VIRIDITOXIN INDUCES SWELLING AND ATPASE BY ACTIVATION OF CALCIUM TRANSPORT IN LIVER MITOCHONDRIA

David T. Wong and Robert L. Hamill

The Lilly Research Laboratories, Eli Lilly and Company
Indianapolis, Indiana 46206

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SUMMARY: Viriditoxin activates ATP hydrolysis (ATPase) and swelling in rat liver mitochondria. The monocarboxylic ionophore of divalent cations, A23187, inhibits both activities at low concentrations of viriditoxin, but does not inhibit the ATPase induced by viriditoxin at concentrations above 2.5 x 10^{-5}M. However, the monocarboxylic ionophore of monovalent cations, monensin, has no effect on the viriditoxin induced ATPase, but inhibits the valinomycin induced activity. Viriditoxin may facilitate the active transport of membrane bound calcium into the matrix of mitochondria

Viriditoxin produced by Aspergillus viridi-nutans is toxic to mice with a LD_{50} of 2.8 mg/kg i.p. and has weak antimicrobial activities (1,2). Weisleder and Lillehoj (2) first reported the chemical structure of viriditoxin as shown in Fig. 1. However, the biological effects of viriditoxin at the subcellular levels have not been studied. In this report, we describe the ability of viriditoxin to induce the hydrolysis of ATP and swelling in rat liver mitochondria. Both of these events caused by viriditoxin are sensitive to the divalent cation ionophore, A23187 (3,4). The possibility of activation of the calcium pump by viriditoxin in mitochondria is suggested.

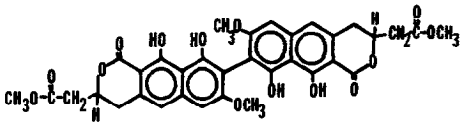

Fig. 1. The chemical structure of viriditoxin.

METHODS AND MATERIALS: Male Sprague-Dawley rats weighing about 150 g were obtained from Harlan Industries, Cumberland, Indiana. Rat liver mitochondria were isolated in 0.25M sucrose and 1 mM EDTA with two washings in 0.25M sucrose (4). Protein was determined by a modified biuret method (5).

Adenosine triphosphatase (ATPase) of rat liver mitochondria was assayed according to the described method (4). Mitochondrial swelling was monitored by a Beckman DB spectrophotometer at 520 nm (6).

Viriditoxin and the ionophores, monensin, valinomycin and A23187, were isolated and crystallized at the Lilly Research Laboratories. All other biochemicals were purchased from Sigma.

RESULTS: Viriditoxin at 5×10^{-5}M activated ATP hydrolysis (ATPase) in rat liver mitochondria (Table 1). The viriditoxin induced ATPase was 50% higher than that induced by $CaCl_2$ but 67%

Table 1

Activation of ATP Hydrolysis by Viriditoxin, Calcium Chloride and Valinomycin in Rat Liver Mitochondria

Activator (M)	Monensin Added (M)	ATP Hydrolyzed µmole/mg protein
Viriditoxin 5×10^{-5}	-	2.81 ± 0.11*
	5×10^{-5}	3.50 ± 0.24
$CaCl_2$, 1×10^{-3}	-	1.92 ± 0.12
	+	1.28 ± 0.30
Valinomycin 1×10^{-6}	-	4.69 ± 0.27
	+	0.79 ± 0.05

*Mean ± S.D.

Mitochondria of 1 mg protein were incubated in a reaction mixture containing 0.25M sucrose, 30 mM Tris-chloride (pH 7.4), 20 mM acetate-Tris (pH 7.4), 1 mM $MgCl_2$, 30 mM KCl and 6 mM ATP-Tris (pH 7.4). Samples also contained viriditoxin, $CaCl_2$ or valinomycin at concentration as indicated. The reaction mixture was incubated at 37°C for 10 min and was then mixed with trichloroacetic acid (TCA) at 5% concentration. Inorganic phosphate in the TCA extract was determined (4).

less than that caused by valinomycin and K^+. Potassium was included in the reaction mixture. Monensin, the polyether monocarboxylic ionophore, abolished over 80% of the valinomycin-K^+ induced ATPase and 30% of the $CaCl_2$-ATPase but not the viriditoxin activated ATPase.

The amount of ATP hydrolyzed increased with the concentration of viriditoxin up to 5 x 10^{-6}M but was not further increased with higher concentrations of viriditoxin (Table 2). The divalent cation ionophore, A23187, inhibited most of the ATPase activities induced by the low concentrations (2.5 x 10^{-6}M and 5 x 10^{-6}M) of viriditoxin and by $CaCl_2$, but not the ATPase activities induced by the higher concentrations of viriditoxin (2.5 x 10^{-5}M and

Table 2

Activation of ATP Hydrolysis by Calcium Chloride and by Various Concentrations of Viriditoxin in Rat Liver Mitochondria and Effects of A23187

Conditions	ATP Hydrolyzed µmole/mg protein
$CaCl_2$, 1 x 10^{-3}M	0.84 ± 0.17
and A23187, 5 x 10^{-6}M	0.28 ± 0.03
Viriditoxin (M)	
2.5 x 10^{-6}	1.10 ± 0.17
5 x 10^{-6}	1.99 ± 0.20
2.5 x 10^{-5}	1.60 ± 0.34
5 x 10^{-5}	1.66 ± 0.34
Viriditoxin (M) and A23187	
2.5 x 10^{-6}	0.19 ± 0.07
5 x 10^{-6}	0.28 ± 0.05
2.5 x 10^{-5}	1.55 ± 0.16
5 x 10^{-5}	1.58 ± 0.18

ATPase was assayed in the same manner as shown in Table 1, except A23187 at 5 x 10^{-6}M and/or viriditoxin at various concentrations was added to sample as indicated.

5 x 10^{-5}M). Since A23187 is known to release endogenous divalent cations, such as Mg^{+2} (3), 1 mM $MgCl_2$ has been included in the ATPase assay.

Mitochondria swell with the accumulation of Ca^{+2} which is supported by either the hydrolysis of ATP or by electron transport (7). In a suspension of ATP-energized mitochondria, the addition of viriditoxin at 8 x 10^{-6}M induced swelling of mitochondria instantly (Fig. 2). The rate of swelling was significantly retarded with a decrease in concentration of viriditoxin to 3.5 x 10^{-6}M. Mitochondria did not swell with the addition of 1.7 x 10^{-6}M viriditoxin until a final addition of 0.33 mM $CaCl_2$ was made.

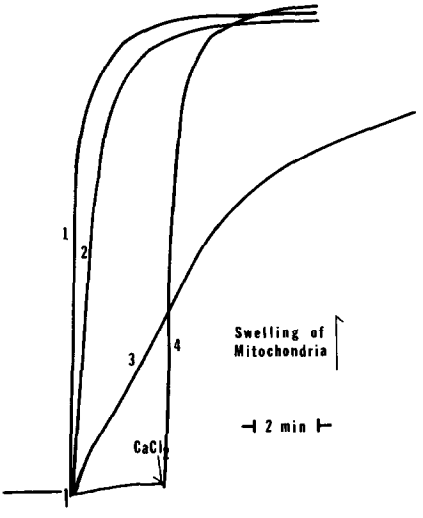

Fig. 2. Mitochondria swell upon the addition of viriditoxin at various concentrations and 0.33 mM $CaCl_2$.

Rat liver mitochondria of 1 mg protein was suspended in 6 ml of reaction mixture containing 0.25M sucrose, 10 mM KCl, 1 mM phosphate-Tris (pH 7.4), 1.7 mM ATP-Tris (pH 7.4) and 3.3 µM rotenone. The mitochondrial suspension was divided into two photo cells. The addition of viriditoxin at 8 x 10^{-6}M(1), 5 x 10^{-6}M(2), 3.5 x 10^{-6}M(3), and 1.7 x 10^{-6}M and 0.33 mM $CaCl_2$(4) are made in one of the cells.

The magnitude of mitochondrial swelling induced by viriditoxin was equal to that induced by the addition of 0.33 mM, $CaCl_2$ (Fig. 3, Trace 1 and 2). The divalent cation ionophore, A23187 at 3.2 x 10^{-6}M, prevented both the $CaCl_2$ and viriditoxin induced swelling in mitochondria (Trace 3). The final addition of ATP was to assure the supply of energy but did not lead to mitochondrial swelling.

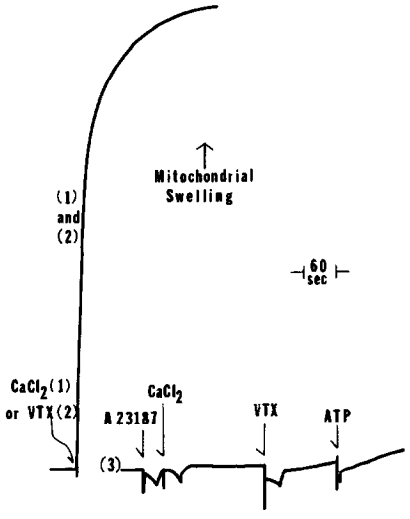

Fig. 3. The divalent cation ionophore, A23187, prevents mitochondrial swelling induced by either $CaCl_2$ or viriditoxin.

Swelling of mitochondria was measured under identical condition as in Fig. 2. Swelling was initiated upon the addition of 0.33 mM $CaCl_2$(1) or 8 x 10^{-6}M viriditoxin (VTX, 2). In a separate experiment(3), the addition of 1 x 10^{-5}M A23187 precede those of $CaCl_2$ and viriditoxin and a final addition of 1.7 mM ATP-Tris (pH 7.4).

DISCUSSION: The viriditoxin activated ATPase has properties similar to those exhibited by the Ca^{+2}-activated ATPase in liver mitochondria. Both ATPase activities are sensitive to the divalent cation ionophore, A23187 (4). The inhibitory effects of

A23187 was overcome by increasing the concentration of viriditoxin. Unlike the valinomycin-K^+ activated ATPase, viriditoxin induced activity was not inhibited by the polyether monocarboxylic ionophore, monensin, while the Ca^{+2}-activated ATPase was only slightly inhibited by monensin. Thus, viriditoxin might have activated the Ca^{+2} dependent ATPase in rat liver mitochondria.

Despite the absence of exogenously added Ca^{+2} ions, viriditoxin is capable of causing swelling in mitochondria. Perhaps, viriditoxin has made membrane bound Ca^{+2} available to the calcium pump which transports Ca^{+2} into the matrix space of mitochondria. Then, the divalent cation diffuses back to the membrane sites. Therefore, the divalent cation is continuously recycled. The divalent cation ionophore, A23187 may deprive the supply of Ca^{+2} to viriditoxin, especially with concentrations of viriditoxin below 1×10^{-5}M.

Viriditoxin has a rather rigid and elongated structure (Fig. 1). It is therefore, not expected to form cyclic metal cation complex as ionophores like monensin or A23187 (8). However, viriditoxin does chelate with metal cations, Mg^{+2} and Ca^{+2} as indicated by their highly fluoresent complexes in ethanol (unpublished data). The 4 hydroxyl groups and 2 keto groups in the molecule are likely responsible for chelation with metal cations. The oxygen atoms of these groups may align themselves in a row and serve as ion exchangers for Ca^{+2} within the membrane. The aromatic hydrocarbon backbone of the molecule provides sufficient lipid solubility in the membrane.

REFERENCES

1. Lillehoj, E. B. and Ciegler, A. (1971) Can J. of Microbiol. 18, 193.

2. Weisleder, D. and Lillehoj, E. T. (1971) Tetrahedron Letter 48, 4705.

3. Reed, P. W. and Lardy, H. A. (1972) J. Biol. Chem. 247, 6970.

4. Wong, D. T., Wilkinson, J. R., Hamill, R. L. and Horng, J. S. (1973) Arch. Biochem. Biophys. 156, 578.

5. Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) J. Biol. Chem. 177, 751.

6. Wong, D. T., Horng, J. S., Hamill, R. L. and Lardy, H. A. (1971) Biochem. Pharmacol. 20, 3169.

7. Chappell, J. B. and Crofts, A. R. (1965) Biochem. J. 95, 378.

8. Pressman, B. C. (1972) in The Role of M embranes in Metabolic Regulation, p. 149, Academic Press, New York.