

## Abstract

Irreversible electroporation (IRE) is the irreversible permeabilization of the membrane through the application of a series of electrical field pulses from micro-to millisecond duration. IRE induces lateral tension in the membrane of giant unilamellar vesicles (GUVs) of sizes similar to biological cells. We investigated the effects of NaCl concentration in the buffer solution on the IRE induced rate constant of pore formation in the membranes of GUVs. The GUVs were prepared by a mixture of anionic lipid dioleoylphosphatidylglycerol (DOPG) and neutral lipid dioleoylphosphatidylcholine (DOPC) in a buffer (10 mM PIPES, pH 7.0 and 1 mM EGTA) containing 62, 162 and 262 mM NaCl concentration using the natural swelling method. The IRE signal (pulsating DC) of frequency 1.1 kHz was applied to the GUVs through a gold coated electrode system. The time course of the fraction of intact GUVs among all the examined GUVs was fitted with a single exponential decay function from where the rate constant of pore formation was calculated. The values of rate constant were obtained  $(1.7 \pm 0.1) \times 10^{-2}$ ,  $(5.4 \pm 0.4) \times 10^{-2}$  and  $(1.6 \pm 0.4) \times 10^{-1} \text{ s}^{-1}$  at electric tensions 4.5, 5.5 and 6.5 mN/m, respectively for 62 mM NaCl concentration in the buffer. The values of rate constant were obtained  $(1.4 \pm 0.2) \times 10^{-2}$ ,  $(4.6 \pm 0.7) \times 10^{-2}$  and  $(1.0 \pm 0.6) \times 10^{-1} \text{ s}^{-1}$  at 6.5, 7.0 and 7.5 mN/m, respectively for 262 mM NaCl concentration. The similar tendency of increasing the rate constant with electric tension was obtained for 162 mM NaCl concentration in the buffer solution. Therefore, the rate constant of pore formation increased with the increase of membrane tension. However, the decreased of NaCl concentration in buffer solution showed the higher rate constant of pore formation at a particular tension. It means that the decreased of NaCl concentration in the buffer solution increase the mechanical stability of membranes. The tension dependent rate constant pore formation was fitted to the theoretical equation and obtained the line tension of membranes, which increased with the decrease of NaCl concentration in buffer. The decrease in the energy barrier of a prepore state with the decreased of NaCl concentration in buffer solution is the main factor to increase the rate constant of pore formation in the membranes of GUVs.