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Electron current recordings in living cells

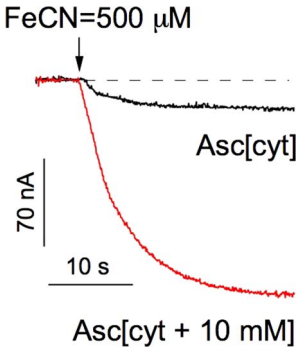
Paolo Trost[a], Cristiana Picco[b], Joachim Scholz-Starke[b], Margherita Festa[c,d], Laura Lagostena[b], Alex Costa[c,d], Francesca Sparla[a], Armando Carpaneto[b,*]

[a] Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Via Irnerio 42, 40126 Bologna, Italy
[b] Institute of Biophysics, CNR, Via De Marini 6, 16149 Genova, Italy
[c] Department of Biosciences, University of Milan, Via G. Celoria 26, 20133 Milan, Italy
[d] Institute of Biophysics, Consiglio Nazionale delle Ricerche, Via G. Celoria 26, 20133 Milan, Italy

HIGHLIGHTS

- Living cells use electricity for a plethora of physiological processes.
- Ion currents in channels and transporters are very well investigated by electrophysiological techniques.
- It is equally possible to record electron currents mediated by specialized redox proteins.

GRAPHICAL ABSTRACT



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ABSTRACT

Living cells exploit the electrical properties of matter for a multitude of fundamental physiological processes, such as accumulation of nutrients, cellular homeostasis, signal transmission. While ion channels and transporters (able to couple ions to various substrates) have been extensively studied, direct measurements of electron currents mediated by specific proteins are just at the beginning. Here, we present the various electrophysiological approaches that have allowed recordings of electron currents and highlight the future potential of such experiments.

1. Introduction

Electrical phenomena are governed by the general principle of electroneutrality: each negative charge must be associated with a positive charge (for a critical view see [1]). Therefore, to separate two charges of opposite sign, physical work must be employed. Because the electric field is conservative, this work is returned when the system is allowed to go back to equilibrium. Living cells exploit this law in various ways. Specialized proteins capable of separating ionic charges at the edges of the plasma membrane create a membrane potential. In the case of animal cells, this task is performed by the sodium/potassium antiporter, which creates a potassium gradient with the cytosolic K^+ concentration (about 100 mM) being higher than the external K^+ concentration (about 5 mM). The opening of potassium channels drives the membrane voltage to hyperpolarised values close to the equilibrium (Nernst) voltage of potassium, i.e. about − 70 mV [2]. In plant cells, the membrane voltage has a different origin, can reach more negative values of up to − 200 mV and is created by the combined action of H^+-

* Corresponding author.
E-mail address: armando.carpaneto@ge.ibf.cnr.it (A. Carpaneto).

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ATPases and potassium channels [3].

1.1. Ion and gating currents mediated by ion-selective channels

A very important class of membrane proteins able to detect variations in membrane potential is represented by voltage-gated cation channels. They have a tetrameric structure [4]; the single monomer is equipped with a voltage sensor, an alpha helix called S4 segment, able to perform significant movements upon physiological changes of the membrane potential. The topological variations of the S4 segment are then relayed to different parts of the protein (segments S5 and S6), which allow the opening and closing of the ion permeation pathway of the channel, a mechanism generally called gating [5]. In voltage-dependent channels, gating and selectivity, i.e. the ability of the channel to discriminate between ionic species, are mediated by different structural parts, the latter being determined by the precise assembly of four short segments (P-loops). In voltage-gated proteins, two distinct types of currents can be measured: 1) currents associated with the movement of the S4 segments, the so-called *gating currents*. This type of current is intrinsically transient, since the voltage sensor is anchored to the membrane, and difficult to detect, given the small amount of charge involved [6]; 2) currents associated with ion permeation, which have been investigated in cells of many organisms, including, for example, sponges [7,8] and mussels [9], marine [10,11] and aquatic [12] plants. These currents are modulated by a multitude of factors, i.e. oxidizing and reducing agents [13–16], polyunsaturated fatty acids [17], antibiotics [18], divalent ions [19–21], accessory proteins [22], in some cases even by differences in the voltage stimulation protocol [23]. The composition of the channel can also play an important role; for example, plant potassium channels are tetramers, which can be homomeric or heteromeric, likely according to the status of the plant, with different functional properties in potassium uptake [24–27]. Finally, fine structural differences can result in opposite functional properties such as inward or outward rectification [28]. Recently, an increasing interest in intracellular channels, namely the channels localised in compartments and organelles inside the cell, has also emerged and novel approaches to study their biophysical properties have been developed (see for example [29–32]).

1.2. Ion transporters

Ion transporters have also been intensively investigated. Differently to ion channels, in which the permeating ions simply follow their electrochemical potential, transporters employ a coupling mechanism between an ion and a specific substrate, which allows the substrate to move against its electrochemical gradient at the expense of the electrochemical potential of the coupled ion. For example, the plant proton/sucrose symporter, essential for phloem loading of sucrose (in special cases also for the unloading), is able, to accumulate up to 1 M sucrose inside the phloem from an external concentration of few millimolar, by using the strong electrochemical gradient for protons (with pH 5.5 in the apoplast and pH 7.2 in the symplast, together with about − 200 mV of membrane voltage) [33]. In transporters, the coupling mechanism is achieved through a conformational change of the protein [34], causing the turnover rate to be usually much lower compared to the values found in ion channels. An ion channel with a single channel conductance of 10 pS, stimulated by a 10-mV potential difference, allows the movement of about 600.000 ions per second, while the turnover rate of the proton/sucrose cotransporter has been estimated to be 500 ions per second [35]. Therefore, the currents mediated by transporters are generally small, even though their expression levels are usually higher than that of ion channels. In transporters, currents also exhibit two components: a transient component, called presteady-state current (Fig. 1a, b), and a stationary phase associated with the ion/substrate cotransport (named transport-associated current, I_{tr}) The presteady-state current usually occurs in the absence of the substrate to which the ion is coupled. The Peres group has proposed an interesting hypothesis about their origin: they would be the manifestation of the ion movement trapped within the carrier in the absence of the substrate; following the application of the substrate the ion would be unlocked and contribute to the transport-associated current [36]. This hypothesis, which arises from the surprising finding that transport-associated current amplitudes in the GAT1 sodium/GABA cotransporter can be accurately predicted from the mere knowledge of the presteady-state current amplitude [37], was also confirmed in the plant proton/sucrose symporter [35] (Fig. 1c).

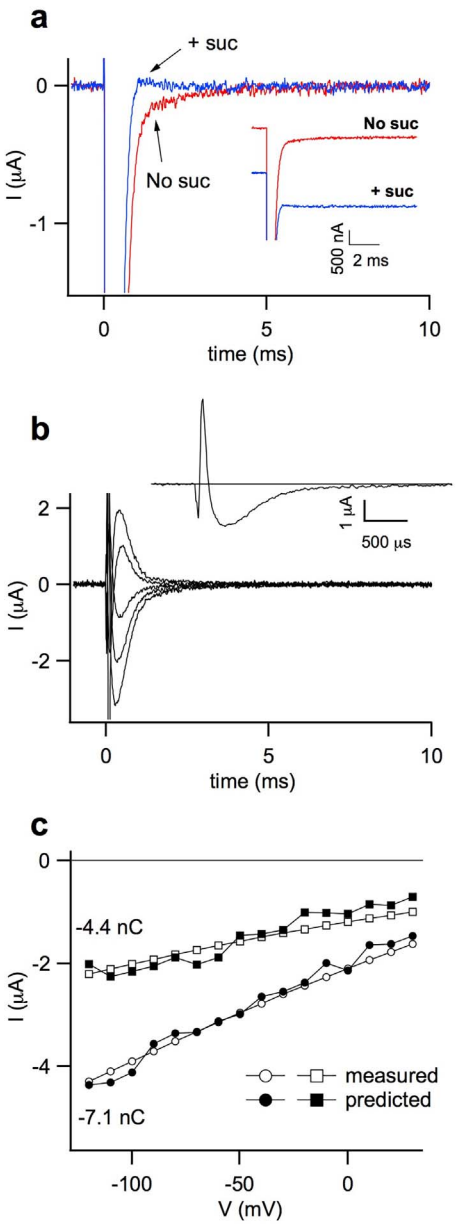

Fig. 1. Stationary and presteady-state currents in the proton/sucrose symporter ZmSUT1. a. In the inset, proton currents recorded in a ZmSUT1-expressing Xenopus oocyte, in the absence of sucrose (No suc) and in the presence of a saturating sucrose concentration (+ suc) are shown. The same currents are displayed in the main panel after forcing their stationary level to zero. Holding and pulse voltages were − 20 and − 80 mV, respectively. b. Presteady-state currents vs time were obtained after subtracting the stationary currents in the absence and in the presence of saturating sucrose. Holding voltage − 20 mV, voltage pulses from + 40 mV to − 120 mV (step − 40 mV). In the inset: presteady-state current elicited by a voltage of − 80 mV. c Measured (empty symbols) and predicted (filled symbols) currents displayed versus voltage for two oocytes with different ZmSUT1 expression levels. From Carpaneto et al. (2010), PLoS One, 5:e12605.