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The influence of the trichorzianin C-terminal residues on the ion channel conductance in lipid bilayers

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Four natural trichorzianin analogues, channel-forming peptaibols, differing in their C-terminal residues (Gln or Glu, Trpol or Pheol) were tested for their macroscopic and single-channel conductances in planar lipid bilayers. The results indicate that, as regards to the voltage threshold, the most efficient analogue is the charged Trpol-bearing one. In addition, Trpol brings about a drastic lengthening of the open channel life-times. This behaviour is attributed to the dipole moment of the end residues and to the bulkiness and hydrogen bonding ability of Trpol.

Most peptaibols, linear antibiotic peptides rich in α-aminoisobutyric acid residues (Aib), the archetype of which is the 20 residue-long alamethicin, adopt a predominant α-helical structure. Ion channels induced by these molecules in planar lipid bilayers, displaying up to six conductance substates with non-integral increments, are best explained by the 'barrel-stave' model, i.e., aggregates of helices, with uptake and release of individual molecules [1,2]. The model implies in a later version Ref. 3 a reorganization of the C-terminal part as a major step in the gating event.

Apart from a requirement for a match between the peptide length and the bilayer thickness in the activated state, different parts of the molecules may be considered with respect to a structure–activity relationship. These are the side-chains involved in the peptide peptide and peptide–lipid interactions, the anchoring groups near the membrane interface and the side-chains lining the inner pore wall. In the particular case of peptaibols, some residues are likely to be crucial determinants, i.e., the Gln located inside the pore at about one-third of the molecular length, the Pro inducing a bent in the helical structure at about two-thirds of that length, the neutral or charged nature of the C-terminal residues, and the physicochemical properties of the uncommon end amino alcohol.

We have tested these two last parameters with four selected peptaibols (Fig. 1) chosen amongst the 16 known 19-residue-long peptides [4,5], members of the trichorzianin family. In trichorzianins III (T III), the C-terminal amino alcohol is Trpol, while Pheol is present in trichorzianins VII (T VII). In each couple, there is a neutral analogue (TA with a glutamine-18) and a charged analogue (TB with a glutamic acid in the same position). Conductance experiments, either macroscopic or unitary, were run on virtually solvent-free lipid bilayers, with 1 M KCl both sides. The lipids were from Avanti Polar Lipids (Birmingham, AL, U.S.A.).

Notice that T III and T VII also differ in position 5, Aib and isovaline respectively, located in the hydrophobic quadrant of the helix. The influence of this replacement upon the ionophore properties could be tested with reference to another available sample (TA VIb, Fig. 1) and was shown to be negligible. Indeed, the single-channel behaviour, and especially the life-times, of TA VIb and TA VII samples were very similar.

The voltage- and concentration-dependences of the macroscopic conductance were assayed at room temperature with current–voltage curves where many channels are present in the membrane. The bilayer is cast over a 120 μm hole in a thin Teflon film sandwiched between two glass half cells by lowering then raising a lipid monolayer on top of the electrolytic solution [6]. The monolayer is made from the mixture 1-palmitoyl-2-oleoylphosphatidylcholine/dioleoylphosphatidylethanolamine (POPC/DOPE, 7:3, 1% in hexane). The membrane is then submitted to a triangular voltage waveform (40 s/period) and after checking for stability and silence of the bare bilayer, the peptide in methanolic stock solution is added to the *cis*- or positive-side. After

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                    1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19
TA IIIc :  Ac Aib Ala Ala Aib Aib Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Ile Gln Gln Trpol
TB IIIc :  Ac Aib Ala Ala Aib Aib Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Ile Gln Glu Trpol

TA VII  :  Ac Aib Ala Ala Aib Iva Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Ile Gln Gln Pheol
TB VII  :  Ac Aib Ala Ala Aib Iva Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Ile Gln Glu Pheol

TA VIb  :  Ac Aib Ala Ala Aib Aib Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Ile Gln Gln Pheol
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Fig. 1. The amino-acid sequences of the five selected trichorzianins; the varying residues are underlined.

allowing between 1 and 2 h, for partitioning equilibrium, three to five voltage sweeps are superimposed and current–voltage curves are recorded. The curves are asymmetric, an exponential current developing in the positive quadrant. In deriving the current density, the membrane area was assumed to be that of the hole since in these bilayers, the solvent annulus is greatly reduced.

For each concentration, a characteristic voltage, V_c, is defined as the voltage where the exponential branch crosses a reference conductance (Fig. 2, for TB VII, as an example), at least 10-times the bare membrane conductance. From the characteristic voltages V_{c1} and V_{c2} at two concentrations, V_a is defined as the V_c shift for an e-fold change in peptide concentration. Similarly, V_c is defined as the voltage increment resulting in an e-fold change in conductance at a given concentration. From these two experimental parameters, the apparent mean number of molecules per channel can be estimated by the ratio $V_a : V_c$ [7].

In spite of relatively large standard deviations, two main trends seem to emerge from the results summarized in Table I: the characteristic voltages or thresholds are lower for the Trpol-bearing peptides on the one hand and within the same couple, T III or T VII, for the charged analogue on the other hand. Thus the most efficient analogue, as regards to voltage threshold and voltage sensitivity, seems to be TB IIIc, the charged Trpol-bearing analogue. This certainly reflects its greater dipole moment of the C-terminal part, while the smaller apparent number of molecules per channel can be tentatively attributed to the bulkiness and hydrogen-bonding ability of the Trpol side-chain.

These trends are confirmed by the analysis at the single-channel level. In these experiments, lipid bilayers were formed at the tip (diameter in the range of 1 to 3 μm) of patch-clamp glass micropipettes [8] allowing a better time resolution than previous classical methods. Indeed, the kinetics of all trichorzianin channels were rather fast and the fluctuations were barely resolved in the usual POPC/DOPE mixture. To slow down these fluctuations, a more complex lipid composition had to be used at 17.5°C, incorporating dipalmitoylphosphatidylserine (DPPS) and cholesterol (Chol) with the previous lipids, namely: POPC/DOPE/DPPS/Chol (5 : 2 : 2 : 1). The peptide concentration was $5 \cdot 10^{-9}$ M in the *cis*-side compartment.

As depicted in Fig. 3 for the example of the TA/TB VII couple, the charged analogue shows sustained continuous current fluctuations, while these latter occur in

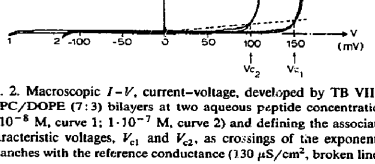

Fig. 2. Macroscopic I–V, current–voltage, developed by TB VII in POPC/DOPE (7 : 3) bilayers at two aqueous peptide concentrations ($5 \cdot 10^{-8}$ M, curve 1; $1 \cdot 10^{-7}$ M, curve 2) and defining the associated characteristic voltages, V_{c1} and V_{c2}, as crossings of the exponential branches with the reference conductance (130 μS/cm², broken line).

TABLE I

Conductance parameters

Macroscopic conductance parameters (averaged from six experiments for each analogue, with the standard deviations) yielding the voltage-dependence and the apparent mean number, N_{app}, of peptide molecules involved in the channels [7]

	C (M)	V_c (mV)	V_a (mV)	V_c' (mV)	N_{app}	$N_{rounded}$
TA VII	$5 \cdot 10^{-8}$	183 ± 6	68 ± 15	9.8 ± 0.4	6.9 ± 1.8	7
	$1 \cdot 10^{-7}$	133 ± 9				
TA IIIc	$5 \cdot 10^{-8}$	117 ± 5	59 ± 11	10.2 ± 1	5.8 ± 1.7	6
	$1 \cdot 10^{-7}$	73 ± 6				
TB VII	$5 \cdot 10^{-8}$	151 ± 5	45 ± 15	8.6 ± 0.6	5.2 ± 1.9	5
	$1 \cdot 10^{-7}$	113 ± 13				
TB IIIc	$5 \cdot 10^{-8}$	99 ± 2	35 ± 10	8.2 ± 0.7	4.3 ± 1.6	4
	$1 \cdot 10^{-7}$	73 ± 13				

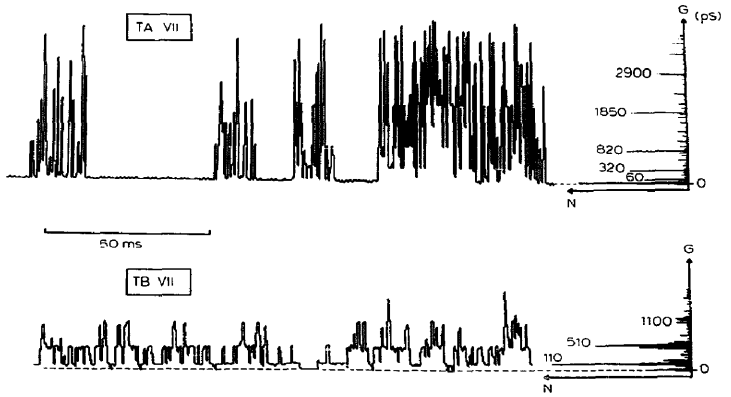

Fig. 3. Single-channel traces compared between the neutral (TA VII) and the charged (TB VII) analogues within the same couple bearing the C-terminal Pheol group. Applied voltages were 250 and 240 mV for TA VII and TB VII, respectively. On the right: the associated amplitude histograms (N, number of events, G, unit conductance).

bursts with the neutral analogue. This is equally true with the couple TA/TB IIIc as previously published, see Ref. 9. Note from the conductance amplitude histogram, that the lower substates are more probable with the charged analogue, in agreement with the slightly smaller apparent number of molecules per aggregate derived from macroscopic conductance data.

If one compares the charged analogues of both groups (for example: TB IIIc/VII, Fig. 4), it is obvious that Trpol induces a drastic lengthening of the life-times of the open channels. This is consistent with the macroscopic conductance results and confirms the stabilizing influence and greater voltage sensitivity brought about by the larger dipole moment of the indole group.

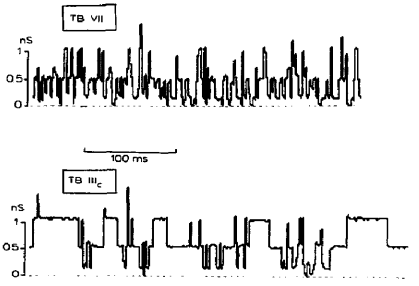

Fig. 4. Single-channel traces compared between the Pheol and the Trpol C-terminal bearing peptides, both charged (Glu-18). Applied voltages were 240 and 190 mV for TB VII and TB IIIc, respectively.

This conclusion may have more general implications. Indeed, although the gramicidin channel is not gated by voltage, a gramicidin analogue, M^-, in which all the four Trp of the native molecule, gramicidin A, were replaced by Phe [10], displays shorter life-times of open single-channels. This kind of study might also bear physiological significance since, for example, the highly voltage-dependent sodium channels found in excitable membranes, consisting of a complex bundle of α-helices, present a high density of Trp residues near the interface or the mouth of the channel, particularly for the inner segments delineating the pore [11].

These results will furthermore have a practical and immediate application. Since we found that the channels induced by the des-Aib-Leu analogue of alamethicin had rather fast kinetics [12], a similar analogue incorporating Trpol, or alternatively Trp, at the C-terminal, instead of the usual Pheol of alamethicin should display more stable active states and thus allow us to test on this peptide, much easier to synthesize than Aib-containing peptides, the above mentioned Gln and Pro influence.

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