Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 1230–1237
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbamem

Sensitized photoinactivation of minigramicidin channels in bilayer lipid membranes

Elena A. Dutseva [a], Yuri N. Antonenko [a,*], Elena A. Kotova [a], Jochen R. Pfeifer [b], Ulrich Koert [b]

[a] *Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119992, Russia*
[b] *Fachbereich Chemie, Philipps-Universitat, Marburg, Germany*

Received 5 October 2006; received in revised form 26 December 2006; accepted 10 January 2007
Available online 19 January 2007

Abstract

The method of sensitized photoinactivation based on the photosensitized damage of gramicidin A (gA) molecules was applied here to study ionic channels formed by minigramicidin (the 11-residue analogue of gramicidin A) in a planar bilayer lipid membrane (BLM) of different thickness. Irradiation of BLM with a single flash of visible light in the presence of a photosensitizer (aluminum phthalocyanine or Rose Bengal) generating singlet oxygen provoked a decrease in the minigramicidin-induced electric current across BLM, the kinetics of which had the characteristic time of several seconds, as observed with gA. For gA, there is good correlation between the characteristic time of photoinactivation and the single-channel lifetime. In contrast to the covalent dimer of gA characterized by extremely long single-channel lifetime and the absence of current relaxation upon flash excitation, the covalent head-to-head dimer of minigramicidin displayed the flash-induced current decrease with the kinetics being strongly dependent on the membrane thickness. The current decrease became slower both upon increasing the concentration of the minigramicidin covalent dimer and upon including cholesterol in the membrane composition. These data in combination with the quadratic dependence of the current on the peptide concentration can be rationalized by hypothesizing that the macroscopic current across BLM measured at high concentrations of the peptide is provided by dimers of minigramicidin covalent dimers in the double $\beta^{5.7}$-helical conformation having the lifetime of about 0.4 s, while single channels with the lifetime of 0.01 s, observed at a very low peptide concentration, correspond to the single-stranded $\beta^{6.3}$-helical conformation. Alternatively the results can be explained by clustering of channels at high concentrations of the minigramicidin covalent dimer.

Keywords: Channel-forming peptide; Planar lipid bilayer; Ionic current; Sensitized photoinactivation; Membrane thickness; Hydrophobic mismatch

1. Introduction

Functioning of biological ion channels is determined by properties of both channel-forming proteins and membrane lipids, in particular, by hydrophobic matching of the proteins and the lipid bilayer [1–3]. With the bacterial porin OmpA, it has been shown that the kinetics of channel folding depends on membrane thickness [4,5]. According to theoretical studies [6], the transition between open and closed conformations of a potassium channel is affected by protein–lipid hydrophobic matching. A series of research works on ion channels formed by pentadecapeptide gramicidin A (gA, see the sequence in Table 1) in planar bilayer lipid membranes have revealed the dependence of open channel lifetime and the conformational preference on membrane thickness [7–13]. The consequences of hydrophobic mismatch are expected to be more pronounced for derivatives of gA with a shortened amino acid sequence [14,15]. Actually channels formed by a truncated gA analogue called minigramicidin (with four amino acids omitted from the N terminus as compared to gA) are substantially stabilized in thinner membranes [14].

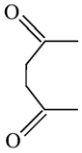
It is generally accepted that the open state of the gA channel represents a hydrogen-bridged, head-to-head transmembrane dimer of gA molecules having the conformation of a right-handed single-stranded β-helix with 6.3 residues per turn [16–

Abbreviations: BLM, bilayer lipid membrane; gA, gramicidin A; $AlPcS_3$, aluminum trisulfophthalocyanine; DPhPC, diphytanoylphosphatidylcholine

* Corresponding author. Fax: +74 95 939 31 81.
E-mail address: antonen@genebee.msu.ru (Y.N. Antonenko).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.01.006

Table 1
Peptides used in the present work

Sequences of peptides	Titles
HCO-L-Val-G-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH2CH2OH	Gramicidin A (gA)
L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OSi$ (tBu) Ph_2	Minigramicidin
 L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OSi$ (tBu) Ph_2 L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OSi$ (tBu) Ph_2	Minigramicidin covalent dimer

23]. In line with this, channels formed by covalent dimers of gA are characterized by very long duration [24,25]. Surprisingly single-channel measurements have revealed modest difference in the mean dwell time of channels formed by minigramicidin and its covalently linked derivative [14]. Rather low stability of single channels formed by the minigramicidin covalent dimer may imply that another channel structure contributes to the conductance induced by this compound. Noteworthy, according to the observations of Stark et al. [26] made under multi-channel conditions, covalent dimers of gA apparently form aggregates being in equilibrium with the conducting state. As shown by Arndt et al. [27], in organic solvents, the minigramicidin covalent dimer can adopt two conformations: the right-handed single-stranded $\beta^{6.3}$-helix and a left-handed double-β-helix with 5.7 residues per turn, the latter structure representing the dimer of covalent dimers. According to the data of [11] and [28], the double-stranded helical dimer of gA can form ion channels only in thick and unsaturated lipid bilayers where this structure is energetically more beneficial. The important role of the double $\beta^{5.7}$-helix in the channel-forming activity can be suggested for minigramicidin, as the problem of the hydrophobic mismatch becomes more acute in the case of this shortened gA analogue [14]. To further elucidate the mechanism of minigramicidin channel operation, we applied here the sensitized photoinactivation method [29]. According to a series of studies, this approach allows to examine the kinetics of gA channels [30–39], in particular, to determine rate constants of gA channel formation and dissociation [29]. The results obtained in the present work show that the properties of the conducting state of the minigramicidin covalent dimer observed under single-channel conditions differ substantially from those examined at high peptide concentrations. The latter conditions obviously promote minigramicidin clustering that may result in the formation of the double $\beta^{5.7}$-helix or other aggregated forms.

2. Materials and methods

Minigramicidin and minigramicidin covalent dimer were synthesized as described in [14]. Planar bilayer lipid membranes (BLMs) were formed from a 2% solution of diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids, Alabaster, AL) in *n*-decane or other solvents (squalene, hexadecane) (Merck, Darmstadt, Germany) by the brush technique on a hole in a Teflon partition separating two compartments of a cell containing aqueous buffer solutions. A cell with a 0.15-mm diameter hole was used in single-channel experiments, and one with a 0.55-mm diameter hole was used in multi-channel experiments. The bathing solution used was 1 M KCl (or 0.1 M KCl), 10 mM Tris, 10 mM MES, 10 mM β-alanine, pH=7.0. The electrical current (I) was measured with Keithley 428 amplifier, digitized by a LabPC 1200 (National Instruments, Austin, TX) and analyzed using a personal computer with the help of WinWCP Strathclyde Electrophysiology Software designed by J. Dempster (University of Strathclyde, UK). A voltage of 30 mV (unless otherwise stated) was applied to BLM with Ag–AgCl electrodes placed directly into the cell. In single-channel experiments, a patch-clamp amplifier (model BC-525C, Warner Instruments, Hamden, CT) was used for current measurements. Minigramicidin monomer (Table 1, see also compound *1* in [14]) was added at two sides of BLM, the minigramicidin covalent dimer (see compound *3* in [14]) was added at one side (both from ethanol solutions). Aluminum trisulfophthalocyanine ($AlPcS_3$) from Porphyrin Products, Logan, UT, or Rose Bengal (Sigma) was added to the bathing solution at the trans-side (the cis-side is the front side with respect to the flash lamp) in the sensitized photoinactivation experiments. BLMs were illuminated by single flashes produced by a xenon lamp with flash energy of about 400 mJ/cm^2 and flash duration <2 ms. A glass filter cutting off light with wavelengths <500 nm was placed in front of the flash lamp. To avoid electrical artifacts, the electrodes were covered by black plastic tubes.

3. Results

It has been shown by Rokitskaya et al. [29] that a flash of visible light in the presence of a photosensitizer provokes a transient decrease in the electric current (I) induced by gA across a DPhPC/decane membrane. The time course of this transient decrease (called sensitized photoinactivation kinetics) is well approximated by single exponential $I=I_0+A\cdot\exp(-t/\tau)$ with τ (the characteristic time of photoinactivation) corresponding to the gA single-channel lifetime. In the case of a DPhPC/squalene membrane, the photoinactivation kinetics of gA becomes biphasic [29,40]: it contains a fast (shorter than 1 ms) phase attributed to sensitized photodamage to the gA conducting form (dimers), apart from the slow phase with τ of the order of 2 s associated with a shift of dimer–monomer equilibrium as a result of photodamage to gA monomers.

In our experiments minigramicidin appeared to be unable to induce the ionic current across a DPhPC/decane membrane even at concentrations higher than 10 μM, whereas it was an active channel former in a thinner DPhPC/squalene membrane, in agreement with the data of [14] where single-channel properties of minigramicidin were firstly described. Upon flash excitation in the presence of a photosensitizer ($AlPcS_3$), the current induced by minigramicidin across a DPhPC/squalene membrane exhibited photoinactivation (Fig. 1A) which was much more pronounced (as judged by its amplitude) than that with gA (Fig. 1B). The minigramicidin photoinactivation