brief communication

Antimicrobial peptide magainin I from *Xenopus* skin forms anion-permeable channels in planar lipid bilayers

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ABSTRACT The ionophore properties of magainin I, an antimicrobial and amphipathic peptide from the skin of *Xenopus*, were investigated in planar lipid bilayers. Circular dichroism studies, performed comparatively with alamethicin, in small or large unilamellar phospholipidic vesicles, point to a smaller proportion of α-helical conformation in membranes. A weakly voltage-dependent macroscopic conductance which is anion-selective is developed when using large aqueous peptide concentration with lipid bilayer under high voltages. Single-channel experiments revealed two main conductance levels occurring independently in separate trials. Pre-aggregates lying on the membrane surface at rest and drawn into the bilayer upon voltage application are assumed to account for this behaviour contrasting with the classical multistates displayed by alamethicin.

I. INTRODUCTION

A number of antibiotic or cytolytic peptides (alamethicin, melittin, δ-lysin, . . .) share a minimum length of ~20 residues and adopt in membranes an amphipathic α-helical structure (1). Comparative studies and accumulation of knowledge concerning the properties, especially the ionophore properties in planar lipid bilayers, of such peptides as related to their chemical sequence and conformations could reveal interesting implications about their activity upon biological membranes.

Two 23 residue-long peptides, magainins I and II (sequence of analogue I shown in Fig. 1 *A*), recently isolated from the skin of *Xenopus laevis*, exhibit a wide spectrum of antimicrobial activity (2) together with other peptides of the amphibian neurosecretory system (3). Surface-active properties were suggested and inspection of the helical wheel representation (Fig. 1 *B*) stresses a hydrophilic sector whose size is comparable to the melittin one but larger than for alamethicin. We report and discuss here conformational studies and ionophore properties induced by magainin I in artificial planar lipid bilayers.

2. MATERIALS AND METHODS

Magainin I was purchased from Bachem (Bubendorf, Switzerland) and its purity determined by high-performance liquid chromatography was 98%. The commercial product (peptide content: 75%) was lyophilized and contained trifluoroacetic acid and water. Alamethicin (Sigma Chemical Corp., St. Louis, MO) was Sigma product No. A 4665.

Circular dichroism conformational studies were performed on a Mark V Jobin-Yvon (Longjumeau, France) dichrograph firstly in phosphate buffer saline (150 mM NaCl, pH:7.4) and then after addition of egg lecithin (Sigma Chemical Co.)-supplemented or not with 1-palmitoyl-2-oleoyl phosphatidylserine (POPS; Avanti Polar Lipids Birmingham, AL)-small or large unilamellar vesicles (respectively SUV or LUV). Several scans between 200 and 250 nm were averaged. The different conformational contents were estimated from published standard ellipticity values (4).

For bilayer conductance experiments, 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and dioleoyl-phosphatidylethanolamine (DOPE) from Avanti Polar Lipids were used as a POPC/DOPE (7/3) mixture 1% or 0.1% in hexane. Virtually solvent-free lipid bilayers in macroscopic and single-channel experiments (with patch-clamp pipettes) were formed as previously described (5). Voltage and current sign conventions are the usual ones, in particular: in the patch configuration, the pipette interior corresponds to the conventional trans-side. Temperature is 17.5°C throughout.

3. RESULTS

3.1 Circular dichroism conformational studies

The circular dichroism spectra of magainin I in phosphate buffer saline and after addition of lipid vesicles were compared with those of alamethicin, used as a reference for ion-conducting α-helical aggregates, in the same conditions.

Fig. 2 stresses obviously different conformational content between the two peptides. The α-helical character of alamethicin is significantly increased, to 30–35%, in agreement with previous studies (6, 7) upon addition of egg lecithin SUV (spectrum *b*, Fig. 2 *A*). By contrast, the same operation with magainin I resulted in a smaller increase in helicity, to ~10% with 70% of random coil and 30% of β-structure. There is a further reduction of the helical conformation when negatively-charged phospholipids are incorporated into lecithin LUV (spectrum *b*,

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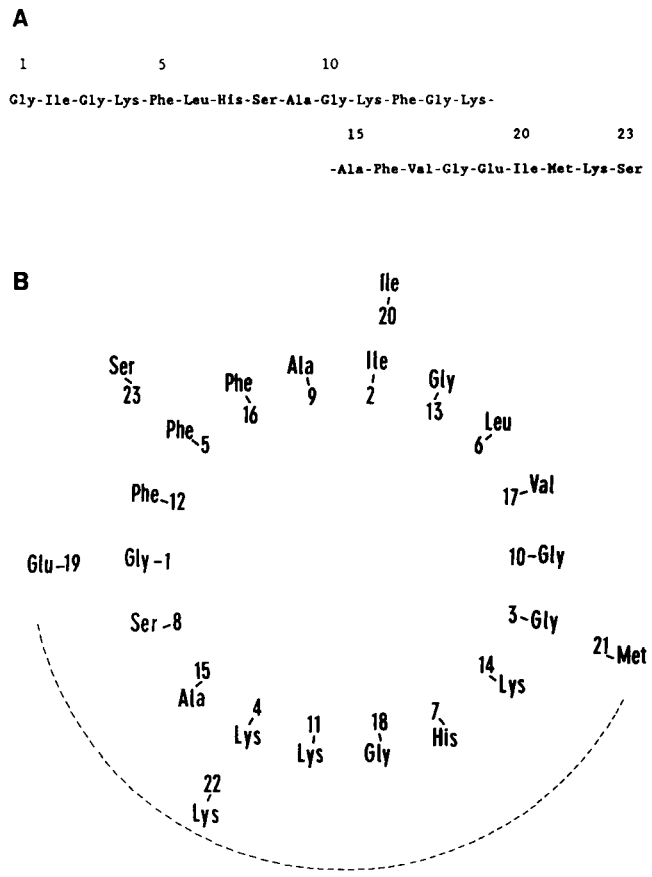

FIGURE 1 (*A*) Aminoacid sequence of magainin I. (*B*) Helical wheel representation (projection on a plane perpendicular to the axis of the α-helix assumed for magainin I). The dotted line points out the hydrophilic sector.

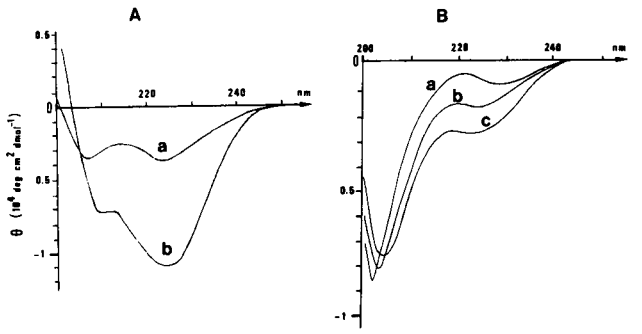

FIGURE 2 Circular dichroism spectra. (*A*) Alamethicin (8 10^{-5} M) in phosphate buffer saline (*a*) and after addition of 10% (vol/vol) 10 mM egg lecithin SUV (*b*). (*B*) Magainin I (9 10^{-5} M) in phosphate buffer saline (*a*) and after addition of 10% (vol/vol) 10 mM egg lecithin SUV (*c*) or egg lecithin + POPS (7/3) LUV (*b*). Room temperature, pathlength: 0.1 cm, 4–5 averaged cycles.

Fig. 2 *B*). The strong electrostatic interaction with positively-charged magainin I molecules are likely not to favor peptide incorporation into the bilayer and we shall see below that conductance properties can be somewhat modulated by these different bilayer compositions.

3.2 Macroscopic conductance

As expected from a previous study of charged alamethicin analogues (8) and from the positive charge at the magainin I C-terminal, macroscopic current-voltage curves were asymmetric and only negative voltages turned on a membrane conductance (Fig. 3 *A*, lower quadrant) when

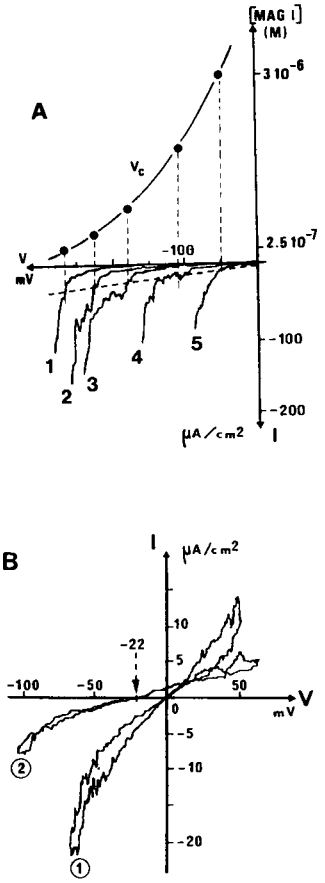

FIGURE 3 Macroscopic current-voltage curves. (*A*) In the lower negative quadrant, for aqueous magainin I concentrations of 2.5 10^{-7} M (*trace* 1), 5 10^{-7} M (*trace* 2), 9 10^{-7} M (*trace* 3), 1.8 10^{-6} M (*trace* 4) and 3 10^{-6} M (*trace* 5) in the *cis*-side only and with 1 M KCl both sides. Above is shown the concentration-dependence of the characteristic voltages Vc (crossings of traces with a reference conductance of 125 μS/cm^2, *dashed line*). (*B*) On a different current scale and with 3 10^{-6} M magainin I both sides, with no salt gradient (1 M KCl both sides, *curve* 1) and with a 100 mM/1 M KCl (*trans/cis*) gradient (*curve* 2). Both positive and negative limbs of the voltage excursion are represented.

magainin I was added to the *cis*-side. The relatively high peptide aqueous concentration reflects an unfavorable lipid/water partition coefficient: the mean hydrophobicity index of magainin I is only 0.05 as compared with 0.50 for alamethicin. An increased peptide concentration both sides of the membrane induced a symmetric curve with a shift of the exponential branch.

About a decade of peptide aqueous concentration could be safely assayed and the resulting macroscopic current-voltage curves are presented in Fig. 3 *A*. From the crossings of these curves with a reference conductance chosen here as 125 $\mu S/cm^2$, characteristic voltages Vc can be defined for the set of aqueous peptide concentrations (8), as depicted in the upper part of Fig. 3 *A*. The concentration-dependence of the conductance is thus quasi-exponential with Va, the voltage shift for an *e*-fold change in concentration, increasing from 60 to 90 mV when going from lowest to highest peptide concentration, i.e., values significantly larger than the one found with alamethicin in bilayers of similar thickness (8). However, the voltage-dependence is much reduced: Ve, the voltage increment producing an *e*-fold change in conductance for a given concentration is 15–20 mV as compared with 4–5 mV for alamethicin (8).

3.3 Anionic selectivity

The hypothesis of an anionic selectivity, deduced from the high content of positive charges in the hydrophilic sector (Fig. 1 *B*), is confirmed by the results presented in Fig. 3 *B*. A peptide concentration of $3\ 10^{-6}$ M and 1 M KCl both sides of the membrane yielded the macroscopic current-voltage curve labeled 1 in the figure. Shortly after a salt gradient—100 mM KCl in the *trans*-side and 1 M KCl in the *cis*-side—was applied to the same membrane, the zero-current voltage E_o (or reversal potential) shifted to −22 mV (curve 2). The application of the Hodgkin-Goldman-Katz equation (9) leads to a permeability ratio $P_{Cl}/P_K = 3$.

3.4 Single-channel behavior

Only one conductance level was observed in a given experiment, rarely two (Fig. 4 *A*). However, this level could differ from one experiment to another. Out of 13 trials, six yielded a conductance level averaging 683 pS and six others at 366 pS. These events were rare and relatively short-lived: for the example shown in Fig. 4 *A*, the probability of opening for the 360 pS level was 0.08 with a mean life-time of the open state of 100 ms. The open state of these channels is flickering and somewhat "inactivating" in smaller steps of 80–100 pS. In addition, this latter level and larger ones at around 1,200 (trace *C*) and 1,900 pS levels were much less frequently observed. The ohmic and nonsaturable character of the two most probable levels over a broad range of voltage is demonstrated by Fig. 4 *D*.

The channel amplitude does not seem to be modulated by the voltage but rather by the peptide aqueous concentration (raising from traces *A* to *B*) and also by the lipid bilayer composition since the incorporation of POPS instead of DOPE in POPC bilayers favored the lowest level (80 pS) which was rapidly fluctuating (not shown). Note that this is matched by a reduced ellipticity (spectrum *b*, Fig. 2 *B*).

DISCUSSION

From the macroscopic conductance data and the mean single-channel conductance, the number of channels in the membrane used in macroscopic conductance experiments (diameter: 125 μm) can be calculated. For a magainin I concentration of $9\ 10^{-7}$ M (curve 3, Fig. 3 *A*), this number (80–100) turns out to be similar to the one derived from experiments with alamethicin at $5\ 10^{-8}$ M. Taking into account the apparent number of monomers per channel, $n = Va/Ve = 3$–6 here for magainin I and 10 for alamethicin (8), the lipid/water partition coefficient can be estimated to be 50–100 times less favorable than the alamethicin one.

From the circular dichroism data, no definitive conclusions can be reached as to the actual magainin I conformation in the conducting state in the bilayer. Note, however, that the circular dichroism spectra were recorded at rest, without applied electric field thought to push the peptide lying on the membrane interface into the bilayer interior and to cross it. The small helicity reported here for magainin I, as compared with alamethicin, in lipid vesicles does not exclude an α-helical conformation for the active species.

A statistical distribution of conductance states from a series of experiments was also encountered with a synthetic 22 residue-long peptide (Pro in position 18), designed to model a transmembrane fragment of an H^+ ATP-ase subunit (10). This contrasts with the highly voltage-dependent multi-state conductance typical of alamethicin, peptaïbols and des-Aib analogues (5), all sharing a Pro in position 13 or 14, and for which the "barrel-stave" model of a channel of varying diameter applies: the pore lumen is delineated by the hydrophilic sectors of a variable number of aggregated α-helices (8, 11, 12). In the case of magainins, able to form long α-helical segments, unperturbed by any Pro and presenting an important hydrophilic and charged sector (Fig. 1 *B*), molecules or preaggregates may lie on the mem-

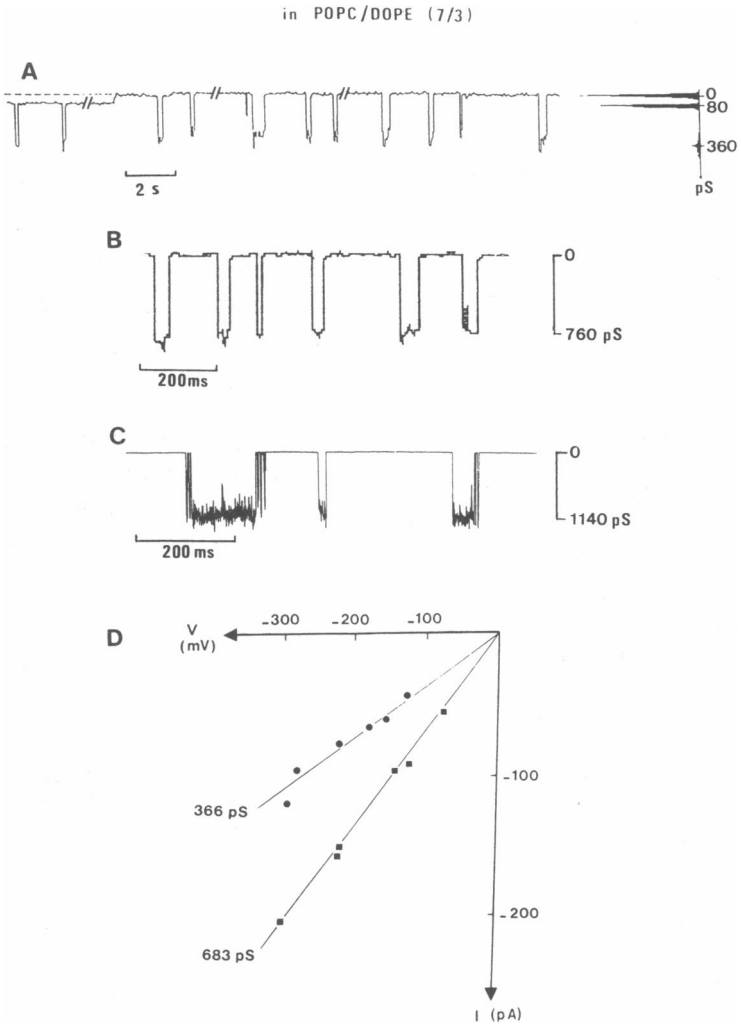

FIGURE 4 Representative examples of single-channel records in three different experiments. Peptide aqueous concentrations (*cis*-side) and applied voltages were $2\ 10^{-7}$ M and -280 mV (*trace A*), $5\ 10^{-7}$ M and -120 mV (*trace B*) and $2.5\ 10^{-6}$ M and -220 mV (*trace C*). Í M KCl both sides and room temperature. (*D*) Single-current-voltage relations for the two most probable levels.

brane surface so that high voltages are necessary to overcome the energetic barrier of the membrane dielectric and to form the channels. The lumen size would then be stable within a set of pre-formed aggregates.

Evidence for anionic permeability induced by peptides is scanty in the literature and it is interesting to note that the anionic/cationic selectivity ratio reported here falls in the range of natural anionic channels, for example in vertebrate twitch muscle (13), and that some of them seem to function as twin gated units: two identical pores in parallel functioning either independently or together (14). Large anion-selective channels adopting any of six open levels of conductance that are integer multiples of 60–70 pS have been reported in pulmonary alveolar epithelial (15). Finally, in cultured cardiac cells, two large conductance systems with an amplitude ratio of 2 were also described and "widely dispersed microclusters forming grouped channels of different sizes" were assumed (16).

Note added in proof: The results reported here are in general agreement with a published abstract concerning magainin II (17) and a more detailed account on channel-forming properties of cecropins (18), peptides related to magainins.

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