Biochimica et Biophysica Acta 1859 (2017) 415–424

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

# Binding of cationic model peptides $(KX)_4K$ to anionic lipid bilayers: Lipid headgroup size influences secondary structure of bound peptides

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ARTICLE INFO

*Article history:*
Received 14 October 2016
Received in revised form 7 December 2016
Accepted 23 December 2016
Available online 26 December 2016

*Keywords:*
Cationic peptides
Anionic lipids
Hydrophobic interaction
Electrostatic interaction
Differential scanning calorimetry
FT-IR-spectroscopy

ABSTRACT

Differential Scanning Calorimetry (DSC) and Fourier transformed Infrared (FT-IR) spectroscopy were used to test the influence of acyl chain length, acyl chain saturation, and chemical structure of anionic phospholipids on the interaction with cationic model peptides $(KX)_4K$, with amino acid X = A, Abu, and L. The lipids used were phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), and cardiolipin (CL). DSC was used to monitor the phase transition of lipid vesicles before and after peptide binding. The electrostatic attraction is the main driving force for binding. The hydrophobicity of the amino acid X influences the binding strength as well as the secondary structure of the bound peptide. Binding of peptides leads to an upshift of the lipid phase transition. Lipids with smaller headgroups show a larger upshift of the main phase transition temperature.
Data from FT-IR spectroscopy show in addition that the stability of the bound β-sheets of $(KX)_4K$ depends on the hydrophobicity of the uncharged amino acid X and on the size of the lipid headgroup. For lipids with large anionic headgroups, such as PS, the antiparallel β-sheet of $(KAbu)_4K$ bound to gel phase bilayers is converted to an unordered structure upon heating through the lipid phase transition. Reducing the size of the headgroup, as in PG, increases the stability of the bound peptide β-sheets. For the smallest headgroups, present in PA and CL, stably bound β-sheets are observed even above the lipid phase transition.



## 1. Introduction

Small cationic antimicrobial peptides (AMPs) are promising drugs against the fight of increasing resistance of bacterial strains towards conventional antibiotics [1,2]. The capability of AMPs to differentiate between bacterial and mammalian cells has its origin in the different composition, hydrophobicity, and charge of the bilayers. Mammalian cells are composed mainly of zwitterionic phospholipids like phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin and contain sterols. Bacterial cells, however, have a high proportion of negatively charged phospholipids, such as phosphatidylglycerol (PG) and cardiolipin (CL) besides the zwitterionic PE, and thus are negatively charged [3,4].

Several models have been proposed for the interaction of cationic peptides with lipid membranes, for instance, the barrel-stave, the toroidal pore, or the carpet model, as well as several other mechanisms [5–8]. The mode of action of the cationic AMPs also depends on other factors like the peptide structure, the lipid to peptide ratio, and the lipid composition of the target membrane [9,10]. We have reported before on the basic features of the binding of short cationic model peptides with the structure $(KX)_4K$ to DPPG monolayers and bilayers [11,12]. After binding of these peptides to DPPG vesicles, a shift of the main phase transition to higher temperatures was found. The hydrophobicity of the uncharged spacer amino acid X in these peptides determined the extent of the upshift of the lipid phase transition. Increased hydrophobicity of X also drives the formation of stable antiparallel β-sheet structures on top of the lipid bilayer [12].

Beside of the peptide sequence, the chemical structure of the anionic lipid headgroup and the nature of the acyl chains can also have an influence on the lipid/peptide interaction. The lipid acyl chain length and saturation determines the transition temperature of the lipid, the thickness of the hydrophobic region of the membrane, and the area per lipid at the bilayer-water interface [13,14]. With a different chain length and thus different transition temperature the altered bilayer properties may lead to other peptide structures bound to the bilayer. The effect of membrane thinning or thickening after peptide binding might then be restricted, so that also a modification of the translocation of the peptide through the bilayer might be the result [15–17].

The negative headgroup charge of the lipids is essential for the binding of cationic peptides. However, the chemical structure of the negatively charged headgroup can be changed. The question then arises, whether not only electrostatic interactions but also additional more specific interactions via hydrogen bonds can play a role for the binding. In a previous study using lipid monolayers composed of anionic phospholipids with different headgroups, we found that the effects of changing the headgroup were marginal. On a reduced surface pressure scale

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http://dx.doi.org/10.1016/j.bbamem.2016.12.019
0005-2736/

all anionic phospholipids displayed similar changes in surface pressure after binding of the peptides [18]. However, in lipid bilayer systems the situation might be different, as the formation of three-dimensional structures can occur, where vesicles are aggregated by the binding of the peptide. This we had shown before using only DPPG as a phospholipid [12].

In this study we therefore focus on lipid bilayer vesicles and changes in lipid structure and its influence on the interaction with small cationic model peptides. The thermotropic behavior of lipid/peptide mixtures was examined by differential scanning calorimetry (DSC) and temperature dependent Fourier transform infrared (FT-IR) spectroscopy. Our main goals were 1) to examine the peptide interaction towards lipids with varying headgroup charges (DMPG, DMPE, DMPC), 2) to test the effect of an altered membrane thickness by changing the chain length from myristoyl up to stearoyl chains in phosphatidylglycerols (DMPG, DPPG, DSPG), 3) to study the effect of an unsaturation in one of the acyl chains (POPG), and 4) to investigate the effect of changing the chemical structure of the negatively charged headgroup using the phospholipids DMPG, DMPA, DMPS and TMCL.

## 2. Materials and methods

### 2.1. Peptides

The custom-synthesized peptides $(KA)_4K$, $(KAbu)_4K$, $(KL)_4K$ were purchased from GeneCust Europe (Dudelange, Luxemburg) with purity above 98 %. For DSC measurements they were used as received as TFA-salts without further purification. For FT-IR spectroscopic measurements, the TFA counter ion was exchanged by addition of DCl to a final concentration of 0.5 mM and by lyophilizing the solution three times [19].

### 2.2. Lipids

The lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), 1,2-stearoyl-*sn*-glycero-3-phosphoglycerol (DSPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), and 1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid (DMPA) were purchased from Genzyme Pharmaceuticals LLC (Liestal, Switzerland). 1,2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were gifts from Lipoid GmbH (Ludwigshafen, Germany). 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS) and 1,1′,2,2′-tetramyristoyl cardiolipin (TMCL) were purchased from Avanti Polar Lipids Inc. (Alabaster, USA). All phospholipids were used as received. Solvents and organic compounds were purchased from Carl Roth GmbH (Karlsruhe, Germany) and used without further purification. $D_2O$ and DCl were purchased from Sigma-Aldrich (St. Louis, USA). For all experiments ultra-pure water with a conductivity of $<0.55\ \mu S\ cm^{-1}$ was used.

### 2.3. Differential scanning calorimetry (DSC)

The pure lipid was suspended in 100 mM NaCl solution, followed by cyclic heating over the phase transition temperature and repeated vortexing. An Avanti Mini-Extruder (Avanti Polar Lipids Inc., Alabaster, USA) was used to prepare liposomes with a monodisperse size distribution by extruding the sample solution through a polycarbonate filter with a pore size of 100 nm for 15 times. Differential Scanning Calorimetry was performed with a Microcal VP-DSC (MicroCal Inc., Northhampton, USA). In all experiments the heating rate was 1 °C/min and a time resolution of 4 s per data point was used. Aqueous lipid and peptide samples were prepared separately, mixed and degassed directly before each measurement. The lipid concentration in the calorimetric cell was always 2 mM and the peptide concentration 0.4 mM. This yields a charge ratio negatively charged headgroup to lysine residue of $R_c = 1{:}1$. A 100 mM NaCl solution was used as a reference. At least three up- and down-scans were performed for each sample, to check for reproducibility of the thermograms. All presented curves originate from the second heating scan.

### 2.4. ATR-FT-IR spectroscopy

Attenuated total reflection Fourier transform infrared (ATR-FT-IR) spectra with a spectral resolution of $4\ cm^{-1}$ were recorded using a Tensor 27 spectrophotometer equipped with an $N_2$-cooled MCT detector and a BioATR II unit (Bruker Optics, Ettlingen, Germany). A total of 256 scans were averaged. As a reference, spectra of a 100 mM NaCl solution in $D_2O$ at each temperature were used. The final absorbance spectra were calculated by $-l\ g\ (I_{sample}/I_{reference})$. The desired temperature was set by a computer-controlled circulating water bath (Haake C25P Phoenix II, Karlsruhe, Germany). Lipids were dispersed in 100 mM NaCl solution in $D_2O$ and sonicated at temperatures above phase transition temperatures in a water bath. Samples were directly prepared on the crystal surface by mixing aliquots of lipid and peptide solutions to obtain a charge ratio ($R_c$) of 1:1 and a lipid concentration of 5 mM at $pD = 7.2$. Before recording spectra, one heating and cooling scan was performed to ensure equilibration. Spectra were recorded in 2 °C intervals after temperature equilibration in a temperature interval ($\Delta T$) of $\pm 0.1$ °C for 15 min. The temperature was measured inside the cover plate of the sample holder by a Pt100 resistor (Omega Newport, Deckenpfronn, Germany). All absorbance spectra were shifted to a zero baseline in a spectral region where no vibrational peak occurred. To determine the position of the vibrational bands in a certain wavenumber interval by calculating the second derivative of the spectra, we used the 'peak picking' function included in the Bruker OPUS software.

## 3. Results and discussion

### 3.1. DSC

#### 3.1.1. Influence of lipid headgroup charge

To test, whether mainly electrostatic interactions drive the binding of the cationic peptides $(KX)_4K$, we changed the chemical structure of the lipid headgroup from negatively charged phosphoglycerol to phosphocholine and to phosphoethanolamine. Thus, the headgroup becomes zwitterionic and the lipids are formally uncharged. In the absence of a net negative charge, the peptide should then bind only due to hydrophobic, dipolar forces, or some specific headgroup/peptide interactions.

Fig. 1 shows the DSC curves of DMPG, DMPC, and DMPE vesicles before and after addition of $(KA)_4K$. It is clear that only the phase transition temperature of DMPG vesicles changes after peptide binding, the transitions of DMPC and DMPE remain unchanged. Without any negative headgroup charge, the binding of the peptide is thus not detectable under our experimental conditions. Even for the more hydrophobic peptide $(KV)_4K$ added to DMPE and DMPC no change in transition temperature was observed. Therefore, a negatively charged lipid headgroup is required for effective binding, electrostatic interactions being the main driving force, as expected and observed before.

#### 3.1.2. Influence of lipid acyl chain length

The influence of different lipid acyl chain lengths on the thermotropic behavior of negatively charged lipids interacting with the cationic model peptide $(KA)_4K$, were tested with the phosphatidylglycerols DMPG, DPPG, and DSPG, respectively. Measurements with DPPG had been performed before to evaluate the influence of the peptide sequence and the influence of the hydrophobicity of the uncharged spacer in the model peptides $(KX)_4K$ on the binding properties [12].

The length of the acyl chain increases in the order DMPG, DPPG, and DSPG. As a result of increased van der Waals interaction the main phase