Notes

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Effect of Fluoride on Ca^{2+}- and Mg^{2+}-Stimulated ATPase in Mitochondria of Rat Lung

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About 45% inhibition of Ca^{2+}-stimulated ATPase activity was observed with 24 mM F^-, and only 50% inhibition of Mg^{2+}-stimulated ATPase was shown with 24 mM F^-. On the other hand, the Ca^{2+}-stimulated ATPase activity in lung mitochondria of rats was significantly elevated by the administration of sodium fluoride (35 mg/kg *i.p.*), while the Mg^{2+}-stimulated ATPase was not affected with the administration.

Keywords——fluoride effect; Ca^{2+}-ATPase; Mg^{2+}-ATPase; lung mitochondria; adenosine triphosphatase

Introduction

The Ca^{2+} transport systems of the red cell[2,3] and sarcoplasmic reticulum[4] are both manifested as a (Mg^{2+}+Ca^{2+})-dependent adenosine triphosphatase(ATPase) (EC 3.6.1.3). The chick intestinal brush border Ca^{2+}-ATPase which increases after vitamin D administration has been identified by Melancon and DeLuca[5] and other workers.[6]

In animal tissues, the lung is an interest organ as a subject for studies on oxidative phosphorylation, water transport and other metabolisms. However, little evidence about the characteristics of ATPases in lung has been obtained.

It has been demonstrated that the fluoride ion caused an increase in permeability and decrease in active transport of sodium in swine red cell membranes.[7] *In vivo* effects of fluoride on (Na^++K^+)ATPase (EC 3.6.1.3) in red cell and on Ca^{2+}- and Mg^{2+}-ATPases in kidney of rat have been investigated by Suketa, *et al.*[8–10] In addition, we have found that Ca contents in several organs such as lung, spleen and kidney of rats were increased by fluoride administration.[10]

This paper describes the effects of Ca^{2+} and Mg^{2+} on mitochondrial ATPase in lung of rats treated with fluoride *in vitro* and *in vivo*.

Materials and Methods

Adenosine-5′-triphosphate, disodium salt was purchased from P.L. Biochemical Inc. (New York, N.W., U.S.A.).

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2) G.H. Bond and D.L. Clough, *Biochim. Biophys. Acta*, **241**, 393 (1971).
3) G.H. Bond and D.L. Clough, *Biochim. Biophys. Acta.*, **323**, 592 (1973).
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Male Wistar albino rats weighing about 100 g were used in this experiment. After being kept on basal diet "MF" purchased from Oriental Yeast Ind. (Japan), for a minimum of one week, animals were given a single intraperitoneal administration of sodium fluoride in a dose of 35 mg/kg. The animals fed the above same diet "MF" and distilled water *ad. libitum*. The rats were anaesthetized with ether and were killed by cardiac puncture 24 hr after dosing. The lungs were removed and immediately placed in ice-cold 0.25M sucrose.

Preparation of Mitochondria——Mitochondria were prepared from rat lungs according to the method of Hogeboom.[11]

ATPase Assay——Disodium ATP was converted to the Tris salt by passage through a chilled column of Dowex 50W—X8-100 cation-exchange resin in the Tris form. The effluent was adjusted to pH 7.5 with addi tional Tris, and the concentration of ATP was calculated from the absorbance at 259 nm.

ATPase activity was assayed according to the method of Killey[12] with minor modification. The reaction system had the following basic composition: 2.0 mM ATP-Tris, 6.0 mM $CaCl_2$ and 60 mM Tris-HCl (pH 8.0). Other additions are indicated in the text. The final volume was 2 ml. The reaction mixtures were incubated at 38° for 30 min and then it was stopped by adding 1.0 ml of 5% $HClO_4$. After centrifugation at 3000 rpm for 15 min, inorganic phosphate liberated in the supernatant was assayed.

Estimation of Magnesium and Calcium——The estimation of Mg^{2+} and Ca^{2+} contents was carried out by the method of Willis[13,14] using a Perkin-Elmer Atomic Absorption Spectrophotometer Model 303.

Protein Determination——Protein was determined by the method of Lowry, *et al.*[15]

Results

Effect of Ca^{2+} and Mg^{2+} on Mitochondrial ATPase in Rat Lung

The effect of Ca^{2+} on the ATPase activity with or without a fixed concentration of Mg^{2+} was studied. As shown in Fig. 1, the activating effect of Ca^{2+} on the enzyme activity in the absence of Mg^{2+} was reached maximum at 3 mM Ca^{2+} with a hill-like shape, but the activity in the presence of a fixed concentration (6 mM) of Mg^{2+} was monotonously inhibited by increasing Ca^{2+} concentration.

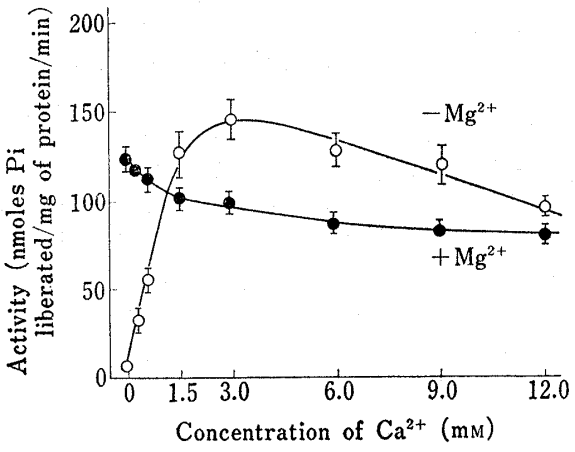

Fig. 1. Effect of Calcium Ion on ATPase Activity in Rat Lung Mitochondria in the Presence or Absence of Magnesium Ion

The reaction mixture (2.0 ml) containing 60 mM Tris-HCl (pH 8.0), 2.0 mM ATP, enzyme (0.2 mg as protein) and Ca^{2+} concentration given on the abscissa in the presence or the absence of 6 mM Mg^{2+} was incubated at 38° for 30 min. Each point represents the average of triplicate. Vertical lines represent ± S.E.

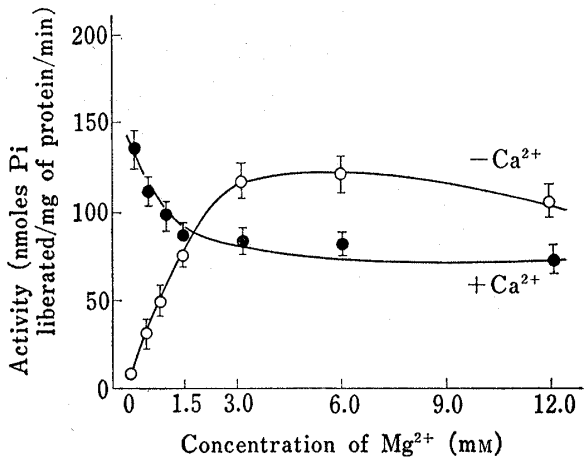

Fig. 2. Effect of Magnesium Ion on ATPase Activity in Rat Lung Mitochondria in the Presence or Absence of Calcium Ion

The reaction mixture (2.0 ml) containing 60 mM Tris-HCl (pH 8.0), 2.0 mM ATP, enzyme (0.2 mg as protein) and Mg^{2+} concentration given on the abscissa in the presence or the absence of 6 mM Ca^{2+} was incubated at 38° for 30 min. Each point represents the average of triplicate. Vertical lines represent ± S.E.

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12) W.W. Kielley, "Methods in Enzymology," Vol. II, ed. by S.P. Colowick and N.O. Kaplan, Acad. Press, New York, 1955, p. 593.
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Fig. 2 shows the effect of Mg^{2+} on the ATPase activity in the presence absence of a fixed concentration (6 mM) of Ca^{2+}. The activation effect of Mg^{2+} in the absence of Ca^{2+} was observed and reached maximum at 3—6 mM Mg^{2+}, but the activity in the presence of a fixed concentration (6 mM) of Ca^{2+} was simply inhibited by increasing Mg^{2+} concentration. The inhibition profile of mitochondrial ATPase activity by Mg^{2+} was similar to that by Ca^{2+} in the presence of a fixed concentration (6 mM) of Mg^{2+} as shown in Fig. 1.

In Vitro and *in Vivo* Effects of Fluoride on Ca^{2+}- and Mg^{2+}-Stimulated ATPase Activity

In the previous investigations,[8–10] we have discussed the ATPase activities in kidney and red cell from fluoride-administered rats in comparison with the acid and alkaline phosphatase activities in the kidney and red cell. Then, the *in vitro* and *in vivo* effects of fluoride on mitochondrial ATPase activity in rat lung were examined in this experiment.

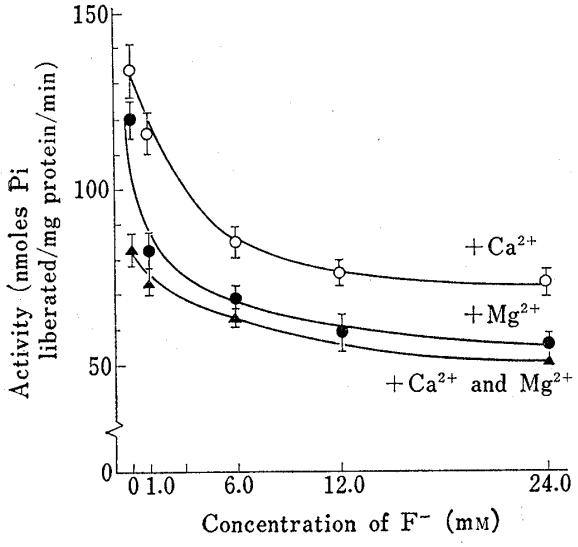

Fig. 3. Effect of Varying Concentration of Fluorine Ion on Ca^{2+}- and Mg^{2}-Stimulated ATPase Activities

The reaction mixture (2.0 ml) containing 60 mM Tris-HCl (pH 8.0), 2.0 mM ATP, enzyme (0.2 mg as protein) and F^- concentration shown on the absscissa was incubated in the presence of 6 mM Ca^{2+}, 6 mM Mg^{2+} or 6 mM Ca^{2+}+Mg^{2+} at 38° for 30 min. Each point represents the average of triplicate. Vertical lines represent ± S.E.

The *in vitro* effect of fluoride on ATPase was examined by incubations with various concentrations of fluoride. As shown in Fig. 3, Ca^{2+}- and Mg^{2+}-stimulated ATPase activity was inhibited by adding sodium fluoride, but it was not complete even at higher concentration (24 mM) of fluoride.

The *in vivo* effects of fluoride administration on the mitochondrial ATPase activities under various conditions were examined as shown in Table I. The enzyme activities were determined in rat lung at 24 hr after administration of fluoride. In the absence of Mg^{2+} and Ca^{2+}, the ATPase activity was significantly decreased by fluoride administration ($p<0.01$), whereas the Ca^{2+}-stimulated ATPase activity was slightly elevated by the administration ($p<0.05$). On the other hand, Mg^{2+}-stimulated ATPase activity was not affected with the administration.

Table I. Effect of Mg^{2+} and Ca^{2+} on the ATPase Activity in Lung Mitochondria of Fluoride-Intoxicated Rats

Conditions	Normal	Fluoride administration
None	10.18±0.23	7.82±0.16[a]
Mg^{2+}	110.34±5.22	115.30±6.45
Ca^{2+}	133.44±7.86	146.57±6.29[b]
Mg^{2+}+Ca^{2+}	81.01±2.90	82.46±4.74

The rats were killed 24 hr after a single intraperitoneal dose of sodium fluoride, 35 mg/kg. The reaction mixture containing 60 mM Tris-HCl (pH 8.0), 2.0 mM ATP, enzyme (0.2 mg as protein) was incubated in the presence of 6 mM Ca^{2+}, 6 mM Mg^{2+} or 6 mM Ca^{2+}+6 mM Mg^{2+} at 38° for 30 min. The enzyme activities are expressed as nmoles Pi liberate mg protein/min. Each value is presented as mean ± S.E. of six animals.
a) Student's t-test: $p<0.01$
b) Student's t-test: $p<0.05$

The effect of fluoride administration on the contents of calcium and magnesium in rat lung was examined as shown in Table II. By fluoride administration, calcium content in rat lung was increased by 1.56-times, but magnesium content did not change.

TABLE II. Contents of Calcium and Magnesium in Lung of Fluoride-Intoxicated Rats

	Calcium[a)]	F/N	Magnesium[a)]	F/N
Normal rats	39.20±5.01	1	145.6±9.80	1
Fluoride-intoxicated rats	61.15±7.01	1.56	138.0±10.6	0.95

The rats were killed 24 hr after a single intraperitoneal dose of sodium fluoride, 35 mg/kg.
a) Calcium and magnesium contents are expressed as μg per g wet weight of tissues. Values are presented as mean±S.E. of six animals.
N: normal rats, F: fluoride-intoxicated rats

Discussion

In the present communication we described the comparison of lung mitochondrial ATPase activity of normal and fluoride-intoxicated rats.

The ATPase activity was increased by increasing the calcium concentrations up to about 6 mM in the absence of Mg^{2+}. Similar results of ATPase activation by Mg^{2+} of 0—6 mM were also observed in the absence of Ca^{2+}. In addition, the ATPase activation by Ca^{2+} was found to be inhibited by about 40% by 6 mM Mg^{2+}, and the ATPase activation by Mg^{2+} to be inhibited by about 40% by 6 mM Ca^{2+}.

On the other hand, Nelson, *et al.*[16] reported that the Ca^{2+}-dependent ATPase in spinach chloroplast was inhibited about 50% by 0.3 mM Mg^{2+}. The specificity of the Mg^{2+}-ATPase for substrates was similar to that of the Ca^{2+}-ATPase in the spinach chloroplast.[16]

In this experiment, it was found that Ca^{2+}-stimulated ATPase and Mg^{2+}-stimulated ATPase in the rat lung mitochondria, respectively were inhibited only about 50% by a high concentration (24 mM) of fluoride, but that the Ca^{2+}-stimulated ATPase activity was elevated by fluoride administration of a large dose, 35 mg/kg *i.p.* On the other hand, the Mg^{2+}-stimulated ATPase was not affected by the administration.

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