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The helical propensity of KLA amphipathic peptides enhances their binding to gel-state lipid membranes

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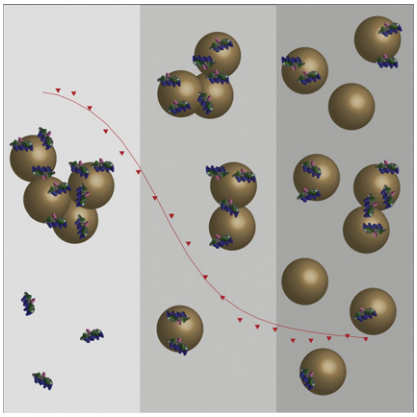
HIGHLIGHTS

- KLA peptides with low helical propensity have high tendency to form beta-structures.
- KLA helical peptides bind more efficiently than beta-structured peptides to gel-state membranes.
- The peptide helical propensity enhances binding to lipid membranes.
- Stoichiometry values from ITC experiments can be used to comment on peptide translocation across membranes.

GRAPHICAL ABSTRACT



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ABSTRACT

The role and importance of the conformation of antimicrobial peptides for their binding and incorporation into lipid membranes as well as for their bioactivity are still not well understood. In this paper, we studied the interaction between four cationic alpha-helical KLA peptides, which differ primarily in their helical propensity, and the anionic gel-state lipid DPPG (1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol). Of particular interest was the influence of the peptide conformation and membrane surface properties on the electrostatic binding process. Dynamic light scattering (DSL) showed that generally the KLA peptides possess high aggregation power but modest solubilization power. Circular dichroism spectroscopy (CD) spectra revealed that the KLA peptides with the low helical propensity tend to form beta-structures at low lipid/peptide ratios. Differential scanning calorimetry (DSC) thermograms showed that the helical KLA peptides stabilize the DPPG bilayer, whereas the beta-structured peptides induce pronounced membrane perturbations. Isothermal titration calorimetry (ITC) isotherms showed that the helical KLA peptides bind more efficiently to DPPG vesicles than

Abbreviations: FDA, Food and Drug Administration; TFE, Trifluoroethanol; ITC, Isothermal titration calorimetry; DLS, Dynamic light scattering; DSC, Differential scanning calorimetry; CD, Circular dichroism spectroscopy; LUVs, Large unilamellar vesicles; SUVs, Small unilamellar vesicles; MIC, Minimum inhibitory concentration; RBC, Red blood cells; Lys, Lysine; Leu, Leucine; Ala, Alanine; Trp, Tryptophan; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; DPPC, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine; DPPE, 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DPPG, 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoglycerol; POPC, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; L/P, Lipid peptide ratio; T_m, Main phase transition temperature; K_{app}, Apparent binding constant; ΔH^o, Standard enthalpy change; ΔS^o, Standard entropy change, ΔG^o, Standard free energy change; ΔC_p, Constant pressure heat capacity change; N, Stoichiometry.

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the beta-structured KLA peptides, and that the binding affinity of the peptides is proportional to the peptide helical propensity and membrane negative surface charge. The stoichiometry values (N) deduced from the ITC isotherms suggest that the helical KLA peptides have a higher capacity to translocate the DPPG lipid bilayer. The new data presented in this study demonstrate the flexibility of KLA peptides in adopting various conformations in response to the surrounding and also how the peptide structuring controls the mode of peptide–membrane interaction.

1. Introduction

Bacterial resistance is a growing problem that threatens our ability to treat infections, particularly as it is not adequately compensated by the development of new generations of antibiotics. For instance, 16 new antibiotics were introduced during 1983–1987 as compared with 5 during 2003–2007 [1]. Moreover, of the 13 new antibiotics approved by the Food and Drug Administration (FDA) between 1998 and 2007, only three were with novel mechanisms of action [2]. This calls for new approaches to treat infections that are less prone to induce bacterial resistance. Bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides are among the most promising candidates [3]. Antimicrobial peptides interact with the lipid components of bacterial membranes, and their non-specific mode of action hampers the development of bacterial resistance. The selective attraction of antimicrobial peptides to bacterial membranes, which contain appreciable amounts of negatively charged lipids like phosphatidylglycerol (PG) and cardiolipin, is furnished by their cationic nature. The peptide amphipathicity is also necessary for its incorporation into lipid membranes [4,5]. It is widely accepted that the final target of antimicrobial peptides is to disrupt the permeability barrier, function, and integrity of bacterial membranes. This may take place by perforating and breaking down the lipid membrane, or interfering with the organization of the lipid species in the membrane via, for instance, peptide-induced lipid demixing, domain formation, and formation of non-lamellar phases [6–15]. The scenario of action depends on the interplay between the peptide macroscopic properties and the composition of the target membrane [6].

KLA peptides are model cationic α-helical peptides that were synthesized to study the structure–activity relationship of antimicrobial peptides [16–19]. The nomenclature and sequences of the KLA peptides used in this study are shown in Table 1, and their properties and activities are shown in Table 2. Except that KLA1 peptide has a slightly lower hydrophobicity, the studied peptides differ only in their helical propensity. The 18-mer KLA peptides posses a nominal charge of +6 provided by five Lys residues and an uncapped N-terminus, whereas the C-terminus is amidated. In an α-helical KLA peptide, the charged residues cover a 90° angle of the peptide interface, whereas the non-polar amino acids (Leu, Ala, Trp) form the hydrophobic part of the amphipathic peptide. The α-helical structure of KLA1 peptide is shown in Fig. 1. Double D-substitution, where two consecutive L-amino acids are replaced with their D-analogues, was utilized to disturb, or sometimes improve, the intrinsic helicity of KLA peptides without affecting other properties. Compared with the all L-peptide KLAL, the double D-substitution in the middle of the KLAL chain pronouncedly disturbed the peptide helicity as in k_9,a_{10}-KLAL and l_{11},k_{12}-KLAL peptides, whereas the double D-substitution at the C-terminus enhanced the peptide helicity, as in k_1, l_2-KLAL peptide [16,19]. Besides the helicity (α), the peptide charge, amphipathicity, hydrophobicity (H), hydrophobic moment (μ) and the hydrophobic/hydrophilic domain ratio (Φ/Ψ) were investigated (see Table 2).

The KLA peptides can adopt different conformations depending on the surrounding and on the experimental conditions. The peptides are unstructured in buffer up to millimolar concentrations, whereas they assume an α-helical structure in trifluoroethanol (TFE) or when added to lipid vesicles. However, the peptides tend to form β-structures at higher peptide concentrations, low lipid to peptide ratios (L/P) or high temperatures [16,20–22]. When bound to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) vesicles at relatively high L/P ratios (230–500), the order of the peptides according to their helical content is k_1,l_2-KLAL (74%) > KLA1 (54%) > k_9,a_{10}-KLAL (48%) ≥ l_{11},k_{12}-KLAL (47%) (see Table 2). The structuring of the bound peptide is induced predominately by the hydrophobic interaction of the peptide residues with the membrane hydrophobic core, whereas the key role of the positive charge of the peptide is to increase its accumulation on negatively charged lipid membranes [16]. The helical structuring, which induces amphipathicity, enhances the incorporation into lipid membranes as well as the antimicrobial and hemolytic activities of the peptide. This enhancement is caused by the greater disturbance of the lipid headgroups and hydrocarbon chains of lipid membranes [4,23]. The KLA peptides interact strongly with anionic lipid membranes, whereas their affinity towards uncharged membranes of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) is modest [16,21,22,24]. The peptides also exhibit pronounced membrane perturbation and permeabilizing activities [16,24].

In this study, we characterized the electrostatic interaction between a set of KLA peptides and anionic gel-state membranes. Of particular interest was the influence of the peptide conformation and membrane surface properties on the binding process. Anionic large unilamellar vesicles (LUVs) were prepared of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG). Circular dichroism (CD) spectroscopy was utilized to determine the conformation of the peptides bound to DPPG, dynamic light scattering (DLS) was utilized to follow the aggregation of the DPPG-peptide complexes, differential scanning calorimetry (DSC) was utilized to determine the phase behavior of the peptide-bound DPPG bilayers, and isothermal titration calorimetry

Table 1
The nomenclature and sequences of KLA peptides.[a]

Peptide	Sequence
KLA1	KLAL KLAL KAW KAAL KLA-NH_2
k_1,l_2-KLAL	klAL KLAL KAL KAAL KLA-NH_2
k_9,a_{10}-KLAL	KLAL KLAL kaL KAAL KLA-NH_2
l_{11},k_{12}-KLAL	KLAL KLAL KAl kAAL KLA-NH_2

[a] The one letter code is used to give the sequence; K: lysine, L: leucine, A: alanine, W: tryptophan. The capital and small letters are used to show L-amino acids and D-amino acids, respectively.

Table 2
The properties and activities of the studied KLA peptides.[a]

Peptide	H	μ	Helicity (α%)			MIC (μM)		EC_{50} (μM)
			TFE	POPG	DPPG	*E. coli*	*S. epidermidis*	RBC
KLA1	−0.025	0.329	73	54[b]	15[d]	5.2	–	11
k_1,l_2-KLAL	−0.016	0.334	68	74[c]	68[d]	4	4	7
l_{11},k_{12}-KLAL	−0.016	0.334	34	47[c]	7[d]	32	32	540
k_9,a_{10}-KLAL	−0.016	0.334	43	48[c]	2[d]	16	16	56

[a] Adapted from Refs. [16,17,19], except the helicity (α%) values in DPPG which were determined as described in Section 2.3. The helicity values (α%) of the peptides in 50% TFE (v/v) or in POPG were determined in Tris buffer using 10 μM peptide concentration.
[b] L/P = 500.
[c] L/P = 230.
[d] L/P = 10–40.