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Formation of Anion-selective Membrane Pores by Texenomycin A, a Basic Lipopeptaibol Antibiotic

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Peptaibols are fungal linear peptides that owe their biological activities to the formation of ion-penetrable pores within biological membranes[1,2]. Our previous investigations suggested that the constituting amino acids determine channel stability, ion selectivity and biological activities[3,4]. Lipopeptaibol antibiotics such as texenomycin A[5] (**1**, Fig. 1) are distinguishable from the common peptaibols due to acylation of the *N*-terminus by a long-chain fatty acid and substitution of the carbon terminus by amino or guanidino moieties. It has been suggested that lipopeptaibols also form pores within biological membranes due to the helixal conformation of their peptide chains. However with the exception of protonophoric helioferin B[6] no information was available. Here we report the membrane activity of texenomycin A (**1**) and compare it with that of lipohexin (**2**) which represents a substructure of texenomycin A[7] (Fig. 1). Both **1** and **2** were reisolated previously from cultures of *Moeszia lindtneri* DSM 11119 as single compounds. Their membrane activities were measured by use of an artificial bilayer membrane (BLM) made from soybean phosphatidylcholin. Shown in Fig. 2 are currents of the single channels formed by 0.1 μM texenomycin A (**1**) presented to the outer side of the artificial membrane suggesting that **1** is a powerful channel-forming agent. At the same concentration no channel formation was observed with lipohexin (**2**) though structure **2** is identical to the left part of **1**. When a higher concentration of texenomycin A (**1**, 0.2 μM) was added the membrane current increased greatly in a time-dependent manner. Fig. 3 displays the dependence of membrane conductances as functions of texenomycin A (**1**) and lipohexin (**2**) concentration. Obviously **1** is much more active in forming ion-penetrable pores within the artificial membrane than lipohexin (**2**) suggesting that the length of the lipopeptide chain of **2** is insufficient to span the whole bilayer membrane. To check the ion selectivity of channels formed by **1** we investigated the membrane potential as function of different KCl concentrations across the membrane. Thus KCl concentration was increased in the *cis* compartment and electrical potential of this compartment was measured with reference to the other compartment. The current was stopped under these conditions if a positive voltage was applied to both electrodes. This result suggested that the channels formed by **1** are more penetrable for anions such as Cl^-than for cations such as K^+.

Obviously the insertion of texenomycin A (**1**) in the membrane increased its positive surface charge due to the basic guanidino group of the arginol moiety substituting the carbon terminus of the peptide chain. Hence cations are repelled from the membrane surface while the access of anions to the membrane channels will be improved.

Texenomycin A (**1**) thus appears as an example of channel-forming basic lipopeptaibol which permeabilizes bilayer membranes especially for anions. The concomitant leakage of the cells and loss of ions thus readily explains

Fig. 1. Structures of texenomycin (**1**) and lipohexin (**2**).

2-Me-3-oxo-TDA-Pro-Aib-Aib-Aib-Aib-R

1 R= Ala-Ala-Aib-ß-Ala-Leu-Aib-ß-Ala-Ala-Aib-ß-Ala-Ala-Aib-Aib-Aib-Ala-Argol

2 R= ß-Ala

Abbreviatins: 2-Me-3-oxo-TDA: 2-methyl-3-oxo-tetradecanoic acid, Aib: α-aminoisobutyric acid, Argol: L-arginol.

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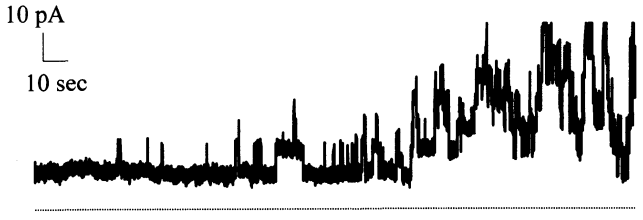
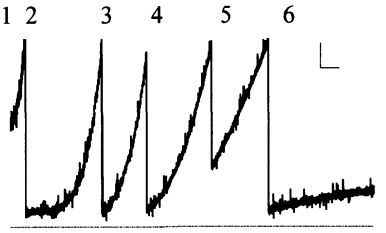
Fig. 2. Currents of the single channels formed by texenomycin A (**1**).



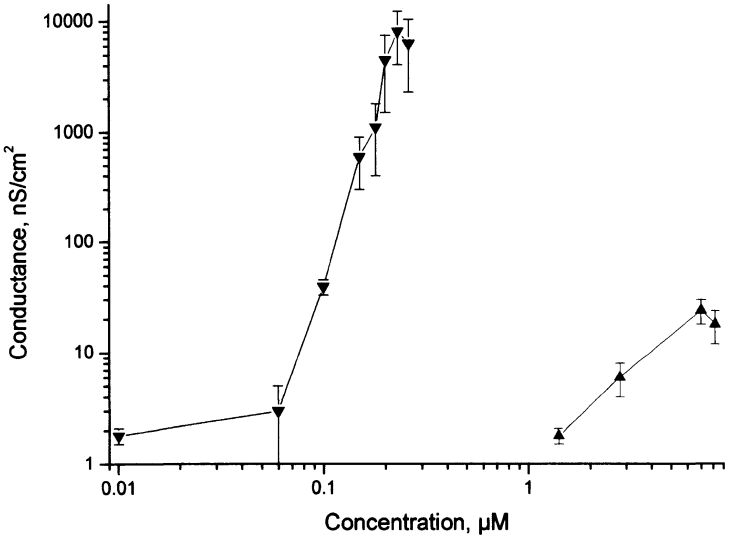
Conditions: 2 M KCl, pH 6.5, membrane voltage 90 mV, texenomycin 0.1 μM.

Fig. 3. Increase of membrane current in time in presence of 0.2 μM texenomycin A (**1**).



Conditions: 500 mM KCl, voltage 50 mV. Calibrations: horizontal 10 seconds and vertical calibration corresponds to: 1 pA for the period of time 1 to 2, 10 pA for 2 to 3, 100 pA for 3 to 4, 1 nA for 4 to 5, 3 nA for 5 to 6 and 30 nA for 6.

Fig. 4. Dependences of the membrane conductance as functions of texenomycin (triangles, basis down) and lipohexin concentration (trangles, basis up).



the observed antibiotic effects.

Experimental

Materials

Texenomycin (**1**) and lipohexin (**2**) were isolated from cultures of *Moeszia lindtneri* DSM 11119 in single peak quality on HPLC[5,7]. The physico-chemical data (MS, NMR) were fully consistent with the data reported for these antibiotics[5,7].

Measurements on Lipid Bilayer Membranes

Artificial membranes were formed from asolectin of soya beans (Sigma P5638). Stock solution was 20 mg/ml in heptane. 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 the BLM. Bilayer lipid membranes were formed by 'painted' method[8,9]. Formation of the BLM was controlled by the use of a binocular microscope. Both the measuring cell (10 ml outside (*cis*)-volume) and and the inner side of teflon cylinder (*trans*-volume; 1 ml) were filled with a solution of

potassium chloride in concentration ranging depending on the type of experiment.

Membrane currents were measured by voltage clamp method[8,9]. A Keathley 301 operational electrometer amplifier was used for the current measuring setup. 1 to 10 μl of the stock solutins of the peptaibols (0.1~1 mg/ml in methanol) were added into the 14 ml glass chamber containing a teflon cylinder with the bilayer membrane. The solution was stirred for 5 minutes by a 10 mm magnetic bar (1 mm lenght) at 500 rotations/minute. Process of bilayer formation and estimation of its area was monitored on screen by the hight of the membrane capacitance current in response to the rectangular shape voltage pulse applied to the membrane. Measurements of the membrane currents corresponding to a given peptaibol concentration were done in 5 minutes after termination of the process of black membrane formation as was marked by the constant hight of the membrane capacitive current.

Acknowledgements

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References

1) TIELEMAN, D. P.; H. J. BERENDSEN & M. S. P. SANSOM: An alamethicin channel in a lipid bilayer: molecular dynamic simulations. Biophys. J. 776: 175~1765, 1999
2) LAVER, D. R.: The barrel-stage model as applied to alamethicin and its analogs reevaluated. Biophys. J. 67: 355~362, 1994
3) GRIGORIEV, P. A.; R. SCHLEGEL, K. DORNBERGER & U. GRÄFE: Formation of membrane channnels by chrysospermins, new peptaibol antibiotics. Biochim. Biophys. Acta 1237: 1~5, 1995
4) GRIGORIEV, P. A.; A. BERG, R. SCHLEGEL & U. GRÄFE: Differences in ion permeability of an artificial bilayer membrane caused by ampullosporin and bergofungin, new 15-membered peptaibol-type antibiotics. Bioelectrochemistry and Bioenergetics 44: 155~158, 1997
5) ARETZ, W.: M. KNAUF, H. KOGLER, W. STAHL, H. STUMP, L. VERTESY, M. VOGEL & J. WINK: Texenomycin A and B, new antifungal peptaibols from *Scleroderma tensene*, Abstracts of the 9th Dechema-Meeting on Natural Products, Irsee, Feb. 21~23, Poster 18, 1997
6) GRIGORIEV, P.; A. BERG, R. SCHLEGEL & U. GRÄFE: Protonophoric activities of helioferin and pamamycin, lipophilic tertiary amino antibiotics from *Mycogone rosea* and *Streptomyces aurantiacus*. Bioelectrochem. and Bioenergetics 39: 295~298, 1996
7) HEINZE, S.; M. RITZAU, W. IHN, H. HÜLSMANN, B. SCHLEGEL, K. DORNBERGER, W. F. FLECK, M. ZERLIN, C. CHRISTNER, U. GRÄFE, G. KÜLLERTZ & G. FISCHER: Lipohexin, a new inhibitor of prolyl endopeptidase from *Moeszia lindtneri* HKI-0054 and *Paecilomyces* sp. HKI-055 and HKI-0096. J. Antibiotics 50: 379~383, 1997
8) GRIGORJEV, & S. M. BEZRUKOV: Hofmeister effect in ion transport: reversible binding of halide anions to the roflamycoin channel. Biophys. J. 67: 2265~2271, 1994
9) KASUMOV, K. M.; M. P. BORISOVA, L. N. ERMISHKIN, V. M. POTZELUEV, A. Y. SILBERSHTEIN & V. A. VAINSTEIN: How do ionic channel properties depend on the structure of polyene antibiotic molecule? Biochim. Biopys. Acta 551: 229~237, 1997