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The Effect of Phospholipase A_2 on Mitochondrial Glycerol-3-phosphate Oxidation

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Summary

Glycerol-3-phosphate oxidation in brown adipose tissue mitochondria of cold-adapted hamster is strongly inhibited by phospholipase A_2 (PLA_2). Our data show that the glycerol-3-phosphate branch of the respiratory chain is sensitive to PLA_2 action more than the succinate branch and that the transfer of reducing equivalents from the glycerol-3-phosphate dehydrogenase to arteficial electron acceptor is especially sensitive to the PLA_2 action.

Key Words

Phospholipase A2 – Free fatty acid – Lysophosphatide – Mitochondrial glycerol-3-phosphate oxidation – Mitochondrial glycerol-3-phosphate dehydrogenase

Introduction

Mitochondrial FAD-linked glycerol-3-phosphate dehydrogenase (EC 1.1.99.5.) is an enzyme embedded in the inner mitochondrial membrane. It catalyzes the transfer of reducing equivalents to the coenzyme Q pool and forms thus one of the branches of the respiratory chain. Mitochondrial glycerol-3-phosphate dehydrogenase (GPDH) together with its cytosolic counterpart, NADH-linked glycerol-3-phosphate dehydrogenase (EC 1.1.1.8.) act as glycerol-3-phosphate shuttle, an important cellular mechanism for oxidation of cytosolic NADH via mitochondria.

Mitochondrial GPDH belongs to the group of membrane-bound enzymes that require phospholipids for maintenance of structural integrity and metabolic function. It was found that isolated enzyme from brain and liver contains cardiolipin (Cottingham and Ragan 1980, Garrib and McMurray 1986) and that cardiolipin plays an important role in regulation of electron acceptor binding sites (Beleznai and Jancsik 1989). Our previous findings also showed that phospholipid environment and changes in the membrane lateral pressure are important for GPDH function, as well as for its hormonal regulation (Beleznai *et al.* 1989, 1990).

Phospholipids are readily catabolized by various phospholipases which may thus influence activity of various phospholipid dependent enzymes. Phospholipase A_2 (PLA_2) hydrolyzes the ester bond which holds the fatty acid to the carbon of glycerol at the position 2 and liberates the free fatty acid and lysophosphatide (monoacyl-sn-glycerol-3-phosphate). Both free fatty acids and lysophosphatides can influence activity of membrane bound enzymes and participate on the inhibitory effects of phospholipase action.

Earlier studies revealed that free fatty acids have an inhibitory effect on the glycerol-3-phosphate oxidation (Houštěk and Drahota 1975, Rauchová and Drahota 1984). This inhibition correlates well with changes of both structural and dynamic parameters of the lipid membrane phase as monitored by steady-state fluorescence anisotropy of hydrophobic fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH) (Amler *et al.* 1986). These data support the idea that free fatty acids act on the enzyme activity indirectly through the modification of lipid microenvironment. However, we have recently found the second independent mechanism: free fatty acids may interact directly with substrate binding sites and inhibit competitively the GPDH (Rauchová *et al.* 1993).

Lysophosphatidylcholine (LPC) which is formed in small amount in cells or tissues is injurious to membranes in higher concentrations. It can affect membrane integrity and change various membrane-bound enzyme activities (Kalous *et al.* 1992).

In attempt to obtain further data concerning the mechanism of modulation of GPDH activity by the phospholipid phase we followed the effect of PLA_2 on the activity of mitochondrial glycerol-3-phosphate oxidation and GPDH (EC 1.1.99.5.) and we tried to differentiate to which extent free fatty acids, lysophosphatides or membrane desintegration are responsible for the inhibitory effect of PLA_2. Contrary to our previous papers (Rauchová and Drahota 1984, Rauchová *et al.* 1985, Amler *et al.* 1986, Beleznai *et al.* 1990, Kalous *et al.* 1992) where we followed the effect of exogenously added free fatty acids or LPC, in the present experiments with PLA_2 we studied the effect of fatty acids and lysophosphatides endogenously produced by PLA_2 action.

Material and Methods

PLA_2 from bee venom, Tris(hydroxymethyl) aminomethane (Tris), bovine serum albumin (Fraction V, fatty acid free), glycerol-3-phosphate and cytochrome c (from bovine heart) were obtained from Sigma (USA), ethylenediamine tetraacetic acid (EDTA) from Serva (Germany) and 2,6-dichlorophenolindophenol (DCIP) from LOBA Chemie (Austria). Other chemicals of the highest purity were obtained from Lachema (Czech Republic).

The experiments were performed on mitochondria isolated from interscapular, cervical and axillary brown adipose tissue of adult male cold-adapted golden hamsters (*Mesocricetus auratus*). Mitochondria were isolated according to the method of Hittelman *et al.* (1969).

The activity of glycerol-3-phosphate oxidation was measured with a Clark-type oxygen electrode (Estabrook 1967). The reaction mixture consisted of 0.25 mol/l sucrose, 1 mmol/l EDTA, 10 mmol/l Tris-HCl pH 7.4, 2 mmol/l $CaCl_2$ and 0.5–2.0 mg of mitochondrial protein. The reaction was started by addition of 25 mmol/l glycerol-3-phosphate.

The activity of GPDH was measured spectrophotometrically at 600 nm at 25 °C in 0.25 mol/l sucrose, 1 mmol/l EDTA, 10 mmol/l Tris-HCl pH 7.4, 2 mmol/l $CaCl_2$, 1 mmol/l KCN and 50 μmol/l DCIP. The maximum protein concentration was 0.1 mg in 1 ml cuvette. The reaction was started by 25 mmol/l glycerol-3-phosphate.

The activities of glycerol-3-phosphate cytochrome c reductase and succinate cytochrome c reductase were measured spectrophotometrically at 550 nm at 25 °C in the same medium as for GPDH, we used 50 μmol/l cytochrome c instead of 50 μmol/l DCIP. The maximum protein concentration was 0.1 mg in 1 ml cuvette. The reaction was started by 25 mmol/l glycerol-3-phosphate or 25 mmol/l succinate.

Protein concentrations were determined according to Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

The effect of various concentrations of PLA_2 on the activity of glycerol-3-phosphate oxidation is shown in Fig. 1. Considerable inhibition (70–80 %) of glycerol-3-phosphate oxidation was found when brown adipose tissue mitochondria were incubated for 2 min with 15–40 μg PLA_2/mg mitochondrial protein. At 25 μg PLA_2/mg protein maximum inhibitory effect was obtained and about 20 % glycerol-3-phosphate oxidation can be detected.

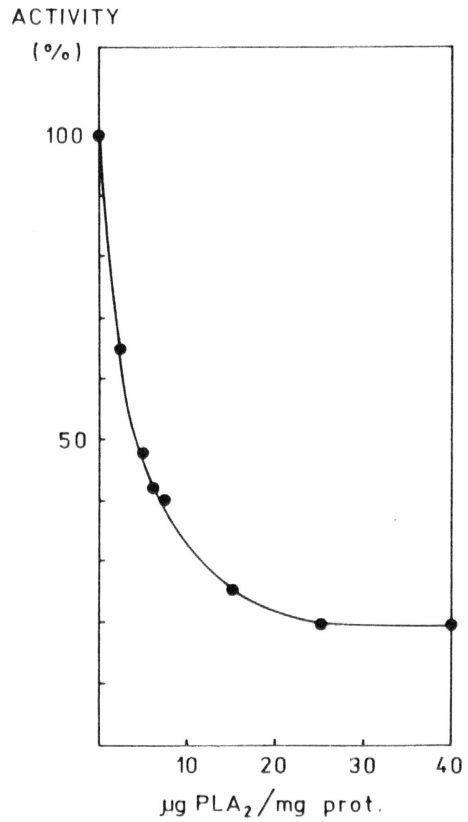

Fig. 1
Effect of PLA_2 on glycerol-3-phosphate oxidation. The preincubation with PLA_2 was performed 2 min in 1 ml final volume of 1 ml at 25 °C. After incubation with PLA_2 substrate was added and the initial respiratory rate was evaluated and expressed in % of the value in the absence of PLA_2. This experiment was performed on 4 different samples of mitochondria.

Bovine serum albumin (BSA) may restore glycerol-3-phosphate oxidation inhibited by added free fatty acids (Houštěk and Drahota 1975, Rauchová and Drahota 1984). We have therefore tested whether BSA can also prevent the inhibitory effect of PLA_2. Data presented in Table 1 demonstrate the inhibitory effect of PLA_2 on glycerol-3-phosphate oxidation under different conditions. Data presented in Table 1 show

that in the absence of Ca^{2+} ions the inhibitory effect of PLA_2 is less pronounced and under these experimental conditions the inhibition could be almost prevented by BSA. In the presence of Ca^{2+} ions the inhibitory effect was more pronounced and could not be prevented by added BSA.

Table 1
Effect of PLA_2 on glycerol-3-phosphate oxidation. The incubation with PLA_2 was performed for 2 min in 1 ml final volume at 25 °C. The amount of PLA_2 was 35 μg per mg of mitochondrial protein. Concentration of Ca^{2+} ions was 2 mmol/l and BSA 0.5 %. The specific activity was expressed as n-atoms of consumed oxygen/mg protein/min. Values indicate means ± S.E. from 5 experiments.

Additions	$-PLA_2$ (A)	$+PLA_2$ (B)	(B/A)
–	159.5 ± 11.7	56.1 ± 6.1	0.35
Ca^{2+}	189.3 ± 17.9	8.3 ± 4.3	0.04
BSA	304.3 ± 21.0	237.2 ± 27.2	0.78
BSA + Ca^{2+}	360.0 ± 19.0	42.7 ± 4.7	0.12

Table 2
Effect of PLA_2 on glycerol-3-phosphate cytochrome c reductase, succinate cytochrome c reductase and glycerol-3-phosphate dehydrogenase activities. The preincubation with PLA_2 was performed 2 min in 1 ml final volume at 25 °C. The amount of PLA_2 was 35 μg per mg of mitochondrial protein. The specific activity was expressed as nmoles/mg prot./min. Values indicate means ± S.E. from 6 experiments.

Enzyme activity	$-PLA_2$ (A)	$+PLA_2$ (B)	(A/B)
Glycerol-3-phosphate cytochrome c reductase	184.06 ± 34.63	47.21 ± 3.71	0.26
Succinate cytochrome c reductase	195.10 ± 23.95	229.58 ± 44.31	1.18
Glycerol-3-phosphate dehydrogenase	132.15 ± 9.57	79.34 ± 8.55	0.60

Table 2 demonstrates the inhibitory effect of PLA_2 in the presence of Ca^{2+} ions on glycerol-3-phosphate cytochrome c reductase, succinate cytochrome c reductase and GPDH activities. Succinate cytochrome c reductase activity was not inhibited by PLA_2 at the concentration that inhibits glycerol-3-phosphate cytochrome c reductase to 74 %. On the contrary, we found a small activation after PLA_2 incubation. Similar results as for oxidation of glycerol-3-phosphate were found when glycerol-3-phosphate cytochrome c reductase activity was tested. However, we can see less pronounced inhibitory effect on glycerol-3-phosphate cytochrome c reductase. GPDH is inhibited only to 40 % in comparison with the glycerol-3-phosphate oxidation which is inhibited to 96 %. The data presented in Table 2 demonstrate that GPDH activity measured as a transfer of reducing equivalents from enzyme to the arteficial electron acceptor (DCIP) shows the lowest inhibition by PLA_2 action.

Discussion

One of the classical approaches to the study of the role of structural phospholipids in maintenance and regulation of func
enzymes is to treat mitochondria with specific phospholipases (Dobiášová *et al.* 1973). In our experiments we used PLA_2 which hydrolyzes the phospholipid molecule to a free fatty acid and a lysophosphatidylcholine (LPC). By this way the membrane structure is effectively changed.

Mitochondrial GPDH is an example of membrane-bound enzyme which activity is modulated by phospholipid phase (Amler *et al.* 1986, Beleznai *et al.* 1990). As was shown in our previous paper (Amler *et al.* 1990) the activity of GPDH decreased when the membrane lateral pressure was higher or lower than that found for proteoliposomes formed without exogenous phospholipids. The enzyme seems to be less sensitive to higher membrane lateral pressure than to the lower one, as was found for phosphatidylcholine-enriched proteoliposomes. It appeared from our previous experiments (Beleznai *et al.* 1989, 1990) that rather the structure of the whole membrane (the nature of the bulk phospholipids) than merely the phospholipid annulus around the enzyme plays a role in maintaining the structural organisation of GPDH molecule and in regulation of its functional activity. However, from the experiments with adriamycin (Beleznai and Jancsik 1989) it was deduced that cardiolipin in vicinity of the enzyme molecule may influence affinity of electron binding sites and thus regulates the enzyme function.

Our results show that besides inhibitory action of free fatty acids also destruction of phospholipids required for maintenance of enzyme function may

inactivate glycerol-3-phosphate oxidation because the changes in membrane lateral pressure (both higher and lower values) have negative effects on the enzyme activity. It is evident from Table 1 that the inhibitory effect of PLA_2 cannot be fully explained by the action of released free fatty acids because the inhibition is not completely reversible by added BSA. The comparison between the effect of PLA_2 on glycerol-3-phosphate cytochrome c and succinate cytochrome c reductase under the same experimental conditions (Table 2) suggest that the most sensitive place is the transfer of reducing equivalents between GPDH and the coenzyme Q pool.

Lysophophatidylcholine, the second product formed by PLA_2 action has inhibitory effect which occurs at high concentrations (about 30-50 fold higher concentration than physiological). LPC does not block more than 30 % of GPDH activity (Rauchová *et al.* 1985, Kalous *et al.* 1992).

Our results suggest that free fatty acids and LPC released by PLA_2 action have inhibitory effects on the molecule of GPDH as well as on the transfer of reducing equivalents from GPDH to the coenzyme Q pool. GPDH could be inhibited both by direct interaction with PLA_2 products and by destruction of endogenous phospholipids required for maintenance of enzyme molecule structure. Our data, however, cannot differentiate to which extent the modification of the bulk phospholipids or modification of phospholipid annulus are involved.

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Reprint Requests

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