MgSO_4$ (△), palmitoyl-CoA + 4 mM $MgSO_4$ (○).



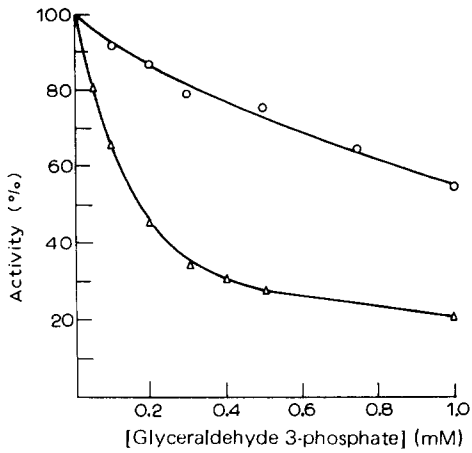


Fig. 7. Inhibition by phospho*enol*pyruvate of α-glycerophosphate dehydrogenase activity in mitochondria from human placental (□), rat skeletal muscle (△) and rat brown adipose tissue (○). Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration was 1 mM.

Fig. 8. Inhibition of α-glycerophosphate dehydrogenase activity in human placental mitochondria by D- (○) and DL-glyceraldehyde 3-phosphate (△). Experimental conditions were as described under Materials and Methods. α-Glycerophosphate concentration was 1 mM.

by phospho*enol*pyruvate. The type of the inhibition of α-glycerophosphate dehydrogenase activity by phospho*enol*pyruvate in the above-enumerated tissues was studied by varying substrate concentration at less than fully-inhibiting amounts of phosphoenolpyruvate. Lineweaver-Burk plots (not shown here) revealed a competitive type of inhibition. The enzyme in brown adipose tissue was about 6 and 18 times less sensitive to phospho*enol*pyruvate (K_i = 9 mM), than the dehydrogenase in rat skeletal muscle (K_i = 1.5 mM) and human placental mitochondria (K_i = 0.5 mM), respectively. We studied also the effect of phospho*enol*pyruvate on α-glycerophosphate dehydrogenase activity in mitochondria from tissues which posses lower activity of this enzyme. The enzymes from rat liver, kidney and brain mitochondria were inhibited by 1 mM phospho*enol*pyruvate by about 40%, 30% and 60%, respectively, under conditions described in Fig. 7.

Among other glycolytic intermediates examined, the most potent inhibitor was glyceraldehyde 3-phosphate. Fig. 8 shows the effect of increasing concentrations of DL- and D-isomer of this compound on the activity of α-glycerophosphate dehydrogenase. The results, expressed as per cent of the activity in the absence of an inhibitor, indicate that the DL racemic mixture inhibited α-glycerophosphate dehydrogenase activity more strongly than the D isomer. Kinetic studies of the effect of these compounds on α-glycerophosphate dehydrogenase activity revealed that this inhibition was competitive with respect to the substrate. The K_i values calculated for the racemic mixture and for the D isomer were 0.12 and 0.95 mM, respectively. This suggests that the L-isomer of the DL-racemic mixture was a more potent inhibitor than the D-isomer of glyceraldehyde 3-phosphate. D-3-phosphoglyceric acid inhibited α-glycero-

phosphate dehydrogenase activity in human placental mitochondria in a competitive manner with K_i about 1.5 mM. 2-phosphoglyceric acid exerted only a slight inhibitory effect on α-glycerophosphate dehydrogenase activity in human placental mitochondria under the same conditions (not shown). Other examined glycolytic intermediates as glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and 2,3-diphosphoglyceric acid were found to be ineffective.

Discussion

The data presented in this paper indicate that several properties of α-glycerophosphate dehydrogenase in human placental mitochondria resemble these of α-glycerophosphate dehydrogenase from other tissues. Estabrook and Sacktor [1] suggested that EDTA itself may be responsible for the inhibition of α-glycerophosphate dehydrogenase activity in mitochondria isolated from blowfly flight muscle. However Hansford and Chappell [16] showed that the activity of α-glycerophosphate dehydrogenase is markedly stimulated by very low concentrations of calcium ion. They showed also that this enzyme exhibits allosteric kinetics with respect to α-glycerophosphate, and that calcium ion stimulates the enzyme activity by lowering the K_m for the substrate. As shown in Fig. 2, the manner in which EGTA exerted the inhibitory effect on α-glycerophosphate dehydrogenase activity is dependent on its concentration. At low EGTA concentration (0.2 mM) competitive nature of the inhibition was observed, whereas in the presence of 4 mM EGTA the enzyme exhibited allosteric kinetics. It is possible that 0.2 mM EGTA may not be sufficient to remove completely endogenous Ca^{2+}. Estabrook and Sacktor [1] showed that only those chelating compounds which possess in addition to chelating properties certain precise steric qualifications are able to inhibit α-glycerophosphate dehydrogenase activity. It is likely that EGTA exerts a dual effect on α-glycerophosphate dehydrogenase activity, on the one hand by chelating calcium ion required for stimulation of the enzyme, and on the other, EGTA itself may be inhibiting the α-glycerophosphate dehydrogenase activity in mitochondria. This explains why the inhibitory effect of EGTA on α-glycerophosphate dehydrogenase activity may be completely overcome either by an excess of calcium ion or by an excess of α-glycerophosphate.

It has been reported that long-chain acyl-CoA esters inhibit the activities of several enzymes in vitro [24—30], however some of these effects could not be ascribed a physiological role, as they were unspecific, irreversible and occured at relatively high concentrations. Some of these effects may be caused by irriversible damage of the enzyme due to the detergent properties of long-chain acyl-CoA esters. Data presented in this paper indicate that the inhibition of the α-glycerophosphate dehydrogenase activity in human placental mitochondria by palmitoyl-CoA was competitive with respect to substrate, could be reversed by Mg^{2+} and Ca^{2+} ions and occured at relatively low concentration of this metabolite. The inactivation of the enzyme due to the detergent properties of palmitoyl-CoA may be ruled out as palmitic acid and palmitoyl-carnitine which posses also detergent properties were ineffective or exerted only slight effect at high concentration of these compounds. It is very interesting that

palmitoyl-CoA strongly inhibited α-glycerophosphate dehydrogenase activity in brown adipose tissue even in the presence of an excess of calcium ion [14]. In human placental mitochondria, however palmitoyl-CoA in the presence of Ca^{2+} or Mg^{2+} ions exerted considerably lower inhibitory effect on α-glycerophosphate dehydrogenase activity than in the absence of these cations. In previous paper we have shown [18] that α-glycerophosphate dehydrogenase in human placental mitochondria is located on the external surface of the inner mitochondrial membrane, in the same way as in mitochondria isolated from other tissues [11,12]. This might facilitate the action of Mg^{2+} ion on α-glycerophosphate dehydrogenase activity as in the intermembrane compartment relatively high level of Mg^{2+} ion is present [31]. In the intact cell the action of palmitoyl-CoA on α-glycerophosphate dehydrogenase activity is also possible as its formation is taking place in the outer mitochondrial membrane [32,33]. In addition, palmitoyl-CoA is oxidized at a low rate in human placental mitochondria [33], hence an increased level of this metabolite in the intermembrane space may appear occasionaly. It seems therefore that the activity of α-glycerophosphate dehydrogenase can be regulated by cytosolic changes of Mg^{2+} ion and palmitoyl-CoA concentrations.

The fact that the pH optimum for α-glycerophosphate dehydrogenase activity in human placental mitochondria varied with substrate concentration and that the K_m for α-glycerophosphate in this reaction also varied with pH may also have a regulatory significance.

The effects of substrate analogues on α-glycerophosphate dehydrogenase activity in human placental mitochondria described in this paper indicate that some of them inhibit also this enzyme in a competitive manner. The most potent inhibitor was DL-glyceraldehyde 3-phosphate (K_i = 120 μM); the D-isomer of this compound was less effective (K_i = 900 μM) similarly to flight muscle [12] and pig brain mitochondria [13]. D-3-phosphoglyceric acid has been also shown to be a competitive inhibitor of α-glycerophosphate dehydrogenase in human placental mitochondria, however the K_i value in this case was ten times lower than the value reported for the mitochondrial enzyme from flight muscle [12]. In the previous paper [19] we reported that α-glycerophosphate dehydrogenase activity in human placental mitochondria was inhibited in a competitive manner by phospho*enol*pyruvate. Comparative studies presented here indicate that the enzyme activity in human placental mitochondria is more sensitive to phospho*enol*pyruvate than the corresponding dehydrogenase in rat skeletal muscle mitochondria. Under the same conditions, this enzyme in mitochondria isolated from rat brown adipose tissue is only slightly affected by phospho*enol*pyruvate. Thus it would appear that the catalytic sites of the α-glycerophosphate dehydrogenases from different tissues have different abilities to bind phospho*enol*pyruvate. In this respect, α-glycerophosphate dehydrogenase from brown adipose tissue differs evidently from human placenta and rat skeletal muscle mitochondrial dehydrogenases.

The data presented in this paper indicate that α-glycerophosphate dehydrogenase activity in isolated mitochondria from human term placenta is controlled by some metabolites. The regulation of α-glycerophosphate dehydrogenase assumes physiological significance in control of lipid synthesis, since α-glycerophosphate is an important substrate for the synthesis of glycerides. The inhibi-

tion of α-glycerophosphate dehydrogenase by phospho*enol*pyruvate and palmitoyl-CoA create a possibility for the cell to keep a significant level of α-glycerophosphate. The precise physiological significance of these findings and of the relatively high activity of α-glycerophosphate dehydrogenase in human placental mitochondria require further investigation.

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