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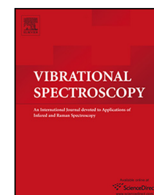

Dynamics of lipid layers with/without bounded antimicrobial peptide halictine-1

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ABSTRACT

Dynamic behavior during the drying process of phospholipid multilayers composed of 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine and 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) in mixtures of 1:4, was investigated by means of attenuated total reflection (ATR) Fourier transform infrared spectroscopy (FTIR). The interaction of the antimicrobial peptide halictine-1 (HAL-1), a linear dodecapeptide isolated from the venom of eusocial bee *Halictus sexcinctus*, with the lipid layer and its influence on the dynamics of the lipid layer was also studied. Analysis of ATR-FTIR spectra of the drying process by principal component analysis (PCA) clearly showed the sensitivity of C=O vibrations at 1737 cm^{-1}, PO_2 vibrations in the region 1000–1250 cm^{-1} and CH stretching vibrations at 2850 and 2950 cm^{-1} to the hydration of the lipid layer. Nevertheless, PCA revealed that the lipid layers periodically oscillate between dehydration/hydration states. The protective influence of HAL-1 on the lipid layer, including the disappearance of dehydration/hydration oscillations, and slowing down of the drying of the system in the presence of the peptide was observed. PCA indicates a two-stage process of the interaction of HAL-1 with lipid layers and as well as the influence of HAL-1 on vibrations of the C=O and PO_2 of lipid groups, whereas CH_2 and CH_3 vibrations remain intact during the binding of the peptide. The peptide binds to phospholipid head groups, changes its structure from a random coil to an α-helix structure, and interacts with the C=O groups of the lipids, staying on the surface of the membrane.

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1. Introduction

Many known antimicrobial peptides (AMPs) from animals and plants possess a considerable therapeutic potential [1–4]. However, their precise mechanism of action remains unclear, and is in fact a matter of controversy [5,6]. The mechanism of antibacterial activity probably includes recognition and specific interaction with bacterial cell membranes, inducing lipid clustering or lipid phase separation, which leads to a breakdown of the membrane and subsequent cell death [7,8]. Several basic models for membrane permeabilization have been suggested: i) the carpet model – peptides bind to phospholipid head groups and align themselves parallel to the membrane surface until a critical threshold concentration for breakdown of the membrane is reached [9]; ii) the barrel-stave model – peptides aggregate in the membrane and finally form transmembrane pores [10]; iii) the toroidal pore model – this postulates a critical concentration of partially buried peptides causing strain and inducing the membrane to curve inward by forming a pore lined with both peptides and lipid headgroups [11]; and iv) the detergent-like model – proposes that peptides intercalate between phospholipid head groups causing curvature strain and micellization [12]. It has been shown that different types of AMPs have different modes of action [2,13].

Recently a number of new AMPs have been identified and isolated from bees by Čeřovský et al. (e.g., [14–20]). Although their biological activities have been thoroughly investigated, the exact mechanism of their antibacterial action remains unclear. For this purpose, it is necessary to conduct studies devoted to mimicking this process in the membrane environment, where cell membranes are formed by phospholipids of various chemical compositions within various spatial structures such as micelles, liposomes, and bilayers [21,22]. For such studies, techniques like differential

Abbreviations: AMP, antimicrobial peptide; ATR, attenuated total reflectance; CD, electronic circular dichroism; FTIR, Fourier-transform infrared; HAL-1, halictine-1; PC, 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine; PCA, principal component analysis; PG, 1,2-dihexadecanoyl-*sn-glycero*-3-phospho-(1′-rac-glycerol).

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scanning calorimetry [23], fluorescence [24], electronic circular dichroism (CD) [25], nuclear magnetic resonance [26] and infrared (FTIR) spectroscopy [27,28] are typically used.

FTIR spectroscopy can be applied as a complementary technique to CD spectroscopy in studies of secondary structure changes to peptides/proteins when interacting with model membranes [27,28]. In addition, the frequencies of methylene vibrations of acyl lipid chains are very sensitive to their conformational state and to lipid phase transitions. Carbonyl stretching vibrations of O-acylated lipids together with vibrations of phosphate PO_2 stretching modes reflect hydrogen bonding, so a hydration of the lipid bilayer can be studied [29]. Thus FTIR spectroscopy can separately provide information about the structural properties of lipids and peptides/proteins.

In our study, we applied FTIR spectroscopy (particularly in ATR mode) to study a phospholipid film to detect the difference between the hydration/dehydration states of the lipid multilayer in the presence or absence of the antibacterial linear dodecapeptide halictine-1 (HAL-1) isolated from the venom of the eusocial bee *Halictus sexcinctus* [15]. These detailed comparisons enable us to observe differences in the dynamics of this process with the aim of shedding light on the mechanism of HAL-1 interaction with membranes and on its biological action. The subtle difference between the hydration/dehydration states of the lipid multilayer in the presence or absence of the HAL-1 peptide were resolved using principal component analysis (PCA).

2. Materials and methods

2.1. Sample preparation

The linear dodecapeptide HAL-1 (Gly-Met-Trp-Ser-Lys-Ile-Leu-Gly-His-Leu-Ile-Arg-NH_2; m.w. 1408 Da) was prepared by standard procedures of solid-phase peptide synthesis using the Nα-Fmoc protocol according to [15]. Trifluoroacetate, which was present in the synthesized peptide, was removed from the sample by peptide lyophilization from a 0.1 N HCl solution (the procedure was repeated several times) [30]. The acquired peptide was dissolved in distilled water at a concentration of 0.125 mg/mL (88.6 μM) for CD measurements and 10 mg/mL (7.09 mM) for FTIR spectroscopy.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (PC) and 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (PG) were purchased from Avanti Polar Lipids, USA. The phospholipid mixtures of PC/PG (1:4) were dissolved in a chloroform/methanol mixture (3:1) and dried in vacuum. The dried lipid layer was hydrated with distilled water and gently stirred. Liposomes, in the form of large unilamellar vesicles, were formed by 31 extrusion passages through polycarbonate membranes with 100 nm pores using a Mini-Extruder (Avanti Polar Lipids, USA). The temperature of the lipid suspension was kept at ca. 60 °C, i.e., above the phase transition temperature (41 °C) of the lipid mixture, throughout the whole hydration and extrusion process. The 100 nm size of the prepared liposomes was verified by light scattering using a Zetasizer Nano ZS (Malvern, UK).

The peptide/lipid concentration ratios (1:8 and 1:15) were chosen with respect to the employed spectroscopic techniques (CD and FTIR spectroscopy), enabling us to study the samples simultaneously under very similar conditions.

2.2. Circular dichroism

CD experiments were performed using a J-815 spectrometer (Jasco Analytical Instruments, Japan). The spectra were collected from 180 to 300 nm at room temperature (20 °C) in 0.1-cm quartz cells (2 scans, 0.5 nm steps, 20 nm/min scanning speed, 8 s response time constant, 1 nm spectral bandwidth). After baseline subtraction, the final spectra were expressed as molar ellipticities θ ($deg \cdot cm^2 \cdot dmol^{-1}$) per residue.

2.3. FTIR spectroscopy

FTIR spectra in the transmission mode were recorded in a Vector 33 spectrometer (Bruker, Germany) using a standard MIR source, KBr beamsplitter, and MCT detector (5000 scans, 2 cm^{-1} spectral resolution, Blackman-Harris 3-term apodization function). The samples were measured at room temperature (20 °C) in a CaF_2-cell with an 8-μm path length and the spectrometer was purged with dry air.

ATR-FTIR measurements were done with the same setup using an ATR-MIRacl™ – a single-reflection diamond horizontal ATR prism (Pike Technologies, USA). For each measurement, the volume of 25 μL of liposomes was deposited on the prism. According to the literature [31–33], the liposomes formed an oriented multilayer structure on the hydrophobic surface of the ATR prism. The ATR-FTIR spectra were collected in continuous mode (120 scans taking 75 s) during the free desiccation of the sample in dried air (75 min). The sample was covered with the ATR-lid (providing a total internal volume of the chamber ~400 μL) at ~25th minute after sample deposition to study the dynamics of the stopped drying process. When investigating the peptide–lipid interactions, 2 μL of the HAL-1 peptide was added ~20 min after the deposition of the liposomes on the prism. The spectra were collected after a brief stabilization of the sample.

The interfering water signal was subtracted using the standard algorithm [34]. Subsequently, the FTIR spectrum of water vapor was subtracted and a baseline was corrected. The data processing was carried out using the software GRAMS/AI (Thermo Electron, USA).

2.4. Principal component analysis

To analyze FTIR data sets PCA, using a singular value decomposition algorithm [35], was applied. Each spectrum of the matrix $Y_i(\nu)$ can be unambiguously expressed as:

$$Y_i(\nu) = \sum_{j=1}^{M} V_{ij} W_j S_j(\nu), \quad (2.1)$$

where W_j is the diagonal matrix of singular values, $S_j(\nu)$ corresponds to the matrix of the orthonormal subspectra (eigenvectors) and V_{ij} is the unitary square matrix of coefficients (representing the strength of the influence of the subspectrum S_j). M represents the number of independent "spectral species", distinct from the spectral noise, found in the analyzed data set. The number of independent subspectra can be estimated from residual errors or from singular values [35]. The calculation of PCA was done using our own software programmed in Matlab™ (MathWorks®, USA).

3. Results and discussion

3.1. HAL-1 in interaction with membranes

The ability of HAL-1 and its biologically active analogs to form the α-helical structure has been demonstrated earlier [15,36]. Here we applied CD spectroscopy only to verify this finding for our membrane-mimicking model, i.e., the liposomes prepared from the PC/PG mixture in a 1:4 ratio (with longer acyl chains). As shown in Fig. 1, HAL-1 in aqueous solution contains mostly an unordered structure characterized by a broad negative band at 198 nm [37,38]. The addition of liposomes (peptide/lipid ratio 1:8 and 1:15) causes an increase in the α-helical content characterized by a strong

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