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Voltage-dependent and multi-state ionic channels induced by trichorzianines, anti-fungal peptides related to alamethicin

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The ionophore properties of two peptaibols of the trichorzianine family have been investigated in planar lipid bilayers and compared to those of alamethicin. Macroscopic conductance experiments reveal voltage-dependent channels only in the thinnest membranes and a greater efficiency of the neutral analog. In single-channel experiments, a multi-state behaviour, consistent with the usual barrel-stave model, is disclosed but the discrete current fluctuations are much more rapid than for alamethicin. The results indicate a stringent requirement for the helix length/bilayer thickness match in agreement with a previous model and suggest the design of new synthetic peptides.

Amphipathic peptide; Peptaibol; Planar lipid bilayer; Membrane conductance; Voltage-dependent channel; Peptide aggregation

1. INTRODUCTION

Peptaibols are linear polypeptides rich in α-aminoisobutyric acid (Aib) and a number of them (alamethicin, suzukacillin, trichotoxin, hypelcin, ...) form voltage-dependent ionic channels in lipid membranes. These channels consist in aggregates of amphipathic (mostly α-) helical monomers whose sequential uptake and release (the 'barrel-stave' model) leads to the multi-level single-conductance pattern [1,2]. According to the model now in favour [3], at rest, the N-terminal part up to Pro 14 is within the membrane [4], without crossing it totally, whilst the remainder of the molecule lies at the interface. The electric field acting on the peptide dipole would align the two parts of the molecule and, drawing the N-terminal towards the opposite interface, would create a pore by the association of several of these monomers.

Such a model places stringent requirements on the length of the helical peptide and the bilayer thickness. The possibility of testing this dependence was offered by the recent isolation and characterization of trichorzianines, another family of anti-fungal peptaibols produced by *Trichoderma harzanium* and which are 19 amino acids long, i.e. one amino acid shorter than alamethicin [5]. A comparative study is also possible within the trichorzianine family since 16 components are now available, the main variations being the presence or absence of a formal negative charge and the nature of the C-terminal (tryptophanol or phenylalaninol). Since trichorzianine A IIIc (neutral) has already been shown to induce leakage of vesicular-entrapped carboxyfluorescein [6], the study was begun with trichorzianines TA IIIc and TB IIIc, its charged analog (sequences shown in fig.1). Ma-

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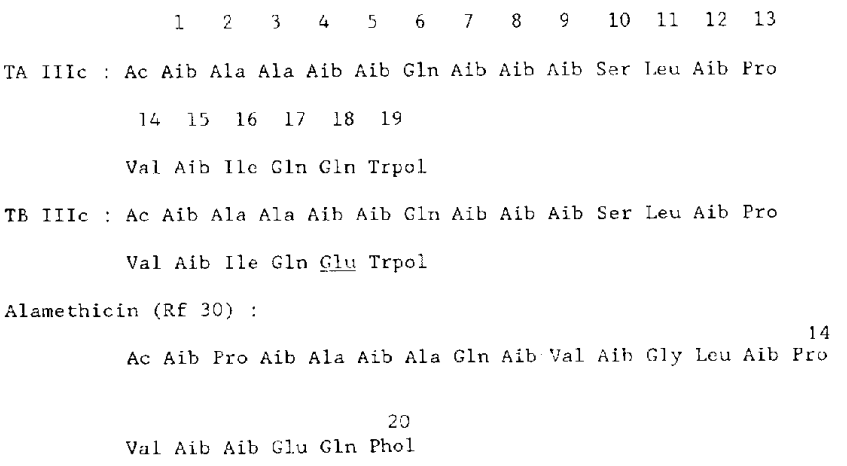

Fig.1. Amino acid sequences of TA IIIc, TB IIIc and alamethicin.

croscopic and single-channel conductances are reported here and compared with those induced by alamethicin.

2. MATERIALS AND METHODS

TA IIIc and TB IIIc were gifts from Dr Bodo and Coll. (Museum d'Histoire Naturelle, Paris) and were extracted from a spore culture of *T. harzanium* and purified by HPLC [5]. Alamethicin RF 30 fraction was purified from the Upjohn product.

For the macroscopic conductance experiments, lipid bilayers were formed by a modified brush technique on a 0.3 mm hole in a thin teflon septum separating two half-chambers filled with a 1 M KCl solution. The lipids, dissolved in *n*-decane to 1–2%, were either bacterial phosphatidylethanolamine (PE, Sigma) or one of the following monoglycerides: monolein and monopalmitolein (Sigma) or monomyristolein (Nu-Check). Bare or doped membranes were tested with a $\pm$ 200 mV triangular voltage waveform (at 40 s/sweep). The voltage and current sign conventions are the usual ones.

For single-channel experiments, virtually solvent-free lipid bilayers were formed at the tip of patch-clamp pipettes [7]. Since bilayers made from the monoglycerides used above were unstable with this technique, phospholipid mixtures 1‰ in *n*-hexane + methanol (99:1) were used for this part of the work. These phospholipids were: 1-palmitoyl-2-oleoyllecithin (POPC), dioleoylphosphatidylethanolamine (DOPE), both from Avanti Polar Lipids and dipalmitoylphosphatidylserine (DPPS) from Sigma. The peptide in a methanolic stock solution was added to the bath.

3. RESULTS AND DISCUSSION

3.1. *Macroscopic conductance*

As either form of trichorzianines IIIc leads to irreversible rupture of PE-decane bilayers at around 150 mV for 5×10^{-5} M aqueous peptide concentration, the study was continued in a series of unsaturated monoglycerides of decreasing chain length. With monoolein the membrane rupture was still observed, whilst in monopalmitolein it was reversible on decreasing the voltage and a temperature shift from 20 to 50°C induced an exponential *I-V* curve. Only in monomyristolein and at room temperature are the results consistent with the normal behaviour of voltage-gated channels, as shown in fig.2. A more pronounced asymmetry in the opposite quadrants is observed with the charged analog (fig.2B). Increasing the cholesterol mole fraction from 0 to 0.5 produced a +25 mV shift of the curves along the voltage axis. This relatively weak effect, when compared to a similar study with alamethicin [8], is in agreement with vesicular leakage experiments (El Hajji, personal communication).

For each analog, two current-voltage curves corresponding to two aqueous peptide concentrations

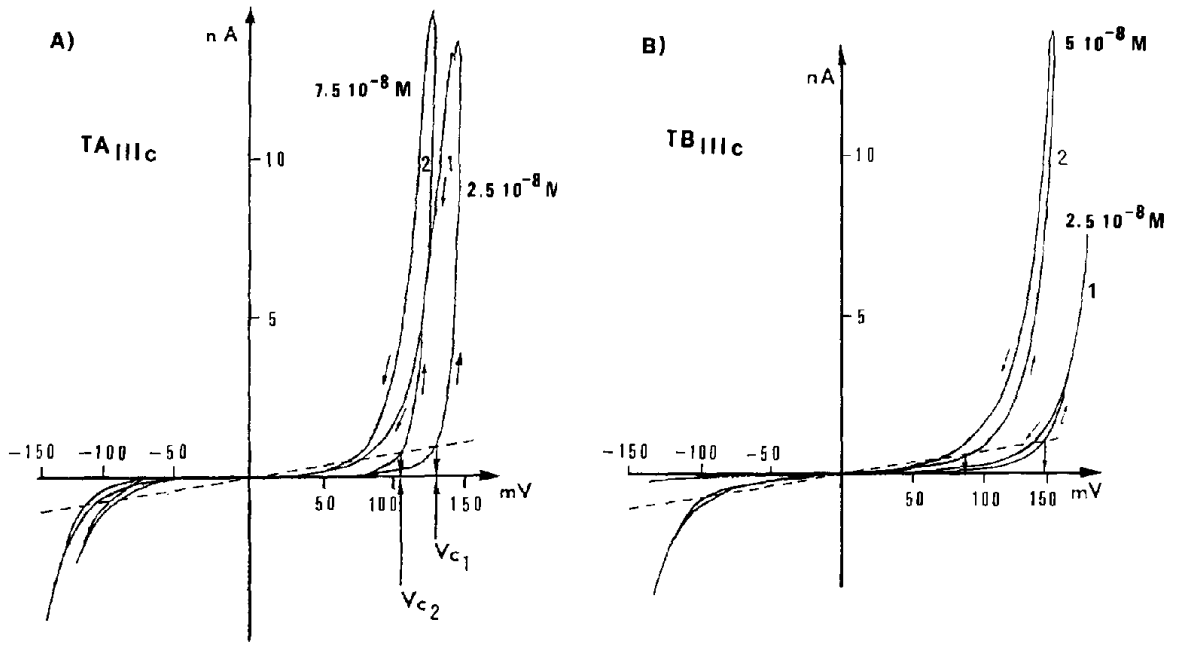

Fig.2. Macroscopic current-voltage curves induced by the two trichorzianine analogs for two aqueous peptide concentrations. 1 M KCl and room temperature. The dashed lines represent 6 nS.

are shown, yielding the characteristic voltages Vc_1 and Vc_2, i.e. voltages at which the curves cross a reference conductance chosen as 6 nS. Applying an analysis previously described [9], the apparent number of monomers per channel can be estimated as $N = Va/Ve$, where Va is the Vc shift for an e-fold change in aqueous peptide concentration and Ve, the voltage increment producing an e-fold change in conductance. The results are summarized in table 1 and compared with those of alamethicin. Although the terminal charge of TB IIIc seems to stabilize larger channels, the conductance displayed by the neutral analog is more voltage-dependent. This unexpected finding, corroborated by previous results on vesicular leakage [6] and antibiotic activity pointing out a greater efficiency of TA IIIc, argues for a predominant role (in the voltage gating) of the dipole moment of the intrapeptide bonds [10] over the formal negative charge.

Table 1

Macroscopic conductance data and the apparent number of monomers involved in the channels

	Vc_1 (mV) (2.5×10^{-8} M)	Vc_2 (mV) (5×10^{-8} M)	Va (mV)	Ve (mV)	N
TA IIIc in monomyristolein: decane	130	112	23	7.5	3
TB IIIc in monomyristolein: decane	152	79	95	16	6
Alamethicin (RF 30)					
In monomyristolein: decane	170	151	26	7.2	4
In bacterial PE: decane	232	192	54	5.5	10

3.2. *Single-channel conductances*

With POPC:DOPE (7:3) membranes at room temperature, rare bursts of very fast fluctuations between three open substrates (up to 700 pS, lasting about 1.8 ms) were observed with trichorzianines IIIc. The resolution was greatly enhanced with POPC:DOPE:DPPS:CHOL (5:2:2:1) membranes and a more complete spectrum of single-channel conductance levels was obtained (fig.3A,B). As for alamethicin, the conductance increments between the sub-levels are increasing so that the barrel-stave model applies for these compounds. When the conductance amplitudes are normalized with respect to the first level, omitting the rare and lowest level (60 or 150 pS), the ratio of conductances from one level to the next gives the sequences shown in table 2. In agreement with the macroscopic experiments, the neutral analog behaves more like alamethicin than the charged one and the voltage needed to trigger single-channel activity is lower than for TB IIIc. This is compatible with the flip-flop model [11], since the energetic barrier for a formal charge to cross the membrane hydrophobic core would have to be overcome by a larger voltage.

Table 2

Sequences of normalized conductance sub-levels

For TA IIIc	1:3.1:6.1:9.3
For TB IIIc	1:2.1:4 :5.7
For alamethicin	1:3.3:6 :8.8

The most striking differences with alamethicin

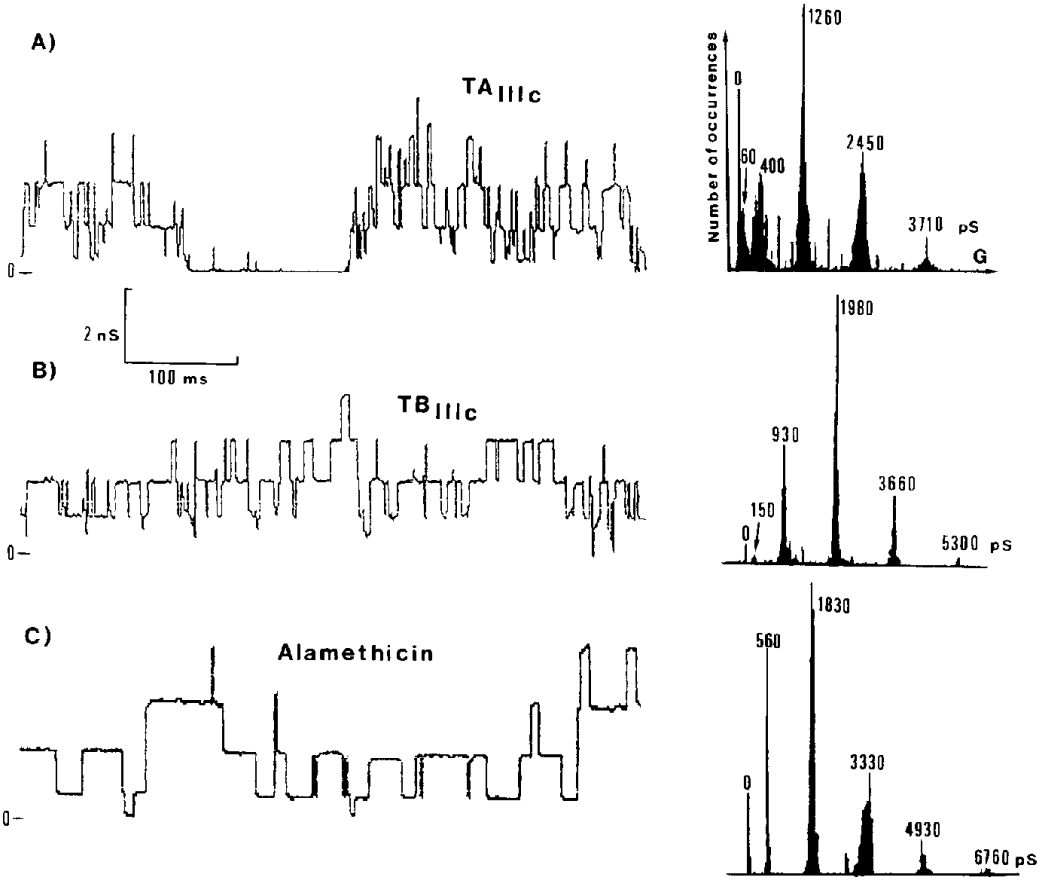

Fig.3. Comparison of single-channel conductance pattern (left) and the associated amplitude histograms (right). The applied voltages were: 128 mV (A), 200 mV (B) and 167 mV (C). 1 M KCl and 17.5°C. Peptide concentrations: 5×10^{-9} M (A,B) and 10^{-9} M (C).

Table 3

Probabilities of opening and mean open lifetimes

	Probability of opening		Mean open lifetime (ms)	
	Most frequent level	Next higher level	Most frequent level	Next higher level
TA IIIc	0.42	0.20	7.5	4
TB IIIc	0.63	0.16	12	5
Alamethicin	0.79	0.29	57	25

are the mean lifetimes of the substates and the lower probability of the highest levels being open (table 3). Apart from the shorter helix length equivalent to about one C-C link in the lipid paraffinic chain, the missing Pro in position 2 might partly account for this different behaviour. This point and the putative role of Ser in position 10 [12] will have to be further tested with the aid of new synthetic peptides.

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