Colloids and Surfaces A: Physicochem. Eng. Aspects 343 (2009) 104–110

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

The affinity of two antimicrobial peptides derived from bovine milk proteins for model lipid membranes

Wanda Barzyk[a], Sylvie Campagna[b,1], Katarzyna Więcław[c,d], Beata Korchowiec[c,d], Ewa Rogalska[d,*]

[a] Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239 Krakow, Poland
[b] URAFPA, Nancy-Université, INRA, Equipe Protéolyse-Biofonctionnalité des Protéines et des Peptides, Boulevard des Aiguillettes, BP239, 54506 Vandoeuvre-lès-Nancy, France
[c] Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Jagiellonian University, ul. Romana Ingardena 3, 30-060 Krakow, Poland
[d] Structure et Réactivité des Systèmes Moléculaires Complexes, BP 239, CNRS/Nancy Université, 54506 Vandoeuvre-lès-Nancy cedex, France

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 5 January 2009
Accepted 30 January 2009
Available online 5 April 2009

Keywords:
1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine
1,2-Dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)
Peptide–membrane interactions
Langmuir films
Phospholipid monolayers

ABSTRACT

In this work, two antimicrobial peptides were studied regarding their capacity to interact with lipid membranes. The peptides subsequently named L-16-Y and N-23-T were derived from the bovine milk α-s2 casein and from the component-3 of proteose peptone (PP3), respectively. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPG) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DPPC) monomolecular films spread at the air–water interface were used, respectively, as model host and bacterial membranes. The surface pressure and surface potential measurements, as well as Brewster angle microscopy (BAM) showed that both peptides interact with the model membranes. However, the higher affinity for DPPG compared to DPPC indicates that these peptides are innocuous for the host membranes.

1. Introduction

Bacteria evolve quickly to counter new antibiotics and treatments for infections [1,2]. Therefore, there is an urgent need for new antibiotics and new strategies to combat bacteria, particularly those showing multidrug resistance. In contrast to conventional antibiotics, the antimicrobial peptides (AMPs) [3] do not appear to induce antibiotic resistance. Therefore, these peptides raise much interest as potential candidates to develop alternative/adjunct therapeutic strategies. AMPs are generated either by *de novo* synthesis or by proteolytic cleavage from antimicrobially inactive proproteins. Most AMPs contain between 12 and 50 residues, including two or more positively charged residues and a large proportion (generally > 50%) of hydrophobic residues [4–6]. The cationic side chains of arginine, lysine and histidine are thought to mediate peptide interactions with negatively charged membranes and/or cell walls of bacteria, including lipopolysaccharide [7]. The ability to associate with membranes is a characteristic feature of AMPs [8]. Many of these peptides fold into their final conformation [9] upon interaction with cell membranes. It was shown recently that some AMPs with longer chains are more effective in killing both gram-negative and gram-positive bacteria compared to smaller peptides [3].

In the present work, lactophoricin, a peptide corresponding to the f(113–135) C-terminal domain of bovine component PP3 (sequence NH_2-NTVKETIKYLKSLFSHAFEVVKT-COOH) called here N-23-T, was studied upon penetration to lipid membranes. In some experiments a shorter chain peptide, subsequently named L-16-Y, corresponding to the residues 164–179 of the bovine milk αs_2-casein (sequence NH_2-LKKISQRYQKFALPQY-COOH) was used and compared with N-23-T. It was shown before that both peptides had antimicrobial activity [10–12]. The structure of L-16-Y was investigated by secondary structure prediction [12], circular dichroism and Fourier transform infrared spectroscopy [13], while the secondary structure of N-23-T was determined by circular dichroism in various conditions [11].

Biological membranes are complex bilayer structures composed mostly of a mixture of phosphoglycerides [14]. A phospholipid monolayer spread at the air–water interface can be considered as a half-membrane and used as a model of biological membranes [15–17]. Lipid monolayers provide a convenient model system to study interactions of different biomolecules with lipid membranes. The density range considered as representative for biological membranes corresponds to the phospholipid monolayer

* Corresponding author. Tel.: +33 03 83 68 43 45; fax: +33 03 83 68 43 22.
E-mail address: rogalska@lesoc.uhp-nancy.fr (E. Rogalska).
[1] Present address: Ifremer, CNRS, Université Montpellier 2, UMR 5119, Ecosystèmes Lagunaires:UMR 5119, Place E. Bataillon, CC80, 34095 Montpellier cedex 05, France.

0927-7757/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2009.01.036

liquid-expanded (LE) or liquid-condensed (LC) phases, or their mixture, LE–LC, in the range $30 \pm 5\,mN\,m^{-1}$ [18]. The packing and charge density of phospholipid monolayers can be easily adjusted to study the effect of surface properties on peptide behaviour. In this study, the insoluble monolayers formed with a zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and a negatively charged 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DPPG) were used as models of host and of bacterial cell membranes, respectively. The surface pressure–area (Π–A) and surface potential–area (ΔV–A) isotherms, as well as Brewster angle microscopy (BAM) were used to study the monolayers. The effects of the stationary penetration, $\Delta\Pi$ and $\Delta\Delta V$, were examined in relation to the initial density of the phospholipid film characterized by the initial surface pressure, Π_{init}. The results obtained show that N-23-T has a higher affinity for the negatively charged DPPG than for the zwitterionic DPPC. These observations help understanding the antibacterial activity of N-23-T as related to the electrostatic interactions with the negatively charged membranes or cell walls of bacteria; by the same token, N-23-T would be innocuous for the host cell membranes. It can be observed that the affinity of L-16-Y for the monolayers is lower compared to N-23-T.

2. Experimental

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; ≥99%; P4329) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) sodium salt (DPPG; ~99%; P9789) were from Sigma. Originally, the peptides were isolated from the sequence of the bovine milk α-s2 casein (L-16-Y; 16 residues peptide; molar weight 2011 g mol^{-1}; p*I* 10.17) and from the bovine component PP3 (N-23-T; 23 residues peptide; molar weight 2683 g mol^{-1}; p*I* 9.40). The peptides used in this study were prepared on a solid-phase peptide synthesizer (model Synergy 432, PerkinElmer) using the standard cycle for 9-fluorenylmethylcarbonyl strategy. Cleavage and side chain deprotections were carried out with trifluoroacetic acid (TFA). The peptides were purified by C18 reversed-phase high performance liquid chromatography using linear gradients of water and acetonitrile (0.1% TFA) and rechromatography with gradients of water and acetic acid was performed to remove bound TFA. The correct synthesis was confirmed by analysis of amino acid composition and mass spectrometry.The aqueous peptide solutions were prepared with MilliQ water, which had a surface tension of 72.8 mN m^{-1} at 20 °C, pH 5.6; at this pH both peptides are positively charged. Spectrophotometric grade chloroform (Aldrich, A.C.S.) was used for preparing phospholipid solutions.

2.2. Compression isotherms and Brewster angle microscopy

The surface pressure (Π) and electric surface potential ((V) measurements were carried out with a KSV 5000 Langmuir balance (KSV Instruments Ltd., Helsinki, Finland). A Teflon® trough (15 cm × 58 cm × 1 cm) with two hydrophilic Delrin® barriers (symmetric compression) was used in compression isotherm experiments. The system was equipped with an electrobalance and a platinum Wilhelmy plate (perimeter 3.94 cm) as a surface pressure sensor. Surface potential was measured using a KSV Spot 1 with a vibrating plate electrode and a stainless steel counter electrode immersed in the subphase. The apparatus was closed in a Plexiglas box, and temperature was kept constant at 20 °C. All solvents used for cleaning the trough and the barriers were of analytical grade. The monolayers were spread from calibrated solutions (concentration around 0.5 mg mL^{-1}) of DPPC and DPPG in chloroform by the use of a microsyringe (Hamilton Co., USA). After the equilibration time of 20 min, the films were compressed at the rate of 2.5 mm min^{-1} barrier^{-1} by two symmetrically moving barriers. A PC computer and KSV software were used to control the experiments. Each compression isotherm was performed at least three times. The standard error was ±0.5 Å^2 with mean molecular area, ±0.2 mN m^{-1} with surface pressure and ±5 mV with surface potential measurements.The morphology of the studied films was visualized using a computer-interfaced KSV 2000 Langmuir balance combined with a Brewster angle microscope (KSV Optrel BAM 300, Helsinki, Finland). The Teflon® trough dimensions were 6.5 cm × 58 cm × 1 cm; other experimental conditions were as described above.

Table 1
Parameters of the phospholipid monolayers at Π = 10.0 mN m^{-1} and at the collapse point.

	Π (mN m^{-1})	A (Å^2)	C_s^{-1} (mN m^{-1})	ΔV (mV)
10.0 mN m^{-1}				
DPPC on water	10.0	55	73	454
DPPC on 1.0 μM N-23-T subphase	10.0	298	122	340
DPPG on water	10.0	49	138	217
DPPG on 1.0 μM N-23-T subphase	10.0	420	208	169
Collapse point				
DPPC on water	55.2	40	230	580
DPPC on 1.0 μM N-23-T subphase	55.1	42	251	430
DPPG on water	53.1	40	513	240
DPPG on 1.0 μM N-23-T subphase	58.1	41	498	220

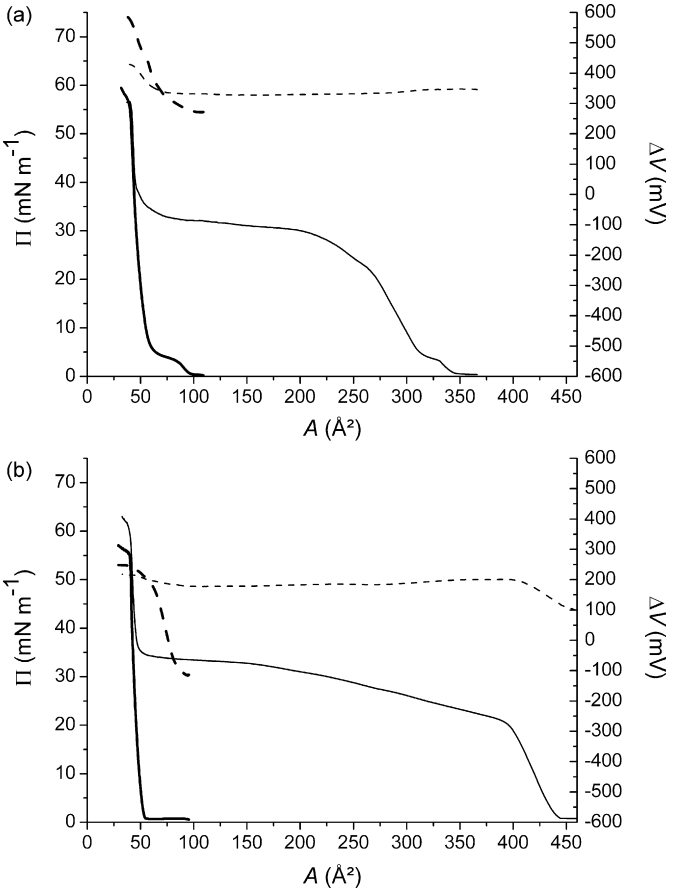

Fig. 1. Compression isotherms of phospholipid monolayers spread in the absence and in the presence of N-23-T. Results obtained with (A) DPPC and (B) DPPG; solid lines: Π–A isotherms; dashed lines: ΔV–A isotherms. Bold curves: pure lipid film spread on pure water subphase; thin curves: pure lipid film spread on 1.0 μM N-23-T aqueous solution. Temperature 20 °C, pH 5.6.