Dielectric energy of orientation in dead and living cells of *Schizosaccharomyces pombe*

Fitting of experimental results to a theoretical model

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ABSTRACT Using the experimental data obtained with killed cells of *Schizosaccharomyces pombe* (1), we have formulated a theoretical model that is able to predict cell orientation for microorganisms with ellipsoidal or cylindrical shapes as a function of the frequency of the electric field and of the conductivity of the external medium. In this model, comparison of the difference in potential energy for both orientations parallel-perpendicular with the thermal agitation energy allows one to interpret the intervals where these orientations occur. The model implies that the conductivity of the cytoplasm is slightly higher than that of the external medium. This assumption is easy to understand taking into account that not all the intracytoplasmic material is released to the exterior during cell death.

INTRODUCTION

The techniques of cell fusion have acquired considerable importance not only because they are a good tool for understanding and interpreting fundamental questions in cell biology (the analysis of the dielectric properties of cells and membranes, etc.), but also because of their practical use in biotechnology. For example, conventional methods (fusions that employ chemical agents) can be improved by the use of electrofusion. Using electroporation it has also been possible to introduce exogenous molecules such as protein, DNA, and RNA into living cells without deterioration of cellular or membrane functions. This permits, for example, transformation with exogenous DNA cells and microorganisms that are refractory to conventional methods (2). However, a fundamental problem inherent to these techniques is that of situating the pair of cells to be electrofused in a predetermined orientation. Thus, the study of cell orientation in electric fields appeared as a central question.

Different authors (1, 3–7) have reported that living, nonspherical cells can orient in several directions in an alternating electric field. The experimental results of our team (1, 8) have shown that in the 1–20 MHz frequency range and for electrical conductivities of the medium between 1 mS/m and 25 mS/m, living cells always become oriented with their longest axis parallel to the field lines. By contrast, in a suspension of dead cells the orientation of the major axis may lie either parallel or perpendicular to the direction of the field, depending on the frequency of the field applied and the conductivity of the medium. In some cases both orientations may coexist (1). The systems studied by us have involved three bacteria (*Escherichia coli, Bacillus subtilis,* and *Bacillus megaterium*), a yeast (*S. pombe*), and a fungal spore (*Phycomyces blakesleeanus*), all of them with a cylindrical or ellipsoidal shape.

In the present work we offer theoretical results on the dependence of orientation on the frequency of the alternating field and on the electrical properties of the cells. The theoretical predictions interpret the experimental results obtained by our team in dead *S. pombe* cells fairly well.

MATERIALS AND METHODS

Yeast strains, growth conditions, dielectrophoretic set up, dielectric conditions, etc., have been described in detail in previous papers (1, 8–11). As relevant to the present work, we should mention that the *S. pombe* cells were killed by different methods: autoclaving at 121°C, permeabilization with toluene-ethanol mixtures (12), treatment with UV light and chemical reagents. In all cases the membrane appeared permeabilized as could be detected by the methylene blue vital staining technique (13). Viability was also assayed by plating cell suspensions onto solid YED and incubating at 24°C for 48 h.

THEORETICAL BACKGROUND

A nonspherical particle immersed in a medium with different dielectric properties tends to become oriented in the electric field. Due to the lack of spherical symmetry, the potential energy of the system will depend on the relative position of the particle with respect to the lines of the electric field. The stabilized equilibrium position will depend on the orientation for which the potential energy is minimal. If the particle were an ellipsoid in shape and its dielectric constants greater than those of the dielectric medium, this position would correspond to the one where the major axis of the particle would be aligned with the lines of the electric field. However, in real circumstances both the medium and the particle should be considered as dielectric but with losses; that is, it is necessary to take into account the electrical conductivity of the media. Then, in the formulation of the problem, complex magnitudes will arise; in particular, the dielectric constant will change from being a real magnitude (ϵ) to a complex number ($\tilde{\epsilon}$):

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$$\tilde{\epsilon} = \epsilon - j\frac{\sigma}{\omega}, \qquad (1)$$

where σ is the electrical conductivity, ω is the frequency of the field, and $j = \sqrt{-1}$. The dependence on energy will be a complex function of the frequency of the alternating field. In other words, in such cases the equilibrium position does not necessarily have to coincide with the major symmetry axis parallel to the direction of the field but rather will correspond to other positions.

Schwarz and co-workers (14) and Saito and co-workers (15) conducted theoretical studies on the stability of a given orientation for ellipsoidal particles with and without shells immersed in a medium with different dielectric properties. The direction of stable orientation is determined by the material involved and also by the frequency of the fields. The change in the stable direction on modifying the frequency and the conductivity of the medium may occur in principle either as a gradual change or as a sudden jump of 90°. Saito and co-workers (15), analyzed the stable orientation for ellipsoidal particles with and without shells by the minimum energy principle. According to their theory, turnover can only occur if the ellipsoidal particles have a shell of symmetrical composition and parameters of biological interest are considered. However, if the field intensities do not have sufficiently high values, this sudden jump may be blurred by friction on the microscope slide, thermal agitation, etc.

Starting from the expression for the potential energy stored in the system proposed by Schwarz and co-workers (14), Saito and co-workers (15) obtained a value for the potential energy of each of the orientations of a revolution ellipsoid with a shell representing a cell membrane (Fig. 1), given by:

$$U_i = \tfrac{1}{2}E^2\lambda_i \quad (i = p_a, p_e), \qquad (2)$$

subindex p_a refers to the case in which the direction of the principal axis coincides with that of the electric field and subindex p_e refers to the case in which the direction of the principal axis is perpendicular to the electric field. E is the strength of the electric field and λ_i depends on the geometry of the particle and on the electrical parameters through the equation:

$$\lambda_i = \frac{4}{3}\pi ab^2\epsilon_{ex}\left[\frac{\epsilon_{ex} - \epsilon_i}{\epsilon_{ex} - (\epsilon_{ex} - \epsilon_i)L_i} + \frac{B_i}{[\sigma_{ex} - (\sigma_{ex} - \sigma_i)L_i]^2 + [\epsilon_{ex} - (\epsilon_{ex} - \epsilon_i)L_i]^2\omega^2}\right] \qquad (3.1)$$

where B_i is:

$$B_i = \frac{2\sigma_{ex}\epsilon_{ex}(1 - L_i) + (\sigma_{ex}\epsilon_i + \sigma_i\epsilon_{ex})L_i}{\epsilon_{ex}^2 - (\epsilon_{ex} - \epsilon_i)\epsilon_{ex}L_i} \times (\sigma_{ex}\epsilon_i - \sigma_i\epsilon_{ex}), \qquad (3.2)$$

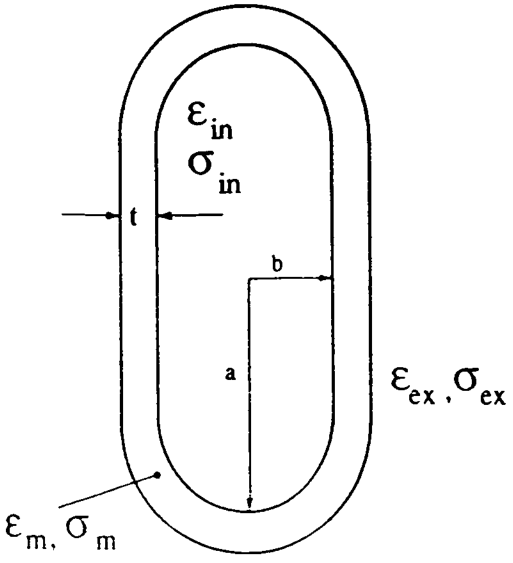

FIGURE 1 One-shell electric model of a revolution ellipsoid. The dielectric permitivity, ϵ, and electric conductivity, σ, in different phases are specified. The subindices: in, m, and ex denote, respectively, cell interior, cell membrane and suspending medium; a and b are major and minor axes of the ellipsoid, and t is the membrane thickness.

where a and b are the major and minor semi-axes, respectively, of the revolution ellipsoid. ϵ_i and σ_i (permitivity and electrical conductivity) are given by the real and imaginary part of:

$$\epsilon_i - j\frac{\sigma_i}{\omega} = \frac{(2 - e_iM_i)\left(\epsilon_{in} - j\frac{\sigma_{in}}{\omega}\right) + e_iM_i\left(\epsilon_m - j\frac{\sigma_m}{\omega}\right)}{e_iN_i\left(\epsilon_{in} - j\frac{\sigma_{in}}{\omega}\right) + (2 - e_iN_i)\left(\epsilon_m - j\frac{\sigma_m}{\omega}\right)} \times \left(\epsilon_m - j\frac{\sigma_m}{\omega}\right) \qquad (4.1)$$

$$M_a = \frac{2}{b^2}; \quad M_b = \frac{1}{a^2} + \frac{1}{b^2}; \quad N_a = \frac{1}{a^2};$$
$$N_b = \frac{1}{b^2}; \quad e_a = 2at; \quad e_b = 2bt, \qquad (4.2)$$

t is the thickness of the membrane and subindices (ex) and (in) refer to the exterior and interior of the cell, respectively; subindex (m) refers to the cell membrane.

RESULTS AND DISCUSSION

Expression 2 for the potential energy of each of the orientations can be used to determine the energy of different biological systems in an alternating electric field. In this work we shall study the theoretical results obtained with equation 2 for the energy when applied to killed cells of *S. pombe*. As an initial approximation, these cells can be considered (from a geometric point of view) as revolution ellipsoids with a shell representing a cell membrane.

The L_i parameters were calculated, by us, obtaining the following expressions:

$$L_b = \frac{ab}{a^2 - b^2}\left[\frac{a}{2b} - \frac{b}{4\sqrt{a^2 - b^2}} \ln\left(\frac{a + \sqrt{a^2 - b^2}}{a - \sqrt{a^2 - b^2}}\right)\right]. \quad (5.1)$$

$$L_a = \frac{b^2}{a^2 - b^2}\left[\frac{a}{2\sqrt{a^2 - b^2}} \ln\left(\frac{a + \sqrt{a^2 - b^2}}{a - \sqrt{a^2 - b^2}}\right) - 1\right]. \quad (5.2)$$

The numerical values used in the calculation programs compiled by us were as follows: geometric parameters (semi-axes of the ellipsoid) $a = 5\ \mu$m; $b = 1.13\ \mu$m, membrane thickness $t = 8$ nm. These parameters correspond to those of the real one of *S. pombe* and were obtained by measuring directly several images obtained with electron microscopy and calculating the mean values (16).

The electric parameters used were those customarily employed in cellular physiology (15, 17). Dielectric permitivity inside the cell was 60 ϵ_0; that of the membrane 11 ϵ_0 and that corresponding to the suspending medium, which was an aqueous salt solution, 78 ϵ_0, $\epsilon_0 = 8.85 \times 10^{-12}$ F/m being the permitivity of the free space. The electrical conductivity of the medium was controlled externally over the 1 mS/m–25 mS/m range. Additionally, the electrical conductivities of the membrane and of the cytoplasm are necessary for computing the energy of each orientation. However, we have no experimental results for these parameters and, to our knowledge, neither are their values to be found in the literature. To determine values for these conductivities, we shall use the experimental results found by our team for the dependence of orientation on the frequency of the electric field and on the conductivity of the external medium in killed *S. pombe* cells (1).

Our computer program provides the coordinates (conductivity of the external medium and frequency of the electrical field) for which the energy of the two orientations (parallel-perpendicular) are equal. This curve (line 0 in Fig. 2) divides the plane into two regions. Each of these corresponds to the orientation favoured by its lower energy. The rest of the curves in Fig. 2 correspond to points of equal energy difference between both orientations. Evidently, it is possible to obtain as many mappings (like those of Fig. 2) as pairs of values that one assigns to the electrical conductivities of the membrane and cytoplasm (σ_m, σ_{in}). For each value of these parameters we assigned an index of correspondence that relates the areas overlapped by the theoretical curve and the experimental curve obtained for the fitting of our data (1, 8). The calculation performed by the steepest descent or gradient method (18) provides an optimization of this index for the values: $\sigma_m = 5 \times 10^{-4}$ S/m and $\sigma_{in} = 4 \times 10^{-2}$ S/m when we choose a value of $\Delta U = 1.4 \times 10^{-19}$ J for an applied electric field of 10 KV/m (1).

In order to determine the sensitivity of the method we programmed line maps of this energy difference ($\Delta U = 1.4 \times 10^{-19}$ J) for different values of σ_m and σ_{in} (Fig. 3). In this way one can establish which modifications of one order of magnitude (10^{-3}–10^{-4}) in σ_m do not have a significant influence on the behavior of the model. By contrast, the effect of the variations on σ_{in} lead the selected value to being critical since a margin of 5×10^{-3} S/m in the conductivity value causes a variation in the result that is greater than those produced by variations in some of the other parameters employed, such as geometric and electric.

In Fig. 2 it may be seen that the qualitative agreement between the results obtained for $\sigma_m = 5 \times 10^{-4}$ S/m and $\sigma_{in} = 4 \times 10^{-2}$ S/m when lines $\Delta U = 1.4 \times 10^{-19}$ J are selected and our experimental results for *S. pombe* is noteworthy. In both, experimentally and with the computer, two clearly differentiated regions can be observed, one of them close to the ordinate axis (where the energy corresponding to the perpendicular orientation is noticeably lower than the parallel orientation energy) and the other region, close to the abcissa axis where the opposite is the case. The stable equilibrium position is that corresponding to the parallel orientation. Finally the region that, under experimental conditions, does not show any defined orientation is modelled by the region between the curves that determine an energy difference with orientations with a value lower than ΔU. This energy is higher than the thermal agitation energy $1/2kT$, where k is the Boltzmann constant and T is the absolute temperature corresponding to the rotation of the particle. This fact seems to show that, under our experimental conditions, other types of dissipative energies (scraping on the slide, convection, etc.) have some influence. This qualitative agreement is satisfactory if we take into account certain aspects that were not considered when we established our physical model of *S. pombe*. Geometrically, *S. pombe* is not very like an equifocal layered ellipsoid. Additionally, the inside the cell is not homogeneus (there is a nucleus, mitochondria, endoplasmic reticulum). However, this simplistic model, together with what has been reported in the work of Saito and co-workers (15) seem to be sufficient to explain, at least qualitatively, our experimental results on orientation with dead cells of *S. pombe*. More refined models, considering aspects that include energy dispersion in the medium such as those described by Sauer (19, 20), can undoubtedly be elaborated.

The orientation values obtained for dead *S. pombe* cells (Figs. 2 and 3) either experimentally or with our computer program take the same aspect as those described by Hatakeyama and co-workers (reference 21, see Fig. 8) for live cells of *Paramecia*. However, two main differences should be noted. The first is that with our experimental devices we were unable to achieve a perpendicular orientation to the field lines with living *S. pombe* cells. The second is that for dead cells we found values for mixed orientation that could also be predicted by our computer program.

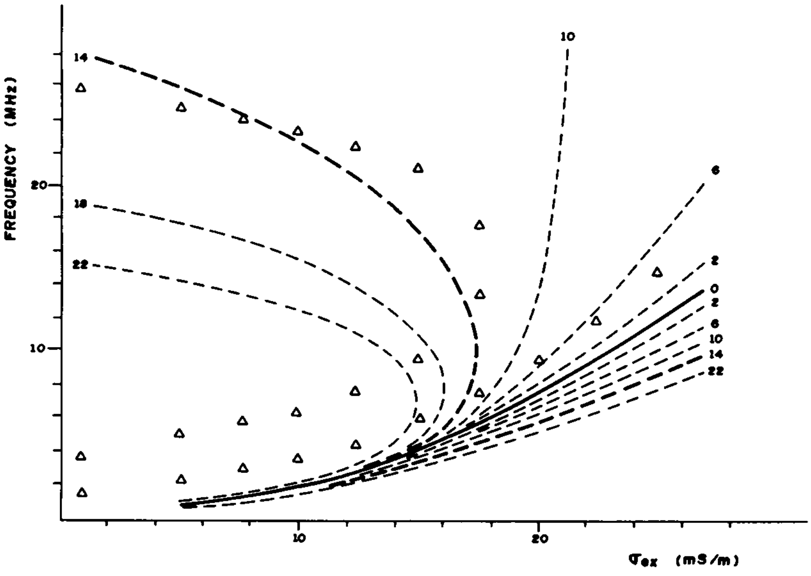

FIGURE 2 Computed orientation of nonliving cells of *Schizosaccharomyces pombe* and experimental results in an alternating electric field as a function of frequency and conductivity of the external medium. The solid line (——) indicates that the energy of the particles is the same in both the parallel and transversal orientation of the field. The broken lines (----) indicate identical energy differences between both orientations (2, 6, 10, 14, 18, and 22 × 10^{-20} J). The thick line (– – –) represents that corresponding to 14 × 10^{-20} J (best fit with our experimental results). (Δ) Experimental results.

It should be noticed that this good agreement between the theoretical and experimental values can only be achieved if a value of 4 × 10^{-2} S/m is chosen for the electrical conductivity inside the microorganism and a value of 5 × 10^{-4} S/m for the electrical conductivity of the cell membrane. The value of 5 × 10^{-4} S/m selected by the calculation program is higher than that used by Pohl for live yeasts (10^{-4} S/m) and by Hatakeyama and co-workers (21) for *Paramecia* (10^{-5} S/m). Our hypothesis is that in killed cells pores occur in the mem-

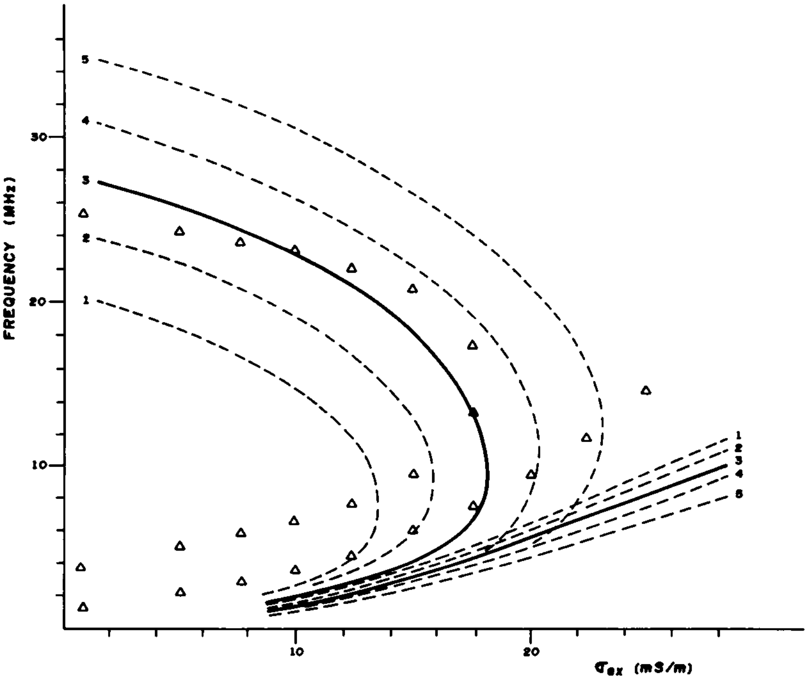

FIGURE 3 Lines of constant energy difference (14 × 10^{-20} J) for different internal conductivities. The thick line (——) represents that corresponding to σ_{in} = 40 mS/m (best fit of our experimental results). Lines: 1, (30 mS/m); 2, (35 mS/m); 3, (40 mS/m); 4, (45 mS/m); and 5, (50 mS/m). (Δ) Experimental results.

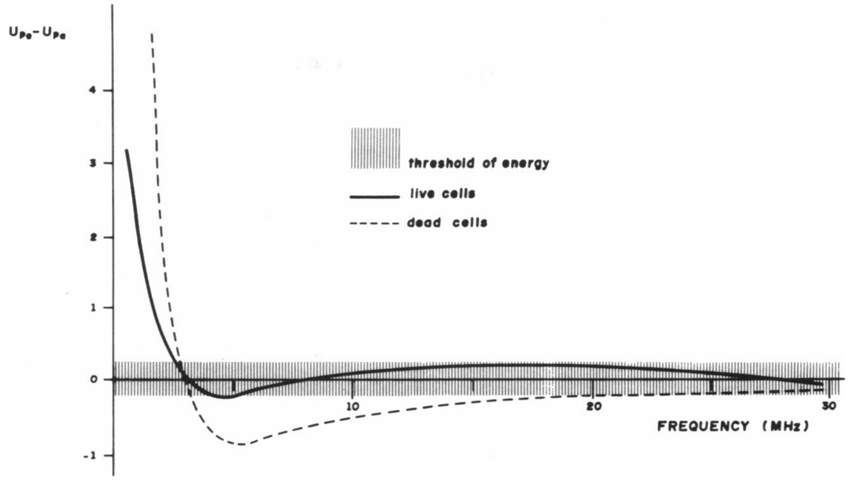

FIGURE 4 Computed energy difference between the parallel and perpendicular orientation as a function of frequency. The conductivity of the external medium is 10 mS/m (——) live and (----) dead cells of *Schizosaccharomyces pombe*. Shaded region corresponds to a threshold energy.

brane and cations may become bound to these. This would lead to ionic bonds that would make the membrane more conductive. On the other hand, the value of 4×10^{-2} S/m chosen for the conductivity of the cytoplasm is similar although slightly higher than that of the external medium but in any case much lower than that usually employed for the interior of living cells (1 to 10^{-1} S/m). Our hypothesis and results are coherent with the notion that permeabilization of the plasma membrane permits a passive flow of ions and metabolites that tends to equalize the conductivity of both media, inside (cell cytoplasm) and outside (suspending medium) (as an example, dead cells are permeable to methylene blue). At this point it could be emphasized that permeabilized cells, at least when dealing with the toluene-ethanol method, retain enzymes inside them that maintain their activity, as could be detected by the corresponding assay. Our data indicated that probably all kinds of molecules with molecular weights above 10,000 are retained in the cell cytoplasm (Domínguez, unpublished work). However, the alterations in the membrane and even those that may occur in the cell wall (e.g., a partial solubilization of galactomannan) permit dead cells of *S. pombe* to keep their shape, as confirmed by light and electron microscopy, (8).

Fig. 4 shows the differences in energies ($U_{Pe} - U_{Pa}$) between the transverse and parallel orientations as a function of frequency for live and dead *S. pombe* cells. The energies for each orientation were calculated according to the theoretical equation 2. For live cells, the values of the different variables participating in the energy equation 2 were taken the same as for dead cells, except regarding the conductivity of the membrane, 10^{-6} S/m, and the conductivity of the cytoplasm, 1 S/m (17). The conductivity of the medium was 10^{-2} S/m.

For live *S. pombe* cells (continuous line in Fig. 4), at frequencies below 3 MHz, there is a clear predominance of the orientation parallel to the electrical field. However, for values higher than this frequency and up to 30 MHz, the difference in energies does not surpass the threshold value corresponding to the thermal agitation energy and hence it is not possible to observe a net cellular orientation perpendicular to the field. This theoretical result could account for the fact that, within the frequency range assayed in our experimental measurements, no change in the orientation of live *S. pombe* cells was found. The fact that no reorientation of living cells is observed in the frequency range studied can therefore readily be attributed to the high conductivity of the cytoplasm, which is far higher than that of the external medium.

For comparative purposes, the discontinuous line shows the values computed for the energies calculated with electrical parameters corresponding to the same microorganism killed. As in the case of live cells, it is possible to observe a parallel orientation below 3 MHz. By contrast, the difference in energies reaches a negative value higher than the threshold in the frequency range between 3 and 30 MHz. The negative value of the difference in energies means that the preferred orientation is transverse or perpendicular to the electrical field lines. Such results obtained for dead cells obviously could have been extrapolated directly from Fig. 2.

In conclusion, our experimental results on cellular orientation with respect to the electrical field can be described theoretically as long as suitable values are chosen for the electrical conductivities of the cytoplasm and the cell membrane. This agreement, for the electrical conductivity of the cytoplasm in dead *S. pombe* cells, demands values slightly higher than that of the conductivity of the external medium and in any case far lower than that usually described for live cells. This suggests that membrane permeabilization permits a flow of metabolites that tends to become equal to the conductivities of both media. It is striking that the agreement achieved cannot be attained if, as suggested by Pohl, a conductivity exactly equal to that of the external medium is used in the calculation.

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