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Synthetic analogues of alamethicin: effect of C-terminal residue substitutions and chain length on the ion channel lifetimes

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In a previous study, a synthetic analogue of the peptaibol alamethicin, in the sequence of which all α-aminoisobutyric acid (Aib) were substituted by leucine residues and the C-terminal residue modified, was shown to display the same single-channel behaviour as alamethicin in planar lipid bilayer, except that the sublevel lifetimes were much reduced. New analogues differing in their C-terminal residue (Phe-NH_2, Pheol, Trp-NH_2) have now been tested for their single channel properties in neutral lipid bilayers. The conductance amplitudes and open channel lifetimes do not differ significantly from the previous analogue. Thus, the nature of the last residue, which may be located near the membrane interface, does not seem to play an important role in the destabilisation of the conducting aggregate observed after the Aib substitution by Leu. Since the deletion of one residue (Glu^{18}) in the 14–20 moiety induces a slight decrease of the increment between the conductance levels, but has no effect upon the channel lifetimes, this residue and the length of this segment do not interfer much with the channel lifetime of peptaibols. In conclusion the factors influencing the aggregate stability may be sought in the helix–helix interactions.

Peptaibols, natural peptides rich in α-aminoisobutyric acid (Aib), form in lipid bilayers multi-level ion channels through a voltage-dependent mechanism [1–5] well described by the barrel stave model [6], a bundle of helices with a varying number of monomers. However, the restricted possibilities of residue variations in this family do not allow testing the role of some key residues in the mechanism of molecular aggregation and ion transport. This, and the presence of the non-proteinous amino acid Aib, led us to synthesize an analogue (L1) of alamethicin (a major peptide among these peptaibols) where all the Aib were substituted by leucine (Leu) residues [7]. From its study, it was concluded that the Aib high content was not a requisite condition for observing an ionophore behaviour similar to the alamethicin one. However the single channel lifetimes observed were drastically shortened in the ratio of about one to ten, whatever sublevel was considered, indicating a reduced stability of the aggregates. But, as actually another modification was introduced in the chemical nature of the C-terminal group, namely an amido instead of a hydroxyl end, several hypotheses could be put forward to account for this result:

(1) the substitution of the terminal hydroxymethyl group of Pheol by the amide end of Phe-NH_2 leads, for instance, to a less efficient anchorage at the bilayer interface;

(2) the substitution of all Aib (a 3_{10}-helical promotor) by Leu (an α-helical promotor) may induce in some way a conformational shortening of the molecule, for instance by increasing the α-helical fraction of the peptide;

(3) the presence of Leu, a residue bulkier than Aib, in the helix-helix contact destabilizes the aggregate.

In this paper we examine the first two hypotheses. Two analogues of the L1 type with terminal Pheol (L2) or Trp-NH_2 (L3) were tested as well as an amidated one shortened by deletion of Glu^{18} (L4) (Table I). The amidated peptides were synthesized by solid-phase technique on a benzhydrylamine resin. The N terminus was acetylated and the HF treatment left the required peptides. For L2 an original procedure was used which is described elsewhere [8]. The products were purified by HPLC on a reverse phase C_{18} preparative column under a methanol/water gradient. After lyophilisation, analytical HPLC allowed to control the purity of each peptide (to be published). Their characterization was

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TABLE I

Amino acid sequences of alamethicin (ALA) and its synthetic or natural analogues

ALA Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Pheol

TA3 Ac-Aib-----Ala-Ala-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Ile-Gln-Gln-Trpol

T40 Ac-Aib-----Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Glu-----Valol

L1 Ac-Leu-Pro-Leu-Ala-Leu-Ala-Gln-Leu-Val-Leu-Gly-Leu-Leu-Pro-Val-Leu-Leu Glu-Gln-Phe-NH2

L2: X= Glu-Gln-Pheol L3: X= Glu-Gln-Trp-NH2 L4: X= Gln-Trp-NH2 X

TABLE II

Molecular FAB-derived and theoretical masses

Products	Experimental	Expected
L1	2200.350	2200.387
L2	2187.371	2187.364
L3	2239.370	2239.379
L4	2210.336	2210.328

then achieved by Fast Atomic Bombardment mass spectrometry (Table II).

For single-channel experiments, the POPC/DOPE * lipid mixture (7:3), dissolved in 1‰ hexane, was used to form virtually solvent-free bilayers at the tip of patch-clamp micropipettes (diameter 1 to 2 μm). Bilayer formation was monitored by the capacitance response. Prior adding the peptide to the *cis*- or positive-side, the bare membrane was tested for 30 min in order to control the absence of channel-like events.

All peptides gave a strongly voltage-dependent multi-state behaviour since potentials up to 220 mV were necessary to trigger the electric signal. Their multi-level conductance behaviour is similar to L1 as can be seen in Figs. 1 and 2 where single-channel conductance fluctuations are compared for L1 and L2 as an example. The corresponding amplitude histogramme for L1 (Fig. 1B) argues for an homogenous channel population. In addition, Table III shows for the four analogues no significant difference in the sublevel amplitudes except for L4.

For L2 and L3, a data processing treatment of the records allows to quantify their channel lifetimes (Table IV), showing no significant difference with the values observed with L1 but which, compared with those of alamethicin, are between five- and 10-times shorter according to the sublevel considered. Thus, the variations on the C-terminus residue (L1: Phe-NH_2, L2: Pheol, L3: Trp-NH_2) do not affect significantly the behaviour of these des-Aib peptides in the binary lipid mixture.

* Lipids (from Avanti Polar Lipids, Birmingham, AL, U.S.A.): POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DOPE, 1,2-dioleoylphosphatidylethanolamine.

TABLE III

Sublevel conductance values in pS of alamethicin analogues in POPC/DOPE membranes at 240 mV in 1 M KCl

Temperature 10 °C and peptide concentration in the 10^{-9} M range.

Products	Sub-level conductance					
	1	2	3	4	5	6
L1	100	500	1130	1870	2840	3870
L2	120	410	1020	1820	2770	3910
L3	130	410	1040	1810	2810	3890
L4	110	390	890	1580	2470	3400

The study of the shortened analogue (L4), as compared to L1, reveals a decrease in the increments between sublevels, but no change in the lifetime values (Table IV). Actually, the deletion of Glu^{18} in the hydrophilic sector is compensated by the Gln^{19} transfer (Fig. 3). The chemical difference between both residues modifies the hydrophilic lumen formed by aggregation of the helical rods, and can account for the small changes observed in the conductance values. These results concerning the lifetime values are inconsistent with previous conclusions [9] stating that the unresolved channel states in trichotoxin A40, (a 18-residue peptaibol lack-

TABLE IV

Mean lifetimes in ms of the different substrates for the four synthetic analogues compared to alamethicin (ALA)

Applied voltage 175–250 mV and temperature 10 °C.

Levels	1	2	3	4	5
ALA	2.8	5.0	3.4	1.6	1.1
L1	0.55	0.49	0.38	0.34	0.31
L2	0.40	0.41	0.34	0.28	0.25
L3	0.43	0.36	0.35	0.32	0.25
L4	0.20	0.48	0.34	0.29	0.24

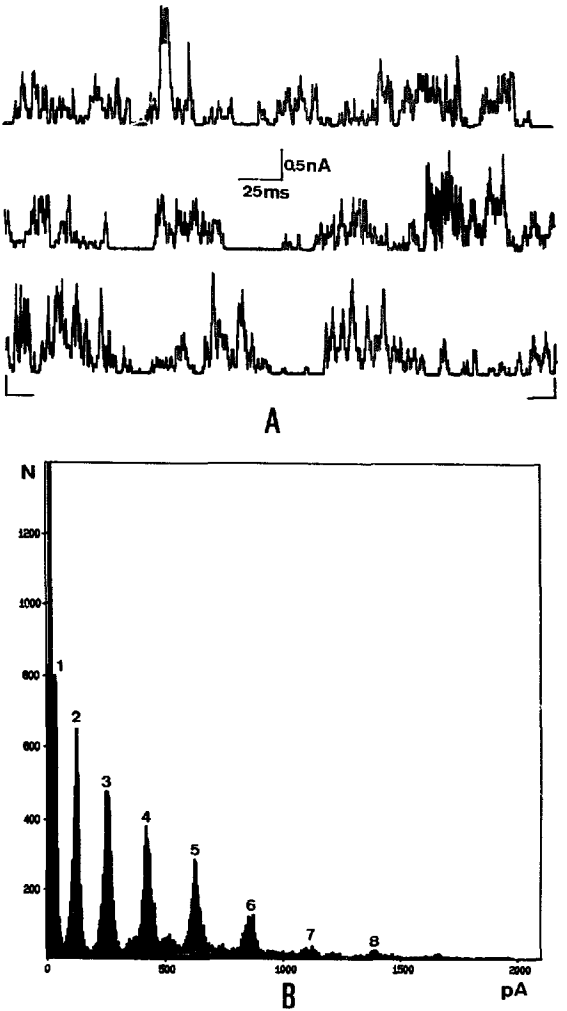
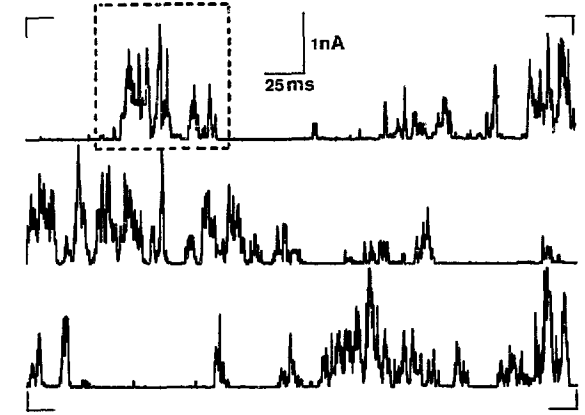


Fig. 1. (A) Single-channel activity induced by the alamethicin analogue L1 in a POPC/DOPE membranes (7:3). 1 M KCl in both compartments and 10°C. The voltage and current sign conventions are the usual ones. Peptide concentration in the 10^{-9} M range and a 226 mV applied potential and (B) associated amplitude histogramme for 18000 events.



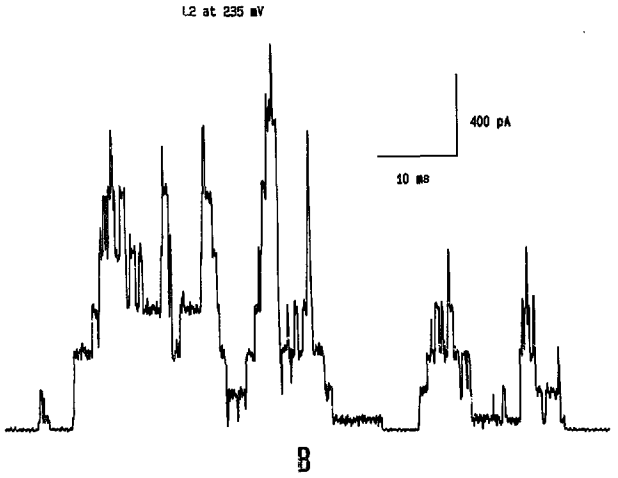


Fig. 2. (A) Single-channel activity induced by the analogue L2 under the same conditions as in Fig. 1, (B) dotted line box at a expanded scale.

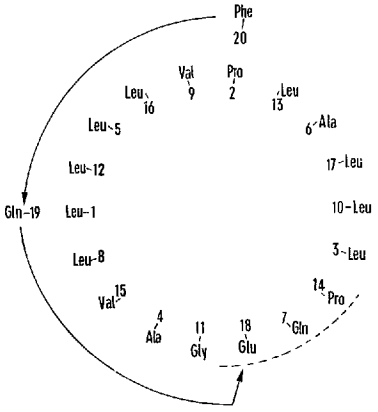


Fig. 3. L2 α-helix projection on a plane perpendicular to the helical axis. The hydrophilic sector is emphasized by dotted lines. In L4 the deletion of Glu^{18} results in a 100 degree anticlockwise rotation of Gln^{19} and $Pheol^{20}$.

ing Pro^2 and Glu^{18}, T40 in Table I) compared with alamethicin was due to the lack of Glu^{18}, supposed to form a stabilizing cluster with Gln^{19}. Our results [10] with different trichorzianins (a 19-residue peptaibol lacking also Pro^2 but not Glu^{18}, TA3 in Table I) show a similar decrease of the channel lifetime. Thus the destabilisation of the conducting aggregates of trichotoxin or trichorzianin as compared to alamethicin is probably due to the shortening of the 1–14 segment subsequent to Pro^2 deletion.

Conversely, one may think that an additional residue in this 1–14 segment should be sufficient to increase the channel lifetimes of our des-Aib-Leu analogues. We have shown previously [11] that the lengthening of this segment by introducing an additional serine located in the hydrophilic sector of L1 does not increase significantly the channel lifetimes [12].

In conclusion, variations on the nature of the C-terminus * and lengthening of the 1–14 segment do not allow the channel lifetime of the des-Aib-Leu analogues to recover values as large as the alamethicin ones. Further work is now in progress to test the hydrophobic interaction zones between helices responsible for the structure of the aggregates.

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* Thus the role of the hydroxyl end group in the peptaibols may be to offer a site for esterification by fatty acids, as is found for gramicidin A [13].