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Inhibition of Mitochondrial ATPase by Dicarbopolyborate, a New Enzyme Inhibitor

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Polyborate anions were found to inhibit mitochondrial ATPase. Mercapto and chloro derivatives of dicarbononaborates showed full inhibition of the enzyme activity at 0.5–0.8 mM. The inhibitory effect of dodecaborates was lower. The inhibition was of competitive type with respect to ATP. The inhibition of soluble F_1-ATPase indicates a direct interaction of the polyborate anion with the catalytic part of the enzyme molecule.

KEY WORDS: Polyborate anions; mitochondria; F_0F_1-ATPase.

INTRODUCTION

Boron cluster compounds are species with a unique molecular architecture (Fig. 1) and unusual chemical properties (Plešek 1992). Polyborates and dicarbopolyborates as lipophylic molecules that easily penetrate across biological membranes were used as membrane potential probes in mitochondria, mitochondrial particles, and liposomes (Bakeeva *et al.*, 1970, Grinius *et al.*, 1970, Jasaitis *et al.*, 1972). Several substituted boron cluster compounds were designed for neutron capture therapy of brain tumors because they accumulate more in neoplastic cells (Hatanaka, 1986, Hatanaka and Sweet, 1975). Pharmacological experimental studies revealed, however, that many of these compounds have severe undesirable side effects (Mareš *et al.*, 1992a, b, Kliegel, 1980). The toxic effect of these drugs was tentatively explained by their interaction with metabolic pathways involving pyridoxal phosphate as cofactor (Kliegel, 1980). However, no systematic study of their biochemical properties has been published, and the molecular mechanism of their toxicity has not yet been clarified.

In the neoplastic cells polyborates accumulate also in mitochondria (Hatanaka and Sweet, 1975). Therefore we studied their toxic effect on cell energy metabolism, especially on mitochondrial ATPase.

The inhibitory effect of mercaptododecaborate designed for neutron capture therapy was compared with a new group of chloro and mercapto compounds of dicarbopolyborates (Plešek *et al.*, 1984, 1985a, b) that showed *in vivo* 5–10 times higher toxicity for mice (Mareš *et al.*, unpublished data).

Our experiments show that polyborate anions inhibit the activity of mitochondrial ATPase and respiratory chain enzymes. In agreement with our *in vivo* measurements the inhibitory effect of dicarbononaborates was higher than that of dodecaborates. Preliminary results were published elsewhere (Drahota *et al.*, 1992).

MATERIAL AND METHODS

Polyborates used in this study included dodecaborates, (Na_2-$B_{12}H_{12}\cdot 2H_2O$, Na_2-5-SH-$B_{12}H_{11}$) and a new group of dicarbononaborates (Na-5-Cl-7, 8-C_2-B_9H_{11}, Na-5, 6-Cl_2-7, 8-C_2-B_9H_{10}, Na-5-SH-7, 8-C_2-B_9H_{11}), synthesized by Plešek *et al.* (1984, 1985a, b). All other chemicals were of analytical grade purchased from Lachema (Czech Republic) or Sigma (USA).

Mitochondria were isolated from rat liver according to Smith (1967). Isolated mitochondria

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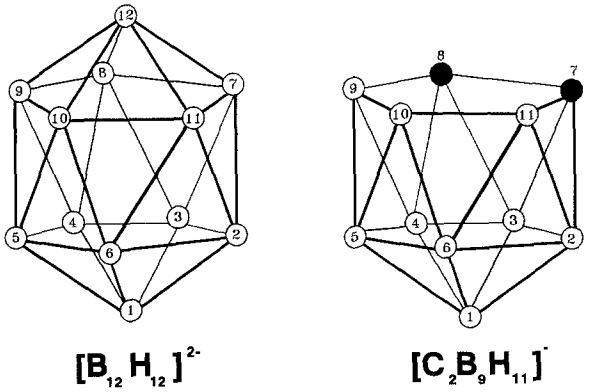

Fig. 1. Molecular structure of dodecaborate $[B_{12}H_{12}]^{2-}$ and dicarbononaborate $[C_2B_9H_{11}]^-$.

were suspended in 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, at pH 7.4 with about 50 mg protein per ml and used immediately or stored at −20°C.

ATPase activity was measured as the release of inorganic phosphate. Mitochondria were incubated for 5 min at 37°C in a medium containing 100 mM KCl, 10 mM Tris-HCl, 3 mM $MgCl_2$, 5 mM ATP, at pH 7.4 and 0.5 mg of mitochondrial protein per ml. Inorganic phosphate was determined as described by Lindberg and Ernster (1956). Succinate-cytochrome c reductase was determined according to Sottocasa *et al.* (1967). Mitochondrial protein was determined according to Lowry *et al.* (1951).

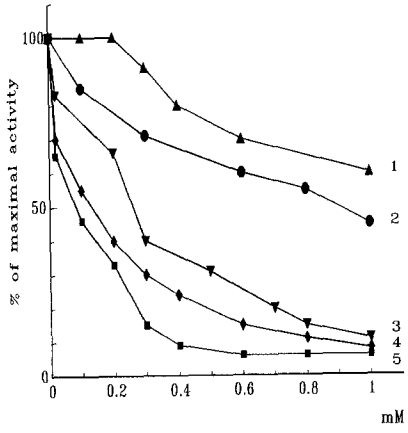

Fig. 2. Inhibition of the mitochondrial ATPase by various polyborate anions. The ATPase activity in the absence of inhibitor (100%) was 0.13 mole P released by 1 mg of mitochondrial protein per min. Inhibitors used: $Na_2B_{12}H_{12} \cdot 2H_2O$ (1), $Na_2SHB_{12}H_{11}$ (2), $Na_2SHC_2B_9H_{11}$ (3), $NaCl_2C_2B_9H_{10}$ (4), and $NaClC_2B_9H_{11}$ (5). The concentration of the inhibitor is expressed as mM. Frozen-thawed rat liver mitochondria were used.

Soluble F_1-ATPase was isolated from sonicated EDTA particles (Lee and Ernster, 1968) by a chloroform extraction procedure (Kopecký *et al.*, 1979).

RESULTS AND DISCUSSION

The inhibitory effect of two dodecaborates and three dicarbononaborates (mercapto, mono, and dichloro derivatives) on the activity of the mitochondrial ATPase is presented in Fig. 2. The inhibitory effect of dicarbononaborates was much higher than that of dodecaborates. Maximum inhibition by dicarbononaborates occurred at a concentration range between 0.5–1.0 mM (see Fig. 2). The inhibitory effect of polyborate anions was the same both in intact and mitochondria disrupted by freezing–thawing.

The inhibitory effect of polyborate anions may be due to interaction with one of the polypeptides of the ATPase molecule or to nonspecific modification of the membrane properties by the lipophylic polyborate anions. Kinetic data show that the inhibitory effect of dichlorodicarbononaborate is of competitive type in the case of ATPase (Fig. 3, Table I) but of non-competitive type in the case of succinate-cytochrome *c* reductase (Table I). At 0.1 mM concentration of dicarbononaborate the succinate-cytochrome *c* reductase is inhibited by 55.8%, and K_M remains unchanged. At 0.4 mM dicarbononaborate the ATPase activity is not changed whereas the K_M

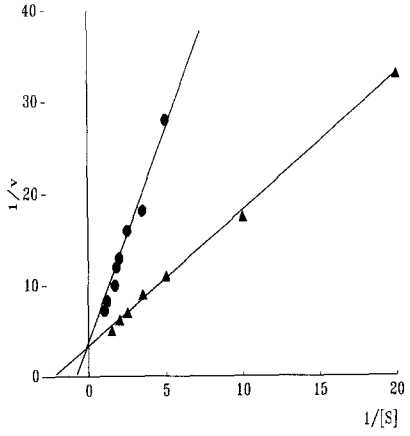

Fig. 3. Lineweaver–Burk plots of dichlorodicarbononaborate inhibition of rat liver mitochondrial ATPase. Circles indicate values without inhibitor, triangles with 0.4 mM inhibitor. [S] is in moles, *v* in mol/min/mg protein.

Table I. The Inhibitory Effect of Dichlorodicarbononaborate on ATPase and Succinate-Cytochrome *c* Reductase Activity[a]

	Control	$+[Cl_2C_2B_9H_{10}]$
	ATPase	
k_{cat}	0.29	0.29
%	100	100
K_M (mM)	0.42	1.42
%	100	338
	Succinate-cytochrome *c* reductase	
k_{cat}	240.0	134.0
%	100.0	55.8
K_M (μM)	40.9	40.8
%	100.0	100.0

[a] Values of k_{cat} are expressed as moles of inorganic phosphate released by 1 mg of mitochondrial protein per min or as nmole of cytochrome *c* reduced by 1 mg of mitochondrial protein per min. For ATPase activity the concentration of dichlorodicarbononaborate $[Cl_2C_2B_9H_{10}]$ was 0.4 mM, and for succinate cytochrome *c* activity it was 0.1 mM. The experiment was repeated three times. Enzyme fitter program was used for calculation of K_M and k_{cat} values.

value is increased 3.4 times (Table I). The inhibitory constant of dichlorodicarbononaborate for ATPase was 0.17 mM as calculated from data in Fig. 3.

These data thus indicate a different mechanism of the inhibitory action of dicarbopolyborate on mitochondrial ATPase and on respiratory chain enzymes. The inhibition of succinate-cytochrome *c* reductase appears to be due to nonspecific modification of membrane properties of mitochondria, induced by lipophylic polyborate anions, e.g., by changes in membrane microviscosity (Amler *et al.*, 1986, 1990), whereas the inhibition of the ATPase activity is due to a direct interaction of the polyborate anion with the enzyme molecule.

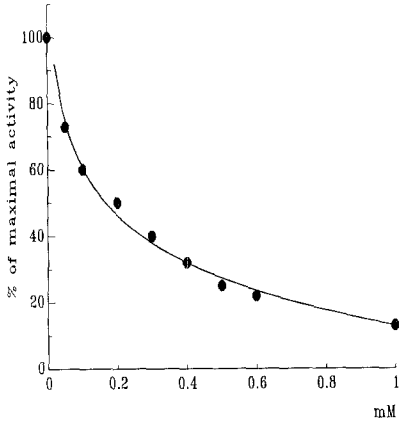

Fig. 4. Inhibition of soluble F_1-ATPase by dichlorodicarbononaborate. The soluble enzyme was incubated under the same experimental conditions as membrane bound enzyme. The incubation mixture contained 0.046 mg of protein per ml. The specific activity of unpurified soluble enzyme was about 5 mol P per min per mg protein. The concentration of the inhibitor is expressed as mM.

To confirm this idea we tested the effect of the polyborate anion on the soluble catalytic part of the mitochondrial ATPase. The results presented in Fig. 4 show that the soluble F_1-ATPase is inhibited by polyborate anions in a similar concentration range as for the membrane bound enzyme. Also for solubilized F_1-ATPase the inhibitory effect is of competitive type (not shown).

Dicarbopolyborate anions are apparently strong inhibitors of mitochondrial ATPase. The kinetic data show that the inhibitory effect results from a direct interaction with the catalytic moiety of the enzyme molecule. However, more experiments will be required for elucidation of the molecular mechanism through which the polyborate anion can modulate functional activity of the enzyme molecule.

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