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Oligoarginine vectors for intracellular delivery: Role of arginine side-chain orientation in chain length-dependent destabilization of lipid membranes

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ABSTRACT

Arginine-rich peptides receive increased attention due to their capacity to cross different types of membranes and to transport cargo molecules inside cells. Even though peptide-induced destabilization has been investigated extensively, little is known about the peptide side-chain and backbone orientation with respect to the bilayer that may contribute to a molecular understanding of the peptide-induced membrane perturbations.

The main objective of this work is to provide a detailed description of the orientation of arginine peptides in the lipid bilayer of PC and negatively charged PG liposomes using ATR-IR spectroscopy and molecular modeling, and to relate these orientational preferences to lipid bilayer destabilization.

Molecular modeling showed that above the transition temperature arginine side-chains are preferentially solvent-directed at the PC/water interface whereas several arginine side-chains are pointing towards the PG hydrophobic core. IR dichroic spectra confirmed the orientation of the arginine side chains perpendicular to the lipid–water interface. IR spectra shows an randomly distributed backbone that seems essential to optimize interactions with the lipid membrane. The observed increase of permeation to a fluorescent dye is related to the peptide induced-formation of gauche bonds in the acyl chains. In the absence of hydrophobic residues, insertion of side-chains that favors phosphate/guanidium interaction is another mechanism of membrane permeabilization that has not been further analyzed so far.

1. Introduction

Cell penetrating peptides (CPPs) display the ability to cross cell membranes and transport cargo molecules inside cells. One of the most representative members of this family of peptides is a short, arginine-rich peptide segment derived from the human immunodeficiency virus (HIV)-1Tat protein. Cellular uptake mechanisms remain nevertheless controversial. For instance, it has been suggested that the TAT peptide translocates with its cargo into eukaryotic cells through a physical mechanism that is not receptor-mediated, without implicating the endosomal pathway (Henriques et al., 2005). This suggests that translocation would require a rearrangement of the lipid bilayer organization and/or packing. In contrast, other studies propose a raft-dependent endocytic pathway, involving macropinocytosis (Wadia et al., 2004). These membrane-permeable peptides share little similarity in their primary and secondary structures except for a high concentration of arginine residues in their sequences. In addition, various arginine-rich oligopeptides display very similar properties in terms of translocation and delivery efficiency, suggesting an obvious correlation between translocation and arginine content (Futaki, 2006). The translocation may be a consequence of the interaction between the hydrophilic moiety of phospholipids and the side-chains of arginine residues as suggested by the arginine-rich peptides' length-dependent internalization and the absence of internalization of peptides with three arginine residues or less (Tung and Weissleder, 2003).

Whereas part of CPPs uptake might involve specific receptors, internalization is observed even in their absence (Richard et al., 2005). Furthermore, these peptides can enter giant unilamellar vesicles made exclusively of lipids (Binder and Lindblom, 2003). All together these data strongly suggest that a specific interaction between the hydrophilic moiety of phospholipids and the side-chains of arginine residues is an important step for peptide internalization.

The interaction between the CPPs and the cell membrane is the first step involved in the uptake mechanism. Even though peptide-induced membrane perturbations has been investigated

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extensively, little information is available about the orientation of the peptide side-chains and backbone with respect to the bilayer that may open the way to a molecular understanding of the peptide-induced membrane perturbations. The fact that the hydrophobic residues present in the sequence of these penetrating peptides favored penetration is possibly a direct consequence of an increased hydrophobicity. The challenge of this work is to deal with highly hydrophilic peptide and to understand how it could nevertheless destabilize a lipid bilayer. Therefore, the main objective of this work is to provide a description of the backbone and side-chain orientation of Arginine oligopeptides (Arg4, Arg7) in the lipid bilayer, in comparison with the isolated amino acid (arg+), using ATR-IR spectroscopy and molecular dynamics simulation and to relate orientation preferences of the peptide with the lipid bilayer packing and organization. In this sense we use zwitterionic PC and negatively charged PG because we want to reveal the mechanism of binding which always precedes a possible translocation, focusing on the influence that peptide binding has on lipid membrane properties. The phosphate moiety was identified in previous studies as a prominent factor in arginine–lipid interaction and therefore DMPG appeared as a valuable biophysical model (Sakai et al., 2005; Tang et al., 2007).

2. Materials

DMPC, DMPG, DOPG were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and used as received. Peptides and amino-acids: Arg7, Arg4 were from AnyGen Co. Ltd., and L-arginine (arg+) was obtained from Sigma–Aldrich (Saint Louis, MO).

2.1. Preparation of liposomes

Lipids were dissolved in chloroform:methanol mixture (3:1 ratio), evaporated under nitrogen flow and desiccated overnight under vacuum to remove any residual solvent. Dried films were vortexed at 40 °C in Tris:NaCl buffer 10:100 mM (pH 7.3).

3. Methods

3.1. Attenuated total reflection Fourier transform IR spectroscopy (ATR-FTIR)

The internal reflection element was a 52 mm × 20 mm × 2 mm trapezoidal germanium ATR plate with an aperture angle of 45° yielding 25 internal reflections. Infra-red spectra were recorded on an IFS55 FTIR spectrophotometer (Bruker, Ettlingen, Germany) purged with N_2. Fifteen microliters of the liposomes sample were deposited under a stream of nitrogen on one side of the germanium. While evaporating, capillary forces flattened the membranes which spontaneously formed oriented multilayer arrangements. Under these conditions a well ordered multilayer stack is formed (Vigano et al., 2000a) it remains stable under a buffer flow (Scheirlinckx et al., 2004). The peptides were added to the lipids at several molar ratios. Spectra were recorded with 2 cm^{-1} spectral resolution between 4000 and 800 cm^{-1} with a broad-band MCT detector provided by Bruker; 128 scans were averaged for one spectrum at each temperature analyzed. A modified continuous flow ATR setup was equipped with a polarizer that can be oriented parallel or perpendicular to the incidence plane. An elevator under computer control made it possible to move the whole setup along a vertical axis. This allowed the crystal to be separated in different lanes. Here, one lane contained the membrane film and the other was used for the background. All spectra were corrected for water vapor contribution and CO_2 and finally apodized at a resolution of 4 cm^{-1}. All the software used for data processing was written under MatLab 7.0 (Mathworks Inc., Natick, MA).

3.2. Lipid bilayer acyl chain conformations

IR spectra were recorded with and without peptides in the 3000–2800 cm^{-1} range, to monitor the lipid state of order and the motional freedom of the methyl groups (Casal and Mantsch, 1984).

3.3. Secondary structure evaluation

DMPC and DMPG liposomes were spread on the diamond crystal and dried under N_2 flow. The peptide film was incubated with arginine peptides of different length and washed with water to remove unbounded peptide. The sample was rehydrated by flushing D_2O-saturated N_2 for 2 h at room temperature. 512 Scans were averaged for each measurement. The determination of the secondary structure was based on the shape of the amide I band (1600–1700 cm^{-1}), which is sensitive to the secondary structure. The analysis was performed on the amide I region of deuterated samples in order to differentiate the α-helical secondary structure from the random secondary structure whose absorption band shifts from about 1655 cm^{-1} to about 1642 cm^{-1} (Goormaghtigh et al., 2006; Oberg et al., 2004; Vigano et al., 2000b).

3.4. Secondary structure orientation

The orientation of different secondary structures was determined as described previously (Bechinger et al., 1999; Grimard et al., 2001). Spectra were recorded with the incident light polarized parallel and perpendicular with respect to the incidence plane. Dichroic spectra were computed by subtracting the perpendicular polarized spectrum from the parallel polarized spectrum. The subtraction coefficient was chosen such that the area of the lipid ester band at 1740 cm^{-1} equaled zero on the dichroism spectrum, in order to take into account the difference in the relative power of the evanescent field for each polarization and also the differences in film thickness as described previously.

An upward deviation on the dichroism spectrum indicates a dipole oriented preferentially near the normal to the ATR plate. Conversely, a downward deviation on the dichroism spectrum indicates a dipole oriented parallel to the plane of the ATR plate.

3.5. Molecular dynamics simulations

MD simulations have been performed with the Gromacs 3.3.1 package (Van Der Spoel et al., 2005) using the Gromos96 43a2 force field (van Gunsteren, 1996) extended with lipid head group (Lensink et al., 2005) and acyl chain parameters (van Gunsteren, 1996). An equilibrated bilayer of 128 POPC molecules was taken as the starting point (Tieleman et al., 1999). From this bilayer a POPG bilayer was created using the same procedure as before (Lensink et al., 2005). An arginine oligomer (Arg7) was initially placed at a distance of 2 nm from the bilayer surface. Various initial conformations were explored (extended, α-helical, β-turn), but found to all converge to a similar unordered structure during and after association with the bilayer. Single point charge water was used (Berendsen et al., 1981). The systems were made electrostatically neutral by adding the required amount of counterions Na^+ or Cl^- and then submitted to an energy minimization and 10 ps MD with position restraints on the peptide heavy atoms and lipid tail atoms. Weak coupling to a temperature (310 K, separate coupling for peptide, lipids and solvent including ions) and pressure (anisotropic, 1.0 bar) bath was employed (Berendsen et al., 1984) using coupling constants of 0.1 and 1.0 ps, resp. Coulomb interactions were treated