Estimation of the Energetic Barrier to Pore Formation and the Pore Radius Corresponding to this Barrier for Different Cell Lines

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ABSTRACT

The aim of this study was to estimate the energy barrier to pore formation at zero transmembrane potential $\Delta W_f(0)$ and the pore radius r_* corresponding to the top of this barrier for four different cell lines. Experiments were performed *in vitro* with two non-tumor cell lines (human erythrocytes and Chinese hamster ovary (CHO) cells) and two tumor cell lines (mouse hepatoma MH-22A and rat glioma C6 cells). Cell electroporation was determined from the extent of the release of intra-cellular K^+.

By comparing the theoretical dependences of the electric field strength required to create a single pore in the cell with the experimental dependences of the electric field strength required to electroporate 50% of cells on the pulse duration, the values of $\Delta W_f(0)$ and r_* were estimated for each cell line. Obtained values were close to each other for erythrocytes, CHO, mouse hepatoma MH-22A, and rat glioma C6 cells and were in the range $\Delta W_f(0)$ = 41–43 kT, and r^* = 0.3–0.35 nm.

Keywords: Electroporation, hydrophilic pore, mouse hepatoma, human erythrocytes, rat glioma

1 INTRODUCTION

The permeability of the cell membrane can be modified by exposing of cells to high-voltage electric pulses leading to the formation of nanometer-sized pores in the cell membrane (electroporation or nanoporation) [1]. This phenomenon is widely used in cell biology, biotechnology and medicine [1,2].

Earlier, optimal electrical parameters for electroporation of cells used to be determined empirically for each particular cell line [3]. Now before real experiments, it is already possible to get theoretical relationships between various parameters the electric field treatment (field strength, duration, number of pulses, etc.) required to electroporate the cell [4]. Just such parameters as the energy barrier to pore formation at zero transmembrane potential $\Delta W_f(0)$ and the pore radius r_* corresponding to to the top of this barrier have to be known.

Unfortunately, these parameters have been estimated just for a few cell lines [5,6], which makes difficult obtaining theoretical predictions for other types of cells because the range of the variations of the values of these parameters between different cell lines is not known yet. Thus, it is still difficult to predict individual responses of different cells to electric treatment [3,7].

The aim of this study was to estimate the energy barrier to pore formation at zero transmembrane potential $\Delta W_f(0)$ and the pore radius r_* corresponding to the top of this barrier for four different cell lines, both cancerous and non-cancerous ones.

2 MATERIALS AND METHODS

2.1 Theoretical Calculations

The energy barrier to pore formation $\Delta W_f(0)$ and the pore radius r_* were estimated by comparing a theoretical dependence of the electric field strength required to create a single pore in the cell with the experimental dependences of the field strength at which 50% of cells become electroporated on the pulse duration.

Theoretical relationships between the parameters of the electric pulse as a result of the exposure to which a single pore has appeared in the cell were obtained from [4]:

$$\int_0^\infty k_{f0}(E,t)dt = 1 \tag{1}$$

where $k_{f0}(E,t)$ is the rate of pore formation at electric field strength E.

For a spherical cell, this rate is can be calculated from [5]:

$$k_{f0}(E_0) = D\int_{-1}^{1} \exp\left[G\left(1.5E_0 ay - \Delta\Phi_0\right)^2\right]dy, \tag{2}$$

where

$$D = \frac{2\pi\nu a^2}{a_l}\exp\left[-\frac{\Delta W_f(0)}{k_B T}\right],$$
$$G = \pi C_m \frac{\varepsilon_w/\varepsilon_m - 1}{2k_B T} r_*^2, \tag{3}$$
$$y = \cos\theta.$$

Here a is the cell radius, $\Delta\Phi_0$ is the resting potential, ν is the frequency of lateral fluctuations of the lipid molecules, a is the cell radius, a_l is the area per lipid molecule, k_B is Boltzmann's constant, T is the absolute temperature, $\Delta W_f(0)$

is the energy barrier to pore formation at zero transmembrane potential and r_* is the radius of the pore corresponding to the top of this barrier, C_m is the specific membrane capacitance, ε_m and ε_w are the specific permittivities of the membrane and water inside the pore respectively, and θ is the angle between the normal to the membrane surface and the field direction.

The transmembrane potential generated by a square-wave electric pulse across the non-conducting membrane of a spherical cell increases over time according to [8,9]:

$$\Delta\Phi_g(t) = 1.5 E_0 a\left[1 - \exp\left(-\frac{t}{\tau_c}\right)\right], \text{ for } 0 \le t \le \tau_i. \quad (4)$$

$$\Delta\Phi_g(t) = \frac{3}{2} E_0 a \exp\left(-\frac{t-\tau_i}{\tau_c}\right), \text{ for } t > \tau_i. \quad (5)$$

Here τ_c is a time constant of the cell plasma membrane charging process. For a spherical cell with the radius a = 1–50 μm, this time constant is within the range of 100 ns – 10 μs [10,11].

Theoretical relationships between the parameters of the electric treatment, required to porate the cell were obtained from (1)-(5). The definite integral in equation (2) was calculated numerically by using Gaussian quadrature technique.

For calculations, the following set of parameters: $\nu = 10^{11}$ s^{-1} [12], $a_l = 0.6$ nm^2 [12], $C_m = 1$ μF/cm^2 [13], $\varepsilon_m = 2$ [14], $\varepsilon_w = 81$, $T = 295$ K, $\Delta\Phi_0 = 10$ mV for human erythrocytes and 40 mV for the rest cells, and $\tau_c = 0.3$ μs for human erythrocytes and 1.0 μs for the rest cells.

2.2 Media and Chemicals

A a medium for electroporation of human erythrocytes, a 9:1 mixture of 154 mM sodium chloride or sodium phosphate buffer (138 mM NaCl, 15 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) and isotonic (272 mM) sucrose solution was used. The sucrose solution was a 9:1 mixture of phosphate-buffered and isotonic (272 mM) sucrose solutions.

The cell culture medium was Dulbecco's modified Eagle's medium (cat. no. D5546, Sigma-Aldrich Chemie, Steinheim, Germany) supplemented with 10% fetal bovine serum (F7524, Sigma-Aldrich Chemie), 1% L-glutamine (G7513, Sigma-Aldrich Chemie), 100 U/ml penicillin, and 90 μg/ml streptomycin (P0781, Sigma-Aldrich Chemie).

Calibration solutions containing 0.2–100 mM KCl were prepared by diluting a stock solution of 100 mM KCl and adding 150 mM sodium chloride and 8 mM sodium benzoate [15].

2.3 Cells

Experiments were performed *in vitro* with two non-tumor cell lines (human erythrocytes and Chinese hamster ovary (CHO) cells) and two tumor cell lines (mouse hepatoma MH-22A and rat glioma C6 cells).

Blood was collected on heparin and the erythrocytes were isolated by centrifugation. The plasma and buffer coat comprising the white blood cells and platelets was carefully removed by aspiration. The cells were then washed three times with isotonic sodium chloride solution (0.9% NaCl) and suspended in the electroporation medium at a volume concentration of 1 or 9%. Erythrocytes were electroporated within the day of preparation.

CHO, MH-22A, and C6 cells were grown in monolayer cultures in 75-cm^2 (250 ml) flasks (Greiner Bio-One, Frickenhausen, Germany) at 37 °C in a humidified 5 % CO_2/95 % O_2 atmosphere in incubator IR AutoFlow NU-2500E (NuAire, Plymouth, MN, USA). Manipulations under sterile conditions were done in vertical laminar flow cabinet Aura Vertical SD4 (BIOAIR Instruments, Siziano, Italy).

When cells reached confluence, they were trypsinazed for 2–10 min with 2 ml of 0.25% trypsin-0.02% ethylenediaminetetraacetic acid (EDTA) solution (cat. no. T4049, Sigma-Aldrich Chemie). When cells detached from the flask bottom, cell suspension was supplemented with 2 ml culture medium to protect cells from further action of trypsin. After centrifugation of the suspension for 5 min at 1000 rpm at room temperature, cells were resuspended in the culture medium at approximately 2–5×10^7 cells/ml and kept for 60–70 min at room temperature (20–21 °C). During this time, the cells restored the normal level of the intracellular concentration of potassium ions [15]. Then the cells were electroporated within 15–20 min.

2.4 Cell Electroporation

A 50-μl droplet of cell suspension was placed between a pair of flat stainless-steel electrodes and subjected to a single square-wave electric pulse. The distance between the electrodes was 2 mm. The pulse duration was varied from 95 ns to 2 ms and the electric field strength – from 0.6 to 15 kV/cm for erythrocytes and from 0.2 to 7.5 kV/cm for CHO, MH-22A, and C6 cells.

2.5 Determination of Cell Electroporation

The fraction of electroporated cells was determined from the extent of the release of intracellular potassium ions [15]. After the electric pulse, cells were immediately transferred to a chilled Eppendorf tube, kept on ice for 5–10 min, and then kept for 30–40 min at 10–11 °C to prevent pores from closing and to allow equilibration between intracellular and extracellular K^+ concentrations. The extracellular potassium concentration was measured by means of a mini K^+-selective and reference electrodes (Diamond Micro Sensors, Ann Arbor, MI, USA) [15]. Potential measurements were made with a pH-meter-millivoltmeter pH-150M (Gomel Factory of Measurement Instruments, Gomel, Belorussia).

3 RESULTS

First, the dependences of the fraction of electroporated cells on the pulse intensity were obtained for the cells exposed to single square-wave electric pulses with the durations of 0.02–2 ms. These dependences obtained for mouse hepatoma MH-22A cells are shown in Fig. 1A. It can be seen that increasing the intensity or the duration of the electric field pulse increased the fraction of electroporated cells (Fig. 1A).

From the relationships of the fraction of electroporated cells on the pulse amplitude obtained at different pulse durations, the pulse amplitude inducing electroporation of 50% of cells, $E_{50\%}$, can be estimated for each pulse length. This way, the dependence of $E_{50\%}$ on the pulse duration can be obtained [17].

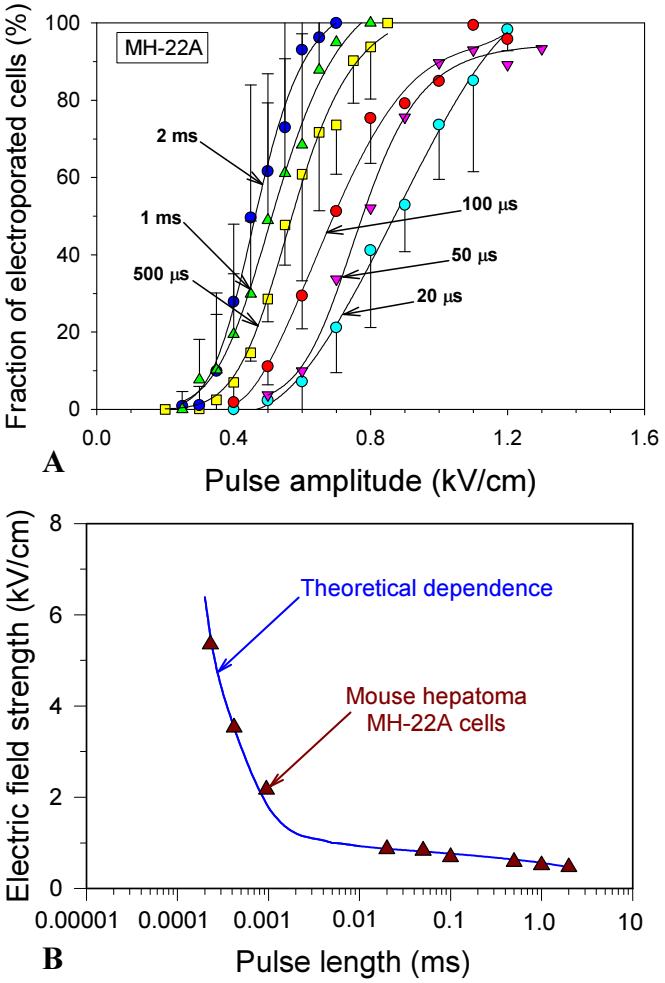

Figure 1: **(A)** The dependences of the fraction of electroporated mouse hepatoma MH-22A cells on the amplitude of a square-wave electric field pulse for various pulse durations. **(B)** Comparison of the theoretical dependence of the electric field strength required to create a single pore in the cell (solid line) with the experimental dependences of the electric field strength required to electroporate 50% of mouse hepatoma MH-22A cells on the pulse duration (triangles). Cell electroporation was determined from the release of intracellular potassium ions [15,16].

Such a dependence obtained for mouse hepatoma MH-22A cells is shown in Fig. 1B. It can be seen that the cell poration time depends on the pulse duration: the shorter the pulse length, the higher the field strength should be (Fig. 1A). This dependence is much more pronounced for short pulses ($\tau_i < 10\ \tau_m$). For long pulses ($\tau_i > 10\ \tau_m$) the electric field strength required for the poration of cell membranes increases significantly slower with decreasing the pulse length (Fig. 1B).

The average cell radii were measured for each cell line. The average radius was 3.0 µm for human erythrocytes, 6.8 µm for rat glioma C6 cells, 7.0 µm for Chinese hamster ovary cells, and 7.7 µm for mouse hepatoma MH-22A cells. When the cell radius is known, the transmembrane potential induced by the external electric field can be estimated [10,17]. The obtained values of the transmembrane potential were in the range of 480–930 mV and decreased with increasing pulse duration. The obtained dependences of the transmembrane potential required to electroporate 50% of cells on the pulse duration were close to each other for all cell lines studied.

Then theoretical dependencies of the electric field strength required to create a single pore in the cell calculated by using Eqns (1)–(6) were compared with the experimental data, as illustrated in Fig. 1B for MH-22A cells. By obtaining the best fit, the energy barrier for hydrophilic pore formation at zero transmembrane potential, $\Delta W_f(0)$, and the pore radius corresponding to the top of this barrier, r_*, were estimated for each cell line. The optimal parameters were found using the least-squares method. The best fit for was obtained with $\Delta W_f(0) = 41\ kT$, and $r_* = 0.3$ nm for human erythrocytes, $\Delta W_f(0) = 41\ kT$, and $r_* = 0.3$ nm for rat glioma C6 cells, $\Delta W_f(0) = 42\ kT$, and $r_* = 0.35$ nm for Chinese hamster ovary cells, and $\Delta W_f(0) = 41.5\ kT$, and $r_* = 0.3$ nm for mouse hepatoma MH-22A cells.

4 CONCLUSION

The estimated values of the energy barrier and the critical pore radius were close to each other for erythrocytes, CHO, mouse hepatoma MH-22A, and rat glioma C6 cells and were in the range $\Delta W_f(0) = 41–43\ kT$, and $r^* = 0.3–0.35$ nm.

5 ACKNOWLEDGEMENTS

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