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REVIEW

BAFILOMYCINS AND CONCANAMYCINS AS INHIBITORS OF V-ATPases AND P-ATPases

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Summary

Bafilomycins and concanamycins, two groups of the plecomacrolide-defined class of macrolide antibiotics, have recently been recognized as important tools for studying the physiological role of vacuolar-type, proton-translocating ATPases (V-ATPases) and ATPases with phosphorylated states (P-ATPases) in animal and plant cells as well as in yeast, fungi and bacteria. The following review will give an account of the classification and function of these antibiotics.

Key words: plecomacrolide antibiotics, V-ATPase, P-ATPase, vacuolar compartments, ATP-binding cassette (ABC) transporter.

Introduction

Most primary ion pumps use the energy provided by the hydrolysis of ATP to energize ion-transport processes across cell membranes. Accordingly, ‘ATPases’ have been found in all organisms studied so far. Several families of ATPases, which can be distinguished by their transport mechanism, their quaternary structure and their sensitivity towards specific inhibitors, have been found in various membranes and cell compartments. In an initial attempt to classify this group of transport proteins, Pedersen and Carafoli (1987) introduced the terms P-type, F-type and V-type ATPases. P-type ATPases (P-ATPases) have a phosphorylated transitional stage; F-type ATPases (F-ATPases) are primarily used in ATP synthesis and, therefore, are usually referred to as ‘F-ATP synthases’; V-type ATPases (V-ATPases) are genetically and functionally related to F-ATPases but function only in ATP breakdown. Recently, it became apparent that the superfamily of ATP-Binding Cassette (ABC) transporters (or traffic ATPases) had to be added to this classification scheme. ABC transporters, which have been found in all living organisms, mediate uptake or efflux of a variety of solutes at the expense of ATP (Higgins, 1992). The use of specific inhibitors has made it possible not only to make quick assignments of unknown ATPases but also to study their function in the corresponding membrane. Thus, *ortho*-vanadate is a well-known inhibitor for P-ATPases, whereas F-ATPases are inhibited by venturicidin. For a long time, dicyclohexylcarbodiimide (DCCD), NO_3^-, *N*-ethylmaleimide (NEM) and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) were the only inhibitors for V-ATPases, although DCCD also affects F-ATPases. With the discovery of bafilomycins as high-affinity inhibitors of V-ATPases (Bowman *et al.* 1988), it became possible to study specifically the function of this type of ATPase. Importantly, this class of antibiotics for the first time allows a clear distinction between the three types of ATPases: F-ATPases are not affected, whereas P-ATPases are inhibited in the micromolar and V-ATPases in the nanomolar concentration range. Interestingly, ATP-binding cassette (ABC) transporters were also found to be sensitive to *ortho*-vanadate and bafilomycin A_1 (micromolar concentration) (Sharom *et al.* 1995; Hunke *et al.* 1995).

History

The discovery of the bafilomycins and concanamycins at the beginning of the 1980s was based on a screening approach that had nothing to do with vacuolar ATPases. Hensens *et al.* (1983) were searching for microbial secondary metabolites that would have effects similar to those of the cardiac glycosides ouabain and digitoxin. The macrolide antibiotic L681,110A_1, later reclassified as bafilomycin C_1, isolated from the culture medium of *Streptomyces* sp. MA-5038, inhibited the target enzyme of the cardiac glycosides, the Na^+/K^+-ATPase (P-type), with a K_i of 11 μmol l^{-1}. However, the compound lacked cardiotonic activity *in vivo* (Huang *et al.* 1984). Further studies revealed that the antibiotic showed anthelmintic activity against *Caenorhabditis elegans*, was active against ticks and tapeworms and also stimulated the release of γ-aminobutyric acid (GABA) from synaptosomes of rat brain (Hensens *et al.* 1983). Independently, Werner *et al.* (1983, 1984) isolated from

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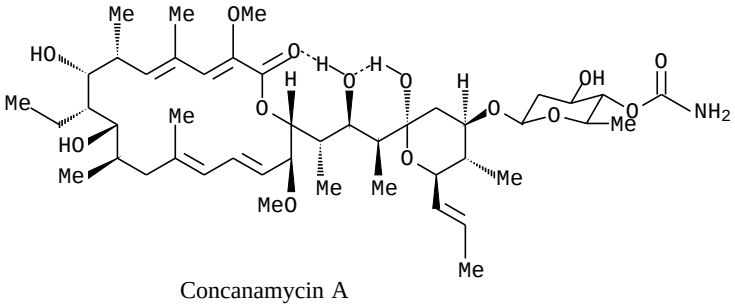
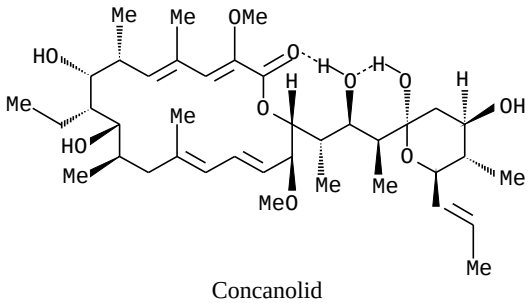
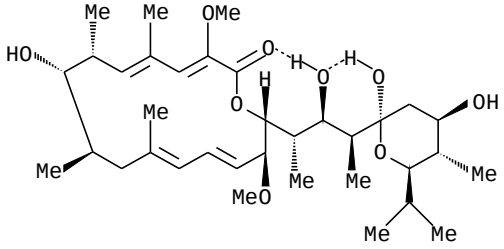


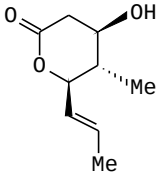
Concanamycin A



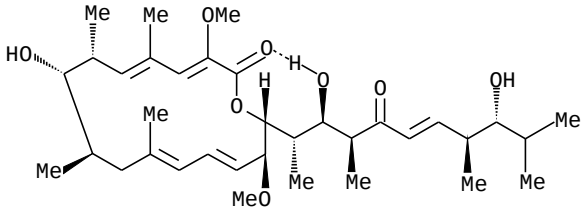
Concanolid



Bafilomycin A_1



Prelactone C



Bafilomycin D

Fig. 1. Structures of different bafilomycins, concanamycins and prelactone C, a shunt product which was isolated from the extract of a concanamycin-producing *Streptomyces* sp.

the mycelium of *Streptomyces griseus* various naturally occurring bafilomycin derivatives, including bafilomycin A_1 (Fig. 1). These derivatives exhibited antibiotic activity against several fungi, yeast and Gram-positive bacteria.

Another group of related macrolides was found in a screening for immunosuppressive compounds (Kinashi *et al.* 1981*a*,*b*). These antibiotics, which were isolated from the culture medium of *Streptomyces diastatochromogenes*, were named concana*mycins* because they inhibited the proliferation of concanavalin-A-stimulated T-cells (Kinashi *et al.* 1984). Further studies revealed that this group of antibiotics exhibited activity against fungi, but not against bacteria. The latter observation can be explained by the additional sugar moiety (Fig. 1), since glycosylation of bafilomycins also abolishes their bactericidal activity (Meyer *et al.* 1985). Even though this group of antibiotics exhibited additional interesting effects, e.g. a naturally occurring bafilomycin derivative showed potential as an insecticide (Kretschmer *et al.* 1985), and bafilomycin A_1, like the concanamycins, inhibited the proliferation of concanavalin-A-stimulated T-cells (Heinle *et al.* 1988), the high toxicity of these compounds was prohibitive for clinical applications. For example, mice die after intraperitoneal injection of 1 mg of concanamycin A per kilogram body mass (Kinashi *et al.* 1984). Only some years later, in the course of an empirical study on different ion-translocating ATPases, was it discovered by Bowman *et al.* (1988) that the bafilomycins inhibit H^+ V-ATPases: inhibition of V-ATPases occurs at nanomolar concentrations of bafilomycin A_1, whereas P-ATPases are affected at micromolar concentrations. ATP synthases of mitochondria and bacteria are insensitive to these macrolide antibiotics. It was later shown that the concanamycins are even more potent inhibitors of V-ATPases than are the bafilomycins (Dröse *et al.* 1993).

Classification and structure

Bafilomycins and concanamycins, which can be distinguished in structure and function from the classical Woodward macrolides, are closely related compounds that belong to the group of 'unusual macrolides' (Õmura, 1984), which are characterized by a 10- to 48-membered macrocyclic lactone ring and which are produced by bacteria. Together with related, highly unsaturated macrolides produced by *Streptomyces* species, bafilomycins and concanamycins encompass the group of plecomacrolides (Bindseil and Zeeck, 1994). The latter authors distinguish three classes within this group of antibiotics. Recently, further concanamycin antibiotics with an 18-membered macrolactone ring have been described (Ishii *et al.* 1995). Of the plecomacrolides discovered so far, only the bafilomycins, concanamycins and elaiophylin have been tested as ATPase inhibitors. Therefore, it is quite conceivable that other inhibitors may still be found in this class of antibiotics. Their most pronounced structural element is the six-membered hemiacetal ring, which is connected to the macrolactone ring by a C3-spacer. This unusual side-chain folding had led to the euonym plecomacrolides (Greek πλεκω, I fold) (Bindseil, 1993; Bindseil and Zeeck, 1994). In addition to this basic structural element, comparable centres of chirality are identical in the macrolactone rings of the 16-membered bafilomycins and the 18-membered concanamycins (Fig. 1;

Table 1. *Effects of bafilomycin A_1 on V-ATPases* in vitro

System investigated	Reference	Method used	I_{50} (nmol mg^{-1})	K_i (nmol l^{-1})
Vacuolar membranes of *Neurospora crassa*	Bowman *et al.* (1988)	ATPase activity	0.4	
Vacuolar membranes of *Neurospora crassa*	Bowman *et al.* (1988)	H^+ transport	0.07	
Vacuolar membranes of *Zea mays*	Bowman *et al.* (1988)	H^+ transport	0.004	
Vacuolar membranes of *Saccharomyces cerevisiae*	Kane *et al.* (1989)	ATPase activity	0.6	0.04
Partially purified vacuolar membranes of *Saccharomyces cerevisiae*	Kane *et al.* (1989)	ATPase activity	1.7	0.1
Tonoplast-enriched fraction from *Zea mays* coleoptiles	White (1994)	ATPase activity	0.043±0.012	0.23
Various animal endomembrane systems, including chromaffin granules	Moriyama and Nelson (1989)	H^+ transport	0.01–0.08	
Reconstituted H^+-ATPase from chromaffin granule membranes (bovine adrenal gland)	Moriyama and Nelson (1989)	H^+ transport	5	
Microsomes from medullary bone	Sundquist *et al.* (1990)	H^+ transport	3	
Vesicles from goblet cell apical membrane of tobacco hornworm (*Manduca sexta*)	Wieczorek *et al.* (1991)	ATPase activity	20±10	
Vesicles from goblet cell apical membrane of tobacco hornworm (*Manduca sexta*)	Wieczorek *et al.* (1991)	H^+ transport	9±2	
Purified H^+-ATPase from goblet cells	Wieczorek *et al.* (1991)	ATPase activity	19±1	
Cyclic-AMP-dependent H^+ secretion by mantle epithelium of the freshwater clam *Unio complanatus*	Hudson (1993)	H^+ transport		7

I_{50}, concentration of inhibitor required to give 50 % inhibition; K_i, inhibitor constant.

Bindseil, 1993). As another common feature, all plecomacrolides possess an intramolecular hydrogen-bonding system between the macrolactone ring, the C3-spacer and the hemiketal ring (Fig. 1). A few representatives of the plecomacrolides have an additional moiety associated with the hemiacetal, e.g. deoxyrhamnose derivatives in the case of concanamycins or fumarate (or derivatives) in the case of bafilomycins B_1 and C_1. The biosynthetic pathway of concanamycin A has been solved by nuclear magnetic resonance spectroscopy of ^{13}C-labelled intermediates (Bindseil and Zeeck, 1994).

Application *in vitro*

The application of bafilomycins as specific inhibitors of V-ATPases led to the discovery and characterization of further vacuolar ATPase complexes in different organisms (see Table 1). In most cases, inhibition of the ATPase activity or of the ATP-driven H^+ translocation was studied. However, the concentration of antibiotic necessary for half-maximal inhibition (I_{50}) depends on the protein concentration (Bowman *et al.* 1988; Hanada *et al.* 1990; White, 1994) and on the purity of the V-ATPase (see Table 1 and references in White, 1994). Therefore, it is difficult to compare directly the published inhibitor constant (K_i) values. Nevertheless, the inhibition of an unknown H^+-ATPase by nanomolar concentrations of bafilomycin or concanamycin can be taken as a criterion for the classification of that enzyme complex as a V-ATPase. An exception to the rule could be a recently discovered V-ATPase from the thermophilic bacterium *Thermus thermophilus* that was insensitive to bafilomycin A_1 up to a concentration of 10 nmol l^{-1} (Yokoyama *et al.* 1994). H^+-ATPases derived from Archaea, which are believed to take an intermediate position between F- and V-ATPases, are inhibited at micromolar concentrations of bafilomycin A_1 (Chen and Konisky, 1993).

Application *in vivo*

Even before bafilomycins and concanamycins were used as inhibitors, it was known that vacuolar ATPases pump protons into the lumen of various endomembrane systems and cellular compartments (Nelson, 1995). Thus, V-ATPases are responsible for the acidification of eukaryotic organelles such as endosomes and lysosomes and the vacuoles of plants and fungi (Mellmann *et al.* 1986; Nelson, 1995). Moreover, V-ATPases have been found in the plasma membrane of kidney (Gluck and Al-Awqati, 1984), macrophages (Swallow *et al.* 1988), osteoclasts (Blair *et al.* 1989) and epididymis (Breton *et al.* 1995). To study the physiological role of acidification for such compartments, acidotropic permeant bases, which accumulate in the lumen in their protonated form and thereby raise luminal pH, were used (Mellmann *et al.* 1986). However, as the accumulation of such bases causes osmotic swelling of the compartments (vacuolation), pleiotropic effects cannot be excluded. With the discovery of bafilomycins as high-affinity inhibitors of V-ATPases *in vitro* (Bowman *et al.* 1988), it was possible to perform more specific inhibition experiments. In cell cultures, the effect of bafilomycins on the acidification of

Table 2. *Effects of bafilomycin A_1 and concanamycin A on V-ATPases* in vivo *(in cell cultures)*

System investigated/effect observed	Reference	Range of inhibition or I_{50}
Inhibition of pinocytosis in *Entamoeba histolytica*/ inhibition of acidification in pinocytic vesicles	Löhden-Bendinger and Bakker-Grunwald (1990)	0.05–0.3 nmol mg^{-1}
Inhibition of lysosomal acidification and the degradation of EGF in mammalian cells	Yoshimori *et al.* (1991)	Partial at 10 nmol l^{-1}, complete at 100 nmol l^{-1}
Inhibition of bone resorption by osteoclasts	Sundquist *et al.* (1990)	1 nmol l^{-1}
Effect on the cytotoxic action of diphtheria toxin, inhibition of Acridine Orange uptake in cultured cells	Umata *et al.* (1990)	Partial at 10 nmol l^{-1}, complete at 500 nmol l^{-1}
Inhibition of *Helicobacter pylori* toxin-induced vacuolation of HeLa cells and inhibition of Neutral Red uptake	Papini *et al.* (1993)	4 nmol l^{-1}
Endocytic and exocytic processes during viral infection of Semliki Forest Virus (SFV)	Palokangas *et al.* (1994)	Approximately 5 nmol l^{-1}
Inhibition of virus entry into animal cells (SFV, influenza virus, vesicular stomatitis virus)	Guinea and Carrasco (1994) Pérez and Carrasco (1994)	5 nmol l^{-1} concanamycin A
Proliferation of cultured cells	Manabe *et al.* (1993)	0.6–8.5 nmol l^{-1}, cell death at 5 nmol l^{-1}
Proliferation of osteoblasts	Hall (1994)	0.5 nmol l^{-1} concanamycin A, cell death after 54 h

I_{50}, concentration of inhibitor required to give 50 % inhibition; EGF, epidermal growth factor.

intracellular compartments was monitored by the uptake of fluorescent compounds, e.g. Acridine Orange (Umata *et al.* 1990; Yoshimori *et al.* 1991). In another set of experiments, the pH-dependent fluorescence change of phagocytized FITC–dextran was recorded in *Entamoeba histolytica* cells (Löhden-Bendinger and Bakker-Grunwald, 1990).

Importantly, it was shown that bafilomycins can inhibit vacuolar ATPases in intact cells without causing vacuolation (Umata *et al.* 1990; Tapper and Sundler, 1995). These initial experiments stimulated the interest of different research groups to study the direct or indirect involvement of V-ATPases in physiological processes. Meanwhile, bafilomycins, and increasingly concanamycins, are routinely used as inhibitors of V-ATPases in different types of cells. We will present a few examples to demonstrate the extent, but also the limits, of their application.

A priori plecomacrolides influence all cellular processes which depend on V-ATPases. If the plecomacrolides are added in 'sufficient' amounts to the culture medium, following a lag phase (Yoshimori *et al.* 1991; Simpson *et al.* 1994), the immediate effect is the inhibition of the vacuolar ATPase in different organelles accompanied by an increase in the luminal pH (Yoshimori *et al.* 1991). The latter effect can secondarily cause changes in the affinity between receptors and ligands, in the conformation of proteins and in the activity of enzymes (for an overview, see Mellmann *et al.* 1986). Subsequently, these indirect effects may be responsible for a variety of further effects (see Table 2 for some examples). Nevertheless, all these effects are based on the inhibition of cellular V-ATPases. However, this universal action of the plecomacrolides on V-ATPases also limits their application since, given in 'sufficient' amounts, the activity of all cellular V-ATPases is impaired. Therefore, all processes that are dependent on acidification through this ATPase complex are affected. In addition, it is by no means easy to identify an unequivocal cause for the observed effects. The outcome of proton pumping by V-ATPases is not necessarily acidification (Harvey, 1992). The lumen of lepidopteran midgut is alkalized to a pH>11 by the combined activity of an H^+ V-ATPase and a K^+/nH^+ antiporter; this process is inhibited by nanomolar concentrations of bafilomycin A_1 (Wieczorek *et al.* 1991).

Within these limitations, it is possible to study certain processes, such as endocytosis and exocytosis. For example, studies on the internalization of epidermal growth factor (EGF) (Yoshimori *et al.* 1991) revealed that, after inhibition of the vacuolar ATPase with bafilomycin A_1, lysosomal degradation of endocytosed EGF was completely abolished, whereas internalization of EGF and its transport into lysosomes was not affected. These results demonstrate that the time point at which the inhibitor is added to the culture can be of significance for the effects observed. Thus, the 'suppression' of the effect of different bacterial toxins, which enter their target cells *via* receptor-mediated endocytosis, is only observed when cells are pretreated with plecomacrolides. These toxins require an acidification of the endosomes for their processing and for the conformational change that is a prerequisite for allowing them to enter the cytoplasm (Umata *et al.* 1990; Williamson and Neale, 1994; Simpson *et al.* 1994). Once the toxins have entered the cell, inhibition of the V-ATPases cannot prevent their cytotoxic actions. Similar effects have been observed for the suppression of invasion of pathogenic viruses in animal cells (Guinea and Carrasco, 1994; Palokangas *et al.* 1994; Pérez and Carrasco, 1994). However, the strongest limitation to the application of plecomacrolides is the fact that cell death occurs upon prolonged incubation of the cells in the presence of the inhibitors (Hall, 1994; Manabe *et al.* 1993; Martinez-

Zaguilan *et al.* 1993; Ohkuma *et al.* 1993). This cytotoxic effect of the macrolide antibiotics is based on the fact that acidification of intracellular compartments is a prerequisite for several essential processes, e.g. membrane turnover, and thus for the endocytotic uptake of nutrients. In this context, Hall (1994) clearly demonstrated the impairment of iron uptake in osteoclasts that have been exposed to concanamycin A. Also the intra-Golgi trafficking or the secretory pathway from the Golgi to the plasma membrane (Yilla *et al.* 1993), the availability of *de novo* synthesized receptors or the 'recycling' of membrane receptors (Reaves and Banting, 1994) and their processing within the Golgi apparatus (Muroi *et al.* 1994) are inhibited. In fact, Kinashi *et al.* (1984) described the general toxicity of concanamycins and demonstrated that the effect of these compounds is not limited to concanavalin-A-stimulated cells. Therefore, their 'immunosuppressive' effect is due to their general cytotoxicity. In view of this complication, it is hard to envisage any clinical benefits from pharmacological application of these substances, be it the suppression of cell invasion by pathogenic viruses (Guinea and Carrasco, 1994; Pérez and Carrasco, 1994), the suppression of the effect of the vacuolating toxin from *Helicobacter pylori* (Papini *et al.* 1993; Cover *et al.* 1993) or the inhibition of acidification by osteoclasts (Sundquist *et al.* 1990) in order to ameliorate osteoporosis (Baron *et al.* 1995).

Even so, useful conclusions can be drawn from long-term *in vivo* studies. After removal of the macrolide antibiotics from cultured cells by a washing step, their inhibitory effects are reversed and cell division resumes (Manabe *et al.* 1993; Yoshimori *et al.* 1991). As bafilomycin A_1 binds very tightly to purified V-ATPase of chromaffin granules (dissociation constant, K_D, approximately 10^{-8} mol l^{-1}; Hanada *et al.* 1990), *de novo* synthesis of enzyme may be responsible for the recovery of activity. In short-term incubations, protein synthesis is not affected by bafilomycins (Umata *et al.* 1990).

The concentrations of the plecomacrolides which have been used for these experiments vary over orders of magnitude (nanomolar to micromolar concentration range). *In vitro* P-ATPases, e.g. the Na^+/K^+-ATPase present in the plasma membrane, are also inhibited by micromolar concentrations of the antibiotics (Bowman *et al.* 1988; Huang *et al.* 1984). Therefore, to exclude effects due to inhibition of cellular P-ATPases, only nanomolar concentrations of the plecomacrolides should be used *in vivo*.

The values given in Table 2 refer to the concentrations of 'free' antibiotics. Because of their lipophilic nature, these compounds are expected to accumulate in the cell membrane, so that their effective concentration may be significantly higher there. From the published values, it can be deduced that the plecomacrolides can easily reach their target, the V-ATPases in mammalian cells. However, higher concentrations of bafilomycin A_1 than those required to block the plasma-membrane-localized H^+ V-ATPase were needed to affect lysosomal pH in macrophages (Tapper and Sundler, 1995). This difference in bafilomycin A_1 sensitivity could be due to a limited accessibility to bafilomycin A_1 in the lysosomal compartment compared with the plasma membrane. An alternative explanation for the observed difference would be that the H^+ V-ATPase of macrophage lysosomes differs from that of their plasma membrane. A different situation may be found in invertebrates. It has been reported (Dijkstra *et al.* 1994) that 10 µmol l^{-1} bafilomycin A_1 is necessary to obtain half-maximal inhibition of the V-ATPase in the Malpighian tubules of an ant. The authors discussed the possibility that the inhibitors may have difficulty in reaching their target sites. In agreement with this notion, the purified V-ATPase from goblet cells of *Manduca sexta* is inhibited with an I_{50} value of 19 nmol mg^{-1} by bafilomycin A_1 (Wieczorek *et al.* 1991), which is in good agreement with the I_{50} of the purified enzyme of chromaffin granules (5 nmol mg^{-1}) (Moriyama and Nelson, 1989). A similar problem has been observed with the inhibition of the vacuolar H^+ V-ATPase in plants, e.g. *Chara* cells. At nanomolar concentrations, bafilomycin A_1 inhibits proton transport in tonoplast vesicles *in vitro*, and at approximately 100 nmol l^{-1} the acidification of the vacuoles by the V-ATPase *in vivo*, if the inhibitor has been applied through the vacuolar perfusion technique from the vacuolar side. However, externally applied bafilomycin A_1 has no influence on the luminal pH of vacuoles (Okazaki *et al.* 1992). By contrast, externally applied concanamycin 4-B (identical to concanolid in Dröse *et al.* 1993) can raise the pH of the vacuoles (Tazawa *et al.* 1995). Note that the vacuolar H^+-pyrophosphatase is not affected by plecomacrolides (Okazaki *et al.* 1992).

Mechanism of action of bafilomycins and concanamycins

Woo *et al.* (1992) reported that concanamycins inhibit the acidification of lysosomes. At the same time, we have carried out studies in collaboration with Dr A. Zeeck and Dr E. J. Bowman on the structure/activity relationship of chemically modified bafilomycins and concanamycins (Dröse *et al.* 1993). The important result was the unequivocal identification of the concanamycins as a further class of specific inhibitors for V-ATPases. Like bafilomycins, concanamycins inhibit P-ATPases at micromolar concentrations (Fig. 2). Our structure/activity studies revealed that the macrolactone ring of the plecomacrolides contributes strongly to the inhibition potential, since derivatives carrying an open hemiketal ring, such as bafilomycin D (Fig. 1), still inhibit V- and P-ATPases; however, the lower affinity of these derivatives indicates that a closed hemiketal ring is necessary for optimal activity (Dröse *et al.* 1993). The participation of the hemiketal ring in the enzyme/inhibitor interaction can also be deduced from the fact that the isolated shunt product prelactone C (Bindseil and Zeeck, 1993) (Fig. 1) and related compounds (Boddien, 1995) inhibit V-ATPases at micromolar concentrations (C. Boddien, S. Dröse, A. Zeeck and K. Altendorf, unpublished results). The additional sugar moiety in concanamycins, which is absent in bafilomycins, does not play an essential role for the inhibitory action (see concanolid, Fig. 2); however, it is responsible for the higher stability of the compound (Bindseil, 1993). The increased sensitivity of V-ATPases for concanamycin A could

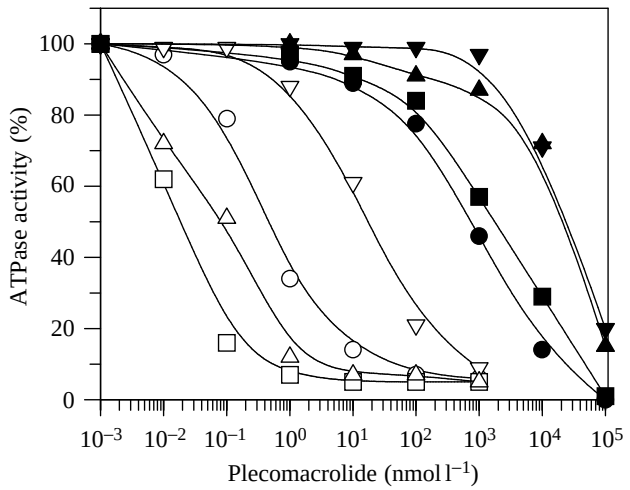


Fig. 2. Inhibitory effects of bafilomycin A_1 (○, ●), bafilomycin D (▽, ▼), concanamycin A (□, ■) and concanolid (△, ▲) on the activity of the K^+-translocating Kdp-ATPase of *Escherichia coli* (filled symbols) and the H^+ V-ATPase from *Neurospora crassa* (open symbols). Mean values of three independent assays are taken from Dröse *et al.* (1993).

be based on the larger flexibility of the 18-membered macrolide ring (Dröse *et al.* 1993), for which an induced fit model has been proposed (Bindseil and Zeeck, 1994). The conclusions drawn from the structure/activity studies are supported by *in vivo* studies by other research groups. Plecomacrolides, in which the macrolide ring has been opened, showed in comparison with the intact compound no inhibitory effect (Heinle *et al.* 1988; Manabe *et al.* 1993; Ito *et al.* 1995). Derivatives with an open hemiketal ring showed a significantly reduced affinity for V-ATPases in cell cultures (Manabe *et al.* 1993; Woo *et al.* 1992; Ito *et al.* 1995). The effect of aglycons of concanamycins is in most cases comparable with that of the intact compound (Woo *et al.* 1992). However, there have been reports that the effect of the aglycon was slightly weaker (Manabe *et al.* 1993) or even stronger (Ito *et al.* 1995) than that of concanamycins.

For V-ATPases, no competition has been observed between ATP and bafilomycin A_1 binding (Bowman *et al.* 1988; Hanada *et al.* 1990). The results obtained for the inhibition of the Na^+/K^+-ATPase (P-type) imply that a non-competitive inhibition mechanism of ATP hydrolysis is present (Huang *et al.* 1984). For the K^+-translocating Kdp-ATPase (P-type) of *Escherichia coli* (Altendorf *et al.* 1992), a similar effect has been found (S. Dröse and K. Altendorf, unpublished results). Since Siebers and Altendorf (1989) did not find any effect of bafilomycin A_1 on the level of phosphorylation of the Kdp-ATPase, they concluded that the antibiotic exerts its effect on the conformational transition from the E_1- to the E_2-form, typical for P-ATPases.

Hanada *et al.* (1990) reported that an excess of isolated and purified V_o complexes, which include the membrane-bound H^+ channel of V-ATPases, protects isolated H^+-ATPase of chromaffin granules from bafilomycin A_1 inhibition in a dose-dependent way. The addition of V_1 complexes, which carry the catalytic centres, had no effect. These studies, which imply a binding of bafilomycin to the V_o complex, have been continued by other research groups. Using [^{3}H]bafilomycin, Mattson and Keeling (1996) reported that this antibiotic inhibits the V-ATPase of osteoclasts by binding tightly but non-covalently to the V_o complex. Crider *et al.* (1994) observed that nanomolar concentrations of bafilomycin A_1 inhibited H^+ translocation through purified V_o in proteoliposomes. In an independent study, these results have been confirmed by Zhang *et al.* (1994), who have narrowed down the binding site to the 116 kDa subunit using extended protection experiments with isolated subunits of the V_o complex. However, several V-ATPases, including the insect plasma membrane enzyme, do not have a 116 kDa subunit (Wieczorek *et al.* 1989). Using bafilomycin-C_1-related affinity chromatography for the purification of the H^+-ATPase of microsomal vesicles from osteoclasts, Rautiala *et al.* (1993) concluded that the 17 kDa subunit (proteolipid) of the V_o complex could be the binding site for the antibiotic. However, the studies of Zhang *et al.* (1994) with isolated proteolipid argue against a high-affinity interaction between bafilomycin and the 17 kDa subunit of the V_o part.

Conclusions

The high specificity of bafilomycins and concanamycins in inhibiting V-ATPases and P-ATPases makes this group of compounds especially useful for the analysis of structure/activity relationships. Chemically modified derivatives have already disclosed some parts of the molecules which are important for their inhibitory action. Further analyses of this type should illuminate additional structural elements that contribute to the high specificity of inhibition.

Intriguingly, the plecomacrolides inhibit structurally unrelated ATPases, although with different K_i values. Since the antibiotics are not covalently bound to the enzyme complex, attempts to explore the binding site(s) without preventing dissociation are doomed to fail. A promising avenue would be the synthesis of azido derivatives that, upon illumination, covalently modify the enzyme complex. Together with the generation of resistant mutants and their molecular analysis, these studies should disclose the binding site(s) and lead to recognition of similar structural elements in these otherwise unrelated enzyme complexes.

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