Activation of Mitochondrial Glycerol 3-Phosphate Dehydrogenase by Cadmium Ions

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Abstract. Mitochondrial glycerol 3-phosphate dehydrogenase (EC 1.1.2.1.) requires Ca^{2+} ions for its activity. Cadmium ions also have activatory effect on the enzyme. They activate the glycerol 3-phosphate dehydrogenase in a very narrow concentration range (1—2 mmol/l). As contrasted with calcium, strong inhibitory effect occurred at higher concentrations (3—4 mmol/l). The inhibition induced by cadmium ions was completely reversible by washing of the mitochondria.

Key words: Mitochondrial glycerol 3-phosphate dehydrogenase — Ca^{2+}, Cd^{2+}, Hg^{2+} ions

Introduction

Mitochondria of the brown adipose tissue show a very high activity of glycerol 3-phosphate dehydrogenase (Houštěk et al. 1975); this activity is localized on the outer surface of the inner membrane (Donnellan et al. 1970; Klingenberg 1979). The high activity of this enzyme enables operation of the glycerol 3-phosphate cycle which plays an important regulatory role in thermogenic activity of the brown adipose tissue (Bukowiecki and Lindberg 1974; Houštěk et al. 1975).

The activity of glycerol 3-phosphate dehydrogenase is entirely dependent on calcium ions (Bukowiecki and Lindberg 1974; Hansford and Chappel 1967; Carafoli and Sacktor 1958; Fisher et al. 1973; Wernette et al. 1981). Hansford and Chappel (1967) observed activatory effect of calcium ions at calcium concentrations as low as 10^{-7} mol/l. Also, other divalent cations, such as strontium (Wohlrab 1977; Wernette et al. 1981), or manganese (Wohlrab 1977) may effect the enzyme activity.

In our experiments we could show that cadmium ions known to affect various enzymes (Chapatwala et al. 1982; Maines et al. 1982) may also activate glycerol 3-phosphate dehydrogenase. However, similarly as in mitochondrial ATPase activation (Rauchová and Drahota 1979) the effect of cadmium ions could be

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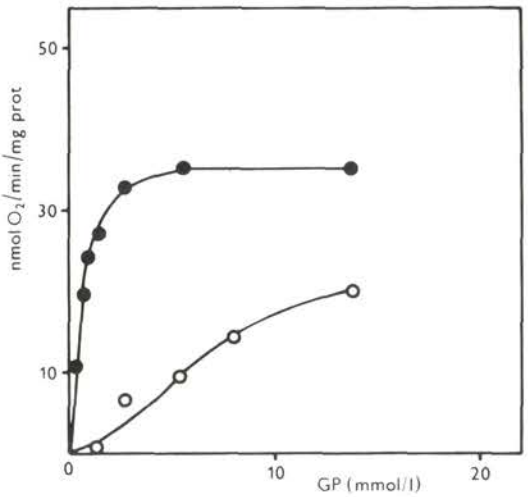

Fig. 1. Activatory effect of calcium ions on glycerol 3-phosphate oxidation in brown adipose tissue mitochondria. The oxidation was measured in a sucrose medium supplemented with 1 mmol/l EDTA and 5 mmol/l EGTA (○), or with 1 mmol/l EDTA and 4.5 mmol/l CaCl (●).

observed with in a narrow concentration range only and strong reversible inhibition occurred at higher concentrations.

Materials and Methods

Mitochondria were isolated from the brown adipose tissue as described by Hittelmann et al. (1969). Glycerol 3-phosphate oxidation was measured in a medium containing: sucrose 0.25 mol/l; tris-HCl 10 mmol/l; pH 7.4. Glycerol 3-phosphate dehydrogenase activity was determined in the same medium supplemented with KCN (1 mmol/l) and phenazinmethosulfate (3 mmol/l) using a Clark oxygen electrode (Estabrook 1967). Glycerol 3-phosphate, EDTA, EGTA or calcium were added as specified in Table 1 and Figs. 1 and 2. Mitochondrial protein was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

Results and Discussion

In agreement with the literary data (Bukowiecki and Lindberg 1974) it was observed (Fig. 1) that in brown fat mitochondria of the hamster, the activatory effect of calcium is dependent on the substrate concentration; this indicates that calcium ions strongly increase the enzyme-to- substrate affinity. At high substrate concentrations, the activatory effect diminished.

At glycerol 3-phosphate concentration of 5 mmol/l giving the maximal rate of glycerol 3-phosphate oxidation in the presence of calcium ions, cadmium ions also activated glycerol 3-phospate oxidation. The rate of glycerol 3-phosphate oxida-

Table 1. The inhibitory effect of mercurials on mitochondrial glycerol 3-phosphate dehydrogenase of the brown adipose tissue

Additions	% Activity	
	− mercaptoethanol	+ mercaptoethanol
—	100	100
0.25 μmol $HgCl_2$/mg prot.	68	102
0.50 μmol $HgCl_2$/mg prot.	47	88
0.25 μmol pCMB/mg prot.	69	77
0.50 μmol pCMB/mg prot.	31	67

Enzyme activities were determined in a medium containing 0.25 mol/l sucrose; 10 mmol/l tris-HCl; pH 7.4; 5 mmol/l glycerol 3-phosphate; 1 mmol/l mercaptoethanol.

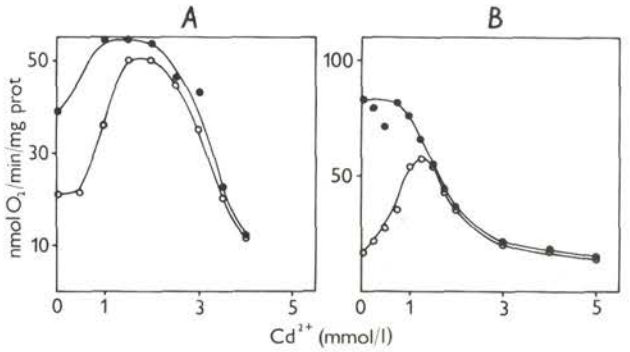

Fig. 2A: Activatory effect of cadmium and calcium ions on glycerol 3-phosphate oxidation in brown adipose tissue mitochondria. The oxidation was measured in a sucrose medium containing 1 mmol/l EDTA; 5 mmol/l glycerol 3-phosphate; and various concentrations of cadmium (○) or various concentrations of cadmium and 2 mmol/l $CaCl_2$ (○). ***B*:** Activatory effect of cadmium and calcium ions on glycerol 3-phosphate dehydrogenase in brown adipose tissue mitochondria. Enzyme activity was measured in a sucrose medium containing 1 mmol/l EDTA; 1 mmol/l KCN; 3 mmol/l phenazin-methosulfate; 2.5 mmol/l glycerol 3-phosphate and various concentrations of cadmium (○), or various concentrations of cadmium and 2 mmol/l $CaCl_2$ (●).

tion was nearly as high as in the presence of calcium ions (Fig. 2*A*). As contrasted with calcium, the effect of cadmium could be observed with in a narrow concentration range only. In the presence of 1 mmol/l EDTA maximum activatory effect was observed at 1.5—2.5 mmol/l cadmium ions, and at higher concentrations (3—4 mmol/l), the respiratory rate was strongly inhibited. At the latter concentration range of calcium ions, the oxidation of glycerol 3-phosphate was not inhibited

at all. The inhibitory effect of higher cadmium concentrations could not be abolished by subsequent addition of calcium ions (Fig. 2*A*).

Fig. 2*B* illustrates the effects of both cadmium and calcium ions on mitochondrial glycerol 3-phosphate dehydrogenase. In this case, maximum activatory effect was observed at 1—1.5 mmol/l cadmium ions.

The maximal rate reached 69 % of that obtained in the presence of calcium ions alone. The inhibitory effect of higher cadmium concentrations was not due to irreversible changes in the enzyme molecule since it could be abolished by centrifugation and resuspension of the mitochondria in a sucrose medium without cadmium ions (not shown).

The inhibitory effect of cadmium ions is not identical with those of $HgCl_2$ or p-chlormercuribenzoate (Table 1) because the former cannot be reversed by mercaptoethanol (not shown).

This indicates that no covalent binding to other polypeptide groups takes place. The data presented in this paper indicate further that the toxic effect of cadmium ions is due to modification of the calcium binding sites in such a way that the inhibitory effect of cadmium cannot be reversed by the excess of calcium ions. Similar results were obtained in comparing the reversibility of the inhibitory effect of cadmium ions on the activity of the mitochondrial ATPase (Rauchová and Drahota 1979).

We may thus conclude that mitochondrial enzymes which require calcium or magnesium ions for their activity may also be activated by cadmium ions. However, calcium ions in the case of glycerol 3-phosphate dehydrogenase and magnesium ions in the case ATPase (Rauchová and Drahota 1979) have an evident biological advantage. Their concentration range over which they do not induce any toxic effects are much wider than that of cadmium. Similarly, the inhibitory effect of mercurials could not be abolished by dilution or washing of mitochondria as in the case of cadmium-induced inhibition; this also indicates that the mechanism of the inhibitory effect of cadmium differs from that of mercurials.

Our data indicate that cadmium ions may be used as a tool for studying molecular mechanism by which divalent cations regulate the functional activity of various enzymes.

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