Short Communication

Differences in Action of Oligomycin and Venturicidin on the H^+-ATPase of *Rhodospirillum rubrum**

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Oligomycin and venturicidin are both antibiotics that inhibit energy-transfer during synthesis and hydrolysis of ATP by mitochondrial and bacterial ATPases. It has previously been suggested that both bind to the same subunit in the F_0 part of the mitochondrial ATPase, the binding site for venturicidin being located within the binding region for oligomycin in yeast[1,2] and in *Neurospora crassa*.[3] However, it has been shown that there are some discrepancies between the action of the two inhibitors on Triton X-100 solubilized ATPases from beef heart mitochondria,[4] on plasma-membrane ATPases of *Paracoccus denitrificans*[5] and on ATPases of chromatophores from *Rhodopseudomonas capsulata*.[6] We present here the results of experiments using the luciferin/luciferase bioluminescense technique for continuous assay of ATP release which reveal dissimilarities in the action of oligomycin and venturicidin on photophosphorylation in chromatophores from *Rhodospirillum rubrum*.

Experimental

R. rubrum, strain S1, was grown and harvested and chromatophores were prepared as in Ref. 7, with the modifications that a Ribi cell fractionator was used to rupture the bacterial cells and that 0.2 M glycylglycine (pH 7.4) was used to wash and suspend the chromatophores. The assay medium was placed in a luminometer (described in Ref. 8). The temperature was maintained at 20 °C and illumination was provided by a 20 W halogen lamp, the light passing through 1 cm of water and through double layers of Wratten 88A gelatine filter which transmits light of wavelengths above 720 nm. ATP-monitoring kit was purchased from *LKB-Wallac (Turku, Finland)*, oligomycin from Sigma (St. Louis, USA) and venturicidin from BDH Chemicals Ltd (Poole, England). Other chemicals were of reagent grade and from commercial sources.

For the experiments of Figs. 1 and 2 the 2 ml assay medium contained 0.2 M glycylglycine (pH 7.5), 100 µl of ATP-monitoring reagent, 50 µM Na-succinate, 50 µM ADP, 2.5 mM Na-Pi, 5 µM P^1,P^5-di(adenosine-5′)pentaphosphate (DAPP) and 125 nM bacteriochlorophyll (Bchl). The medium was illuminated for 15 s to record the activity corresponding to 100 % activity. Inhibitor was then added, still during illumination, and activity was recorded continuously. Before the start and end of illumination, the assay was calibrated twice using an ATP standard.

For the experiment of Fig. 3, the 1.5 ml assay medium contained 0.2 M glycylglycine (pH 7.5), 100 µl of ATP-monitoring reagent, and 75 µM Na-succinate. After 1 min of incubation, 3.2 mM Na-Pi was added [For (e), incubation time was 1 or 3 min; see Table 2]. The first three traces were recorded at 50 mV and the last three at 2 V. Standard ATP additions were 10 pmol and 0.4 nmol of ATP, respectively.

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Results and discussion

The following results were obtained: (1) The inhibitory action of oligomycin showed strong time-dependence and was much slower than inhibition by venturicidin (Fig. 1). After a 10 min incubation the inhibitory effect of oligomycin was still not maximal, whereas venturicidin inhibition reached its maximum within 2 min. (2) Oligomycin inhibition was much stronger when the chromatophores were incubated in light than when incubated in the dark (Table 1). This was not the case with venturicidin, which suggests that oligomycin is dependent on an energized membrane to exhibit its full inhibitory effect (as was also found for rat liver mitochondria[9]). (3) On turning off the light, oligomycin inhibition was partially reversed (Fig. 2), again indicating the need for an energized membrane for maximal inhibition. When the chromatophores were illuminated a second time, the activity was five times greater than when the light was turned off, or slightly more than 25 % of the uninhibited rate. In contrast, venturicidin inhibition was unaltered when the light source was turned off and then turned on again. (4) When no exogenous ADP was

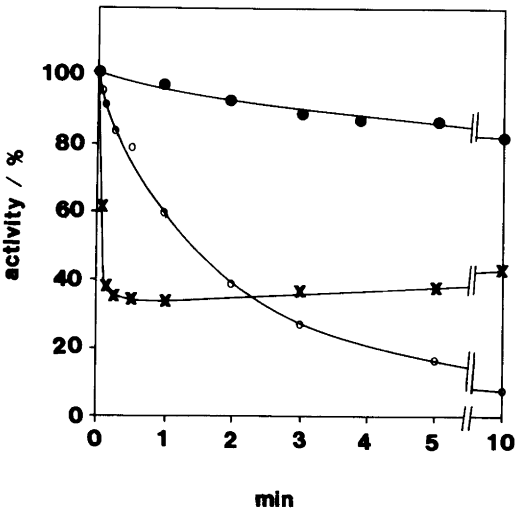

Fig. 1. Time vs. activity diagram for chromatophores incubated with 100 ng ml^{-1} of oligomycin (○——○) and 100 ng ml^{-1} of venturicidin (×——×). (●——●) shows the control chromatophores without any addition. Each point represents the mean value of three different recordings.

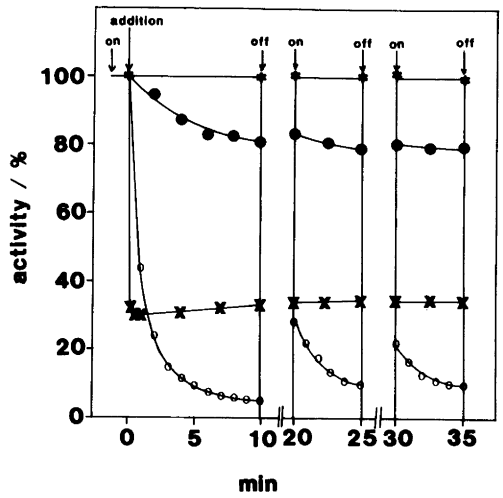

Fig. 2. Time vs. activity diagram showing the reversibility of the oligomycin inhibition. The conditions were as for Fig. 1. (✳——✳) are the controls of the luciferin/luciferase system without chromatophores and without addition of inhibitor. The arrows indicate when light was turned on and off and when addition was made.

added to the assay medium, a small, very slow release of ATP was observed, as detected by the assay system, provided inorganic phosphate was present (Fig. 3). This ATP may arise from photophosphorylation of ADP present in low concentration in the twice-washed chromatophore preparation. It may also arise from phosphorylation of ADP bound at the active site, or from release of bound ATP. As can be seen in Table 2, this

Table 1. The dependence of inhibition on membrane energization. 100 % activity corresponds to the phosphorylation rate in the absence of inhibitor. The conditions were as for Fig. 1, and the medium was incubated with inhibitor for 10 min in the dark or incubated with inhibitor and illuminated for 10 min.

Inhibitor	Amount/ng ml^{-1}	Activity/%	
		Light	Dark
Oligomycin	100	8	83
	1500	0.5	27
Venturicidin	100	43	40
	1500	8	10

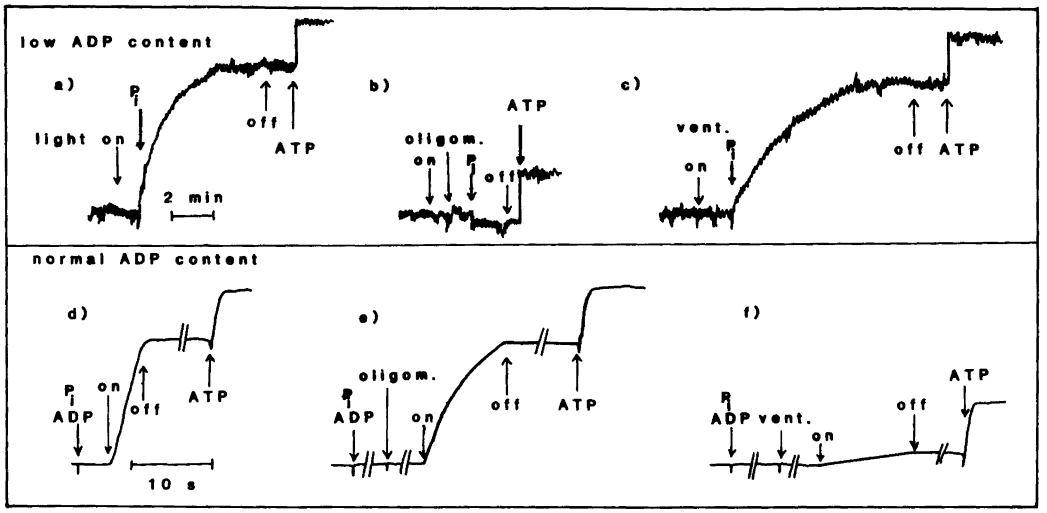

Fig. 3. Diagram showing typical traces for the different actions of oligomycin and venturicidin when: (a)–(c) no ADP was added, and (d)–(f) 75 μM ADP and 7.5 μM DAPP were added. For (a) and (d) no further additions were made; for (b) and (e) the chromatophores were incubated with oligomycin (3.5 μg ml^{-1}); samples (c) and (f) had venturicidin (3.5 μg ml^{-1}) added.

slow phosphorylation/ATP-release was completely inhibited by oligomycin concentrations which only partially inhibited photophosphorylation at higher ADP levels. Venturicidin acted in a different manner: concentrations which only partially inhibited this slow ATP-release almost totally inhibited photophosphorylation when ADP was added.

Table 2. Photophosphorylation with and without added ADP. Medium was as for Fig. 3. The inhibitor concentration in the sample (where added) was 3.5 μg ml^{-1}. Unless otherwise stated, the medium was incubated with inhibitor for 1 min before Pi was added.

Addition	Amount ATP released/%	Initial rate/%	Time for all ATP to be released/%
−ADP			
None	100[a]	100[b]	100[c]
Oligomycin	0	0	0
Venturicidin	91	80	160
+ADP			
None		100[d]	
Oligomycin		65; 30[e]	
Venturicidin		10	

100 % corresponds to: [a]27.9 nM nucleotide in the assay medium or 31.9 mmol ATP (mol Bchl)$^{-1}$; [b]21.7 mmol ATP (mol Bchl)$^{-1}$ min^{-1}; [c]5 min; [d]$10.7 \cdot 10^3$ mmol ATP (mol Bchl)$^{-1}$ min^{-1}. [e]Incubation time with oligomycin was 3 min.

These results suggest that even though oligomycin and venturicidin seem to interact with the same domain of the proteolipid in the membrane part of the ATPase, the mode of action of these antibiotics is different. Oligomycin seems to be most potent as an inhibitor when the protein has a particular conformation induced by energization, and when the ADP level is low, whereas venturicidin inhibition is strongest when all catalytic sites are occupied by nucleotides, and weaker when the ADP level is low. This most likely reflects a need for different conformations of the ATPase for the two inhibitors to be most effective.

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