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Bioelectrorheological Model of the Cell. VI. Experimental Verification of the Rheological Model of Cytoplasmic Membrane

Piotr Pawlowski,* Irena Szutowicz,‡ Stefan Różycki,§ Jerzy Zieliński,‡ and Magdalena Fikus*
*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland, ‡Electrotechnical Research Institute, 04-703, Warsaw, Poland, and §Ministry of Environmental Protection, Natural Resources and Forestry, 00-922 Warsaw, Poland

ABSTRACT The susceptibility of the *Neurospora crassa* (slime) cellular membrane to electroporation and electrodestruction in an alternating electric field was further investigated. The results were analyzed according to the dynamic rheological model of the cytoplasmic membrane. Based on an analytical description of membrane susceptibility to electroporation, the rheological parameters of the foregoing process in *N. crassa* cellular membrane were found: they closely resemble those previously determined for the membranes' destruction. This suggests that both processes are temporally related and are induced within the same structures of the membrane. The dependence of the destruction of the membrane on the time of application and the frequency of the electric field was theoretically predicted and experimentally confirmed.

INTRODUCTION

In an external alternating electric field the cytoplasmic membrane is subjected to different types of mechanical stress generated by the electric field. Depending on the electrical and geometrical parameters either shear (Poznański et al., 1992) or extensil stress (Pawlowski and Fikus, 1993; Pawlowski et al., 1993) dominates in the system. The intensity of a given stress strongly depends on the location of the examined site (sector) on the spherical membrane with respect to field direction.

Whereas shear stress applied to the cell results in the deformation of the cell's shape, extensil stress leads to the transient and often reversible destabilization of the membrane, manifested as electroporation, electrofusion, and electrodestruction. It is obvious that the critical energy specific for each of the above phenomena has to be supplied to the system.

A rheological model of a viscoelastic shell endowed with mass was previously proposed for analysis of electroporation, electrodestruction, and electrofusion of mouse myeloma (Tib9) cells, *Neurospora crassa* (slime), and human erythrocytes. The susceptibilities of cells to poration, $s[p]$, and to destruction, $s[d]$, both defined as reciprocals of the extensil stress giving rise to poration, $\sigma_0^e[p]$, or destruction, $\sigma_0^e[d]$, have been calculated (Pawlowski et al., 1993).

We show that the above model could be applied as well for a quantitative description of electroporation of *N. crassa* spheroplasts within a wide range of field frequencies. The predictions of the model concerning the dependence of threshold stress for electrodestruction on time of field application under fixed field frequency were verified experimentally. Once again these results suggest the universal character of the model.

MATERIALS AND METHODS

N. crassa slime spheroplasts were used previously in our laboratory in various experiments on electric field effects (Fikus et al., 1985, 1987; Poznański et al., 1992).

In the present studies the experimental setup and the procedure used in the experiments were as previously described (Pawlowski et al., 1993). Electroporation was examined by the fluorescence of propidium iodide bound to the nucleus, as described by Marszalek et al. (1990). Cells were suspended in a 10% sorbitol solution and were subjected to 2-s pulses of the periodic electric field in the range of frequencies of 10^2–10^5 Hz.

In electrodestruction experiments under fixed field frequency the field amplitude was applied within the range 5–20 $V_{p\text{-}p}$, and the field action was studied within the range of t = 2–60 s.

The dependence of the probability of electroporation on field amplitude was established, and for fixed field frequency the stress value, $\sigma_0^e[p]$, resulting in poration of 50% of cells was estimated. Subsequently, mean values of susceptibility to poration, $1/\sigma_0^e[p]$, were calculated.

Similarly, the stress, $\sigma_0^e[d]$, resulting in 50% probability of cell destruction was estimated, and corresponding mean values of susceptibility to destruction, $1/\sigma_0^e[d]$, were calculated.

In an analytical conversion of the potential applied to the electrodes into the mechanical extensil stress, σ_0^e, generated on cell "poles" and into the corresponding susceptibility ($1/\sigma_0^e$) of the membrane to electroporation and electrodestruction, use was made of the electrical (field frequency and amplitude, dielectric permittivities and electric conductivities of media) and geometrical (initial cell radius, dimensions of the measuring chamber) parameters of the system (cf. Figs. 1 and 2 and Pawlowski et al., 1993).

RESULTS AND DISCUSSION

Electroporation

The results were analyzed as previously described (Pawlowski et al., 1993). Preliminary computer simulations were aimed at the reduction of errors in the final fitting of the model to the experimental data obtained for electroporation of *N. crassa* slime cells (Fig. 1). As a result, independent combinations of physical parameters of the model were obtained (Table 1). It was assumed that K_{eb} (∞) = K_{eb} (0) and that m, a measure of

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Address reprint requests to Dr. Magdalena Fikus, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland. Fax: 49-39121623; E-mail: magdaf@ibbrain.ibb.waw.pl.

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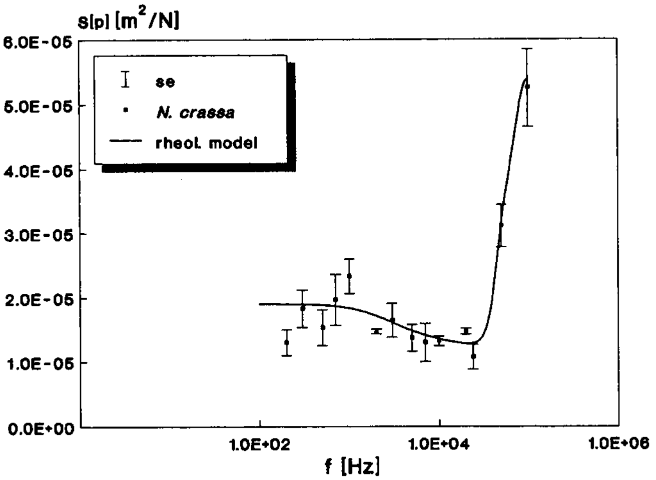

FIGURE 1 Electroporation susceptibility $s[p] = 1/\sigma_0^e[p]$ of *N. crassa* cells exposed to 2-s pulses of alternating electric field; data for 80 cells. Propidium iodide 2 μM, membrane thickness 9×10^{-9} m. Conductivity and dielectric permittivity of the external medium were 0.012 S/m and 80, those of the membrane were 0 S/m and 7.9, and those of the internal medium were 0.229 S/m and 45, respectively (Fikus et al., 1987). Data were evaluated by analysis of the distribution of the density of probability of electroporation, as described by Pawlowski et al. (1993).

membrane inertia, is independent of the type of the analyzed process (electrodestruction and electroporation); constant values of the resonance frequency, $f_r = 10^5$ Hz, and $n = 4$ were assumed.

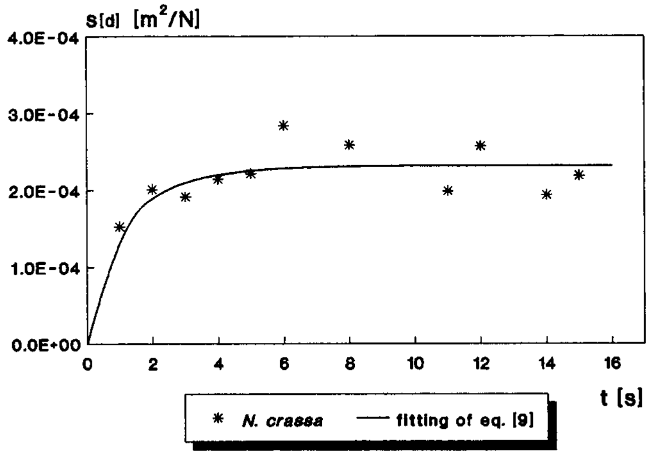

FIGURE 2 Temporal characteristics of susceptibility to electrodestruction $s[d] = 1/\sigma_0^e[d]$ of *N. crassa* cells exposed to a periodic electric field. The conductivity of the external medium (10% aqueous solution of sorbitol) was 5 mS/m; other parameters were as in Fig. 1. Data for 45 cells. Each point represents the averaged susceptibility at a given duration of predestruction field action. The continuous curve represents theoretical predictions. (By analogy with previously investigated electroporation of Tib9 cells (Pawlowski et al., 1993), it can be assumed that the susceptibility of *N. crassa* cells to electrodestruction depends on the ionic strength of the medium. The low ionic strength of the sorbitol solution, like that used in the experiments illustrated in Fig. 2, may be affected by the presence of cellular debris in suspension. For technical reasons this was not directly measured in an experimental chamber.)

Fitting reliability was checked by the χ^2 test, which showed that the hypothesis of the consistency of the experimental data with theoretical predictions could not be rejected (significance level $\alpha = 0.001$).

Data presented in Table 1 prove that the values of rheological parameters of the *N. crassa* cellular membrane subjected to electroporation are fairly close to those previously found when the membrane's electrodestruction was investigated. Energy density that was estimated according

$$e[p] = \gamma_{\mathrm{init}} \frac{\Delta \approx S^{\max}}{S} [p], \tag{1}$$

where ($\Delta \approx S^{\max} [p]/S$ is the maximal value of the relative variations of membrane surface in the absence of heat production and $\gamma_{\mathrm{init}} \approx K_{\mathrm{eb}}$ (0), and by taking data from Table 1 amounts to $(1.05 \pm 0.65) \times 10^5$ J/m^3. It is lower than the chemical bond energy and the van der Waals interactions (calculated per one lipid molecule) but is adequate for that calculated for pore formation (radius $r = 2 \times 10^{-8}$ m) (Weaver and Powell, 1989).

Consistently with the rule of energy equipartition, heat supplied to the system does not favor any one of its degrees of freedom. However, those related to movements of molecules (regarded as a whole) within the membrane are important from the standpoint of membrane integrity. We used the data from Table 1 and applied the type of analysis developed earlier (Pawlowski et al., 1993). Then

$$e'[*] = \frac{K_{\mathrm{eb}}(0)}{2} \left[\frac{\Delta \approx S^{\max}}{S} [*] \right]^2, \tag{2}$$

$$\kappa'' = \frac{e'[*]}{e[*]} \alpha\kappa. \tag{3}$$

Under the assumption that $\kappa \simeq$ t (κ is the effective time of heat accumulation by the membrane and t is the duration of the experiment (=2 s)), the phenomenological value $1/\alpha$ was found to be $\simeq 7 \times 10^2$. This value, in qualitative approximation, can be compared with the number of atoms in a molecule that take part in thermal motions; thus it is of the order of magnitude expected for a lipid molecule.

Electrodestruction

It has been shown that under high electric field frequencies the balance of energy is dominated by heat and that under low frequencies work prevails (Pawlowski et al., 1993).

Thus, the model predicted that at low frequencies membrane susceptibility to destruction ($1/\sigma_0^e[d]$ or $s[d]$) is independent of the time of field action on the membrane. This theoretical prediction was checked experimentally at $f = 50$ Hz and at $f = 5 \times 10^3$ Hz. Under the full range of applied parameters (see Materials and Methods) there was no dependence of the susceptibility to destruction on the time of field action. The destruction process, if observed, manifested itself in the 2nd second from the start.

TABLE 1 Physical parameters of the *N. crassa* cell electroporation, estimated according to the rheological model of cellular membrane

Parameters	*N. crassa* Electroporation	*N. crassa* Electrodestruction	Tib 9 Electroporation
$\mathrm{Re}\{{}^{e}_{k}\}$ (S/m)	0.012	0.012	0.014
$\Delta \approx S^{\max}[p]/S$	$(1.66 \pm 0.59) \times 10^{-2}$	$(2.5 \pm 0.5) \times 10^{-2}$	$(8.0 \pm 1.8) \times 10^{-4}$
$e'[p]$ (J/m^3)	$(8.7 \pm 3.9) \times 10^{2}$	$(2.0 \pm 0.8) \times 10^{3}$	$(7.1 \pm 1.6) \times 10$
K_{eb} (0) (N/m^2)	$(6.3 \pm 1.7) \times 10^{6}$	$(6.3 \pm 1.7) \times 10^{6}$	$(2.2 \pm 0.5) \times 10^{8}$
η_{eb} (0) (Ns/m^2)	$(2.4 \pm 1.9) \times 10^{2}$	$(3.5 \pm 2.6) \times 10^{2}$	$(1.6 \pm 0.4) \times 10^{3}$
η_{eb} (∞) (Ns/m^2)	(5 ± 6)	(3 ± 3)	$(4.6 \pm 1.1) \times 10$
τ (s)	$(2.4 \pm 0.4) \times 10^{-6}$	$(4.2 \pm 0.4) \times 10^{-6}$	$(2.891 \pm 0.021) \times 10^{-6}$
κ' (s)	$(2.3 \pm 2.1) \times 10^{-5}$	$(1.7 \pm 0.9) \times 10^{-4}$	$(1.461 \pm 0.019) \times 10^{-6}$
m (kg/m)	$(8.6 \pm 1.1) \times 10^{-6}$	$(8.6 \pm 1.1) \times 10^{-6}$	$(2.6 \pm 0.6) \times 10^{-5}$
n	4	4	2

In experiments with *N. crassa* $f_r = 1 \times 10^{-5}$ Hz; in those with Tib9 cells $f_r = 2.31 \times 10^{-5}$ Hz. Columns 2 and 3 taken from Pawlowski et al. (1993) for comparison.

At high frequencies, when no potential element was considered, the susceptibility to destruction $s[d]$ is expressed as function of the period of time of field action:

$$s[d] = A(1 - \exp(-t/B)^{1/2}, \tag{4}$$

$$A = (\alpha B/e[d])^{1/2} \times \text{function}(K_{eb}(2\omega), \eta_{eb}(2\omega), m, \omega), \tag{5}$$

$$B = 1/\nu, \tag{6}$$

where K_{eb} (2ω) and η_{eb} (2ω) are the real parts of the complex elastic modulus and the modulus of viscosity of the membrane, respectively, $\omega/2\pi$ is the electric field frequency, and ν is the heat exchange rate.

Equation 4 was fitted to the experimental results on electrodestruction of *N. crassa* cells at a field frequency of 10^5 Hz, which is the resonance frequency for this and related processes (Pawlowski et al., 1993) (Fig. 2). The agreement between the theoretical curve and the experimental points was evaluated by Fisher's test. The statistical analysis showed that the assumption about the exponential character of cellular destruction under the conditions employed could not be rejected.

On the basis of the fitting procedure, the value of B was estimated. Subsequently, the rate of heat loss by the membrane was calculated from Eq. 6 to be 0.59 ± 0.22 (1/s).

The values of rheological parameters K_{eb} (0), η_{eb} (0), η_{eb} (∞), n, and τ calculated for *N. crassa* cytoplasmic membrane on the basis of its susceptibility to poration and destruction are so close (Table 1) that the assumption that both phenomena are induced in the same regions of the membrane, with poration preceding destruction, seems justified. This conclusion is further reinforced by the considerably different values of the above parameters determined by the same approach for cells of different origin, namely, mouse myeloma cells, Tib9 (Table 1).

The rheological model and the postulated kinetics of heat conduction in the membrane satisfactorily describe experiments on cellular destruction during various periods of time of electric field action. The experimentally determined ν value confirms the validity of the previous assumption about $\kappa \approx t$.

The results of the present experiments complement the data published earlier on electroporation and electrodestruction of cells (Pawlowski and Fikus, 1993; Pawlowski et al., 1993), supporting the notion about the general character of the proposed bioelectrorheological model of the cellular membrane.

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