Journal of Electroanalytical Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Channel-forming activity of lactophoricins I and II in mercury-supported tethered bilayer lipid membranes

Lucia Becucci[a,b], Giovanni Aloisi[a], Andrea Scaloni[c], Rolando Guidelli[a,*]

[a] Department of Chemistry, Florence University, via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy
[b] 1st Grade Secondary School "Giuseppe Pescetti", Via Gramsci 390, 50019 Sesto Fiorentino, Firenze, Italy
[c] Proteomics & Mass Spectrometry Laboratory, ISPAAM-National Research Council, 80147 Naples, Italy

ARTICLE INFO

Keywords:
Antimicrobial peptide
Tethered bilayer lipid membrane
Voltage-gated channel
Electrochemical impedance spectroscopy
Carpet mechanism

ABSTRACT

Lactophoricin-I (LPcin-I), an antimicrobial cationic peptide with 23 amino acid residues isolated from bovine milk, and LPcin-II, which lacks six N-terminal amino acids with respect to LPcin-I, were investigated at a mercury-supported tethered bilayer lipid membrane (tBLM) consisting of a 2,3,di-*O*-phytanyl-*sn*-glycerol-1-tetraethylene-glycol-D,L-α lipoic acid ester thiolipid (DPTL) with a dioleoylphosphatidylcholine (DOPC) or dioleoylphosphatidylserine (DOPS) monolayer on top. The two LPcins were incorporated via scans of electrochemical impedance spectra (EISs) regularly distributed from − 0.30 to − 1.00 V and vice versa. During the pristine negative-going EIS scan the distal lipid monolayer is disrupted via a carpet mechanism, as revealed by an EIS exhibiting inductive behavior, whereas it heals during the positive-going scan. LPcin-I forms ion channels yielding voltage-gated cyclic voltammograms (CVs) at pH 3 and 5.4 in DPTL/DOPC tBLMs, but only at pH 3 in DPTL/DOPS tBLMs; no ion channels are formed at pH 6.8. LPcin-II does not form ion channels in DPTL/DOPC tBLMs at the three pH values investigated; however, in DPTL/DOPS tBLMs it forms ion channels at pH 5.4 and 6.8. This overall behavior is explained by dual electrostatic interactions between the zwitterionic polar heads of DOPC or DOPS and neighboring pairs of oppositely charged residues in the peptide chain.

1. Introduction

Antimicrobial peptides are essential for the innate immune system of all living species [1]. Differently from non-peptide molecules, they generally do not seem to induce antibiotic resistance. Lactophorin, also known as component PP3 or glycosylation-dependent cell adhesion molecule 1, is a phosphoglycoprotein made of 135 amino acids [2,3], which was originally isolated from bovine milk and whose exact function in vivo is still uncertain. This protein contains two distinct domains: a negatively charged N-terminal region and a C-terminal part displaying an amphipatic character. A peptide spanning protein consisting of residues 98–135 was shown to form an amphipathic membrane-binding α-helix, which is oriented in plane with the membrane surface when incorporated in planar phospholipid bilayers [4]. On the other hand, two cationic peptides corresponding to portions 113–135 and 119–135, named lactophoricin I (LPcin-I) and lactophoricin II (LPcin-II) respectively, have been demonstrated to penetrate into phospholipid monolayers spread at the air/water interface, up to the equilibrium spreading pressure [5] (Scheme 1). They exhibit similar charge ratios and the same hydrophilic/hydrophobic sectors. Their affinity for the negatively charged palmitoyloleoylphosphatidylglycerol (POPG) seems higher than that for the neutral palmitoyloleoylphosphatidylcholine (POPC), with the longer LPcin-I showing a higher interaction than LPcin-II. Furthermore, it was demonstrated that LPcin-I inhibits the growth of both Gram-negative and Gram-positive bacteria, but has no hemolytic activity at concentrations lower than 200 μM [6]. Conversely, LPcin-II was reported not having antibacterial activity [7,8]. Circular dichroism studies highlighted an α-helical conformation for both LPcin-I [9] and LPcin-II [7] in the presence of neutral and/or negatively charged micelles. The 3D structure of LPcin-I and LPcin-II was determined in phosphatidylcholine bicelles by solid-state NMR; the former is characterized by a slightly curved α-helix, whereas the latter exhibits a straight helical conformation [8]. This structural difference was tentatively associated with the different antimicrobial properties of the two peptides. On the other hand, discrete single-current fluctuations induced by LPcin-I were recorded on planar bilayer lipid membranes (BLMs) made of POPC/dioleoylphosphatidylethanolamine (DOPE) 2:1 and of POPC/DOPE/dioleoylphosphatidylglycerol (DOPG) 2:1:1, whereas no counterparts were measured with LPcin-II [7].

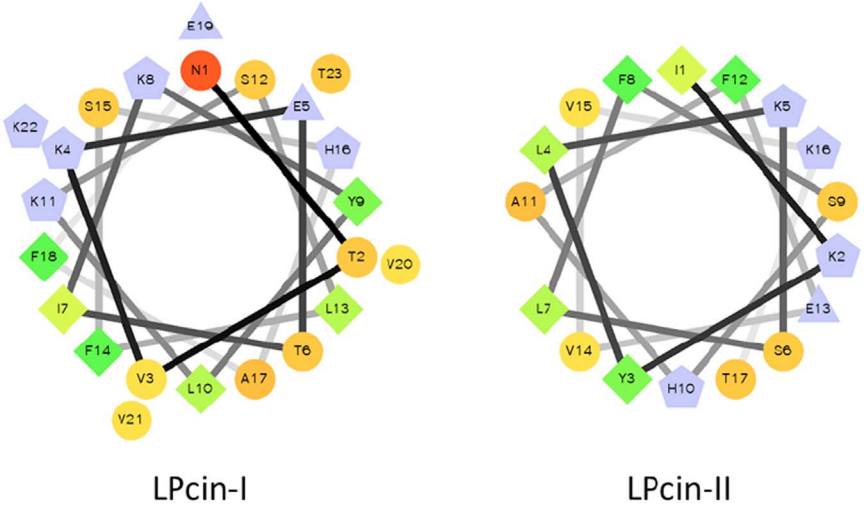
The present work aims at investigating the behavior of LPcin-I and

* Corresponding author.
E-mail address: rolando.guidelli@libero.it (R. Guidelli).

http://dx.doi.org/10.1016/j.jelechem.2017.08.030
Received 9 June 2017; Received in revised form 4 August 2017; Accepted 14 August 2017

LPcin-I ^{113}NTVKETIKYLKSLFSHASEVVKT^{135}

LPcin-II ^{119}IKYLKSLFSHASEVVKT^{135}



Scheme 1. Primary structure of LPcin-I and of LPcin-II. Positively and negatively charged residues are colored in blue and red, respectively. The figure also displays the corresponding helical wheel diagrams.

LPcin-II in Hg-supported lipid bilayers made of dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylserine (DOPS). The variation of the lipid composition intends to simulate the different lipid films that LPcin-I and LPcin-II may encounter in vivo, with the aim to verify the possible effect of the lipid nature on the activity of these two peptides. Use of phosphatidylserine (PS) as an anionic lipid was preferred to that of phosphatidylglycerol (PG), since the former is the most abundant negatively charged phospholipid in eukaryotic membranes, including those of yeasts and fungi. The presence of three ionizable groups in its polar head imparts unique properties to PS self-assembled monolayers (SAMs) and is responsible for its positive charge at pH 3, neutral character at pH 5 and negative charge at pH > 6 [10]. The effect of pH changes in aqueous 0.1 M KCl was also evaluated.

Hg-supported tethered bilayer lipid membranes (tBLMs) have been extensively used in our laboratory for the investigation of ion channels generated by antimicrobial peptides [11–13]. The Hg-supported lipid bilayer consists of a monolayer of a thiolipid, namely 2,3,di-*O*-phytanyl-*sn*-glycerol-1-tetraethylene-glycol-D,L-α lipoic acid ester thiolipid (DPTL), with a DOPC or DOPS monolayer on top of it. DPTL contains a tetraethyleneoxy hydrophilic chain, called spacer, ending at one side with a lipoic acid residue for anchoring to the mercury surface [14,15]. At the other side, it is covalently linked to two phytanyl chains that mimic the hydrocarbon tails of a lipid. Hydrophobic interactions between the phytanyl chains and the overlying phospholipid monolayer give rise to a lipid bilayer interposed between the tetraethyleneoxy chain and the bulk aqueous solution, commonly referred to as tBLM. Hg provides a defect free, fluid and readily renewable surface to tBLMs. Moreover, it imparts to the lipid molecules of the whole film a lateral mobility comparable with that of biomembranes.

2. Material and methods

Water was obtained by an inverted osmosis unit; it was then distilled once and redistilled from alkaline permanganate. Suprapur® KCl (Merck, Darmstadt, Germany) was baked at 500 °C before use in order to remove any organic impurities. HCl from Sigma-Aldrich (St. Louis, MO, USA) and K_2HPO_4 and KH_2PO_4 from Merck were used without further purification. DOPC and DOPS in chloroform solution were purchased from Avanti Polar Lipids (Birmingham, AL, USA). DPTL was provided by Prof. Adrian Schwan (Department of Chemistry, University of Guelph, Canada). Solutions of 0.2 mg/mL DPTL in ethanol were prepared from a 2 mg/mL solution of DPTL in ethanol. Stock solutions of this thiolipid were stored at − 18 °C. LPcin-I and LPcin-II were purchased from NovoPro Bioscience Inc. (Shanghai, China). Peptide purity was monitored by HPLC analysis and corresponded to 97.2% (LPcin-I) and 98.3% (LPcin-II), respectively. Peptide nature was evaluated with a LTQ-XL mass spectrometer (Thermo, USA), performing in both cases MS and tandem MS measurements.

All measurements were carried out with a homemade hanging mercury drop electrode (HMDE) described elsewhere [16]. A homemade glass capillary with a finely tapered tip (about 1 mm in outer diameter) was used. Capillary and mercury reservoir were thermostated at 25 ± 0.1 °C in a water-jacketed box to avoid any changes in drop area due to temperature changes. The HMDE acted as the working electrode in a three-electrode system, with an Ag/AgCl (0.1 M KCl) reference electrode and a platinum coil counter electrode.

Hg-supported tBLMs were obtained by tethering a DPTL monolayer to a HMDE upon keeping the mercury drop immersed in a 0.2 mg/mL DPTL solution in ethanol for about 20 min. The Hg drop was then extracted from the solution, and ethanol was allowed to evaporate under N_2 atmosphere. A lipid monolayer was subsequently self-assembled on top of the DPTL monolayer by first spreading a DOPC or DOPS solution in pentane on the surface of a buffered or unbuffered 0.1 M KCl aqueous solution, in an amount corresponding to about five phospholipid monolayers. The lipid film so formed was a monolayer at its equilibrium spreading pressure (about 45 mN m^{-1}), in equilibrium with the bulk phase of the surfactant. After allowing the pentane to evaporate, the DPTL-coated HMDE was immersed into the aqueous solution across the lipid film, causing a lipid monolayer to self-assemble on top of the DPTL monolayer. The tBLM so obtained was subjected to repeated potential scans over a potential range from − 0.20 to − 1.20 V, while continuously monitoring the curve of the quadrature component of the current at 75 Hz against the applied potential (*E*) using AC voltammetry, until a stable curve was attained.

Incorporation of peptides into mercury-supported tBLMs takes place with a greater difficulty than into conventional BLMs, at least over the range of physiological values of the transmembrane potential, ϕ_m, which is defined as the potential difference across the lipid bilayer moiety of the tBLM. This is due to the fact that tBLMs replace one of the two semi-infinite aqueous phases that bath BLMs with a monomolecular hydrophilic spacer. On the other hand, the resistance of tBLMs to electric fields is much higher than that of BLMs, thus permitting peptide