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Differences in ion-channel formation by ampullosporins B, C, D and semisynthetic desacetyltryptophanyl ampullosporin A

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Abstract

Peptaibol-type ampullosporins B (**2**) and D (**4**) are capable of forming ion-conducting pores in planar lipid bilayer membrane prepared from soybean phosphatidylcholine in a similar manner as it was shown for ampullosporin A (**1**). However, the isomeric ampullosporin C (**3**) was required in 20-fold higher concentration to afford a comparable effect. In contrast to **1**, **2**, **3** and **4**, the desacetyltryptophanyl ampullosporin A (**5**) failed to form ion channels. The results suggest that the sequence of amino acids especially at positions 8–10, the nitrogen-terminal acetyl residues and tryptophane are major factors determining ion-channel formation within bilayer membranes. The differences in membrane activities were comparable to the observed biological activities.

Keywords: Peptaibol-type ampullosporins A, B, C and D; Bilayer lipid membrane; Ion channels; Dependence on structure

1. Introduction

Peptaibols are microbial peptides containing up to 21 amino acids [1,2]. Due to their nonribosomal formation, even nonprotein amino acids such as α-aminoisobutyric acid can be constitutive parts of their linear peptide chain [2]. Usually, the peptaibols form helices, which aggregate in artificial bilayer membranes to form ion-conducting pores [3,4]. As a prerequisite of pore formation, the peptaibols display an alcoholic group at the carbon terminus and the nitrogen terminus is usually acetated [3,4]. Pore formation has been discussed as the reason for the antimicrobial and other activities of peptaibols [5]. Although more than 200 peptaibol-type compounds were reported so far, up to now only few information is available about the influence of structural variations on pore formation, stability of membrane pores, ion selectivity and biological activity.

Recently, we described the discovery of ampullosporins A (**1**), B (**2**), C (**3**), and D (**4**) (Fig. 1) as new peptaibols in cultures of the fungal strain of *Sepedonium ampullosporum* [6,7] and their activities as inducers of fungal pigment formation and neuroleptic drugs. In contrast to ampullosporins A, B and D (**1**, **2** and **4**), the isomeric compound ampullosporin C (**3**) displayed a much lower activity against the fungus *Phoma destructiva* as inducer of pigment formation, and the observation of neuroleptic activity in mice required much higher concentration of **3** [7]. It has been suggested that the observable differences among the ampullosporins could be due to alterations in membrane channel formation. In this paper, we report the membrane activity of **2**, **3** and **4** in comparison to ampullosporin A (**1**) and semisynthetic desacetyltryptophanyl ampullosporin A (**5**, Fig. 1) [8].

2. Experimental

2.1. Materials

Ampullosporins A (**1**), B (**2**), C (**3**) and D (**4**, Fig. 1) were prepared by preparative HPLC as pure compounds from the culture extract of *S. ampullosporum* as was described [6,7]. Desacetyltryptophanyl ampullosporin A (**5**) was prepared from ampullosporin A (Fig. 1) by sequence-specific chemical cleavage of the peptide bond between the terminal L-tryptophane and L-alanine moieties using *N*-bromosuccini-

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Ac-Trp[1]-Ala[2]-Aib[3]-Aib[4]-Leu[5]-Aib[6]-Gln[7]-X-Gln[11]-Leu[12]-Aib[13]-Gln[14]-Leuol[15]

1 X = Aib[8]- Aib[9]- Aib[10]
2 X = Ala[8]-Aib[9]-Ala[10]
3 X = Aib[8]-Ala[9]-Aib[10]
4 X = Aib[8]-Aib[9]-Ala[10]
5

Ala[1]-Aib[2]-Aib[3]-Leu[4]-Aib[5]-Gln[6]-X-Gln[10]-Leu[11]-Aib[12]-Gln[13]-Leuol[14]
X=Aib[7]-Aib[8]-Aib[9]

Fig. 1. Chemical structures of ampullosporins A (**1**), B (**2**), C (**3**) D (**4**) and desacetyltryptophanyl ampullosporin A (**5**). All amino acids possess L-configuration.

mide as cleaving agent. Desacetyltryptophanyl ampullosporin A (**5**) was thus obtained in 20% yield from **1** after purification by preparative HPLC.

2.2. Measurements employing lipid bilayer membranes

Planar bilayer lipid membranes (BLM) were prepared from soya bean phosphatidylcholine (Sigma, P5638; 20 mg/ml *n*-heptane) by the 'painted' method [8]. The measuring glass cell (25 ml of total volume) was equipped with a Teflon cylinder (1 cm diameter), which contained a hole of 0.5 mm diameter to harbour (BLM). Formation of the BLM was controlled by the use of a binocular microscope. Both the measuring cell (10 ml outside (*cis*)-volume) and the inner side of Teflon cylinder (*trans*-volume, 1 ml) were filled with a solution of potassium, sodium or rubidium chloride ranging from 100 to 1000 mM depending on the type of experiment.

Membrane current was measured by the voltage-clamp method. The current measuring device consisted of an operational amplifier model Keathly 301 (USA). Amplitude current noise of the amplifier was less than 10^{-13} A in the frequency range 0.1–20 Hz. The output of the operational amplifier was connected to the pen chart $X-Y$ plotter (Endim-622, Germany). Ampullosporins B (**2**), C (**3**), D (**4**) and desacetyltryptophanyl ampullosporin A (**5**) (1–10 μl of 0.5 mg/ml in DMSO stock solution) were added into 10 ml constantly stirred volume of electrolyte, at the *cis* side of BLM. To avoid the noise caused by the BLM vibration, the magnetic stirrer was switched off during recording of the single ion channel currents.

In general, the membrane current started to increase within about 1 min after addition of the antibiotic and reached a relatively stable level after approximately 3–4 min, whereby it changed less then 10%/min. The values of the membrane currents were used for plotting the conductance versus elaiophylin concentration dependence.

The currents of single channels were recorded at 0.2–0.4 μg/ml of the antibiotic concentration at *cis* side of the BLM.

3. Results and discussion

The concentration dependence of macroscopic conductance induced by ampullosporins B (**2**), C (**3**), D (**4**) and desacetyltryptophanyl ampullosporin A (**5**) is shown in Fig. 2. In comparison with the results reported for ampullosporin A (**1**) [8], the peptaibols **2**, **3** and **4** displayed the same channel-forming properties as shown in Fig. 2 for ampullosporin B (**2**). It can be suggested that transitions of single channel current between different levels, as shown in Fig. 3, correspond to a barrel-like structure of the ampullosporin channel [5,8].

However, ampullosporin C (**3**) was required in a 20-fold higher concentration to afford the same effect as the isomeric compounds **2** and **4** (Fig. 2; open circles). The power of the semilog plot of the membrane conductance as function of concentration (see cf. Fig. 2; open and filled circles) amounted to $n = 10-15$, which means that the conducting oligomeric pores include at least 10 monomer units.

In contrast to **1**, **2**, **3** and **4**, no formation of membrane channels was observed with the semisynthetic desacetyltryptophanyl ampullosporin A (**5**). These results suggest that formation of channels in an artificial bilayer membrane by ampullosporins is strongly dependent on both the quality and sequence of amino acids and the presence of an *N*-acetylated nitrogen terminus. These findings are comparable with available results on other peptaibols showing the influence of structural characteristics on membrane activity [10–12]. The observed relations in membrane activities of compounds **1**, **2**, **3**, **4** and **5** coincided well with their biological activities. Thus, compounds **2** and **4** induced pigment formation by the fungal strain of *P. destructiva* in the same concentration as ampullosporin A (**1**) [6,7]. However, compound **3** was needed in approximately 20-fold higher concentration to afford a positive effect during the

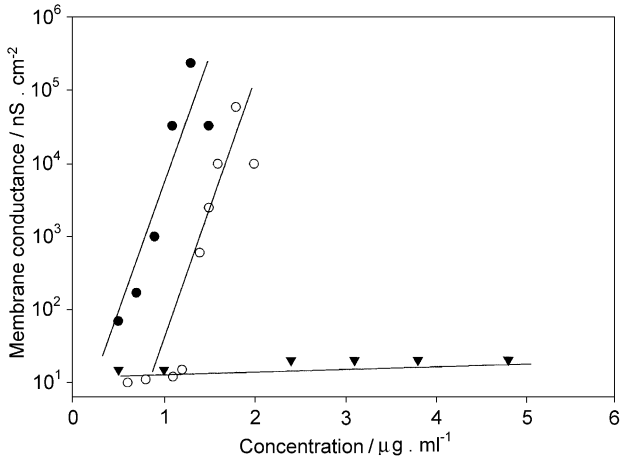

Fig. 2. Membrane conductance as a function of ampullosporins **1**, **2**, **3**, and **4** and desacetyltryptophanyl ampullosporin A (**5**) concentration. Conditions: 200 mM NaCl, pH 7,unbuffered solution, membrane voltage: −40 mV, samples were added at *cis* side of the bilayer. Filled circles: **1**, **2** and **4**; open circles: **3**; and filled triangles: **5**.

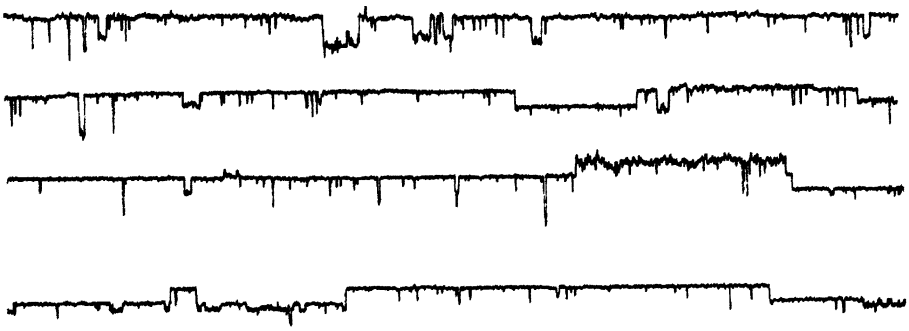

Fig. 3. Traces of single-channel current induced by ampullosporin B (**2**). 4 M NaCl, pH 7, unbuffered, membrane voltage 60 mV. Calibrations: vertical bar 30 pA, horizontal bar 20 s.

agarplate diffusion assay [6,7]. The same quantitative differences among **1**, **2**, **3**, **4** and **5** were found in neuroleptic activity as measured with mice after intraperitoneal administration [6,7]. Compound **5** was completely inactive both against *P. destructiva* and mice. The results are thus comparable with the view that membrane activities of ampullosporins and their chemical derivatives are correlated to their observable biological effects as inducers of fungal pigment formation and neurolepsy in mice.

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