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Ionic channels formed by a primary amphipathic peptide containing a signal peptide and a nuclear localization sequence

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Abstract

The peptide SP-NLS (Ac-Met-Gly-Leu-Gly-Leu-His-Leu-Leu-Leu-Ala[10]-Ala-Ala-Leu-Gln-Gly-Ala-Lys-Lys-Lys-Arg[20]-Lys-Val-NH-CH_2-CH_2-SH) is composed of a hydrophobic signal sequence (SP, Met-1 to Ala-16) followed by a polycationic nuclear localization sequence (NLS, Lys-17 to Val-22) terminated by a cysteamide group. Designed to act as drug carrier this primary amphipathic peptide proved cytotoxic and bactericidal when used at high concentrations, probably by inducing the formation of ion channels. In this work, we show that indeed SP-NLS exhibits a pore-forming activity when incorporated into planar lipid bilayers and *Xenopus laevis* oocyte plasma membranes, with conductance values of 25 pS in 0.1 M NaCl. In both membranes, the insertion of the peptide was voltage-triggered whereas the induced conductances proved almost voltage-independent. Moreover, SP-NLS ion channels were selective for monovalent cations ($K^+ > Na^+ > Li^+ >$ tetraethylammonium$^+ >$ choline$^+$). The ion channel activity of this type of peptides thus provides some insight on their toxicity but also on the mechanism involved for their membrane crossing process.

Keywords: Planar lipid bilayer; Patch-clamp; Cation selective channel; Voltage dependence

1. Introduction

Peptides are powerful tools that can be used as carriers to facilitate the cellular uptake of drugs. In this field it has been shown that peptides containing an oligolysine sequence [1] or peptides corresponding, or related to, the homeodomain of Antennapedia can be easily taken up by cells [2,3]. In order to improve the efficiency of such drug carriers, we have designed a series of peptides with a primary amphipathic sequence based on the association of a hydrophobic sequence, i.e. a signal peptide (SP) [4] or sequence issued from a viral fusion protein [5,6] with a hydrophilic sequence derived from a nuclear localization sequence (NLS) [7,8]. They act as carriers for drugs linked to the peptides either by a covalent bond or by formation of a hydrophobic or electrostatic complex. When incubated with fibroblast cells, most of these peptides are rapidly (less than 3 min) taken up by the cells with a final localization which is mainly nuclear [9–11]. However, at high concentrations (> 10 μM), the peptides of the SP-NLS family are toxic for the cells and have an antibacterial activity. This latter property, analysed using mollicutes

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as target [12], appears to be due to changes in membrane potential and pH gradient thus suggesting the formation of transmembrane ionic channels as already observed for melittin [13], peptaibols or related peptides [14,15].

For the study reported in this paper we have selected one of the most efficient peptides with respect to its effect on the membrane potential of mollicutes. The sequence of this peptide, which will be referred to hereafter as SP-NLS, is composed of 22 residues: Ac-Met-Gly-Leu-Gly-Leu-His-Leu-Leu-Leu-Ala^{10}-Ala-Ala-Leu-Gln-Gly-Ala-Lys-Lys-Lys-Arg^{20}-Lys-Val-NH-CH_2-CH_2-SH. The 16 N-terminal residues are hydrophobic and correspond to the signal peptide of *Caiman crocodylus* Ig(v) light chain [4] where one valine has been deleted and the six C-terminal residues arise from the SV40 large T antigen [7,8]. Furthermore this peptide is C-terminated by a cysteamide group allowing post-synthesis linking either to a drug or to a fluorescent probe through its –SH function. We describe here the ion channel properties induced by incorporation of this peptide into lipid bilayers using two different systems. The first deals with the membrane of *Xenopus* oocytes in order to provide information for the understanding of the antibacterial and toxic activity induced by this peptide. The second refers to artificial planar lipid bilayers which allow an unambiguous assignment of the origin of ionophore activity. Indeed, the channels detected in this latter system will thus reflect an intrinsic property of the peptide and no activation of constitutive channels of the oocyte membrane. We also describe the ion selectivity of the channels together with their formation and activation.

2. Methods

2.1. Synthesis, purification and characterization of the SP-NLS peptide

The peptide was previously synthesized by solid phase peptide synthesis using the Fmoc strategy, purified by semi-preparative HPLC with a Nucleosil 300, C8, 5 μm column and identified by Electrospray mass spectrometry, amino acid analysis and NMR [9]. No impurities (peptides where some residues were lacking) could be detected.

2.2. Xenopus oocyte isolation and injection

Ovaries were surgically removed from *Xenopus laevis* females (Elevage de Lavalette, Montpellier, France) anaesthetized using a 0.2% MS222 solution (Sigma, Laverpillere, France). After mechanical dissociation and extensive washing using the OR-2 solution, (containing in mM: NaCl, 100; $MgCl_2$, 2; KCl, 2; HEPES, 10), oocytes were isolated by a 2 h enzymatic dissociation using 1 mg/ml Collagenase IA (Sigma) dissolved in OR-2. Oocytes were then washed several times with OR-2 and selected in ND96 solution (containing in mM: NaCl, 96; $MgCl_2$, 2; KCl, 2; $CaCl_2$, 1.8; HEPES, 10; pyruvic acid, 2.5; gentamicin, 50 μg/ml; neutralized at pH 7.2 using NaOH).

2.3. Electrophysiological recordings

Whole cell currents were recorded as described by Charnet et al. [16] under a two-electrode voltage-clamp using the GeneClamp 500 amplifier (Axon, Burlingame, CA). Current and voltage electrodes were filled with CsCl, 2.8 M, BAPTA, 10 mM, and the pH adjusted at 7.2 with CsOH. The bath-clamp headstage was connected to the bath using two agar bridges filled with 2% agar in 3 M KCl. Before each recording, electrode resistance was checked to be less than 1 MΩ, and liquid junction potential was zeroed. The typical recording solution (NA100) had the following composition (in mM): NaCl, 100; HEPES, 10; $MgCl_2$, 2; pH 7.2 with NaOH. Ionic selectivity of SP-NLS-induced channels was tested using a set of solutions described in Table 1. Currents were filtered and digitized using a DMA-Tecmar labmaster, and subsequently stored in a computer using version 6.02 of the pClamp software (Axon). Voltage ramps (from −60 mV to +50 mV at 275 mV/s; total duration 400 ms) were applied from the holding potential every 5 s.

From a fresh stock solution at 0.5 mM in water, the peptide was then diluted in the appropriate solution at 2 or 20 μM and added directly (100 μl) to the recording chamber (volume 150 μl with no dilution). Gravity-driven perfusion was sometimes made without any significant difference. Reversal potentials were measured as the zero-current potential. Correction for non-linear leak-current was obtained by sub-

Table 1
Reversal potentials under different ionic conditions

	Sol. 1	Sol. 2	Sol. 3	Sol. 4	Sol. 5	Sol. 6
Main ion	NaCl: 100	KCl: 100	LiCl: 100	TEA-Cl: 100	Choline-Cl:100	Na-acetate: 100
$MgCl_2$	2	2	2	2	2	2
pH	7.2 NaOH	7.2 KOH	7.2 NaOH	7.2 TEAOH	7.2 NaOH	7.2 acetic acid
Mean E_{rev} (±S.E.M.)	−10.7±1.6	−7.1±1.1	−13.2±1.5	−15.4±1.7	−18.1±1.9	−5.2±1.3
Junction pot. (/3 M CsCl)	+1	+1	+1	+1	+1	−2
Number of oocytes	12	7	5	9	8	5

tracting traces recorded before peptide application. Junction potential between the different solutions used was less than 2 mV and neglected except when mentioned.

For single channel recordings, the vitelline membrane of the oocyte was mechanically removed after a 10 min immersion in a hyperosmotic solution (NaCl, 300; HEPES, 10; $MgCl_2$, 2). Cell-attached patch-clamp was performed using the Geneclamp 500 (Axon) with a pipette of 10–12 MΩ (borosilicate, KG-33 Gardner) as described previously by Charnet et al. [17]. The holding potential was between −100 and +100 mV. All-point amplitude histograms were fitted to multiple Gaussians using the routines of pStat (pClamp 6.02).

2.4. Peptide reconstitution into planar lipid bilayers

Using the Montal and Mueller technique [18], virtually solvent-free planar lipid bilayers were formed by the apposition of two monolayers on a 100–120 μm diameter hole in a thin Teflon film (10 μm) pretreated with hexadecane/hexane (1:40, v/v). Measurement compartments were glass cells. Bilayer formation was monitored by the membrane capacitance response to a ±10 mV triangular signal. The current fluctuations were recorded using a BLM 120 amplifier (Biologic) and stored on a digital tape recorder (DTR 1202, Biologic). The stored signals were transferred to a computer for analysis (currents, amplitude histograms) using a software from Intracell (Royston, UK).

In macroscopic current experiments, doped membranes were subjected to triangular voltage sweeps (duration 20 s). Transmembrane currents were fed to a Keithley amplifier (model 427). Current-voltage curves were recorded on an X-Y plotter.

Diphytanoylphosphatidylcholine (DPhPC) or a mixture of palmitoyloleoyl-phosphocholine/dioleoylphosphoethanolamine (POPC/DOPE, 7:3, w/w) or palmitoyloleoyl-phosphocholine/dioleoylphosphoserine (POPC/DOPS, 9:1, w/w) was used as lipid (Avanti Polar, Birmingham). The electrolyte solutions were 0.1 M or 1 M NaCl and 1 M KCl, buffered with 10 mM sodium phosphate (pH 7.4). The peptide was diluted in water buffered with sodium phosphate (pH 7.4). It was added to both compartments, typically at 10^{-13}–10^{-11} M for single channel experiments or 10^{-10}–10^{-7} M for macroscopic measurements. Selectivity experiments were carried out imposing a NaCl or KCl gradient through the lipid bilayer: from 0.1 M on the *cis* side to 1 M on the *trans* side. The zero-current potential (E_{rev}) was cor-

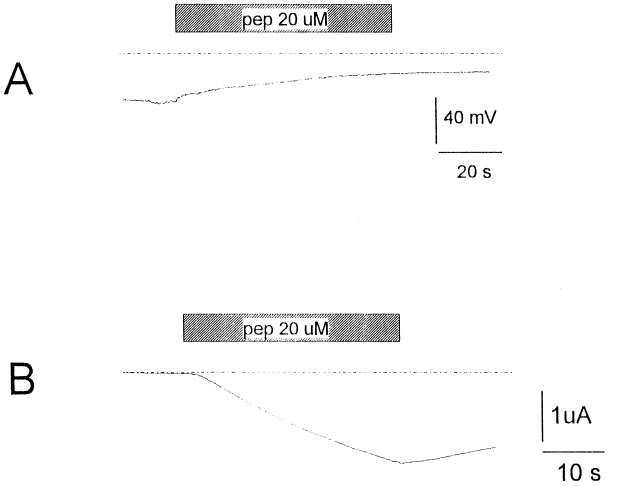

Fig. 1. Single microelectrode recording of *Xenopus* oocyte membrane potential. (A) Perfusion of 20 μM SP-NLS peptide induced a slowly developing depolarization of the membrane potential which stabilized around −15 mV; dashed line: zero voltage. (B) Same experiments as in A but in a voltage-clamp configuration. Application of peptide (20 μM, dashed box) leads to the generation of a marked (>1 μA) inward current that very slowly recovers after peptide washout. Dashed line: zero-current.

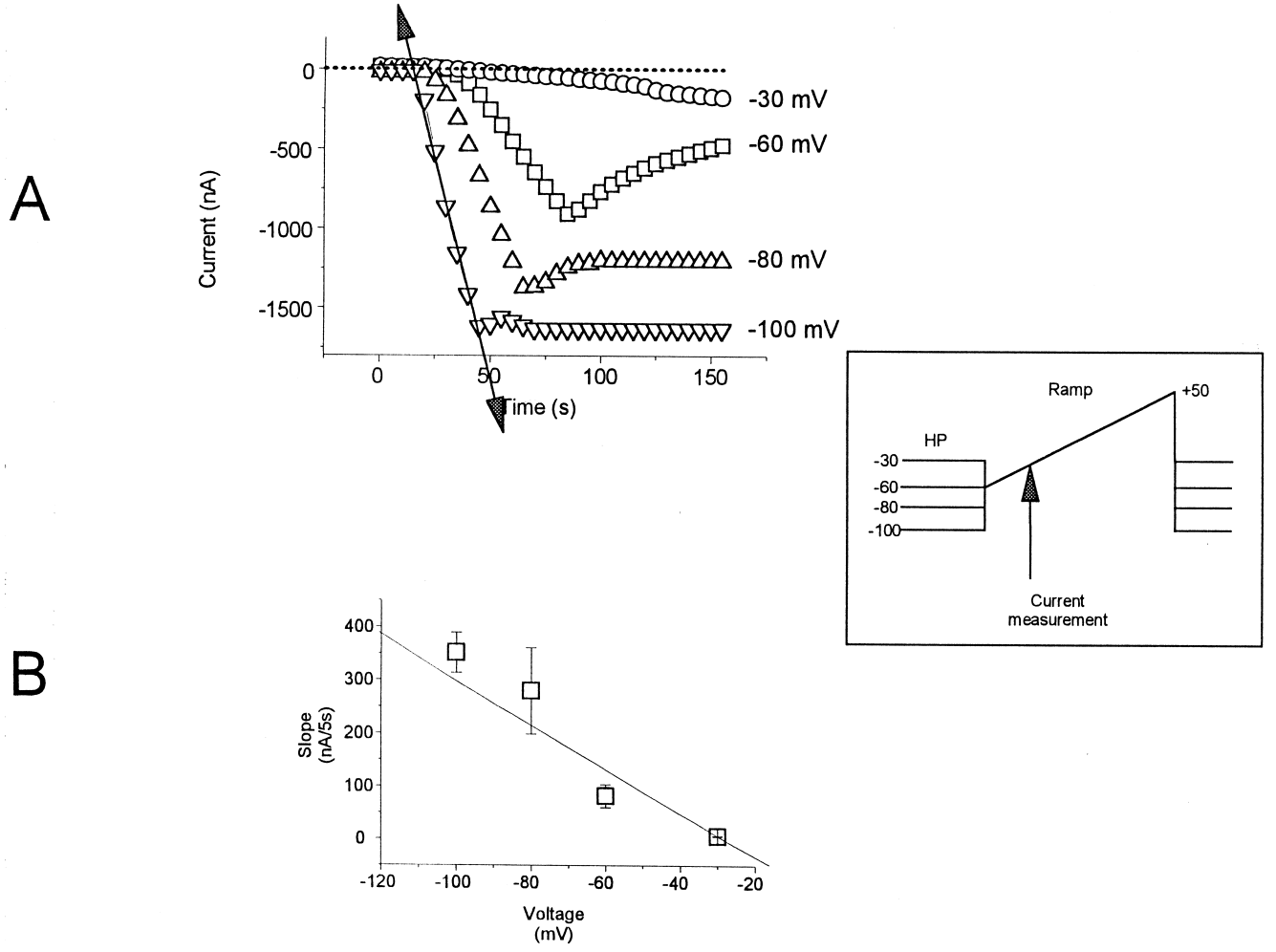

Fig. 2. Voltage dependence of SP-NLS peptide incorporation. (A) Effect of varying the holding potential on peptide incorporation. Voltage ramps (−60/+50 mV, 275 mV/s) were applied every 5 s. The holding potential between these ramps was set to either −30, −60, −80 or −100 mV and the current was arbitrary zeroed before applying the ramp. Peptide was always added after 20 s. Currents recorded at −40 mV are displayed. In each case, peptide-induced currents rapidly overcome the compliance of our amplifier, so that no steady state effect could be obtained. The speed of the increase in membrane current is, however, clearly dependent on the holding potential. The stop in current increase is due to the end of perfusion followed by a washout, otherwise a continuous increase of the current occurs. For positive holding potential no transmembrane current could be detected probably due to a lack of peptide incorporation. (B) Plot of the slope of the increase in membrane current against the holding potential. The slope was calculated, for each voltage, as the tangent of the maximal increase in membrane current in A. Peptide concentration was 20 μM. The smooth line represents the best fit of the data using a linear regression.

rected by subtracting the asymmetric potential due to the salt gradient ($E_{rev} = V_{i=0} - V_{asymmetric}$).

3. Results and discussion

3.1. Macroscopic current measurements

We first added the peptide directly to *Xenopus* oocytes bathed on the Na100 solution (final concentration of 20 μM), and followed the membrane resting potential. Under these conditions, a few seconds after peptide addition, a rapid depolarization of the membrane occurred and the oocyte membrane potential (around −40 mV in control conditions) decreased and stabilized at about −15 mV (Fig. 1A). The same experiments were also performed in voltage clamp configuration (Fig. 1B). As expected,

when the membrane potential was held at −60 mV, addition of peptide (final concentration 20 μM), clearly induced a marked transmembrane current that can reach several μA. These results suggest that the peptide addition induces a change in the transmembrane currents recorded in these conditions.

In order to test the possibility of modulation of this current by voltage, we performed a series of voltage pulses (ramps from −60 to +50 mV every 5 s) using a constant peptide concentration but at different holding potentials (−30, −60, −80 and −100 mV, see inset of Fig. 2). In all cases, the membrane currents were measured during the voltage ramps at −40 mV. At hyperpolarized membrane holding potential, the ability for the peptide to permeate the membrane was markedly increased, as revealed by the rapid development of the current after injection of the peptide (compare traces at −30 and −100 mV in Fig. 2A). The voltage dependence could be calculated by plotting the actual speed of current induction (arrow in Fig. 2A) versus the holding potential (Fig. 2B). The almost linear variation indicates that the peptide insertion in the membrane is proportional to the applied potential.

In order to rule out any possibility of activation of endogenous channels in oocyte membranes, the same type of experiments were carried out on artificial lipid bilayers. Fig. 3 shows two current-voltage (I-V) curves separated by a 10 min interval and recorded when applying a triangular voltage ramp (±200 mV at 10 mV/s) to highly doped bilayers (10^{-9} M). A further increase of the peptide concentration up to 10^{-7} M generates formation of leaky and unstable membranes. Strong differences between the two traces are obvious. Indeed, for the first applied ramp, an exponential development of membrane current above voltage threshold is observed, thus characterizing voltage dependence (trace 1). V_e, the voltage resulting in an *e*-fold increase in membrane conductance is 25 mV for this concentration [19]. This value, which is close to that of melittin (27 mV [20]) and significantly higher than that of alamethicin (5 mV [21]), indicates a low voltage dependence of the activity of SP-NLS. After 10 min the value of V_e is increased up to 60 mV indicating a decrease of voltage dependence (trace 2). This phenomenon has also been observed for lower concentrations of the SP-NLS peptide (10^{-10} and 10^{-11} M). Furthermore, this feature occurs whatever the delay between addition of the peptide and the first applied ramp (between 0 and 60 min) or the nature of lipids (POPC-DOPE (7:3) or DPhPC). It should also be noted that the attainment of a final voltage independent conductance always required preliminary voltage ramps.

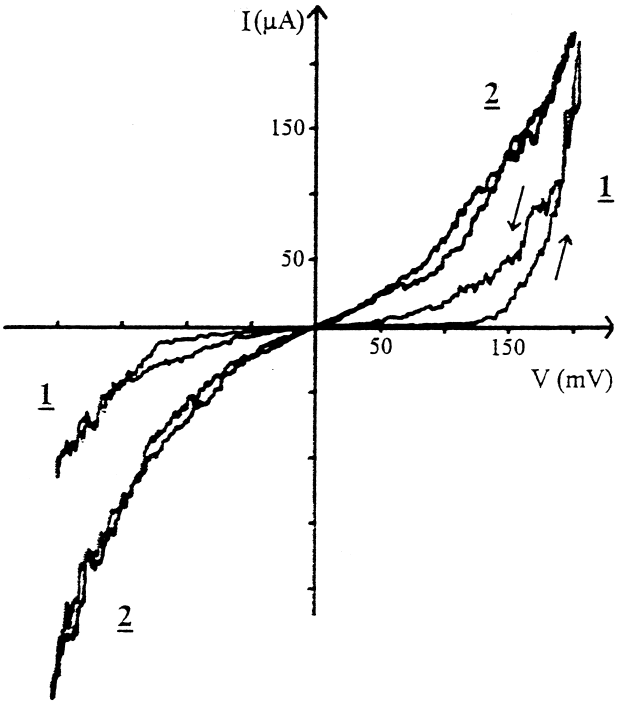

Fig. 3. Macroscopic current-voltage (I-V) curves of the SP-NLS peptide in DPhPC bilayers. 1, first voltage ramp (ramp sweep, 20 mV/s) when 10^{-9} M peptide is added in both sides and with KCl 1 M, 10 mM sodium phosphate (pH 7.4) as electrolyte. 2, second voltage ramp after 10 min.

These results observed in oocytes membranes and planar lipid bilayers indicate a voltage dependent insertion for the peptide which is followed by a voltage independent conductance.

3.2. Single ion channel experiments

Single channel recordings (Fig. 4) on oocytes were obtained on cell-attached patches using a peptide concentration of 20 μM in the pipette solution. A few minutes after patch formation, discrete transmembrane current fluctuations characterizing single channel events could be recorded. Recordings at different holding potentials in Na100 solution revealed

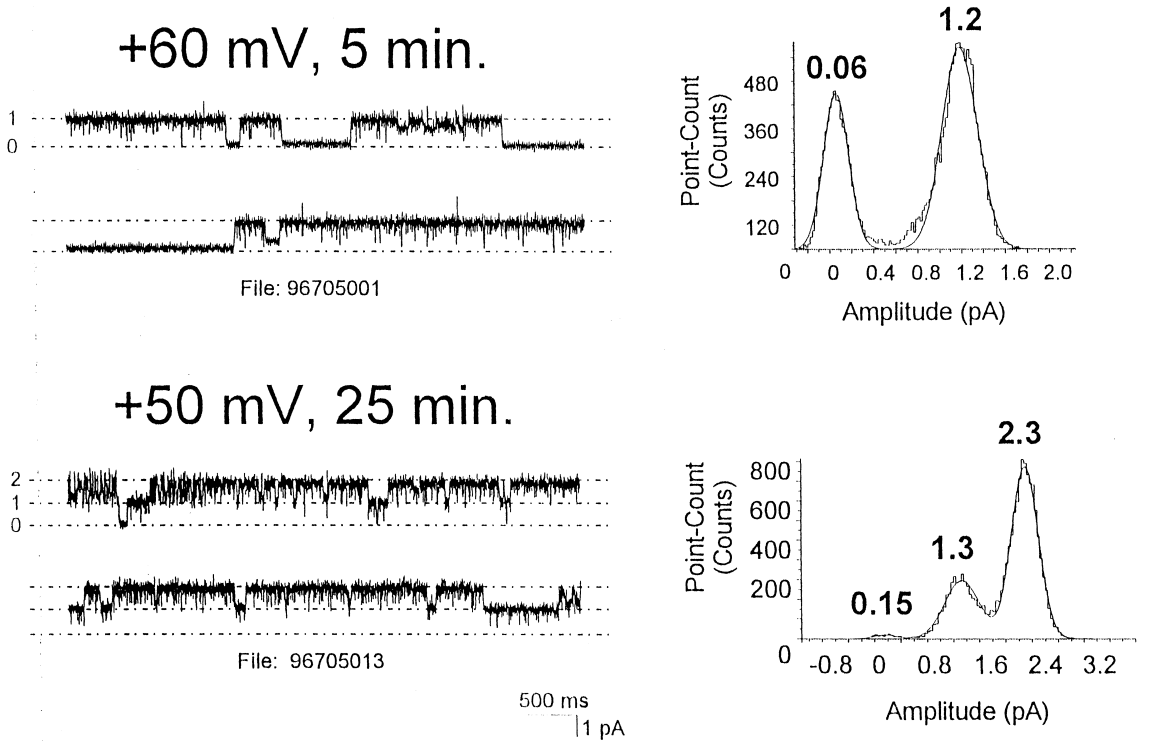

Fig. 4. SP-NLS peptide-induced pore formation at the single channel level. Cell attached, single channel recordings were performed using 20 μM peptide in the pipette solution (Na100). (Left) Current traces recorded a pipette potential of +50 mV, 5 (top) and 25 (bottom) min after seal formation. In the latter case, two levels of opening can be seen. (Right) All-points amplitude histogram of the current traces displayed above. In both cases the smallest peak (0.06 and 0.15 pA) represents the amplitude of the baseline. Conductances are 23.4 and 42.8 pS, and 22.8 pS respectively.

a main open state with a single channel conductance of 23 ± 1 pS without any obvious voltage dependent gating. For cell attached patches lasting for several minutes, occurrence of new channels in the same patch was often observed (level 2 in Fig. 4), indicating a continuous insertion of the peptide into the membrane patch.

Analogous to the experiments carried out on oocyte membranes, single channel events could be detected in planar lipid bilayers. Indeed, when doped with a low peptide concentration (10^{-13} M), discrete fluctuations of the transmembrane current were generated upon applying a potential as observed in Fig. 5A. Examination of the associated histogram gives a major channel conductance of 295 ± 30 pS in a 1 M KCl medium. When replacing the electrolyte KCl by NaCl the behaviour induced by the presence of the peptide is very similar (conductances of 250 ± 25 pS in 1 M NaCl, data not shown). Also, whatever the nature of lipids (DPhPC, a mixture of POPC/DOPE or of POPC/PS), the conductance values were conserved. In order to compare the results obtained using planar lipid bilayers with those obtained on oocyte membranes, single channel experiments were performed in 0.1 M NaCl (Fig. 5B). The main conductance value was about 60 pS, but occasionally poorly resolved channels (due to the resolution limit of the setup used for these experiments) with a smaller conductance value about 30 pS were also detected, as already observed in the oocytes experiments (25 pS).

These similar conductance values seem to indicate the same mechanism of ion channel formation in both cases. This peptide does not display the typical multi-level channel behaviour previously observed with alamethicin [20,22–24], i.e. 'the barrel-stave model' with conductance increments between substates that are increasing. On the contrary, Fig. 5 shows two identical levels of conductance which suggests that the SP-NLS peptide would form conducting aggregates by association of the same number of monomers. This behaviour is reminiscent of that

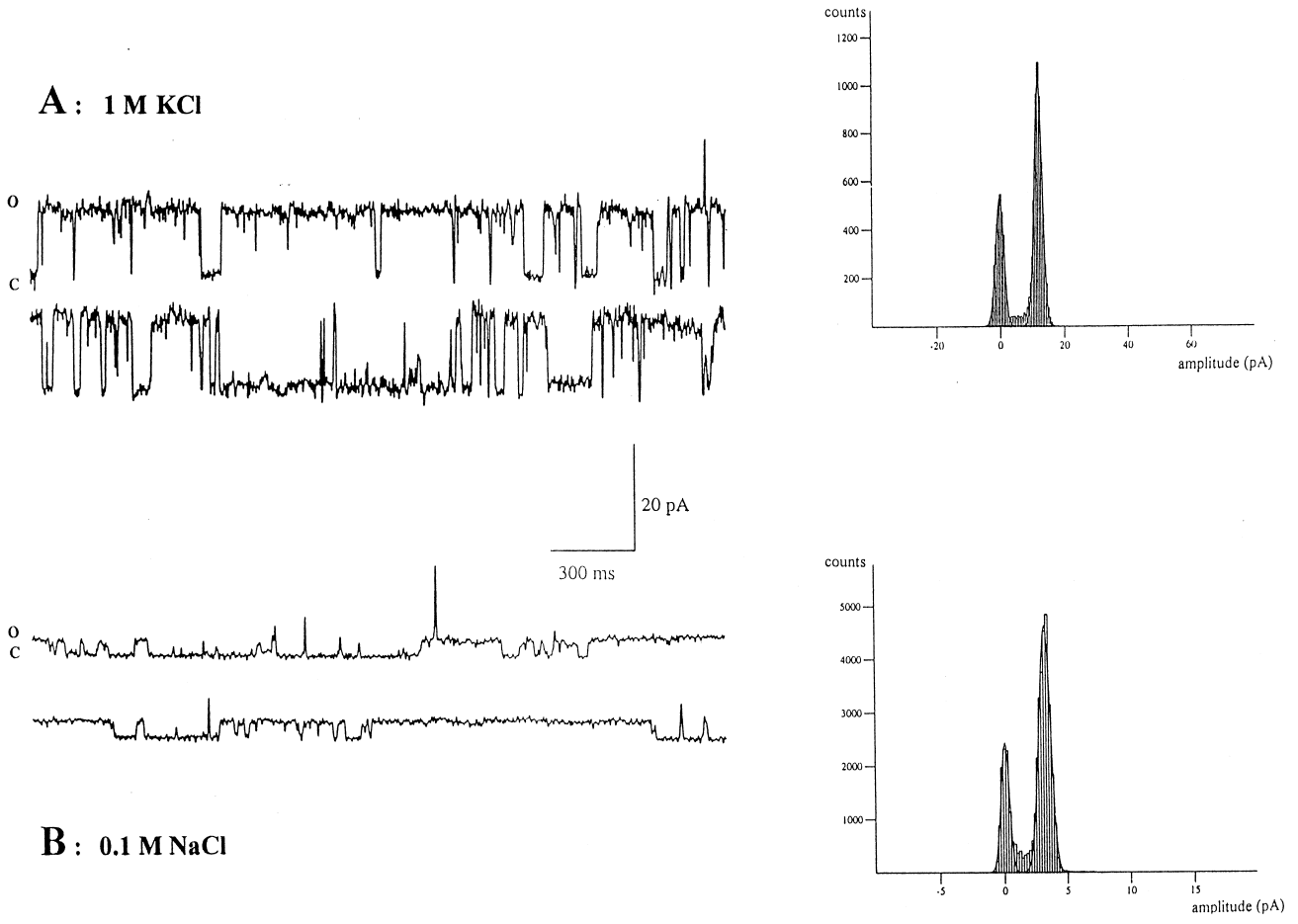

Fig. 5. Single channel recordings induced by SP-NLS in the DPhPC bilayer in different electrolytes with their associated amplitude histogram. o, open state; c, closed state. (A) In 1 M KCl. Peptide concentration: 10^{-13} M; applied voltage: 40 mV; digitization rate: 3000 Hz; filter: 300 Hz. The amplitude histogram with a Gaussian fitting shows a conductance level of 295 pS. (B) In 0.1 M NaCl. Peptide concentration: 10^{-13} M; applied voltage: 50 mV; digitization rate: 3000 Hz; filter: 300 Hz. The corresponding amplitude histogram shows a major conductance level of 60 pS.

found for magainin which induces two main conductance levels occurring independently in separate trials and suggesting the formation of preaggregates on the membrane surface [25].

Nevertheless there is a strong difference of bulk peptide concentration between the artificial membrane experiments and oocyte reconstitution. This difference required in concentration to promote membrane conductance may find its cause in the relation between membrane composition and the length of the α-helices building the channel. Indeed, the cation selectivity of the channel (see Section 3) indicates that the positively charged residues of the NLS sequence do not participate in the channel function and therefore that, at maximum, the 16 N-terminal residues are engaged in the channel structure. Such a length is sufficient [26] but can be considered as a lower limiting length spanning a bilayer membrane to form a channel and requires some membrane adaptation which can occur more freely in artificial lipid bilayers than in natural membranes.

3.3. Ionic selectivity experiments

The effect of the peptide on the oocytes membrane potential recorded at the steady state, i.e. no applied potential (−15 mV, see Fig. 1A), suggests that several ionic species carry this current since, in Na100, it does not match a known equilibrium potential. To characterize more precisely the ion selectivity of the channels involved in the generation of these currents, we have performed a series of current measurements

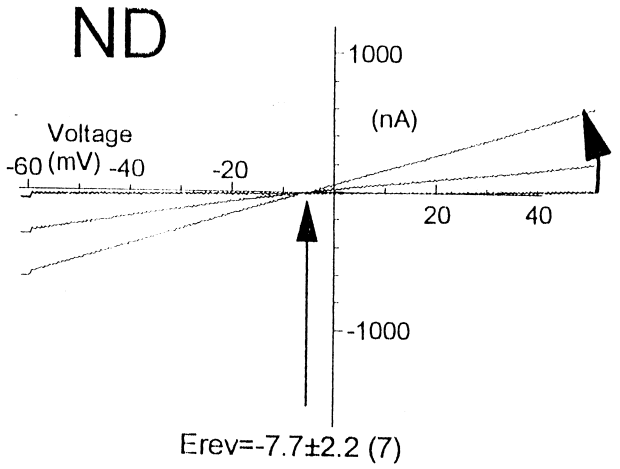

Fig. 6. Ionic selectivity at the macroscopic level for the SP-NLS peptide. Current traces were recorded under voltage ramps (−60 to +50 mV) applied at different times after addition of 20 μM peptide to the bath solution. The bent arrows indicate the chronology of the recordings. The vertical arrow gives the reversal potential (−8 mV in the Na100 solution).

during voltage ramps applied from −60 to +50 mV (every 5 s), and using different ionic conditions. Changes in membrane conductance, after addition of peptide to the bath solution (concentration of 20 μM), can be measured as the slope of the current-voltage curves (Fig. 6). Under these conditions the crossing point (−10.7 mV in Na100) is representative of the current reversal potential and is related to the intracellular and extracellular concentrations of permeating ions [27] and to the selectivity of the channel. Similar measurements performed in six different solutions show that this current reversal potential (see Table 1) was mainly carried by monovalent cations with the following rank of permeation: $K^+ > Na^+ > Li^+ > TEA^+ > choline^+$ (−7.1 to −18.1 mV). This scale represents the relative permeabilities and their absolute values are not given since the composition of the internal solution is not precisely known. Moreover, the fact that the corrected reverse potential changed when Cl^- (solution 1, $E_{rev} = -10.7$ mV) was replaced by acetate (solution 6, $E_{rev} = -5.2$ mV) also indicated the existence of a difference in the relative permeability between the anions. In order to determine the real permeability ratio between cations and anions, planar lipid bilayer experiments were performed.

Thus in planar lipid bilayers the ionic selectivity of the channel formed by the peptide was analysed as reported by Hille [27] by measuring the zero-current potential after installation of a salt gradient (0.1 M/1 M KCl or NaCl, *cis*/*trans*). These measurements indicate that the channel is cation selective with a cation/anion permeability ratio of 4.3 ± 0.4 and 4.7 ± 0.7 for Na^+/Cl^- and K^+/Cl^- respectively (for P_{Na}/P_{Cl}, $E_{rev} = 29$ mV; for P_K/P_{Cl}, $E_{rev} = 28$ mV), as calculated by the Goldman-Hodgkin-Katz method.

Interestingly, these results compared to the weak cationic selectivity of the neutral hydrophobic antibiotic alamethicin [28] indicate that the positively charged C terminus of the SP-NLS peptide does not participate in the channel activity. This is also consistent with the anion selectivity of melittin which is probably due to the positively charged lysine in the hydrophobic domain, oriented toward the lumen of the channel [13].

In conclusion, the present study has shown that a primary amphipathic peptide where the hydrophobic domain corresponds to that of a signal peptide can induce channel formation when incorporated into lipids. Since the ion channel properties observed in both natural and artificial membranes are similar, all the properties described in this paper reflect true properties of the peptide and indicate that both systems are appropriate models for the study of ionic channel formation. The observations reported here may also provide an explanation for the different activities [9,10,12] induced by this type of peptide which was designed de novo. Indeed ion channels induced by these primary amphipathic peptides seem to be a transitory step in the mechanism of their membrane crossing process (before reaching their nuclear target). It can also originate their toxicity at high concentrations owing to their accumulation in membranes where they could provoke a leak of cell compounds or a membrane destabilizing effect. However, this study also questions the biological role of signal peptides, when engaged in a protein, and their possible participation in the exportation process of proteins.

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References

[1] J.P. Leonetti, G. Degols, B. Lebleu, Bioconjugate Chem. 1 (1990) 149–153.
[2] D. Derossi, A.H. Joliot, G. Chassaing, A. Prochiantz, J. Biol. Chem. 269 (1994) 10444–10450.
[3] J. Brugidou, C. Legrand, J. Mery, A. Rabié, Biochem. Biophys. Res. Commun. 214 (1995) 685–693.
[4] M.S. Briggs, L.M. Gierasch, Adv. Protein Chem. 38 (1986) 109–180.
[5] M. Rafalski, J.D. Lear, W.F. DeGrado, Biochemistry 29 (1990) 7917–7922.
[6] V.A. Slepushkin, S.M. Andreev, M.V. Sidorova, G.B. Melikyan, V.B. Grigoriev, V.M. Chumakov, A.E. Grinfeldt, R.A. Manukyan, E.V. Karamov, AIDS Res. Hum. Retroviruses 8 (1992) 9–18.
[7] D. Kalderon, W.D. Richardson, A.F. Markham, A.E. Smith, Nature 311 (1984) 33–38.
[8] D.S. Goldfarb, J. Gariépy, G. Schoolnik, R.D. Kornberg, Nature 322 (1986) 641–644.
[9] L. Chaloin, P. Vidal, A. Heitz, N. Van Mau, J. Méry, G. Divita, F. Heitz, Biochemistry 36 (1997) 11179–11187.
[10] L. Chaloin, P. Vidal, P. Lory, J. Méry, N. Lautredou, G. Divita, F. Heitz, Biochem. Biophys. Res. Commun. 243 (1998) 601–608.
[11] M.C. Morris, P. Vidal, L. Chaloin, F. Heitz, G. Divita, Nucleic Acids Res. 25 (1997) 2730–2736.
[12] L. Béven, L. Chaloin, P. Vidal, F. Heitz, H. Wrõblewski, Biochim. Biophys. Acta 1329 (1997) 357–369.
[13] M. Tosteson, J. Levy, L. Caporale, M. Rosenblatt, D. Tosteson, Biochemistry 26 (1985) 6627–6631.
[14] C. Kaduk, H. Duclohier, M. Dathe, M. Beyermann, G. Molle, M. Bienert, Biophys. J. 72 (1997) 2151–2159.
[15] M. Sansom, Q. Rev. Biophys. 26 (1993) 365–421.
[16] P. Charnet, E. Bourinet, T.P. Snutch, J. Nargeot, FEBS Lett. 344 (1994) 87–90.
[17] P. Charnet, C. Labarca, H.A. Lester, Mol. Pharmacol. 41 (1992) 708–717.
[18] M. Montal, P. Mueller, Proc. Natl. Acad. Sci. USA 69 (1972) 3561–3566.
[19] I. Vodyanoy, J.E. Hall, T.M. Balasubramanian, Biophys. J. 42 (1983) 71–82.
[20] W. Hanke, C. Methfessel, H.U. Wilmsen, E. Katz, G. Jung, G. Boheim, Biochim. Biophys. Acta 727 (1983) 108–114.
[21] J.E. Hall, I. Vodyanoy, T.M. Balasubramaniam, G.R. Marshall, Biophys. J. 45 (1984) 233–247.
[22] L.G. Gordon, D.A. Haydon, Biochim. Biophys. Acta 436 (1976) 541–556.
[23] G. Baumann, P. Mueller, J. Supramol. Struct. 2 (1974) 538–557.
[24] G. Boheim, G.J., Membr. Biol. 19 (1974) 277–303.
[25] H. Duclohier, G. Molle, G. Spach, Biophys. J. 56 (1989) 1017–1021.
[26] S. Wada, A. Iida, K. Asani, T. Fujita, Bioorg. Med. Chem. Lett. 6 (1996) 2275–2278.
[27] B. Hille, in: B. Hille (Ed.), Selective Permeability: Independence. Ionic Channels of Excitable Membranes, Sinauer Associates, Sunderland, MA, 1984, pp. 226–248.
[28] W. Hanke, G. Boheim, Biochim. Biophys. Acta 596 (1980) 456–462.