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Imaging potassium-flux through individual electropores in droplet interface bilayers☆

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ABSTRACT

Using total internal reflection fluorescence microscopy of droplet interface bilayers containing the potassium-sensitive fluorophore APG-4, we imaged the ionic flux through individual electropores. We are able to monitor up to 30 individual pores in parallel and show voltage dependent responses in fluorescence that corresponds to the measured ionic current. These experiments help quantify the scope and current limitations of optical single channel recordings of potassium flux. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

1. Introduction

Potassium channels represent a key therapeutic target [1] with over 90 genes coding for the α subunit of potassium channels [2]. Specific malfunctions in potassium channels have been linked to a wide range of important diseases including neonatal epilepsy, deafness, and long QT syndrome [3–6]. However, a major challenge in screening for new compounds that modulate ion channels is in scaling conventional methods to achieve high throughput [7]. The shortage of effective methods for screening potassium channels stands in stark contrast to their abundance and importance for all living organisms.

Optical measurements of ionic flux provide an alternative to patch-clamping when studying the functional properties of ion channels [8–11]. In particular, optical single-channel recording (oSCR) enables straightforward simultaneous parallel measurement of the single-channel electrical activity from many channels [9]. This is in contrast to the specialized electronics required for parallel electrical recording. For optical recording, parallel signals are obtained by simply imaging the lipid membrane using a camera. In addition to electrical activity, imaging also provides the location of individual channels in the membrane. In oSCR a fluorogenic ion-sensitive probe is used to create a localized fluorescence signal at the site of an individual ion channel. Much work in this area has focused on calcium channels, due to the excellent selectivity of calcium-responsive fluorophores [12]. For example, 'optical patch-clamping' of Ca^{2+} fluxes have been used to measure simultaneous currents from hundreds of nicotinic acetylcholine receptors with a temporal resolution of 2 ms [13]. Most recently the same technique was used to image calcium flux through pores formed by β-amyloid [14]. We recently combined these methods with droplet interface bilayers (DIBs) in order to achieve simultaneous optical and electrical measurements from individual protein pores [15] in vitro. DIBs are formed from the contact of two monolayers surrounding water droplets immersed in an oil/lipid solution. They provide giga-ohm seals in a bilayer that is stable, easy to form, and straightforward to image. We have recently used arrays of DIBs in order to demonstrate high-throughput screening of the total ion flux through many bilayers in parallel [11] and to image pores created by the antimicrobial peptide alamethicin [16].

Optical single transporter recording also uses bilayers formed over microfabricated compartments to image the buildup of molecules within the compartment caused by transport through proteins present in the bilayer [17]. For example, sequential electrical and optical measurements of calcium-flux through alpha-hemolysin have been realized [18]. Membrane-covered compartments can also be used to establish an ionic gradient across a bilayer and proton flux through pores or channels can be monitored using pH-sensitive dyes [19].

To our knowledge, oSCR has so far not been applied beyond imaging Ca^{2+} and pH changes. The major obstacle in their application to potassium channels is the lack of indicator dyes that are able to detect small changes in the concentration of potassium while also showing high ion-selectivity [10]. Imaging methods with surrogate ions such as thallium or rubidium are the only alternatives when measuring the activity of potassium channels with fluorescence. While there are many examples where thallium-flux assays have been successfully

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applied [20–22], health and safety risks and low solubility in Cl^- buffers associated with thallium are significant drawbacks.

Here we use the indicator dye Asante Potassium Green 4 (APG-4) (Teflabs, Austin TX) to explore the potential for imaging potassium flux in DIBs (Fig. 1A). Rather than imaging ion-channels, we choose to first focus on electroporation of the bilayer. Electroporation is the permeabilization of the membrane due to an applied transmembrane electric field [23]. Electroporation is widely used as a method to introduce molecules into cells, particularly for transfection [24,25], however the details of its mechanism are still relatively poorly understood. Electroporation is thought to proceed via the formation of toroidal pores in the membrane [26]. As such it can produce pores with high (~1–13 nS [27]) and controllable conductance in comparison to a typical potassium channel (e.g. 50–100 pS for KcsA [28]). This approach provides us with the flexibility to quantitatively assess the sensitivity of potassium flux imaging from individual pores.

2. Materials and methods

2.1. Materials

All lipids were purchased from Avanti Polar Lipids, Inc. The cryptand-based potassium indicator dye APG-4 was purchased from Teflabs (dissociation constant 6.5 mM (personal communication, Teflabs)). All other components were purchased from Sigma Aldrich.

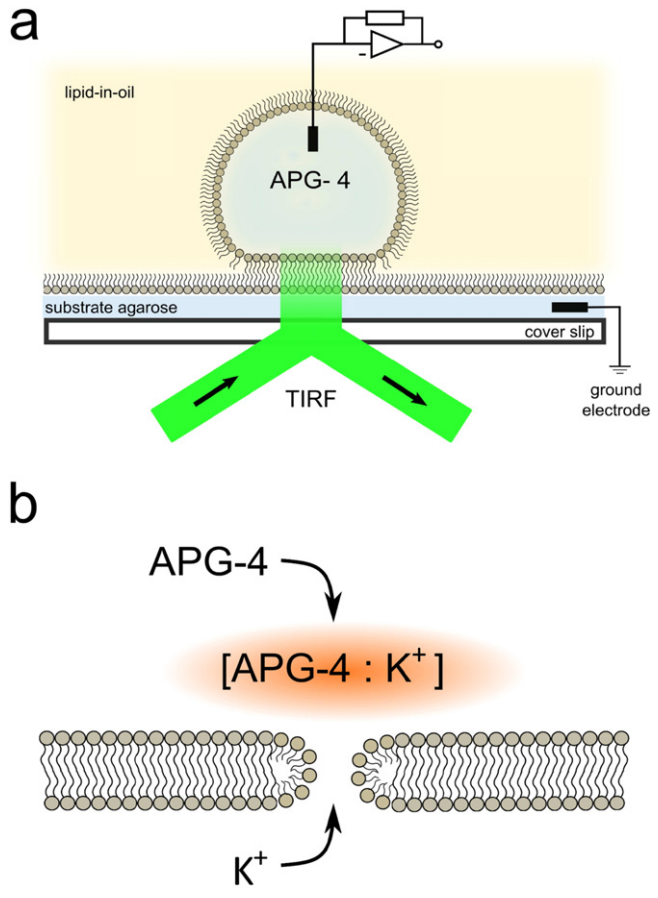

Fig. 1. (*a*) *Schematic of experiment*. A droplet interface bilayer forms when lipid monolayers on an agarose substrate and an aqueous droplet are brought into contact in a solution of phospholipids in hexadecane. Following application of a potential difference across the membrane electropores can form. The presence of the potassium indicator dye APG-4 enables the fluorescence from individual electropores to be detected as a fluorescent signal using TIRF illumination of the bilayer. (*b*) *Cartoon of electropore formation* and configuration for imaging with APG-4.

2.2. Ensemble fluorescence

Conditions for optimized APG-4 response were determined using fluorescence spectroscopy. Excitation is at 520 nm and the fluorescence was monitored at 550 nm with slit widths of 5 nm. The effect of both KCl and kryptofix-222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-(8.8.8)-hexacosane) on APG-4 response were assessed by titration. Kryptofix-222 is a chelator for potassium, and we originally added it in with the intention of controlling the spatial distribution of fluorescence in a similar manner to how EDTA is used in optical recording of calcium flux [13].

We first titrated a solution of 3 M KCl in 10 mM HEPES into an APG-4 solution (1.75 μM APG-4, 3 M NaCl, 10 mM HEPES, pH 7) containing kryptofix-222 concentrations ranging from 0 to 25 mM (Fig. 2A). We

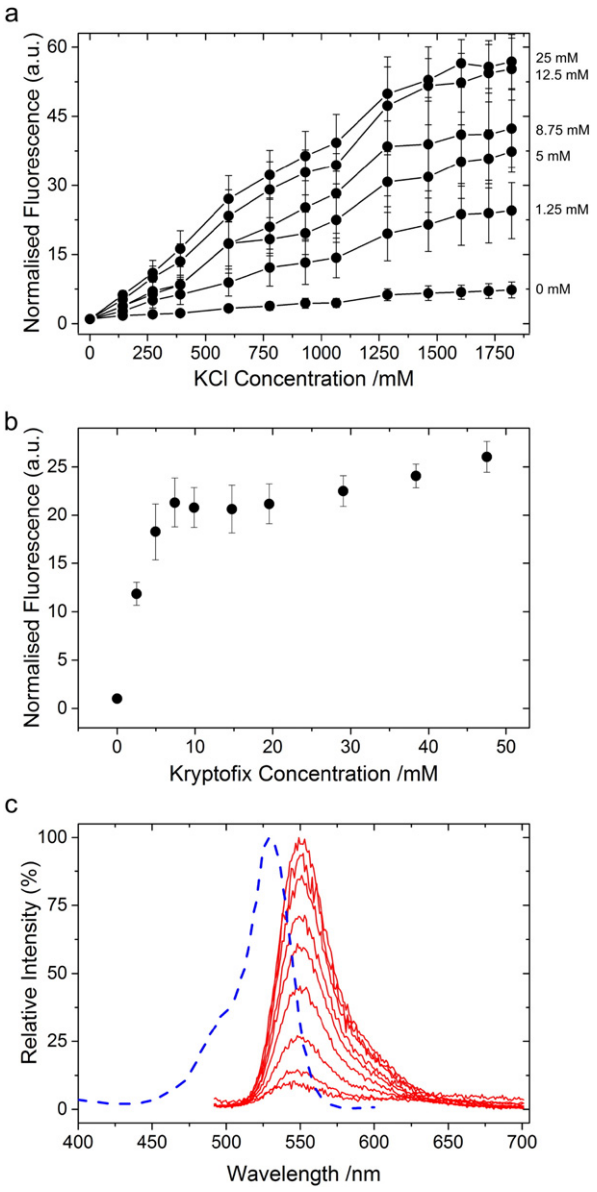

Fig. 2. *Response of APG-4 to* K^+ (*a*) KCl was titrated into a solution of APG-4 as kryptofix-222 was varied, to assess the sensitivity of the indicator dye. The signal to background ratio improves with increasing kryptofix-222 concentration. At 0 mM kryptofix-222 the maximum normalized response of APG-4 to K^+ is 7-fold, whereas at 12.5 mM the response reaches a maximum of 55-fold. Data points are normalized to the respective fluorescence signal without any added potassium. Excitation is at 520 nm, emission at 550 nm (*b*) Titration of kryptofix-222 into 3 M KCl, 10 mM HEPES and 1.75 μM APG-4 shows dye response is maximized above 10 mM kryptofix-222. (*c*) Excitation and emission spectrum of APG-4 normalized to maximum intensity, as K^+ is varied from 36 mM to 3 M.