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Inhibition of vacuolar H^+-ATPases by fusidic acid and suramin

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The vacuolar system of eukaryotic cells is energized by a few ATP-driven ion pumps. One of these, the H^+-ATPase, plays a major role in providing the protonmotive force for several organelles, as well as maintaining the proper pH inside the organelles. Formation of the protonmotive force in organelles isolated from the vacuolar system was inhibited by fusidic acid. The inhibition results from a combination of uncoupling the proton pumping and inhibition of the H^+-ATPase activity. Suramin is also a potent inhibitor of the H^+-ATPase from chromaffin granules. A possible connection between these activities and inhibition of HIV infection is pointed out.

H^+-ATPase; Fusidic acid; Suramin; Enzyme inhibitor

1. INTRODUCTION

Proton ATPases are among the most important ion pumps in nature. They play a crucial role in respiration and photosynthesis, as well as energizing several organelles in eukaryotic cells [1–3]. One of the proton ATPases, the vacuolar-type H^+-ATPase, is present in organelles connected to the vacuolar system of eukaryotic cells. It functions by regulating the pumping of protons into the organelles, which results in acidification of the organelles and generates a protonmotive force that can be utilized for secondary uptake processes. The controlled proton pumping also maintains the proper environment for the maximal activity of specific enzymes and receptors which reside in the organelles.

Regulation of proton pumping into organelles of the vacuolar system is illustrated by the different proton gradients generated during endocytosis [4,5]. In primary endosomes, the ΔpH may be maintained at 0.5 units, while in secondary endosomes, it may increase to about 1 unit, and finally at the lysosomal level, a ΔpH of about 2 is maintained. The pH modulation is obtained by changes of the proton pumping activity of the vacuolar H^+-ATPase. Among the known modulators of the proton pumping activity are various anions, which enhance or inhibit the activity, the phospholipid composition of the membrane and the electrochemical potential across the organelle membrane [6–10]. In vitro, the proton uptake activity can be influenced by several inhibitors such as *N,N'*-dicyclohexylcarbodiimide and thiol reagents, such as *N*-ethylmaleimide. These inhibitors are not specific for the vacuolar H^+-ATPase, the former is a potent inhibitor of the eubacterial-type enzyme and the latter inhibits the plasma membrane H^+-ATPase from *Neurospora crassa* [1–3]. Weak base type uncouplers collapse the proton gradient of organelles in vivo [11,12], however, they are most active in organelles with a large ΔpH, such as lysosomes. It would be useful to find reversible and non-toxic inhibitors of vacuolar H^+-ATPases. In this communication, we report inhibition of vacuolar H^+-ATPases by fusidic acid and suramin.

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2. MATERIALS AND METHODS

Fusidic acid was obtained from Sigma. Suramin was purchased from Mobay Chemical Corp., FBA Pharmaceuticals. These reagents were dissolved in distilled water and kept at −20°C until use. [γ-^{32}P]ATP and [^{32}P]orthophosphate (carrier free) were purchased from Amersham.

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Clathrin-coated vesicles from bovine brain [13], chromaffin granule membranes from bovine adrenal medullae [8,14,15], rat liver lysosomal membrane vesicles [16], vacuolar membranes from red beet [7], chloroplasts from spinach leaves [17], rat liver mitochondria and submitochondrial particles [16] were isolated by published procedures. The H^+-ATPase from chromaffin granule membranes was purified and reconstituted as described previously [8–10].

ATP-dependent proton uptake was assayed by following absorbance changes of acridine orange at 492–540 nm by Aminco DW-2a spectrophotometer as previously described [8–10]. ATPase activity was assayed by measuring the liberation of $^{32}P_i$

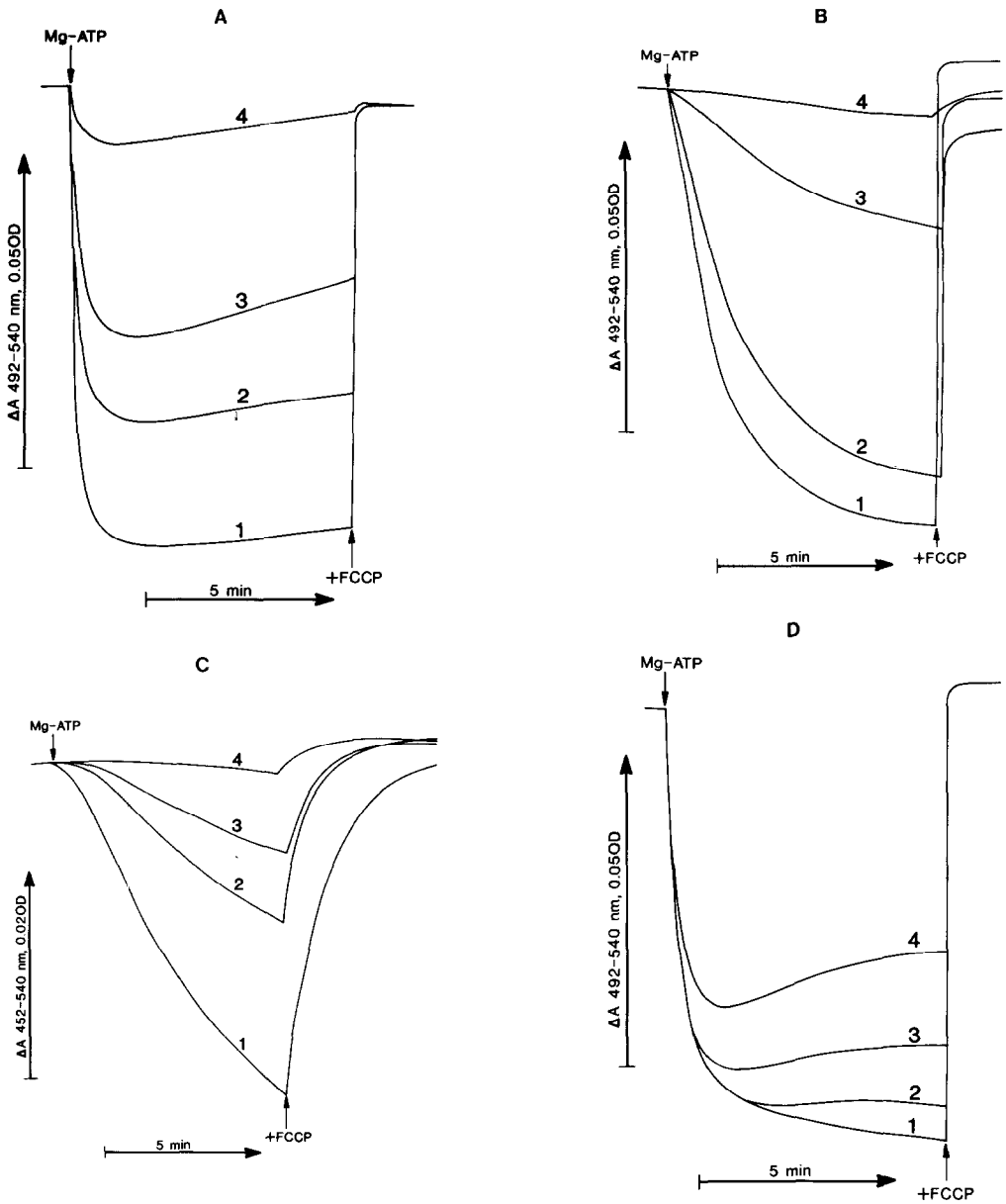

Fig.1. Effect of fusidic acid on the ATP-dependent proton uptake into various organelles of the vacuolar system. The reaction was initiated by the addition of 1 mM Mg-ATP and when specified 1 μM FCCP was added. Clathrin-coated vesicles containing 250 μg protein were assayed in (A), chromaffin granule membranes containing 50 μg protein in (B), lysosomal membranes containing 48 μg protein in (C) and red beet vacuolar membranes containing 54 μg protein in (D). The following amounts of sodium fusidate were included in the reaction mixture: 1, 0 mM; 2, 0.25 mM; 3, 0.5 mM; 4, 1 mM.

from [γ-^{32}P]ATP as previously described [8–10]. Oxidative phosphorylation in mitochondria was assayed by the published procedure [18].

Light-triggered ATPase activity of isolated chloroplasts was assayed as follows: chloroplasts containing 1 mg chlorophyll were added into 2 ml solution containing 22.5 mM Tricine-NaOH (pH 8), 22.5 mM NaCl, 3 mM $MgCl_2$, 3.8 mM DTT, 0.75 mM ATP and 30 μM phenazine methosulfate. After illumination by a slide projector for 5 min, aliquots of 0.1 ml were assayed for ATPase activity in 1 ml reaction mixture containing 30 mM Tricine-NaOH (pH 8), 30 mM NaCl, 4 mM $MgCl_2$, 5 mM DTT, 1 mM [γ-^{32}P]ATP. Sodium fusidate at the specified concentrations was also added. After incubation for 10 min at room temperature the liberation of $^{32}P_i$ was assayed as previously described.

3. RESULTS AND DISCUSSION

The effect of fusidic acid on the ATP-dependent proton accumulation of various organelles of the vacuolar system is depicted in fig.1. In chromaffin granules, lysosomes and clathrin-coated vesicles, the formation of ΔpH was substantially inhibited by fusidic acid at concentrations below millimolar. Smaller inhibition was observed in the plant vacuoles at the same concentrations. This effect may be the result of increased proton efflux, by either uncoupling or acceleration of a specific proton carrier as well as by direct inhibition of the H^+-ATPase of the organelles. As shown in fig.2, a similar effect was observed when fusidic acid was added to purified H^+-ATPase from chromaffin granules, reconstituted into phospholipid vesicles. This experiment eliminates the possible involvement of a specific proton carrier. Fig.2 also shows that fusidic acid inhibits the ATPase activity of purified H^+-ATPase from chromaffin granules, however, higher concentrations of fusidic acid were required to inhibit the ATPase than the proton uptake activity. The higher sensitivity of the proton pumping activity to inhibitors is a general property of the vacuolar proton pumps [6–8].

Fig.3 shows the effect of fusidic acid on the light-triggered ATPase activity of chloroplast membranes. At millimolar concentrations, fusidic acid accelerated the ATPase activity of the chloroplasts, suggesting an uncoupling effect on the system. Fusidic acid also inhibited oxidative phosphorylation in isolated mitochondria, suggesting that it is also an uncoupler of oxidative phosphorylation. The ATPase activity of the mitochondrial and chloroplast enzymes was less sensitive to inhibition by fusidic acid than that of the vacuolar H^+-ATPases.

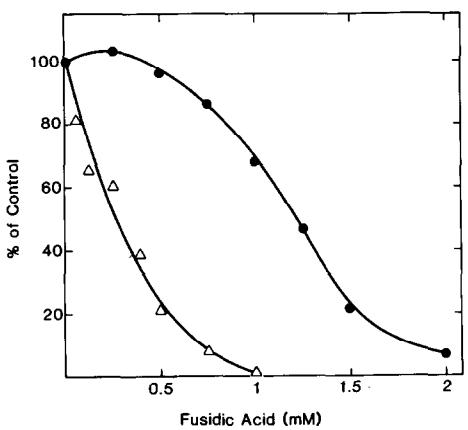

Fig.2. The effect of fusidic acid on the proton uptake and ATPase activities of reconstituted H^+-ATPase from chromaffin granules. ATP-dependent proton uptake (Δ—Δ) and ATPase activity (●—●) were assayed as described in section 2. The control activity for proton uptake was $\Delta A_{492-540nm}$, 0.04 A units per min. The control ATPase activity was 3.7 μmol/min per mg protein.

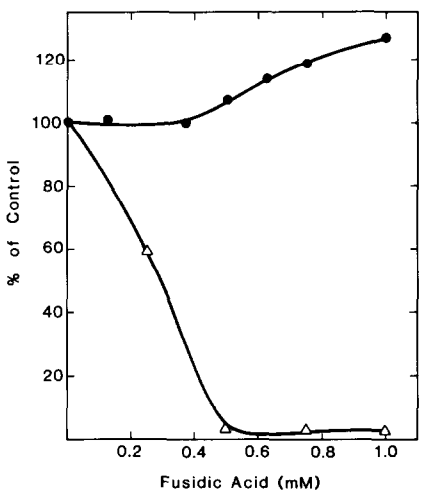

Fig.3. The effect of fusidic acid on light-triggered ATPase activity of isolated chloroplasts and oxidative phosphorylation of isolated mitochondria from rat liver. Light-triggered ATPase activity of isolated chloroplasts (●—●) was measured as described in section 2. The control activity was 7.3 μmol P_i/mg chlorophyll per h. Oxidative phosphorylation of rat liver mitochondria in the presence of fusidic acid (Δ—Δ) is also shown. The substrate was 10 mM glutamate and the phosphate concentration was 10 mM. The control value was 0.31 μmol ATP/mg protein per min.

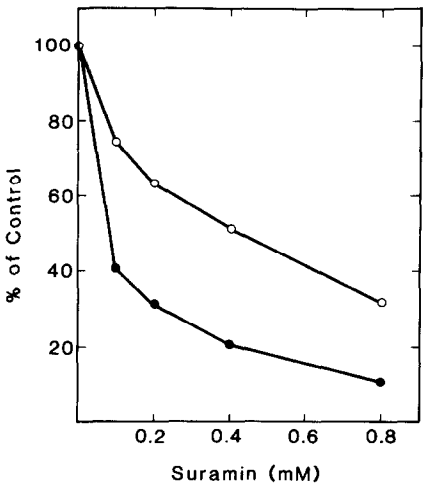

Fig.4. Suramin inhibits both chromaffin granule H^+-ATPase and mitochondrial H^+-ATPase. ATPase activities of reconstituted H^+-ATPase from chromaffin granules (●—●) and of submitochondrial particles from rat liver (○—○) were assayed as described in section 2. Sodium suramin at the specified concentration was added 5 min prior to the assay.

Suramin is being used as an antitrypanosomal agent. Although the drug has been used for many years, the mechanism of its action is not clear. Suramin was shown to inhibit several enzymes including DNA and RNA polymerases [19], and ATPases such as Ca^{2+}-ATPase and Na^+/K^+-ATPase [20]. As shown in fig.4, suramin appears to be a potent inhibitor of the H^+-ATPase from chromaffin granules. About 10-fold higher concentrations of suramin were required to obtain similar inhibition of the H^+-ATPase of rat liver mitochondria. About 50 μM suramin was required to obtain 50% inhibition of the ATPase activity of the H^+-ATPase from chromaffin granules. Addition of bovine serum albumin (1 mg/ml) in the assay mixture prevented the inhibition by suramin, suggesting that the inhibition is a reversible process. In contrast with the effect of fusidic acid, the proton uptake activity had similar sensitivity to suramin as the ATPase activity. This suggests that the site of suramin action is on the catalytic sector of the enzyme and not the proton conducting sector, which may be the primary target of fusidic acid.

It is interesting to note that both suramin and fusidic acid were reported to have effects on the infectivity of HIV and other retroviruses [21–23]. While suramin was shown to inhibit the activity of reverse transcriptase, fusidic acid has no effect on this enzyme. It will be important to study a possible connection between the inhibition of the vacuolar H^+-ATPases and the decrease in HIV infectivity.

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REFERENCES

[1] Mellman, I., Fuchs, R. and Helenius, A. (1986) Annu. Rev. Biochem. 55, 663–700.
[2] Bowman, B.J. and Bowman, E.J. (1986) J. Membr. Biol. 94, 83–97.
[3] Nelson, N. (1987) BioEssays 7, 251–254.
[4] Anderson, R.G.W. and Pathak, R.K. (1985) Cell 40, 635–643.
[5] Marsh, M., Bolzau, E. and Helenius, A. (1982) Cell 32, 931–940.
[6] Johnson, R.G., Beers, M.F. and Scarpa, A. (1982) J. Biol. Chem. 257, 10701–10707.
[7] Bennet, A.B. and Spanswick, R.M. (1984) Plant Physiol. 74, 545–548.
[8] Moriyama, Y. and Nelson, N. (1987) J. Biol. Chem. 262, 9175–9180.
[9] Moriyama, Y. and Nelson, N. (1987) J. Biol. Chem. 262, 14723–14729.
[10] Moriyama, Y. and Nelson, N. (1987) Biochem. Biophys. Res. Commun. 149, 140–144.
[11] Poole, B. and Ohkuma, S. (1981) J. Cell Biol. 90, 665–669.
[12] Maxfield, F.R. (1982) J. Cell Biol. 95, 676–681.
[13] Nandi, P.K., Irace, G., Van Jaarsveld, P.P., Lippoldt, R.E. and Edelhoch, H. (1982) Proc. Natl. Acad. Sci. USA 79, 5881–5885.
[14] Cidon, S. and Nelson, N. (1983) J. Biol. Chem. 258, 2892–2898.
[15] Cidon, S. and Nelson, N. (1986) J. Biol. Chem. 261, 9222–9227.
[16] Moriyama, Y., Takano, T. and Ohkuma, S. (1984) J. Biochem. (Tokyo) 95, 995–1007.
[17] Nelson, N. (1980) Methods Enzymol. 69, 301–313.
[18] Ernster, L. and Nordenbrand, K. (1967) Methods Enzymol. 10, 86–94.
[19] Ono, K., Nakane, H. and Fukushima, M. (1988) Eur. J. Biochem. 172, 349–353.
[20] Fortes, P.A.G., Ellory, J.C. and Lew, V.L. (1973) Biochim. Biophys. Acta 318, 262–272.
[21] Faber, V., Dalgleish, A.G., Newell, A. and Malkovsky, M. (1987) Lancet 10, 827–828.
[22] Mitsuya, H., Popovic, M., Yarchoan, R., Matsushita, S., Gallo, R.C. and Broder, S. (1984) Science 26, 172–174.
[23] Broder, S., Collins, J.M., Markham, P.D., Redfield, R.R., Hoth, D.F., Groopman, J.E., Gallo, R.C., Yarchoan, R., Lane, H.C., Klecker, R.W., Mitsuya, H., Gelmann, E., Resnick, L., Myers, C.E. and Fauci, A.S. (1985) Lancet 21, 627–630.