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Review

Interactions of amphipathic CPPs with model membranes

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Abstract

We have investigated the interactions between two carrier peptides and model membrane systems as well as the conformational consequences of these interactions. Studies performed with lipid monolayers at the air–water interface have enabled identification of the nature of the lipid–peptide interactions and characterization of the influence of phospholipids on the ability of these peptides to penetrate into lipidic media. Penetration experiments reveal that both peptides interact strongly with phospholipids. Conformational investigations indicate that the lipid–peptide interaction govern the conformational state of the peptides. Based on the ability of both peptides to promote ion permeabilization of both natural and artificial membranes, we propose a model illustrating the translocation process. For MPG, it is based on the formation of a β-barrel pore-like structure, while for Pep-1, it is based on association of helices.

Keywords: Cell-penatrating peptide; Amphipatic peptide; Interactions with membrane; Conformation; Spectroscopic analysis

Contents

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Primary amphipathic peptides are the result of the sequential assembly of hydrophobic and hydrophilic domains. The first one is required for membrane anchoring and for complex formation with hydrophobic cargoes. The hydrophilic domain is required to address a subcellular compartment, to improve the solubility of the vector and for complex formation with hydrophilic negatively-charged molecules [1].

There are three major families of primary amphipathic peptides: those derived from a signal peptide (SP) [2,3], from a fusion peptide (FP) as found in the MPG family [3,4], and the tryptophan-rich sequences forming the Pep family [5]. These three families have a common hydrophilic domain, the Nuclear Localization Sequence (NLS) of SV40 large T antigen: PKKKRKV [6]. All these peptides bear a WSQ sequence which acts as a linker between the hydrophilic and hydrophobic domains, thereby maintaining their integrity. The selected SP sequence corresponds to that of the light chain of the immunoglobulin of *caiman crocodylus* while the FP of MPG was chosen from HIV-1 fusion protein gp41. For Pep peptides, the hydrophobic sequence was selected from the dimerization motif at the interface of HIV-1 reverse transcriptase. All of these peptides are acetylated and bear a cysteamide group at their N- and C-termini, respectively.

GALFLGFLGAAGSTMGAWSQPKKKRKV for MPG
KETWWETWWTEWSQPKKKRKV for Pep-1

The peptides of the SP family enter cells very rapidly at 4 °C as well as at room temperature. Since endocytosis inhibitors have no effect on cell entry, the endocytosis pathway has been ruled out to explain the mechanism of internalization. These peptides have been used for the intracellular delivery of oligonucleotides [3] or porphyrin [7] derivatives which are covalently linked to the carrier though the cysteamide moiety. However, they have proved to be toxic when used at concentrations higher than 10 μM due to pore formation which generates membrane depolarization [8].

Attempts to overcome this property were made by designing the MPG family based on the association of a fusion sequence to the above-mentioned NLS and linker. Several variants of the parental peptide have been tested. The first one (MPG-W) is the result of a W^7 to F substitution and results in efficient nuclear localization in fibroblasts [1,3,9]. This behavior prompted us to synthesize other variants in order to control the addressed subcellular compartment for nucleic acid cargoes. This was achieved by a K to S substitution in position 23 corresponding to the second lysine of the NLS sequence, generating the vector peptide MPG-Δ^{NLS}. This substitution was made to reduce nuclear addressing by decreasing the number of positive charges, and thereby also reducing the stability of the complex formed with the negatively charged nucleic acids. MPG-Δ^{NLS} was shown to efficiently transfer single- and double-stranded oligonucleotides, RNAs [4,10], siRNAs [11,12], double-stranded phosphorothioate oligonucleotides [13] and more sophisticated oligonucleotides such as $N^{3'} \rightarrow P^{5'}$ thio-phosphoramidates without formation of any covalent linkage [14,15].

The peptides of the Pep family differ from MPG in the nature of the hydrophobic sequence and were designed with the aim of delivering peptides, proteins or PNAs [5,16]. Pep-1, the leader peptide of the Pep family, forms complexes with proteic cargoes which are rapidly delivered into a great variety of cell lines and applied *in cellulo* and in vivo [17,18].

1. Mechanisms of translocation

Identification of the mode of action of CPPs is crucial for the design of future generations of CPPs. Investigation of the mechanism of internalization requires identification of several physicochemical properties of the carrier peptides. First, it is crucial to elucidate the type of interaction that they can elicit in the presence of membrane components, mainly the phospholipids. It is also necessary to identify the structural criteria, mainly the peptide primary and secondary structures, which can influence the internalization process. Finally, three main entry mechanisms can be examined: direct penetration into the membrane, translocation through formation of a transient structure and an endocytosis-mediated entry.

With respect to the interactions of CPPs with the plasma membrane, most data stress the role of positive charges, which allow direct electrostatic interactions with phospholipid headgroups. However, some differences can be noticed depending on the nature of the CPP. As an example, penetratin differs from transportan in that the former interacts preferentially with negatively charged membranes whereas interactions of the latter do not depend on charges [19]. Furthermore, comparison of internalization properties of all-L with those of all-D peptides [20,21] indicate that this process is not receptor-mediated as confirmed by the use of giant unilamellar vesicles [22]. Other investigations point out the importance of the heparan sulfates (HS) present at the cell surface. Indeed, internalization of Tat, penetratin and polyarginines is inhibited upon degradation of the heparan sulfates, by addition of heparin or sulfated polysaccharides or HS deficient cell lines [23–25]. However, other authors claim that Tat and penetratin can enter cells independently of the presence of heparan sulfates [22,26].

A last mechanism has recently been proposed for internalization mediated by peptides belonging to the family of primary amphipathic peptides, namely MPG and Pep-1. On the basis of physico-chemical investigations, including CD, FTIR, and NMR spectrometry [27–30] associated with electrophysiological measurements and investigations dealing with the use of systems mimicking model membranes such as monolayers at the air–water interface and transferred monolayers, two very similar models have been proposed. Both are based on the formation of transient pore-like structures. The main difference between the model proposed for MPG and that proposed for Pep-1 is found in the structure giving rise to the pore structure. For MPG, it is formed by a β-barrel structure [29], while that of Pep-1 depends on association of helices [30]. For both peptides, strong hydrophobic phospholipid–peptide interactions have been detected and in both models the folded parts of the carrier molecule correspond to the hydrophobic domain while the rest of the molecule (linker+NLS) remains unstructured.

Here, we gather the main physicochemical approaches which were used and enabled us to the proposal of pore-based models accounting for the cellular internalization process of MPG and Pep-1.

2. Conformational investigations and physicochemical approaches

2.1. Pep-1

2.1.1. Structural characterization of Pep-1 in solution and in the presence of a SDS

For concentrations ranging between 0.1 and 0.3 mg/mL, the CD spectrum of Pep-1 in solution in water shows a single negative band centered at 202 nm associated with a shoulder around 220 nm, suggestive of a non-structured or poorly ordered structure. Interestingly, increasing the concentration of Pep-1 up to 3 mg/mL promotes a dramatic modification which leads to a spectrum characterized by a minimum at 205 nm, a pronounced shoulder at 221 nm, and a maximum at 190 nm, representative of a helical conformation [30]. This tendency for Pep-1 to adopt, at least in part, a helical structure is confirmed by observations in SDS-containing media. Indeed, above the CMC of the detergent, a spectrum with two minima at 207 and 222 nm and a maximum at 192 nm is obtained, characterizing a helical conformation [31]. The helical folding of Pep-1 is confirmed by NMR investigations. The sequential and medium range NOEs observed for Pep-1 in H_2O and Pep-1 in the presence of SDS. The observed NOEs for Pep-1 in H_2O in the segment 4–13 are consistent with the existence of a helical secondary structure in this part of the sequence. In the presence of SDS, the observed NOEs are characteristic of a helix for the same segment (residues 4 to 13). In addition, several NOEs detected at the beginning of the sequence are indicative of a 3_{10} helix. The extension of the helical structure to the N-terminus of Pep-1 in medium containing SDS is the only difference observed between the conformation of Pep-1 in H_2O and Pep-1 in SDS micelles. Interestingly, the NLS moiety of Pep-1 remained unstructured in both media [30].

2.1.2. Structural characterization of Pep-1 in the presence of phospholipids

We have also characterized the structural states of Pep-1 in the presence of phospholipids. As shown by CD, successive addition of vesicles of DOPC/DOPG 80/20 to a dilute solution of Pep-1 in water induced a structural transition. Owing to the existence of isodichroic points around 220 nm, the conformational transition is the result of a transition between two states. The first state corresponds to a non-structured form, while the second is more difficult to identify unambiguously due to the presence of 5 Trp residues which are most likely involved in the contribution at 228 nm. Nevertheless, since the spectra always show a maximum at about 191 nm and a minimum located in the 206–208 nm range, with no minimum between 215 and 220 nm, the presence of a β-sheet structure can be ruled out, and these data therefore also suggest that the conformation of Pep-1 is helical in the presence of phospholipids. The helical structure adopted by Pep-1 in the presence of phospholipids is confirmed by CD observations performed on peptide-containing transferred monolayers (Fig. 1) [32]. In all cases, with or without any type of lipid, whether neutral or negatively charged, the spectra exhibit two minima at 206 and 222 nm and one maximum around 190 nm, indicating that a helical structure is the major structural component. From the FTIR point of view, all spectra showed a complex contour of the Amide I band. In the absence of lipids and at low lipid/peptide ratio (DOPC), two distinct contributions were observed, one at 1625–1630 cm^{-1} and the other centered at 1655–1660 cm^{-1}. An increase of the DOPC/peptide ratio generated a significant decrease of the 1625 cm^{-1} contribution associated with a broadening of the 1655–1660 cm^{-1} contribution [33]. The same behavior was obtained with DOPG instead of DOPC (data not shown). The finding of a broad band is in full agreement with the NMR data obtained in SDS (see above) and indicates that two helical forms are maintained in a lipidic medium. (1655 cm^{-1}, α-helix; 1665 cm^{-1}, helix 3_{10}) [34,35].

2.1.3. Studies at the air–water interface—adsorption at lipid-containing interfaces

The ability of Pep-1 to penetrate into lipidic media was determined by the monolayer approach. This requires first the determination of the saturating surface pressure induced by the peptide at a lipid-free air–water (0.154 M NaCl) interface. The variation of the surface tension as a function of the peptide in the subphase shows that saturation is obtained at a concentration of 5×10^{-7} M Pep-1 and that the corresponding surface tension is rather low (4 mN/m) compared to other cell-penetrating peptides studied previously in our laboratory (>15 mN/m) [2,36]. Such a low increase of the surface tension indicates that Pep-1 has a weak amphipathic character. The understanding of the mechanism through which Pep-1 penetrates into membranes was performed through penetration experiments using phospholipids in the liquid expanded (DOPC and DOPG) or liquid condensed (DPPC and DPPG) states. In the case of liquid-expanded monolayers, a strong increase in the surface pressure is observed. The most important characteristics of these experiments lie in the fact that (i) both DOPC and DOPG yielded identical cpi (45 mN/m)

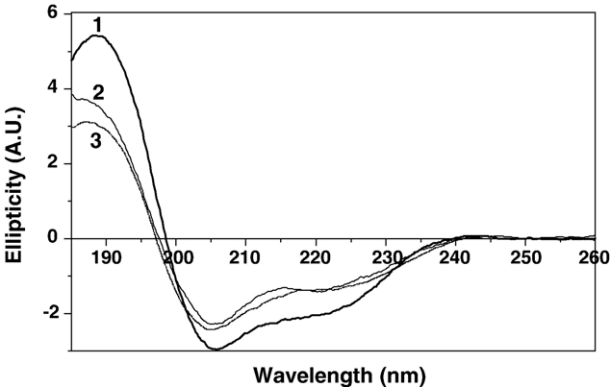

Fig. 1. Far UV CD spectra of transferred Pep-1 containing monolayers. Pure peptide (spectrum 1) and peptide in the presence of DOPC or DOPG at a peptide/lipid ratio of 0.25 (spectra 2 and 3, respectively).

and that (ii) extrapolations at zero initial pressure were high (16 and 32 mN/m for DOPC and DOPG, respectively), and significantly different from that measured in the absence of lipid (Fig. 2). For the two other phospholipids, DPPC and DPPG, again the cpi is high (33 mN/m) and does not depend on the nature of the headgroups. The high values found for the cpi suggest that the Pep-1 can spontaneously insert into natural membranes [37]. Extrapolations at zero initial pressure provide a good indication that strong peptide–lipid interactions can occur at least in monolayers, with all lipids except DPPC.

2.1.4. Penetration into phospholipid vesicles

The ability of Pep-1 to penetrate into phospholipid bilayers was investigated by fluorescence spectroscopy. The peptide contains five Trp residues in its hydrophobic moiety, which represent sensitive probes for monitoring interactions and changes in its environment upon penetration into phospholipid bilayers by intrinsic fluorescence. Successive additions of phospholipid vesicles to a solution of Pep-1 induce significant changes in the fluorescence spectrum. Indeed, a continuous shift of the fluorescence maximum (from 350 to 328 nm) accompanied by quenching of fluorescence occurs when the lipid/peptide ratio increases from 0 to 5. The blue shift of fluorescence is consistent with a change in the environment of the Trp residues from polar to nonpolar in the presence of phospholipid vesicles [38]. This is a good indication that the Trp residues are embedded in the lipidic core when Pep-1 encounters a membrane. In addition, quenching is suggestive of a transfer between Trp residues which are engaged in clusters when the peptide adopts a helical structure.

2.1.5. Formation of carrier/cargo complexes and structural consequences

Finally, the study of the ability of Pep-1 to interact with P-A, a 32-mer cargo peptide was investigated by intrinsic tryptophan fluorescence spectroscopy together with the structural consequences of this interaction. Binding of P-A peptide to Pep-1 induced a marked quenching of the intrinsic tryptophan fluorescence of Pep-1, with a saturating value of 36%. Moreover, the blue shift of the fluorescence emission maximum of 11 nm, (from 350 to 339 nm, data not shown), suggests that the Trp residues of Pep-1 interact directly with the cargo peptide P-A. Saturation is reached at a concentration of 200 nM, that is, 5-fold lower than that of the Pep-1 (1 μM), suggesting that the cargo peptide interacts with more than one molecule of Pep-1 peptide vector. The interaction between Pep-1 and P-A remains stable at high salt concentrations (300 mM NaCl), revealing that binding to Pep-1 mainly involves hydrophobic contacts. Taking into account the number of Pep-1 molecules bound to the cargo peptide P-A, the dissociation constant was estimated to be in the range of 120–150 nM, indicating that when Pep-1 is mixed with a peptide in solution they associate rapidly into noncovalent stable complexes through hydrophobic interactions. This experiment was repeated using P-B whose sequence strongly differs from that of P-A and led to the same conclusion. This indicates that the binding is not specific for a given peptide. As to the conformational consequences of these interactions, CD observations indicate that the formation of a complex does induce any structural modification of Pep-1. Finally, to complete our understanding of the relationship among the interactive ability of Pep-1 with its cargo, its penetrating capacity, and its conformational state, we examined whether the presence of phospholipids induced any structural modifications on Pep-1 complexed to its cargo, similar to those already detected for Pep-1 in its free form. Upon addition of vesicles made of DOPC/DOPG (80/20) to a preformed Pep-1/P-A complex, the modifications of the CD spectrum show the same trend as those obtained for free Pep-1 to which lipid vesicles were added. This result indicates that the particle formed by complexes of Pep-1 and its cargo interacts with lipid bilayers and that this interaction promotes, at least in part, a conformational transition of Pep-1 to an α-helical form.

P-A=RGTKALTEVIPLTEEAELELAENREILKEPVH and P-B=MEFSLKDQEAKVSRSGLYRSPSMPENLNRPRLKQ-VEKFKDNTIPDKKKC.

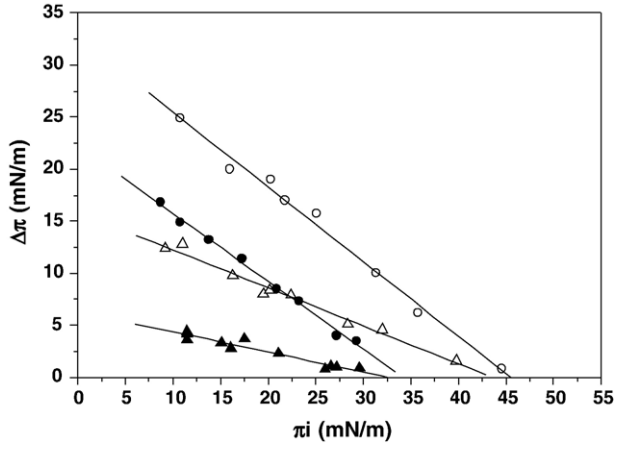

Fig. 2. Variation of the surface pressure as a function of the initial surface pressure of the phospholipid monolayer for Pep-1. (Δ) DOPC, (○) DOPG, (χ) DPPG, and (α) DPPC. Extrapolation at zero initial pressure gives the critical pressure of insertion.

2.1.6. Electrophysiological measurements

When Pep-1 (10 μM) is applied to voltage-clamped oocytes, a marked increase in membrane conductance is noted. This increase is best visualized by an increase in membrane current recorded during voltage ramps applied from −80 mV (the usual holding potential) to +80 mV (see Fig. 3). Taken together, these results suggest that the permeabilizing capabilities of Pep1 are due to the formation of membrane ion channels.

2.2. MPG

2.2.1. CD investigations

When in solution in water, peptide MPG exhibits a CD spectrum with a single minimum at 198 nm, characteristic of a nonordered structure. The spectrum remains unchanged either when the concentration of peptide is varied or when phosphate buffer is added. For spectra obtained on transferred monolayers made of pure peptides, the spectrum of peptide MPG strongly differs from that obtained when in solution in water. It is now

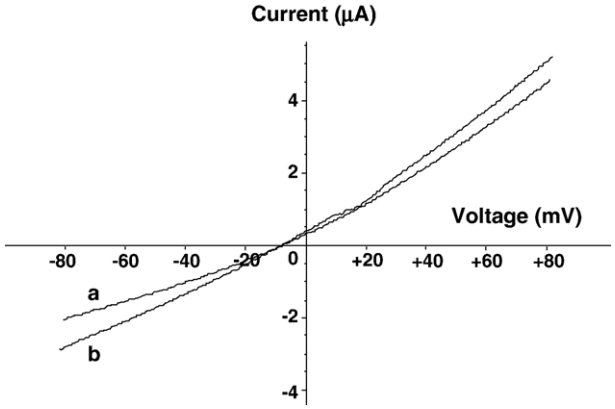

Fig. 3. Macroscopic current-voltage curve measured during application of Pep-1 or MPG peptide using a voltage-ramp from −80 to +80 mV. The current is displayed after digital subtraction of current recorded before peptide application. Trace a corresponds to the current induced by Pep-1 while trace b was obtained using MPG.

characterized by a single minimum centered at 218 nm associated with a maximum at 191 nm. Such a spectrum is characteristic of a sheet structure.

2.2.2. FTIR investigations in the presence of phospholipids

The influence of phospholipids on the conformation of the peptides was examined by FTIR. These experiments were carried out using the same procedure as reported previously [39]. For peptide MPG, the FTIR spectra show that in the absence and in the presence of phospholipids (DOPC, DOPG, DPPC, and DPPG) at any peptide/lipid ratio, above 0.05, the sheet structure, as characterized by the presence of a major amide I band component centered around 1625 cm^{-1} is favored. The existence of a broad contribution around 1650 cm^{-1} suggests the presence of unfolded domains as already observed by NMR studies of similar peptides engaged in micelles of SDS where the C-terminal NLS sequence always remains unfolded [2,9]. It must be noted that neither the nature of the lipid nor the peptide/lipid ratio modify the conformational state of peptide MPG as reported previously for a peptide containing the same hydrophobic sequence but with slight extensions at the C- and N-termini.

2.2.3. Studies at the air–water interface—adsorption at lipid-containing interfaces

The ability of peptide MPG to insert into phospholipid monolayers spread at the air–water interface was monitored by measuring variations in surface tension using different initial pressures and by injecting a given peptide concentration in the subphase. The selected concentration is slightly less than the equilibrium spreading pressure of the peptide [40]. Various penetration experiments were carried out using four different phospholipids where the nature of the headgroups (zwitterionic or negatively charged) and the physical state (liquid expanded or liquid condensed) were varied. The various plots of pressure variation versus the initial surface pressure are reported in Fig. 4. In the case of peptide MPG, although still high, the critical pressure of insertion increases from 30 to 38 mN/m for DPPC and DPPG, respectively, indicating a better uptake by negatively charged phospholipids (Fig. 4). For lipids in the liquid expanded (LE) state, insertion depends on both the initial conformational state of the peptide and the nature of the headgroups. In all cases, the critical pressures of insertion are high (see Fig. 4). Further examination of Fig. 4 also provides information on the interactions occurring between the peptide and the phospholipids considered here. These are based on the pressure value measured for an air–water interface at low lipid content (i.e., at low initial pressure). The finding of surface pressures higher than these obtained for the pure peptides at saturation is indicative of strong peptide–lipid interactions [41]. This is the case of MPG particularly in the presence of negatively charged phospholipids.

2.2.4. Conformational investigations in the presence of phospholipids

In water, the CD spectrum of MPG exhibits a single minimum centered at 198 nm, which is typical of that expected for a non-structured peptide [42]. Upon addition of phospholipid vesicles made of DOPG or DOPC/DOPG (80:20), drastic changes in the CD spectrum are observed. These modifications are characterized by a strong decrease in the intensity of the negative band at 198 nm associated with a slight increase in the contribution centered at 223 nm. To identify the nature of the lipid-induced conformation, comparison with the structural behavior of MPG was carried out in the presence of SDS. Addition of SDS generates two different processes depending on the concentration of SDS in the medium. The first process occurs at low SDS concentrations (below 2.2×10^{-3} M) and can be interpreted as a random coil–β sheet transition. Indeed, the spectrum of MPG obtained in pure water is typical of that of a non-structured peptide, while that at 2.2×10^{-3} M SDS suggests that the peptide adopts, at least in part, a sheet structure. As to the second process, which occurs at higher SDS concentrations, i.e., around the critical micellar concentration, it can be

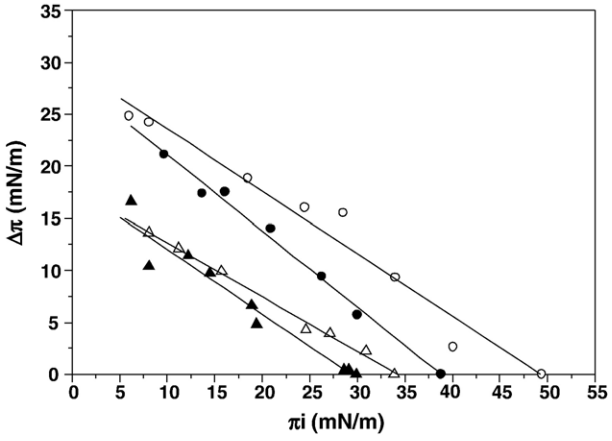

Fig. 4. Variation of the surface pressure as a function of the initial surface pressure of the phospholipid monolayer for MPG. (Δ) DOPC, (○) DOPG, (χ) DPPG, and (α) DPPC. Extrapolation at zero initial pressure gives the critical pressure of insertion.

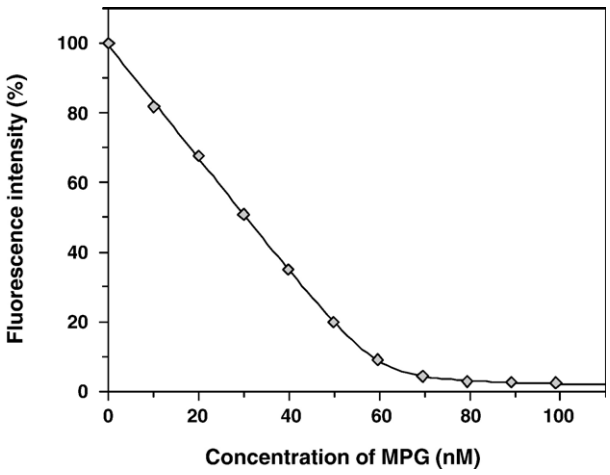

Fig. 5. Variation of the relative fluorescence intensity (maximum of the emission spectrum) of HEX-labeled *bcl*-2 oligonucleotide (solution containing 10 nmol) as a function of the concentration of MPG peptide.

attributed to a transition from the above mentioned structural form to a form which is, at least in part, helical (one maximum around 192 nm and two minima at 206 and 222 nm), the NLS remaining unstructured. Therefore, due to the strong similarity to the spectra detected at low concentration of SDS, the lipid-induced conformational change can be assigned to a conformational transition with formation of a sheet structure, at least in part, and rules out the existence of any helical structure. The existence of a sheet structure as the major structural component is confirmed by FTIR observations which reveal, in the presence of phospholipids, the existence of a major Amide I band around 1625 cm^{-1} associated with a broad shoulder at 1655 cm^{-1} [43]. On the basis of NMR data obtained in solution in water, with and without micelles of SDS [36], it can be stated that the hydrophobic sequence of the peptide corresponds to the folded domain while the remainder corresponding to the NLS is not structured.

2.3. Polynucleotide binding and induced conformational changes

2.3.1. Polynucleotide binding

Fluorescence and gel shift assays were used to monitor formation of a peptide/oligonucleotide complex. A solution of HEX-labeled oligonucleotide (18-mer) was titrated with MPG. Changes in fluorescence intensity were monitored and are reported in Fig. 5, showing an abrupt linear decrease the extrapolation of which reveals a ratio of about 7 peptides per oligonucleotide. In terms of charges, this result indicates that the positive/negative charge ratio is 2 for the complex in good agreement with previous results obtained with a double-stranded DNA [44]. An identical stoichiometry for the complex was obtained by titration of the peptide by the oligonucleotide. Moreover, determination of the equilibrium constants from both experiments indicates that the peptide exhibits high affinity for the oligonucleotide, respectively, 6 and 5 nM.

In gel shift assays, oligonucleotide was incubated with the peptide at different peptide/oligonucleotide ratios and then submitted to gel migration. For a charge ratio (+/−) of 1 or lower, the band corresponding to the free form of the oligonucleotide can be detected, while an increase of this ratio leads to disappearance of this band, accompanied by an increase in the amount of precipitated material remaining in the injection well. Taken together, these data indicate that the

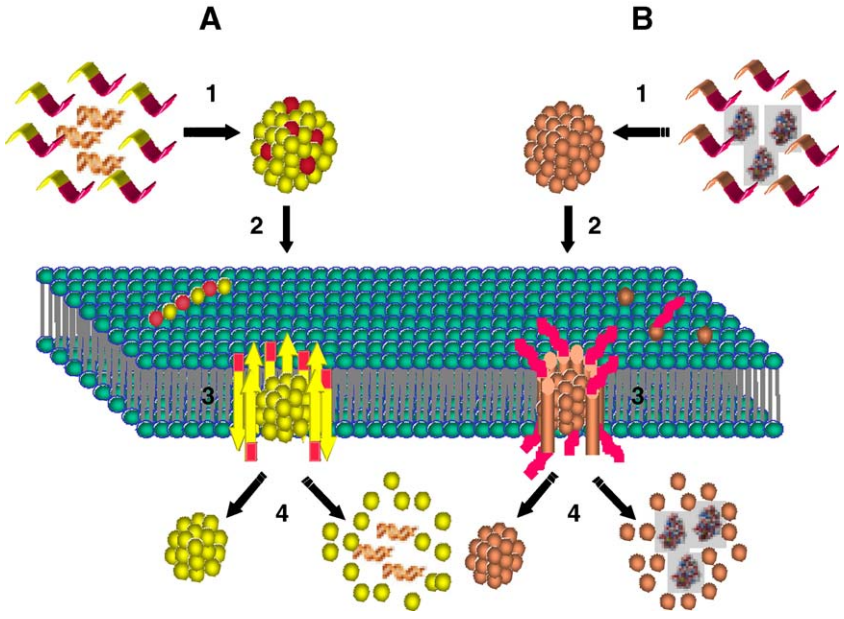

Fig. 6. Model for the translocation process of Pep-1/cargo and MPG/cargo complexes through phospholipid bilayers. For both carriers, the four steps correspond to (1) formation of the complex, (2) membrane uptake of the complex, (3) translocation through the bilayer, and (4) release into the cytoplasmic side. (colour convention: red corresponds to the NLS of both carrier, yellow and orange to the hydrophobic domain of MPG and Pep-1, respectively).

carrier peptide MPG strongly interacts with an oligonucleotide cargo to form a complex containing most probably more than 7 peptide molecules per oligonucleotide.

2.3.2. Induced conformational changes

Addition of the oligonucleotide to a solution of MPG in water in an oligonucleotide/peptide ratio ranging from 0 to 0.05 promotes changes in the CD spectrum. These spectral variations indicate a decrease in the amount of unfolded peptide to the benefit of sheet structures. Interestingly, the CD spectra obtained for oligonucleotide/peptide ratios ranging from 0 to 0.1 indicate formation of a small but significant amount of β-sheet structure. The mixtures corresponding to the ratios previously used in transfection assays (0.1 or 0.2) [4,10,12,45] were used for further experiments related to the influence of phospholipids on the structure of the peptide engaged in a complex with oligonucleotide. Typical observed variations of the CD spectrum reveal that addition of DOPG induces spectral modifications that are similar to those observed in the absence of oligonucleotide, thereby indicative of formation of a β-sheet-based structure.

2.3.3. Peptide-induced ionic leakage

When the peptide (30 μL of a 10-μM peptide solution) in its free form or engaged in a complex at an oligonucleotide/peptide ratio of 0.1 was applied to voltage-clamped oocytes, marked increases in membrane conductance were observed (Fig. 3). The presence of nucleic acids has no effect on the reversal potential (−12 mV) but appears to reduce the current amplitude. We cannot decide now whether this decrease in the current arises from an artificial decrease of the peptide concentration because it is engaged in the complex, or to modification of the transmembrane current characteristics. Nevertheless, electrophysiological measurements strongly resemble those obtained for Pep-1 and for other channel-forming cell-penetrating peptides [8,30], suggesting thus, that the peptide-induced membrane permeabilization properties are due to formation of an ion channel.

3. The models

On the basis of physicochemical investigations, including CD, FTIR, and NMR spectrometry associated with electrophysiological measurements and investigations dealing with the use of systems mimicking model membranes such as monolayers at the air–water interface and transferred monolayers, two very similar models have been proposed. Both are based on the formation of transient pore-like structures. The main difference between the model proposed for MPG and that proposed for Pep-1 is found in the structure giving rise to the pore structure. For MPG, it is formed by a β-barrel structure (Fig. 6), while that of Pep-1 depends on association of helices (Fig. 6). Such a model accounts with the expected length of the helical transmembrane domain of Pep-1. In the case of MPG, the situation is less clear. Indeed, a sheet structure with 17 residues is much longer than the thickness of a bilayer (about a 2-fold). Therefore, in that case, the possibility of a hairpin-like structure favoring an antiparallel arrangement, as suggested by the infrared observations, cannot be rule out.

4. Conclusions and perspectives

Although, some of the cell-penetrating peptides described above begin to receive applications mainly in research laboratories, some weaknesses are still encountered, in particular due to the lack of specificity toward targets. This implies that some modifications of their sequences or the introduction of non-natural amino acids bearing side-chains that can improve their specificity is required. However, it is now known that modifications in their sequences, although minor, can strongly modify their ability to act as drug carrier. It is therefore necessary to identify precisely the criteria which can define an efficient cell-penetrating peptide with a high degree of drug transfer. This requires the understanding of the interactions of these carrier peptides with all the membrane components in association with the structural consequences of these interactions. These criteria have to be satisfied and will help in the design of the next generation of carrier peptides.

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