

Large amplitude density fluctuations in a lipid bilayer, induced by picosecond electric pulses, and their possible biotechnological applications

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Abstract. Electroporation refers to changes in permeabilization of lipid membranes induced by external pulsed electric fields. Diverse applications of electroporation in biotechnology include gene transfer, modulation of gene expression, cell proliferation, seed germination, sterilization in the food industry, and extraction of chemical compounds. We argue that the formation of large amplitude, subnanometer density fluctuations in lipid bilayers, induced by high-intensity pulsed electric fields with picosecond durations, may produce lipid nanopores and change transmembrane transport, which trigger cellular signalling pathways with subsequent cellular responses and changes in cell physiology, thus, could represent perspectives regarding the development of electroporation biotechnologies.

1 Introduction

Electroporation or electropermeabilization refers to transient or permanent changes in permeabilization of lipid membranes induced by external pulsed electric fields. These changes can be due to the formation of aqueous lipid pores, pH changes, ion imbalance, and other biophysical and biochemical mechanisms [1, 2]. Electroporation of biomembranes in living cells may involve membrane proteins [1], cytoskeletal scaffold, and oxidation of membrane lipids [3].

Milli- and microsecond electric pulses were routinely used in electroporation-based procedures. In the 1990s, a technique was developed to produce high-intensity pulsed electric fields with a duration of ~500 ns [1]. Recently, a new technique based on an impulse radiating antenna instead of electrodes was developed to produce an electric pulse with a width of ~500 ps [4]. Modern pulsed power technology is now so advanced that the electric pulse widths can be between about 1 ps and 1 ms [5]. Though still being under development, picosecond electric pulse devices may be widely used in the near future.

Nanosecond and picosecond electric pulses have several important advantages in electroporation-based procedures over the conventional micro- and millisecond pulses including low level of heating and reduced electrochemical reactions, which makes them

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attractive for use in biotechnology and food industry [6]. Nanosecond and picosecond electric pulses can be considered as the first non-invasive technique affecting cell organelles [1].

The delivery of a series of pulses of high electric fields with nanosecond and picosecond durations into cells or biological tissues is a non-invasive and cheap technology that can manipulate cellular membranes and transmembrane proteins. Diverse applications of this technique in biotechnology and food technology include gene transfer, modulation of gene expression, cellular phenotype manipulation [1], cell proliferation [7], fermentation processes [8, 9], seed germination [10], microbial inactivation for food preservation [11], and extraction of chemical compounds [12].

We earlier described one possible electroporation mechanism that consists in the formation of large amplitude density fluctuations on subnanometer length scales in the hydrophobic region of a lipid bilayer due to the impact of electric pulses [13, 14]. Here, we argue that the formation of large amplitude, subnanometer density fluctuations in lipid bilayers, induced by high-intensity pulsed electric fields with picosecond durations, may produce lipid nanopores and change transmembrane transport with subsequent cellular responses, which could be of interest for the development of electroporation biotechnologies.

2 Results and discussion

Chen et al. [15] suggested that a theory for permeation of water molecules through the lipid bilayer would have to involve large amplitude density fluctuations. Two models in this direction were developed in [13, 14] to propose a possible electroporation mechanism that consists in the formation of large amplitude density fluctuations on the subnanometer length and subpicosecond time scales in the hydrophobic core of a lipid bilayer due to changes in the structure factor of the system. The results of [13, 14] indicate that high-intensity picosecond electric pulses can produce lipid nanopores through the formation of these fluctuations.

The amplitude of density fluctuations can be considered as proportional, in some sense, to the static structure factor $S(k)$ of the lipid bilayer, where k is the wavenumber of the density fluctuations. Here, we calculate $S(k)$ for several values of an externally applied electric field E using the formulas obtained in [13, 14] and data for a typical lipid bilayer given in [13, 14].

Figure 1 illustrates the behavior of $S(k)$ characterizing the enhancement of the amplitude of density fluctuations with increasing externally applied electric field.

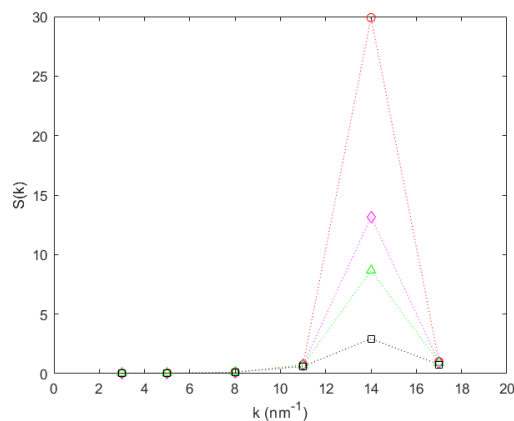


Fig. 1. The value of $S(k)$ characterizing the enhancement of density fluctuations in the hydrophobic core of a lipid bilayer for the electric field $E =$ (squares) 0, (triangles) 3.0, (diamonds) 3.25, and (circles) 3.5 V/nm. The dotted lines are guides to the eye.

Figure 2 illustrates the divergence of the amplitude of density fluctuations when the externally applied electric field approaches to its critical value.

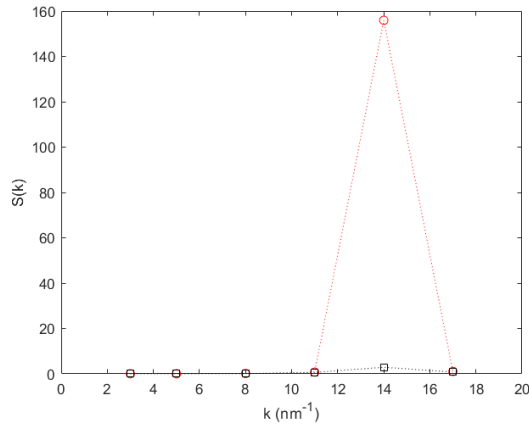


Fig. 2. The value of $S(k)$ characterizing the enhancement of density fluctuations in the hydrophobic core of a lipid bilayer for the electric field E = (squares) 0 and (circles) 3.65 V/nm. The dotted lines are guides to the eye.

The critical values of electric fields for electroporation obtained in [13, 14] are of ~ 3 V/nm. However, significant increase of the amplitude of density fluctuations is possible also for subcritical electric fields [14]. These results agree with experimental and simulation data for picosecond pulses. Indeed, it is known that the critical electric field for dielectric breakdown increases with the decrease in pulse duration. According to an empirical formula proposed to estimate the critical electric field for different pulse durations, the critical field is somewhat inversely proportional to the cube root of the pulse width [16]. As mentioned in [16], the critical electric field in the nanosecond time scale can exceed 1–2 V/nm. Also, from the available data one can conclude that, in the common case, the electric field that is needed to induce electrostimulation increases with the decrease in pulse width. Thus, computer simulations for 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes showed that the electric field of 0.2 V/nm was needed to induce a nanopore after 13.6 ns of simulation [17], while the porating fields for lipid bilayers were estimated as ~ 1.5 – 2.5 V/nm on the time scale of ~ 5 – 10 ps [18]. Similarly, the voltage sensing domain of a voltage-gated Ca^{2+} channel, inserted in phospholipidic membranes with high cholesterol content, undergoes major conformational changes under an electric pulse of 0.2 V/nm with a duration of 50 ns [19]. Also, cell stimulation was observed for pulsed electric fields of ~ 0.02 V/nm with a duration of 500 ps [20], which is significantly higher than for longer durations.

Thus, the mechanism of large amplitude density fluctuations induced by high electric fields with picosecond durations seems to be possible. This mechanism accompanies the movement of charged species under the influence of an external electric field. It seems, however, that at least on the picosecond time scale, this movement is not a dominating factor (cf. [21]).

The primary effect of nanosecond and picosecond electric pulses is the formation of permeability pores of nanometer or subnanometer size in the cell plasma membrane [22, 13, 14].

Nanopores created by high-intensity nanosecond and picosecond electric pulses in living cells demonstrate complex conductive properties (voltage sensitivity, inward rectification, and ion selectivity), which indicates that these pores could be protein structures rather than simple lipid pores [3]. Thus, membrane proteins are potential targets of electroporation.

High-intensity picosecond electric pulses may cause conformational changes which may lead to function loss and to pore formation within the protein itself. The voltage sensor domain of voltage-gated ion channels can be influenced by high voltages, with the activation of the ion channels. A direct effect of the applied field on the pore gate, bypassing the shift of the voltage sensor, is also possible [20].

One of the main cellular consequences of the formation of nanopores and activation or opening of voltage-gated ion channels is the increase in the cytoplasmic concentration of Ca^{2+} , which activates multiple signaling pathways [20, 23-25]. This leads to various consequences including proliferation and differentiation. The formation of nanopores can cause also activation of phosphoinositide signaling [26], and apoptotic or necrotic cell death [27]. In addition, a persistent depolarization of the resting membrane potential was observed [28].

As to the detailed secondary molecular mechanisms, proteomic analysis for microalgae [29], for example, suggested that cell proliferation induced by the application of nanosecond electric pulses may be caused by the activation of stress response pathways. It was shown that the protein $\text{Na}^+/\text{Ca}^{2+}$ exchanger/integrin- $\beta 4$ was overexpressed after the application of nanosecond electric pulses. Integrins are known to be related to growth stimulation. It was concluded that Ca^{2+} signaling could play a key role in the regulation of cell proliferation by ultra-short electric pulses.

3 Conclusion

This scenario makes a wide range of biotechnological applications possible, including modulation of gene expression, cell proliferation, fermentation processes, food technology, and seed germination. There are a series of examples of the corresponding effects, induced by ultra-short electric pulses, which are likely mediated by Ca^{2+} mobilization and diverse signaling pathways [7, 8, 9, 29]. First, cyanobacteria cell growth can be increased by application of repeated nanosecond electric pulses [7]. Second, treatment with nanosecond electric pulses enhanced product recovery in cell fermentation and reduced the time needed for reaching a plateau in the fermentation process [8, 9]. Third, sustainable and economical microalgae-based biorefineries might be improved by using treatments with nanosecond electric pulses [29]. We also note that application of nanosecond electric pulses can reduce total bacterial contamination in microalgae cultures without compromising the microalgae (a novel food ingredient) [30]. In addition, treatment with nanosecond electric pulses can significantly affect seed germination and pre-growth of plants [10].

In view of the above, one can expect that the theoretically predicted formation of large amplitude, subnanometer density fluctuations in lipid bilayers, induced by high-intensity pulsed electric fields with picosecond durations, may produce lipid nanopores and change transmembrane transport, which trigger cellular signalling pathways with subsequent cellular responses and changes in cell physiology, thus, could represent perspectives regarding the development of electroporation biotechnologies.

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