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A gramicidin analogue that exhibits redox potential-dependent cation influx

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Abstract

A synthetic analogue of gramicidin A, gram-2-(nicotinamidyl)ethyl carbamate (gAN) was found to regulate the influx of potassium ions depending on its redox state. At potentials less than −0.52 (±0.05) V, the nicotinamide group in gAN is reduced and allows charge- and size-selective influx of cations. In the oxidised state, charge repulsion between the positively charged nicotinamide group and the cations prevents any influx of cations. Chronoamperometric experiments were conducted to determine permeabilities of Eu^{3+}, Tl^{+}, K^{+} ions in a BLM porated with gAN. Scanning electrochemical microscopy (SECM) experiments were conducted with two ions: permeant K^{+} ions and the larger $(CH_3)_4N^{+}$ ion which blocks the channel. The switching potential for gAN, at which the channel allows flux monovalent cations, is shown to be potential and pH-dependent and temperature-independent.
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Keywords: Voltage-regulated ion channel; Gramicidin analogue; Scanning electrochemical microscopy (SECM); pH; Bilayer lipid membrane (BLM); Chronoamperometry

1. Introduction

The study of the interaction of channel forming synthetic and natural peptides within phospholipid membranes is an area of increasing interest [1–3]. The pore-forming mechanisms and ion-selectivities of several peptide antibiotics have been extensively investigated using a variety of techniques, amongst the most prevalent of which are fluorescence microscopy, FT-IR, electrophysiology, AFM and NMR. The dynamics of charge-transfer in bilayer lipid membranes (BLMs) is usually probed by patch-clamp amperometric microelectrodes to probe ion fluxes in biological membranes [4–6].

In this work we initially used chronoamperometric measurements, where a gAN ion channel-porated membrane was inserted on a gold working electrode (WE) surface (*vide infra*) and the potential was switched back and forth from an initial potential at which the nicotinamide group in the ion channel was reduced to a step potential, where the nicotinamide group was oxidised to ascertain the voltage-dependent properties of gAN. Following these studies scanning electrochemical microscopy (SECM) using ultra microelectrodes was used for further investigations.

The development of scanning electrochemical microscopy (SECM) techniques in the late 1980s by Bard and Mirkin [7], resulted in several publications on the use of this technique in probing the kinetics of ion-transfer (IT) [8–10] and electron-transfer (ET) processes [11–16] on monolayers at the interface between two immiscible electrolyte solutions (ITIES). Tsionsky et al. [15] used SECM to probe the kinetics of ion-transfer processes on the surface of BLMs using approach curves. Amemmiya and Bard used voltammetric ion-selective microelectrodes to study K^{+} transfer through gramicidin channels in horizontal BLMs prepared by the brush technique [8,10]. Mauzeroll et al. [17] used SECM to monitor the transport of thallium (I) ions across gA half channels inserted in a dioleoylphosphatidylcholine (DOPC) monolayer supported on a thallium amalgam hanging mercury drop electrode (HMDE) and obtained an apparent rate constant of $k_{het} = 2.8\ (\pm 0.1) \times 10^{-4}\ cm\ s^{-1}$.

Nelson and co-workers have used chronoamperometry and cyclic voltammetry to study the reduction of Tl(I) at gA-treated DOPC-coated mercury electrodes [18,19]. The Tl(I)/Tl(Hg) reduction process is known to occur very rapidly at uncoated

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mercury electrodes [20] meaning the rate limiting step is the diffusion of Tl^+ through the gA channel in the system studied. Chronoamperometry was performed by applying potential steps in the potential range and more negative of the Tl(I)/Tl(Hg) redox process. The resulting current–time plots showed two distinct regions—the first of which, an exponential current decay, was attributed to inactivation of the monomer channel conduction and the second region, a current quasi-steady-state, was due to diffusion of ions to the surface reaction. Normalised current-potential plots demonstrate a linear relationship between Tl^+ permeation and gA concentration and show that, in contrast to the behaviour of the Tl^+ permeant ion, Cd^{2+} ions are unable to pass across the gA channel. At potentials more negative than −0.7 V the membrane becomes non-selectively permeable due to an increase in structural defects. It was also reported that incorporation of aromatic or conjugated compounds into the DOPC monolayer caused an increase in the reduction current by decreasing the rate of inactivation of the channels and increasing their stability.

Gramicidin A (gA), a naturally occurring peptide antibiotic of sequence formyl-V-G-A-D-L-A-D-V-V-D-V-W-D-L-W-D-L-W-D-L-W-ethanolamine is a well-characterised membrane-active peptide. In membranes, as a result of the unusual alternating sequence of D- and L-amino acids, the peptide adopts a $\beta^{6.3}$ right-handed helical conformation [21]. The monomer is approximately 10.5 Å in length, less than the diameter of a single lamella in most membranes. Cation-selective channels are formed when gA monomers diffuse laterally in the membrane and form head-to-head (N-terminal-to-N-terminal) dimers. Previous studies have demonstrated that modification of the C-terminal ethanolamine with carbamate groups produces channels with modified conductance properties. In particular, single molecules of gramicidin A-ethylenediamine (gE), in which the terminal amino group is positively charged under physiological conditions, showed multiple conductance levels when studied by patch-clamp techniques [28]. In the open form, gE displayed a maximum conductance that was approximately half that of gA, with additional conductance levels that were ∼90%, ∼50% and ∼40% of this maximal value. These conductance levels were attributed to *cis*/*trans* isomerism of the carbamate group, with the positively charged ammonium group obstructing the channel entrance and/or exit in the *cis* isomer, but lying away from the channel axis in the *trans* form. The four possible *cis*/*trans* combinations were then responsible for the four observed conductance levels. It was additionally observed in control experiments, that the positively charged group was essential for production of this effect. We reasoned therefore, that the nicotinamide derivative gAN was a potentially interesting target, as in the oxidised form, the molecule would possess a positive charge in the same location as gE, and should therefore display similar conductance properties. In the reduced form however, this charge would be removed, and the channel should conduct, providing a means for producing redox-controlled channel activity. In effect gAN should provide a means of very precise regulation of the ion channel at the redox potential of the nicotinamide moiety in contrast to gE where the ion channel permeability varies with the four possible *cis*/*trans* combinations.

1.1. Ion influx

A biological membrane inserted with an ion channel can be compared to an electric circuit. The dielectric constant is a measure or the relative membrane capacitance which is related to the capacitance $C = Q/E$ where Q is the charge on the capacitor and E is the potential. The lipid membrane has a dielectric constant, $\varepsilon \sim 2.0$ compared to ∼80 for water at room temperature [22]. Insertion of gA creates a more polar environment, increasing the dielectric constant and allowing passage of hydrophilic cations. The effective dielectric constant, ε_{eff}, is assumed to be ∼20 [23].

When a porated insulating BLM membrane is inserted between two ionic solutions, the ionic current is limited by the transfer of permeable ions across the membrane [24].

The difference between the concentration of cations on either side of the membrane induces a diffusion flux, J, of ions which is dependent on diffusion coefficients, D, in the ion channel and the concentration gradients on either side of a membrane porated with ion channels. The diffusion is also dependent on the potential gradient on either side of the membrane. These effects are shown by the following equations:

$$\Delta J_i = -\Delta D \left(\frac{\partial \Delta c_{(1-2)}}{\partial x} \right) = \nu_i + \frac{\mathrm{d}\tau_i}{\mathrm{d}t} \tag{1}$$

where ΔD is the difference in diffusion coefficients of the permeable cations in the aqueous phase and in the membrane ion channel, ν_i the ion-transfer rate and τ_i is the concentration of ions on the membrane. The charge per unit time is the current, which splits into capacitive and a Faradaic term [25]

$$i = \sum z_i F A J_i = \sum z_i F A \nu_i + \sum z_i F A \left(\frac{\mathrm{d}\tau_i}{\mathrm{d}t} \right) \tag{2}$$

$$i = I_{\mathrm{F}} + \frac{\mathrm{d}Q}{\mathrm{d}t} = I_{\mathrm{F}} + I_{\mathrm{C}} \tag{3}$$

$$\nu_i = k_i^{1\to 2} \left((c_{i1} - c_{i2}) \exp \left[-z_i \frac{F^{(\Delta\phi_1 - \Delta\phi_2)}}{RT} \right] \right) \tag{4}$$

where A is the effective area of the membrane, ϕ_1 and ϕ_2 the electrochemical potentials in phases 1 and 2 and c_{i1} and c_{i2} the concentrations of permeable ions in phases 1 and 2, respectively and $k_i^{1\to 2}$ is the potential-dependent rate constant of ion-transfer from phases 1 to 2 across the membrane.

As the capacitance of the membrane is fixed, the ionic current flows until the charge on the capacitor is satisfied. An excess of electrolyte solution satisfies this condition. As increasing potential gradient is applied, an increasingly polar environment is created within the ion channels. In contact with ions of appropriate radii and charge, an increase in current is expected as ions pass through the channels [23,26].

In our experiments, egg phosphatidylcholine bilayer lipid membrane (BLM) formed over a microporous polycarbonate membrane support [23] was inserted between two aqueous electrolyte solutions containing equal and excess concentrations of the redox active $Fe(CN)_6^{3-}$ ion to maintain an equal potential on either side of the membrane and allowing the electrode to remain