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Emergence of a large pore subpopulation during electroporating pulses

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ABSTRACT

Electroporation increases ionic and molecular transport through cell membranes by creating transient aqueous pores. These pores cannot be directly observed experimentally, but cell system modeling with dynamic electroporation predicts pore populations that produce cellular responses consistent with experiments. We show a cell system model's response that illustrates the life cycle of a pore population in response to a widely used 1 kV/cm, 100 μs trapezoidal pulse. Rapid pore creation occurs early in the pulse, followed by the gradual emergence of a subpopulation of large pores reaching ~30 nm radius. After the pulse, pores rapidly contract to form a single thermally broadened distribution of small pores (~1 nm radius) that slowly decays. We also show the response of the same model to pulses of 100 ns to 1 ms duration, each with an applied field strength adjusted such that a total of 10,000 ± 100 pores are created. As pulse duration is increased, the pore size distributions vary dramatically and a distinct subpopulation of large pores emerges for pulses of microsecond and longer duration. This subpopulation of transient large pores is relevant to understanding rapid transport of macromolecules into and out of cells during a pulse.

1. Introduction

Electroporation (EP) is a striking non-thermal response of lipid bilayer regions of cell membranes to an electric field pulse. In biological research, biotechnology development and clinical applications, EP is widely used to deliver molecules, large DNA in particular, into living cells. Most in vitro protocols involve mixing DNA with the cell preparation before pulsing. This usually leads to adsorption of large DNA to cell membranes prior to EP, followed by pulsing and subsequent movement of DNA through membranes [1,2]. After decades of study, however, understanding of EP remains incomplete even though a basic concept endures: Transient aqueous pores are hypothesized to form, expand, and contract in lipid regions of cell membranes in response to transmembrane voltage, U_m, which transiently rises well above normal physiologic values during an electroporating pulse.

In addition to well-established research activity, several papers have recently sought to reinvigorate a potentially important EP research topic: The possible role of horizontal gene transfer (HGT) by lightning-mediated EP in microbial evolution. This thirty year old idea is that lightning can facilitate foreign DNA transfer into bacteria in the sea or soil, with the DNA likely originating from nearby dead bacteria [3]. A subsequent paper describes a system for carrying out experiments on a laboratory scale [4]. Together these papers provide both a motivation and a means for sustained research in this area.

1.1. Continuum and molecular dynamics modeling insights

Both continuum theoretical models and molecular dynamics (MD) models show pores initially appearing with radius, r_p, of about 0.5 nm, followed by expansion while U_m remains elevated [5–9]. MD provides exquisitely detailed descriptions of individual pores within a small membrane area for times less than a microsecond [8,9]. Due to computational limitations, however, MD does not account for cell level features, such as pore populations evolving over a closed, curved cell membrane, flattening of the U_m angular profile in the region of EP, restricted spatial extent of EP, and distributed cell membrane EP asymmetry. MD models by themselves are therefore inadequate to describe cell-level behavior. However, future developments in cell system modeling are likely to incorporate functional behavior described by MD simulations, which can provide more detailed, and often membrane-composition specific behavior. Cell system models are particularly important for behavior during a pulse, when pore creation, evolution of complex pore populations, and rapid molecular transport all take place.

1.2. Family of electroporation models

Here we use a cell system model that belongs to a family of EP models which have been developed over three decades by several

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groups, as discussed in reviews [10,11] and original publications [6,7,12–16]. This family consists of planar membrane EP models (1D) [5,10,17–21] and curved cell membrane models (2D and 3D) [6,7,11,13–15,22,23]. The system model response involves electrical interactions in which local membrane nodes are coupled through the network of nodes that simultaneously describe conductive and dielectric charge transport. As a result of coupling within the system, the spatially-distributed electrical response of the model and the dynamic behavior of pore populations feed back into each other and yield complex emergent behavior [23].

Despite this complexity, the results of electroporation models with dynamic pores [5–7,24] exhibit some common features. Specifically: (1) An electroporating pulse yields a transient peak in U_m, followed by a drop to ~0.5 V for trapezoidal (or square) pulses [6,24] and for exponential pulses [5,7]. (2) During an applied electric pulse, some pores tend to contract to (or remain at) the minimum-size pore radius, r_{min}, while other pores expand significantly beyond r_{min}. An important modeling paper [6] analyzes the evolution of large and small pores for a set of classic experiments with a 1 ms ideal, rectangular pulse [25–27]. Those modeling results are in general agreement with ours for a 1 ms pulse, but the present paper includes a wide range of additional EP pulse durations and associated pore populations.

1.3. Experiments generally cannot determine pore size and number during pulsing

Several experiments with intact cells have measured electrical behavior, particularly U_m, during a pulse [25–29]. However, determination of U_m alone does not yield pore size or pore number. To our knowledge, there is only one experimental report of pore number and size during a pulse, which estimated the expansion of pores to radii as large as 55 nm, amongst numerous small pores [30]. This experiment involves unusually long pulses, 0.1 to 1 s duration ramps.

The use of whole cell patch clamp methods is an established approach for quantitative determinations of transmembrane voltages for normal physiologic conditions. It can also be used for measurements during electroporating pulses for plant protoplasts outer membranes [31], which involve larger U_m. The traditional method is based on electrical measurements alone, but can be supplemented by an important optical method based on a voltage-sensitive dye [32].

However, whole cell clamp conditions differ significantly from conditions based on field pulse application. Some signature EP events, such as flattening of the transmembrane voltage profile, do not occur for patch clamp protocols, essentially by definition. Additionally, almost all EP research and application protocols involve field pulses, and these mainly report effects after pulsing (e.g. molecule uptake or non-thermal cell death). Most methods also have insufficient temporal and spatial resolution to quantitatively describe pores while U_m is elevated. Knowing U_m does not allow direct determination of pore population characteristics, but quantitative determination of U_m does add to the presently sparse quantitative information about membrane behavior during and after a pulse, and provides constraints.

1.4. Modeling can treat both “during” and “after” pulsing

Cell system modeling can, however, provide approximate, quantitative descriptions of dynamic pore population and electrical behavior during and after a pulse. Based on both pore and molecule size, rapid, direct transport of macromolecules is expected only during a pulse when transient large pores develop. Large pores have more extensive focusing fields that can guide charged, macromolecules into a pore [33]. A macromolecule may interact strongly with a pore as it approaches, enlarging the pore further as it transits through [34]. These implications for molecule transport motivate the present study of pore populations during a pulse.

2. Methods

2.1. General model features

Our curved cell membrane model is a recent version [23] of an EP model belonging to the above cited family of models. Cell EP involves spatially distributed highly nonlinear and hysteretic interactions throughout a cell system model (Fig. 1A), and can only be solved computationally [6,7,11,13–15].

2.2. Specific model features

The cylindrical plasma membrane (PM) has 10 μm radius, 13.3 μm length, and 5 nm thickness (Fig. 1A). We use a meshed transport network [14,35] at the 100 μm scale, with electric fields applied via idealized electrodes at upper and lower boundaries. The anode-facing and cathode-facing sides of the cell are marked by red ‘+’ and black ‘−’ symbols, respectively (Fig. 1A). Features of the present model [23] include mesh creation (Fig. 1B–C) for a 600 node-pair plasma membrane and 9267 nodes for the surrounding electrolyte, as well as charge transport and storage between mesh nodes.

Pore creation, destruction, and evolution are governed by the pore energy landscape, $W(r_p,U_m)$ (Fig. 2), and two rate equations. Pore creation and destruction are described by absolute rate equations [23,36] and pore expansion and contraction are described by the Smoluchowski equation using a mathematical analogy with electrodiffusion [37]. The pore energy landscape has a local minimum at $r_{p,min} = 1$ nm for $U_m = 0$ V, but with increasing U_m, the barrier to pore creation decreases, the slope in pore energy becomes increasingly negative, and the local minimum diminishes as it shifts to larger radii. For example, $W(r_p,0.5\ V)$ is relatively flat and results in relatively little change in pore radius, while $U_m < 0.5$ V favors pore contraction towards the local minimum and $U_m > 0.5$ V favors expansion to larger radii.

Our energy landscape (Fig. 2) is developed from prior landscapes [6,7,13,20], with parameters as follows. The location of the barrier to pore creation is $r_p^* = 0.65$ nm, with $r_{p,min} = 1$ nm defining the pore energy landscape minimum for $U_m = 0$ V before EP (Fig. 2) [23]. We use $\Delta\delta_c = 45$ kT and $\Delta\delta_d = 20.3$ kT (barriers to pore creation and destruction, respectively, at T = 298 K and $U_m = 0$). Altogether these parameters yield pore lifetime, $\tau_p = 4$ s (typical for a particular mammalian cell PM [23]). The electroporation model is semi-empirical, with key model parameters, including the pore lifetime, determined by comparisons to experiments [38,39] with mammalian cells based on uptake of fluorescence solutes [23]. We use $D_p = 2 \times 10^{-13}$ m^2/s, for the diffusion coefficient in pore radius space. Additionally, here we use maximum pore radius, $r_{p,max} = 60$ nm [30] in recognition of non-lipid membrane molecules and structures of cell membranes that limit maximum pore size [40]. Other parameters for describing membrane EP within local membrane areas (regions associated with a transmembrane node pair) and adjacent aqueous media are given elsewhere [36].

As in previous models [6,7,13,41,42], we use a resting potential source instead of a fixed resting potential (physiologic transmembrane voltage of an unperturbed cell) to generate the resting transmembrane voltage, $U_{m,rest} = -50$ mV. This feature allows depolarization of the PM to be accounted for once ion-conducting pores shunt the resting potential source. As a result, we obtain transmembrane voltage behavior that reflects experimental results much more closely than passive models, which may predict very high transmembrane voltages in the absence of EP. The largest estimated temperature rise for the results presented here is 2.5 K (0.94 kV/cm, 1 ms pulse), consistent with the