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Measurement of the permeability and resealing time constant of the electroporated mammalian cell membranes

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Abstract

In this study a new method is presented for measuring the transient permeability of mammalian cell membranes to sugar and electrolyte molecules based on the volumetric response of cells subjected to electroporation. The time constant of membrane resealing was determined independently by flow cytometry using a fluorescent dye as the reporter molecule. The volumetric and dye uptake data were analyzed with a model relating the cell volume changes to the solute transport across the reversibly permeabilized cell membrane. The experimental approach developed here might be useful for estimating the amount of electroinjected molecules, which are difficult to measure directly.

Keywords: Membrane permeability; Electropermeabilization; Saccharide; Cryoprotectant

1. Introduction

The disaccharide trehalose is found at high concentrations in organisms that are capable of withstanding harsh environmental stresses including dehydration and cold. This cryo- and lyoprotectant is increasingly being exploited in biotechnology as a very efficient stabilizer of frozen and dry macromolecules, phospholipid membranes and whole cells. The disaccharide is required on both sides of the cell membrane for maximum protection efficiency [1–3]. Depending on the cell type, 150–200 mM intracellular trehalose is necessary for improved survival of frozen mammalian cells after thawing [4]. In contrast to conventional membrane permeable cryoprotectants, such as glycerol and dimethyl-sulfoxide (DMSO), the permeability of mammalian cell membranes to trehalose is extremely low. Therefore, efficient techniques for the intracellular delivery of this natural cryoprotectant are necessary.

Electropermeabilization (also known as electroporation or injection) provides a well-proven tool for gene and cytosol manipulation via the introduction of various membrane-impermeable xenomolecules (such as drugs, hormones, proteins, plasmids, etc.) into living cells as well as the controlled release of intracellular substances. Electropermeabilization is based on the temporary increase of the membrane permeability due to reversible electric breakdown of the plasma membrane upon application of external high-intensity field pulses of very short duration [5]. This field pulse technique has gained common acceptance because it is more controllable, reproducible and efficient than alternative (chemical or viral) methods [6–8]. In the field of biomedical cryopreservation, the electroporation technique appears to be the method of choice for efficient introduction of membrane impermeable cryoprotectants (such as trehalose) into fragile mammalian cells.

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Nomenclature

a	cell radius
C	concentration of species (mol/m^3)
E	electric field strength (V/cm)
E_{cri}	critical field strength to induce breakdown voltage of ~1 V across the membrane
J	flux through plasma membrane
K_a	equilibrium constant of PI binding to mammalian cell DNA ~200 m^3/mol
M	intracellular (i.e., nucleic acid) binding site for PI
N_0	total number of PI binding sites in single Sp2 cell ~10 fmol/cell
n_k	number of cells with fluorescence intensity PI'_k
P	effective membrane permeability (m/s)
Pi	amount of PI bound to nucleic acid per cell (mol/cell)
P_0	effective initial membrane permeability immediately after electropulsing
PI′	fluorescence intensity of PI bound to nucleic acids
S_0	cell surface area ($\sim 4\pi a^2$) in isotonic 300 mOsmol/kg medium
S_{cell}	projected area of a single cell after electropulsing (time dependent)
S_{cell0}	projected cell area ($\sim 2\pi a^2$) before electropulsing
t	time (s)
V_0	isotonic cell volume $\sim 4/3\pi a^3$
V	cell volume after electropulsing (time dependent)
β	normalized cell volume, V/V_0
δ	pulse length (μs)
τ	resealing time constant (s)

Subscripts

l	living cell
d	dead cell
i	non-electrolyte (i.e., trehalose or inositol)
e	electrolyte (i.e., KCl)
PI	propidium iodide
0	initial value

Superscripts

i	intracellular
o	extracellular

To quantify the intracellular concentration of electroinjected trehalose and also to design optimal pulsing conditions for trehalose delivery into cells, experimental approaches to measuring the membrane permeability to the disaccharide after electroporation are necessary. The amount of intracellular trehalose or other sugars taken up by electroporation of small-sized mammalian cells is difficult to determine directly in a non-invasive way. Although a measuring method of membrane permeability after electroporation was reported by authors [9], it involves fluorescent labeling of substances, which is not possible in the case of small disaccharide molecules. Another method reported by Russo et al. [10] involves the labeling of carbon in sucrose with radioisotope and the radioactivity measurement to estimate the amount of intracellular sucrose. This method, however, requires complicated technique and high cost.

Here we report a simple method for measuring the transient membrane permeability to sugar molecules based on the volumetric response of mammalian cells subjected to reversible electropermeabilization. Until now the volumetric method has been widely used to determine the membrane permeability to permeable cryoprotectants [11,12]. An advantage of the volumetric method is that it can be performed in laboratories without access to radioisotope equipment. In this study, mouse myeloma cells (Sp2 line) were suspended in pulse media containing either 300 mM sugar (trehalose or inositol) and KCl (5 or 25 mM) as the major osmoticum and electrolyte, respectively. All pulse media also contained 25 μM fluorescent dye propidium iodide (PI), which was used to determine the resealing time constant of the plasma membrane. The cells were subjected to single electric pulses of moderate field strengths. The volumetric response of electroporated cells was measured and analyzed with a theoretical model that allowed the quantitative evaluation of the temporal changes in the intracellular sugar and electrolyte concentrations. The analysis revealed that the permeability of trehalose through the electropermeabilized cell membrane was lower by about a factor of ten than that of inositol and KCl. Nevertheless, the cells could be loaded with few hundreds mM trehalose without significant loss of cell viability.

2. Measuring methods and theories

2.1. Measurement of relaxation time of membrane resealing and membrane permeability to PI after electric pulse application

2.1.1. Fluorescent flow cytometry of intracellular PI

The murine myeloma cell line Sp2/0-Ag14 which was cultured in RPMI 1640 complete growth medium (CGM), supplemented with 10% (v/v) fetal calf serum

(FCS; PAA, Linz, Austria), at 37 °C under 5% CO_2 was used as the sample cell. Isotonic trehalose or inositol medium contained 300 mM trehalose or inositol (Sigma, Deisenhofen, Germany) and 5 mM (= 0.84 mS/cm) or 25 mM (= 3.08 mS/cm) KCl as the major osmoticum and electrolyte, respectively. Osmolarity and conductivity were determined to be 300 mOsmol/kg and 0.9–1.1 mS/cm (at 22 °C) by means of a cryoscope (Osmomat 030, Gonotec, Berlin, Germany) and a conductivity meter (Knick, Berlin, Germany), respectively. pH of the medium was about 7.3. Cell diameter, $2a$, was 13–15 μm which was measured using an electronic Coulter-type particle counter (CASY 1: Schärfe system, Germany). All the pulse mediums were supplemented with 25 μg/ml (≈30 μM) propidium iodide (PI, Sigma, USA) as the fluorescent reporter molecule. This membrane impermeable cationic dye reveals strong red fluorescence after binding to nucleic acids. PI served two purposes: (1) As a vital stain it distinguished living cells from dead ones. (2) As an indicator of the transient and reversible electropermeabilization it allowed the determination of the resealing time constant from the temporal changes of the membrane permeability to PI. To keep the cell viability higher than 80%, moderate pulsing conditions given in Table 1 were used according to our earlier results with this cell line [13].

Before field pulse exposure, about 10^6 cells/ml were suspended in 200–400 μl pulse medium and transferred into commercial electroporation cuvettes purchased from Eppendorf AG (Hamburg, Germany). Electric fields of 2–3 kV/cm intensity and 20 μs duration (Table 1) were applied to the two plane electrodes of the cuvettes. 50 μl of cell suspension was removed from the cuvette and diluted in 200 μl isotonic phosphate buffer solution—PBS (Sigma, USA) at around every 60 s intervals after field pulse application. PI staining of cells was analyzed in a flow cytometer Epics XL system (Beckman Coulter, Fullerton, CA, USA) equipped with a 15 mW 488 nm argon laser using a band pass filter of 675/15 nm. Cellular red fluorescence (RF) signals from samples containing about 5000 cells were presented as one-dimensional frequency histograms. Dilution of pulsed cells in the isotonic PBS decreases the PI influx into cells, which makes it possible to measure the amount of bound PI at different time intervals after pulse exposure. The short-term viability in electropulsed cell samples was evaluated 10–15 min after electropulsing. Because of a large difference in their RF intensity, the populations of viable and dead cells could easily be distinguished in the RF histograms, for detail see [14]. The viability was defined as the ratio of the count of weakly fluorescent living cells to the total cell number. Unpulsed cell suspension was used for the control of living cells, and the suspension including 0.2 wt% saponin (Sigma, USA), which totally permeabilizes plasma membrane, was used for the control of damaged cells.

Table 1
Estimated effective membrane permeabilities to saccharides, PI and electrolyte after electropermeabilization and the membrane resealing time constant of Sp2

	No.	KCl (mM)	Pulse intensity, E (kV/cm)	Pulse length, δ (μs)	Initial effective membrane permeability to trehalose, P_{sac} (m/s)	Initial effective membrane permeability to electrolyte, P_e (m/s)	Initial effective membrane permeability to PI, P_0 (m/s)	Resealing time, τ (s)
300 mM Trehalose	1	5	2	20	2.27×10^{-9} SE	6.74×10^{-9} SE	5.50×10^{-10} SE	265 SE 23.7
					5.67×10^{-10}	1.68×10^{-9}	3.36×10^{-11}	
	2	25	3	20	1.53×10^{-8} SE	2.15×10^{-8} SE	1.38×10^{-10} SE	289 SE 15.7
					1.26×10^{-9}	1.76×10^{-9}	5.86×10^{-12}	
	3	25	2	20	8.93×10^{-11} SE	4.39×10^{-9} SE	9.29×10^{-11} SE	191 SE 20.3
					1.71×10^{-10}	8.42×10^{-9}	8.57×10^{-12}	
300 mM Insitol	4	25	3	20			1.53×10^{-10} SE	283 SE 34.7
							1.33×10^{-11}	

2.1.2. Theory for estimating the resealing time constant (τ) and permeability of PI (P_{PI})

The theoretical analysis presented here accounts for the two steps involved in the cell staining with PI. These are (1) the diffusion-driven uptake of PI through the electropermeabilized cell membrane (Eqs. (1) and (2)); (2) PI binding to intracellular nucleic acids (Eqs. (4) and (5)), which gives rise to a strongly fluorescent complex. The model is based on the assumption that during resealing the permeability of the plasma membrane to PI, P_{PI}, decreases exponentially with time [15]:

$$P_{PI} = P_{PI0}\exp(-t/\tau). \tag{1}$$

The quantity P_{PI} represents the *effective* permeability of the electroporated plasma membrane to PI averaged over the whole cell surface.

The ruling equation for the flux of PI, J_{PI}, through the electroporated cell membrane during the resealing phase is

$$J_{PI} = \frac{d(C^i_{PI}V_0)}{S_0\,dt} = P_{PI0}\exp(-t/\tau)(C^o_{PI} - C^i_{PI}), \tag{2}$$

where $C^o_{PI} = 25$ μM is its external concentration.

Because the rate of resealing is not sensitive to the cell volume, the shrinkage of cells induced by electroporation (see below) was neglected in the analysis of the cell staining with PI. Assuming both V_0 and S_0 to be invariable, the following expression for the free intracellular PI concentration can be obtained by integrating Eq. (2) over time:

$$C^i_{PI} = C^o_{PI}\left[1 - \exp\left(\frac{S_0P_{PI0}\tau}{V_0}(\exp(-t/\tau) - 1)\right)\right]. \tag{3}$$

Equilibrium is further assumed for the binding of the intracellular PI to the nucleic acids:

$$PI + M \leftrightarrow PI\cdot M, \tag{4a}$$

$$K_a = \frac{[PI\cdot M]}{[PI][M]_{free}} = \frac{Pi}{C^i_{PI}(N_0 - Pi)}, \tag{4b}$$

where $[PI\cdot M] = Pi$ is the concentration of the bound (i.e., fluorescent) dye; $[M]_{free} = (N_0 - Pi)$ is the number of free bound sites. Eqs. (4) can be rearranged to obtain an expression for the bound intracellular PI:

$$Pi = \frac{K_aN_0C^i_{PI}}{1 + C^i_{PI}K_a}. \tag{5}$$

Substituting Eq. (3) into Eq. (5) gives the following result for the time-dependent concentration of the dye PI that (1) crossed the electropermeabilized cell membrane and (2) subsequently intercalated into the cellular DNA, which in turn gave rise to a strong fluorescent cell staining:

$$Pi = \frac{N_0C^o_{PI}K_a[1 - \exp\{(S_0P_{PI0}\tau/V_0)(1 - \exp(-t/\tau))\}]}{C^o_{PI}K_a - (1 + C^o_{PI}K_a)\exp[(S_0P_{PI0}\tau/V_0)\{1 - \exp(-t/\tau)\}]}. \tag{6}$$

Fitting Eq. (6) to the PI uptake data gave the estimates for two unknown parameters: τ and P_{PI0}.

Concentration of bound Pi can be defined from RF intensity of PI measured by flow cytometry:

$$Pi = N_0\frac{\sum_{k=l}^{d}\{(PI'_k - PI'_l)n_k\}}{(PI'_d - PI'_l)\sum_{k=l}^{d}n_k}, \tag{7}$$

where PI'_l stands for the mean RF intensity for living cell control; PI'_d stands for the mean RF intensity for damaged cell control. Essentially, Eq. (7) is a weighted mean of PI, which was normalized by living and damaged cell controls.

For these calculations, we used the value $K_a \approx 200$ m^3/mol for the equilibrium constant of PI binding to mammalian cell DNA reported in the literature [16,17]. The mean number of PI binding sites in Sp2 cells, $N_0 \approx 10$ fmol/cell, has been determined earlier by fluorimetric titration [18].

2.2. Measurement of membrane permeability to electrolyte, trehalose and inositol after electric pulse

2.2.1. Cell volumetry

The values of membrane permeability to electrolyte and saccharides were estimated from the kinetics of the cell volume change induced by electropermeabilization of Sp2 cells in isotonic saccharide media. Observations were made with a microscope (BX51 Olympus, Hamburg, Germany) using transmitted light to examine the volumetric cell response to electropulsing. Microphotographs were taken before and every 1 min after electropulsing using a high-resolution digital camera (ColorView 2, Soft Imaging System, Münster, Germany) attached to the microscope. These experiments were performed in a chamber consisting of two parallel cylindrical electrodes (see Fig. 1) that enabled microscopic observation of the electropulsed cells.

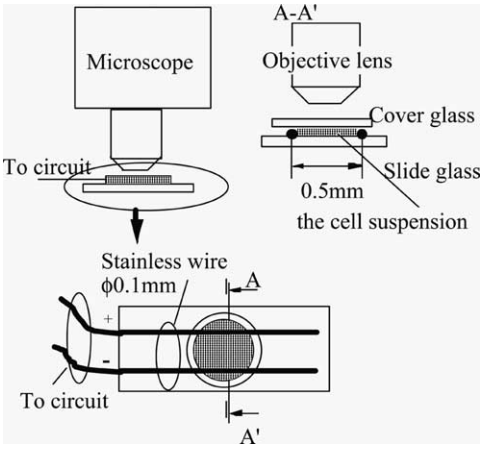

Fig. 1. Experimental setup for electropermeabilization and volumetric measurements under a microscope.

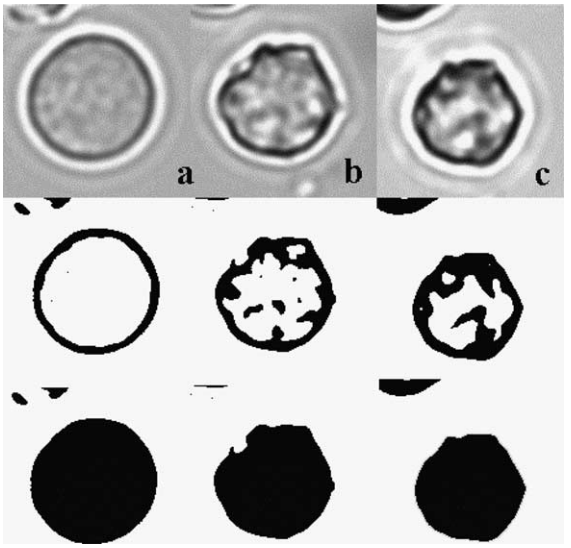

Fig. 2. Selected frames from a video sequence demonstrating cell shrinkage induced by a single electric field pulse of 3 kV/cm strength and 20 μs duration. Frames a, b and c show the same cell shortly before, 6 and 15 min after electropulsing, respectively. The middle and bottom rows illustrate the principle of cell sizing by evaluating the projected cell area.

A sample of cell suspension (5 μl, 10^5–10^6 cell/ml) was added to the chamber between the two electrodes, and a coverslip was placed over its center. The field strength was calculated as the applied voltage divided by the distance between the electrodes (Table 1). From the microphotographs taken before and at various time intervals after electropulsing, the volume of single cells in the course of time was determined from the projected cell area, assuming spherical geometry. The processed images in the middle and bottom rows of Fig. 2, highlighting the cell boundary and projected cross-area S_{cell}, respectively, illustrate the principle of cell size measurement by means of quantitative image analysis. The cell volume was normalized with the cell volume before electric pulsing as follows:

$$\beta = (S_{\text{cell}}/S_{\text{cell0}})^{3/2}. \tag{8}$$

Volume of 10–20 cells was measured for each condition. Volume change for intact cells was also measured as a control.

2.2.2. Theory for estimating membrane permeabilities to trehalose, inositol and electrolyte

Upon electropulsing in isotonic saccharide medium, myeloma cells changed their volume in course of time because of membrane permeabilization. To analyze this membrane transport effect, we used the following set of equations:

$$(C_{\text{e}}^{\text{o}} - C_{\text{e}}^{\text{i}}) + (C_{\text{i}}^{\text{o}} - C_{\text{i}}^{\text{i}}) = 0, \tag{9}$$

$$\frac{\mathrm{d}(C_{\text{i}}^{\text{i}}V)}{\mathrm{d}t} = P_{\text{i0}}\exp(-t/\tau)(C_{\text{i}}^{\text{o}} - C_{\text{i}}^{\text{i}})S_0, \tag{10}$$

$$\frac{\mathrm{d}(C_{\text{e}}^{\text{i}}V)}{\mathrm{d}t} = P_{\text{e0}}\exp(-t/\tau)(C_{\text{e}}^{\text{o}} - C_{\text{e}}^{\text{i}})S_0, \tag{11}$$

where Eq. (9) describes the osmotic equilibrium between the cytosol and suspending medium. Eq. (9) implies that the membrane permeability to water is much higher than its permeability to the osmolytes and that only two osmolytes, saccharide and electrolyte (KCl), maintain the osmotic equilibrium. Eq. (10) relates the flux of saccharide (subscript "i") across the membrane to the difference of its concentration $(C_{\text{i}}^{\text{o}} - C_{\text{i}}^{\text{i}})$ on the two sides of the membrane. Eq. (11) is the corresponding flux relation for the electrolyte KCl (subscript "e"). For simplicity, the reflection coefficients equal to unity are assumed for both osmolytes.

As with the PI electrouptake (treated in the former paragraph), the exponential terms $P_{\text{i0}}\exp(-t/\tau)$ and $P_{\text{e0}}\exp(-t/\tau)$ represent, respectively, the effective permeabilities of the electroporated plasma membrane to saccharide and electrolyte averaged over the cell surface. Since the electroporated cell membrane resealed within few minutes at room temperature, both permeabilities are assumed to decrease exponentially from their initial values P_{i0} and P_{e0}, with the same resealing time constant τ.

In order to solve the system of equations (9)–(11), the following assumptions were applied. The initial intracellular concentrations of saccharide $C_{\text{i}}^{\text{i}}(t \to 0) = 0$ and KCl C_{e}^{i} $(t \to 0) = 300$ mOsmol/kg. Because of the very low cell density used in this study, changes in osmolyte concentrations in the suspending medium were neglected: $C_{\text{i}}^{\text{o}} = 300$ mOsmol/kg and $C_{\text{e}}^{\text{o}} = 5$ or 25 mOsmol/kg (i.e., both external concentrations are invariable). The system of equations (9)–(11) was solved analytically as outlined below.

Combining Eqs. (9)–(11) leads to the following equation:

$$\frac{1}{P_{\text{i0}}}\frac{\mathrm{d}(C_{\text{i}}^{\text{i}}V)}{\mathrm{d}t} + \frac{1}{P_{\text{e0}}}\frac{\mathrm{d}(C_{\text{e}}^{\text{i}}V)}{\mathrm{d}t} = 0. \tag{12}$$

Substituting C_{e}^{i} in Eq. (12) by the expression $C_{\text{e}}^{\text{i}} = C_{\text{i}}^{\text{o}} - C_{\text{i}}^{\text{i}} + C_{\text{e}}^{\text{o}}$ (see Eq. (9)) gives

$$\frac{\mathrm{d}(C_{\text{i}}^{\text{i}}V)}{\mathrm{d}t} = -\frac{P_{\text{i0}}(C_{\text{i}}^{\text{o}} + C_{\text{e}}^{\text{o}})}{P_{\text{e0}} - P_{\text{i0}}}\frac{\mathrm{d}V}{\mathrm{d}t}. \tag{13}$$

Taking into account that at $t = 0$ the intracellular saccharide concentration is zero, $C_{\text{i}}^{\text{i}} = 0$, the integration of Eq. (13) over time t leads to

$$C_{\text{i}}^{\text{i}}V = -\frac{P_{\text{i0}}(C_{\text{i}}^{\text{o}} + C_{\text{e}}^{\text{o}})}{P_{\text{e0}} - P_{\text{i}}}(V - V_0), \tag{14}$$

where V_0 is the initial cell volume $V_0 = 4\pi a^3/3 = 1.4$ pl. Eq. (14) can be rewritten as

$$V = \frac{AV_0}{C_i^i + A}; \quad \text{with } A \equiv P_{i0}\frac{C_i^o + C_e^o}{P_{e0} - P_{i0}}. \tag{15}$$

Substitution of Eq. (15) into Eq. (11) and integration of the differential equation (Eq. (16)) over time gives finally an algebraic expression (Eq. (17)) for the time dependence of the volume of the transiently permeabilized cells during the resealing phase:

$$\frac{A^2V_0}{(C_i^i + A)^2}\frac{dC_i^i}{dt} = P_{i0}S_0\exp(-t/\tau)(C_i^o - C_i^i), \tag{16}$$

$$t = -\tau\ln\left[1 + \frac{A^2V_0}{P_{i0}S_0\tau}\left\{\frac{\beta - 1}{A(C_i^o + A)} + \frac{1}{(C_i^o + A)^2}\ln\left|\beta - \frac{A}{C_i^o}(1 - \beta)\right|\right\}\right]. \tag{17}$$

Since no explicit expression for the cell volume V as function of time could be achieved, the inverse function given by Eq. (17) was fitted to the volumetric data, such as shown in Fig. 4, using the least-square method (see Section 3).

3. Results and discussion

3.1. Relaxation time of membrane resealing and membrane permeability to PI after electropulsing

The kinetics of PI-uptake by electroporated cells together with the cell viability changes in isotonic 300 mOsmol/kg trehalose medium are shown in Fig. 3 (for experimental conditions, see Table 1). The highest PI uptake was observed in cells suspended in low-salinity medium (5 mM KCl) after application of a 2 kV/cm pulse of 20 μs duration (open squares in Fig. 3). Longer pulse durations (>20 μs) resulted in a marked decrease of cell viability to 60%. The P_{PI0} and τ values calculated by the least-square method are shown in Table 1. The continuous curves in Fig. 3 are analytical solutions of Eq. (6) fitted to the corresponding experimental data points (open symbols). For all cases, the fitted curves show good agreement with the experimental data ($R > 0.99$).

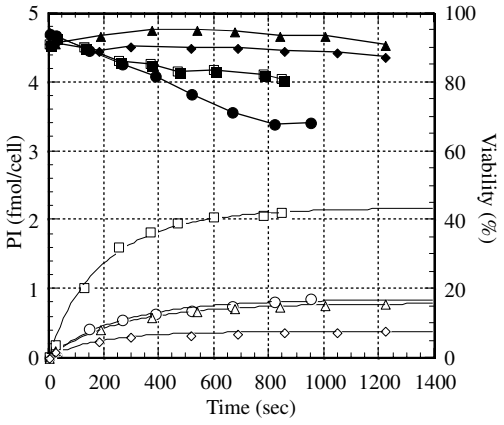

Fig. 3. PI uptake and viability of Sp2 cells suspended in 300 mOsmol/kg-saccharide solution after electropermeabilization. Filled symbols denote viability, and open symbols denote the amount of PI uptake. Squares, triangles, diamonds and circles denote condition Nos. 1–4 in Table 1, respectively.

The PI uptake by cells grew with increasing field strength (compare the data obtained with the 2 and 3 kV/cm pulses in 25 mM KCl given in Table 1 and Fig. 3). The dye uptake also grew with increasing resistivity of pulsing medium (compare the data obtained in 5 and 25 mM KCl media in Table 1 and Fig. 3), which is in good agreement with the earlier reported results [18]. However, the cell viability decreases as the electric field intensity increases [18]. It is interesting that the resealing time constant of Sp2 cells reduced with decreasing medium resistivity (compare the 5 and 25 mM KCl data in Table 1).

There is little difference in both τ and P_{PI0} values between the sample cells suspended in trehalose or inositol solution of the same conductivity and under the same pulsing conditions (3 kV/cm, 20 μs, see Table 1). This suggests an important fact that the type of saccharide affects neither the degree of electropermeabilization nor the resealing process. It is noteworthy that cell viability in trehalose solution (~90%) was significantly higher than in inositol (~70%). The ability of trehalose to improve cell survival after electropulsing has been reported earlier [14].

The PI uptake (Propidium cation: MW = 414) suggests that electropermeabilization of membranes also allows the uptake of the smaller membrane-impermeable sugar molecules such as inositol (MW = 180) or trehalose (MW = 342).

3.2. Membrane permeability to trehalose, inositol and electrolyte

Fig. 4 summarizes the volumetric behavior of Sp2 cells reversibly permeabilized by a single electric pulse (for the field parameters, see Table 1). Within about 10 min upon electropulsing, the cells suspended in trehalose medium shrank to 60–70% of their initial volume (Fig. 4, filled symbols). In contrast, the volume of cells electropulsed in inositol remained nearly unchanged within this time interval (Fig. 4, open circles). Unpulsed control cells did not exhibit any significant volume changes in both trehalose and inositol media (data not shown). The continuous curves through the filled symbols are the analytical solutions of Eq. (17), which were calculated using the fitted parameters given in Table 1.

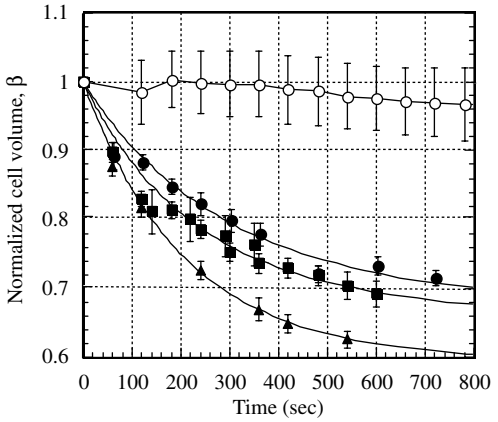

Fig. 4. Volume change of Sp2 cells suspended in 300 mOsmol/kg-saccharide after electropermeabilization The open symbols denote condition No. 4 in Table 1. Filled triangles, squares and circles denote condition Nos. 1, 2 and 3 in Table 1, respectively.

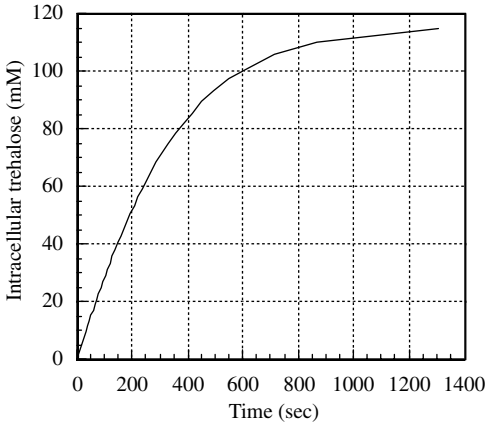

Fig. 5. Typical trehalose uptake kinetics calculated from the volumetric data using the model and τ values.

Taken together the PI-uptake data (Fig. 3) and the volumetric cell response (Fig. 4) revealed that electropulsing rendered the plasma membrane transiently permeable to both intracellular electrolyte (mainly KCl) and extracellular sugar molecules. Taking account of the concentration gradients across the plasma membrane (Fig. 4), the electric pulse caused the uptake of the extracellular saccharide by cells and the efflux of the intracellular electrolyte from the cytosol. As the osmotic equilibrium and the constant extracellular osmolarity are assumed in the proposed model, the cells shrink if the molecular efflux exceeds the influx, and swell if the uptake exceeds the outflow. The former condition corresponds to the case when the sugar permeability is higher than that of the electrolyte ($P_{i0} > P_{e0}$), and the latter corresponds to the case when the membrane permeability to electrolyte is higher than that to saccharide ($P_{i0} < P_{e0}$).

The observed shrinkage of cells suspended in trehalose (Fig. 4, filled symbols) indicates that the membrane permeability to trehalose was lower than that to electrolyte. In contrast, the absence of volume changes in inositol medium ($\beta \approx 1$, Fig. 4, open circles) suggests that the membrane permeability coefficients for inositol and electrolyte were equal ($P_{i0} = P_{e0}$). It has to be noted that the model given by Eqs. (15) and (17) cannot be used for calculating the permeability coefficients when the cell volume remains unchanged upon electropulsing (i.e., in the case of inositol and KCl).

Table 1 summarizes the membrane permeability coefficients for sugars and electrolyte together with the resealing time constants, τ. The kinetics of the intracellular trehalose concentration during the resealing phase can be calculated from the volumetric response data (β, Fig. 4) and the membrane permeability coefficients given in Table 1 by applying the following equation:

$$C_i^i = A(1-\beta)/\beta, \tag{18}$$

which is obtained by transforming Eq. (15). Fig. 5 illustrates a typical kinetics of the trehalose uptake by cells after application of a pulse (2 kV/cm, 20 μs) in trehalose medium containing 5 mM KCl. Under these conditions, about 100 mM were delivered into the cells by electropermeabilization.

4. Conclusions

In the present paper, the transient membrane permeability of electroporated murine myeloma cells (Sp2) was measured by means of cell volumetry. The time constant of membrane resealing was determined by flow cytometry using the fluorescent dye propidium iodide as the reporter molecule. The data were analyzed with a theoretical model that allowed the quantitative evaluation of the kinetics of intracellular sugar and electrolyte concentrations. We reached the following conclusions:

(1) The transient permeability of inositol and electrolyte (mainly KCl) through the electropermeabilized membrane of Sp2 cells were similar.
(2) The permeability of trehalose was lower by about a factor of 10 than that of inositol and KCl. Nevertheless, the cells could be loaded with few hundreds mM trehalose without significant loss of cell viability.
(3) The resealing time constant was independent of the sorts of saccharide.

The measuring method presented here might be useful for estimating the amount of electroinjected molecules, which are difficult to measure directly.

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