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Dielectric properties of yeast cells as determined by electrorotation

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Key words: Electrorotation; Cellular spin resonance; Spin resonance; Electric property; Dielectric spectroscopy; (Yeast)

Electrorotational spectra of yeast cells, *Saccharomyces cerevisiae* strain R XII, were measured over a frequency range of nearly 7 decades. The physical properties of distinct cell parts were simultaneously determined for individual cells by comparison with an electrical two-shell model: The conductivity of the cytoplasm, cell wall and cytoplasmic membrane of living cells were found to be 5.5 mS/cm, 0.1 to more than 0.5 mS/cm and less than 0.25 nS/cm to 4.5 μS/cm, respectively. The conductivity of the cytoplasmic membrane was dependent on the conductivity of the medium. Membrane behaviour is interpreted as an opening of membrane channels when the environment becomes more physiological. The specific membrane capacitance was determined to be 1.1 μF/cm² and the thickness of the cell wall was calculated as 0.11 μm. Heat treated cells showed an increased membrane conductivity of more than 0.1 μS/cm (at 25 μS/cm medium conductivity) and a drop in cytoplasmic conductivity to between 0.1 and 0.8 mS/cm, depending on the length of time the cells were suspended in low conductivity water (25 μS/cm), indicating a perforation of the membrane. A slightly decreased spinning speed scaling factor for dead cells suggests a modification to the cellular surface, while the principal structure of the cell wall appears to be uneffected. It can be demonstrated by these observations, that cellular electrorotation permits the simultaneous investigation of the different cellular compartments of individual cells in vivo under various environmental conditions.

Introduction

Cellular electrorotation [1–3] is a useful method for the non-invasive investigation of physiological phenomena such as fertilization, virus-cell interaction, cell fusion and the influence of drugs and toxins on individual cells. Since the development of electrodynamical models for electrorotation, in particular for the frequency range 10 Hz to above 1 GHz [4,5], measurements of distinct cellular compartments have been possible. In the majority of previous studies, only one of the two or three relaxations proposed has been investigated to reveal information about the cytoplasmic membrane. This is due to a restriction to field frequencies below 35 MHz [2]. The intention of the work described here was to extend the accessible frequency range of rotation experiments in order to investigate the validity of present electrorotation theories at higher frequencies. It has also been possible to carry out further investigation of cellular organelles from these experiments.

Materials and Methods

Cells of the yeast *Saccharomyces cerevisiae*, strain R XII, were used, because details about the properties of distinct cellular constituents are available from the literature and the observation of their rotation is facilitated by their ellipsoidal shape. Strain R XII was chosen as its metabolism is well known within this institute and its behaviour has been tested in many biophysical experiments. Cells were grown in a liquid medium ('complete medium': 10 g/l yeast extract, 5 g/l peptone, 20 g/l glucose) at 30°C and harvested in the stationary phase after 2 days. The suspension was diluted by 10^4 with double-distilled water, without the need for additional washing. The conductivity of the cell suspensions was adjusted mainly by the addition of ionic salts such as KCl and measured with a conductiv-

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ity meter (LF Digi 550, WTW, Weilheim, FRG). Dead cells were prepared by a stepwise dilution in double-distilled water including a step at 75°C for 5 min. The cells were observed under a microscope (Olympus BH 2) combined with a video camera (Philips EL 8000) and recorder (JVC HR 3660 EG).

The measuring chamber (Fig. 1) consisted of four spherical platinum electrodes in a square arrangement, dipped into a hanging drop of the cell suspension. By using spherical electrodes a more homogeneous field could be achieved than with straight electrodes [6], so reducing any lateral movement of cells caused by dielectrophoretic forces. The technique of the hanging drop was chosen for a number of reasons: Firstly, adhesive friction forces are avoided allowing a better quantitative interpretation of the experiments. Secondly, dielectrophoretic forces are partly compensated by gravitational ones due to the curvature of the drop. Thirdly, centering the cells is possible by displacing the cover glass, and fourthly, cleaning of the chamber is facilitated.

The phase shifted AC voltages are conveyed to the electrodes via four coaxial cables. To minimize standing waves the cables are terminated by 51 Ω resistors, and the length of the platinum wires is kept to 16 mm. To further reduce errors resulting from signal reflections, the voltage at one electrode was measured by a radio frequency rectifier mounted directly onto the microscope slide.

The application of several techniques was necessary to generate the four sinusoidal voltages, each phase shifted by 90°, over a frequency range of nearly 7 orders of magnitude [7]: allpass circuits using operational amplifiers, inductively coupled oscillators [8]; and phase shifts by a switchable RC bridge and delay lines. Electrical parameters such as amplitude, frequency, phase shift and harmonic distortion were measured using an oscilloscope (Philips PM 3266), up to about 50 MHz. Above this the frequency was measured with a digital counter (Digicount 312, HEB Digitaltechnik, Hemmingen, FRG), which had been modified to handle frequencies up to 1.2 GHz. The phase shifts were adjusted with a vector voltmeter (Hewlett Packard

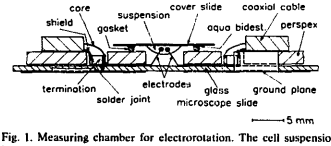

Fig. 1. Measuring chamber for electrorotation. The cell suspension hangs as a drop on the cover glass. A moistened rubber seal prevents evaporation of the drop. The impedance of the coaxial cables is terminated using 51 Ω resistors.

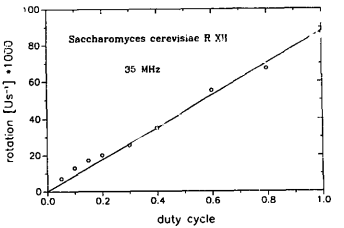

Fig. 2. Relation between the duty cycle of the modulated electric field and the observed rotation speed. Field frequency 35 MHz, frequency of the modulating square wave 125 Hz. The rotation is given in revolutions per second and for conveniency multiplied by 10^3.

8405A) and distortions measured by a spectrum analyzer (Tektronix A7704, plug-in unit 7L12), revealing amplitudes of the third and fifth harmonic of 3% and 2%, respectively, compared with the ground wave.

Results and Discussion

To prove the usually presumed direct relation between the torque exerted by a rotating electric field and the rotational speed of the object, the field was continuously switched on and off at a rate of 125 Hz and with a varying duty cycle (Fig. 2). The above relationship was found to be linear (correlation coefficient $r = 0.998$), implying that nonlinear effects such as adhesive friction were excluded by the hanging drop technique, even for rotation speeds of less than 0.5 revolutions per min.

The most detailed theories for electrorotation simplify the cell as a sphere surrounded by one [5] or two [4] concentric, homogeneous shells. Each compartment is defined by its thickness or diameter, conductivity and permittivity. In this work the two-shell model was chosen to analyse the experimental data since, in a first approximation, yeast cells can be assumed to consist of three compartments: the cytoplasm, the cytoplasmic membrane and the cell wall [9]. The theory proposes three relaxation peaks of the general form

$$N = \Sigma_k \{c_k \cdot f_{ck} \cdot [1 + (f/f_{ck})^2]^{-1}\}$$

(N: torque; k: 1,2,3: c_k: constant; f_{ck}: characteristic frequency) each defined by the two parameters size (c_k) and position (f_{ck}). So these six parameters allow maximal six cellular properties to be determined simultaneously from one electrorotational spectrum. For the calculations, the following relative permittivities were

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GHz [23,24]. Empirical equations for the corresponding frequency dependent conductivities and permittivities between 10 MHz and 18 GHz are given by Foster and Schwan [24]. Both this formula and the influence of the ion concentration on the permittivity of the aqueous compartments [25] were included into the theory to fit the experimental data (Table I). By taking into account values of the membrane permittivity at various frequencies [4,10,16], it was presumed that the permittivity logarithmically decreased by 50% from 1 kHz to 1 GHz. All these modifications lead to a reduction of the value for the cytoplasmic conductivity to 5.5 mS/cm, a value still much higher than those reported for impedance measurements. However, Foster and Schwan [24] report a similar increase in the conductivity of tissues and other biological materials by 4 to 5 mS/cm between 0.1 and 1 GHz.

The scaling factor, which was introduced to compare the absolute values of the experimental and theoretical rotation rates, strongly depends on the presumed electrical field strength at the location of the measured object and thus is influenced by the shape of the electrodes. For the centre of the chamber used in these investigations, a correction for the field strength by a factor of 0.76 relative to a homogeneous field was assumed according to the field distributions measured by Gimsa et al. [6].

The resulting scaling factor of 0.81 ± 0.05 for single cells can be explained by the increased hydrodynamic resistance due to their ellipsoidal shape (semiaxis ratio 0.7–0.8) instead of the spherical symmetry presumed in the Stokes approach of the theory. Furthermore, friction due to the roughness of the cell wall and bud scars are not considered in the models. Scaling factors of 0.6 and 0.9 can be supposed for other rotational experiments [15,16] taking into account, that the friction on the bottom of the chambers contributed to about 20% of the overall friction [16].

The thickness of the cell wall was found to be 0.11 μm, in good agreement with permeability measurements yielding 0.12 μm [26] and 0.16 μm [27,28]. Electron micrographs [29] reveal local variations in thickness of between 0.10 and 0.26 μm for the same cell, resulting in an average thickness of 0.13 μm, this may also vary with the cell's life cycle. When comparing these values it should be considered, that the effective wall thickness for electrical measurements may not be the same as that observed by electron microscopical staining. Also the surface of the cell wall is diffuse due to its composition of polysaccharides and proteins [9], so allowing the diffusion of inorganic ions [10]. Arnold et al. [12] calculated a wall thickness of 0.3 μm from its 15% contribution to the whole cell volume, but possibly omitted a factor of $\frac{1}{2}$, since, on repeating their calculations a yield of 0.16 μm was obtained.

The cell wall conductivity proved to be strongly dependent on the conductivity of the suspension, increasing from 0.1 mS/cm at a medium conductivity of 10 μS/cm to above 0.5 mS/cm at 300 μS/cm. For higher conductivities, the properties of the cell wall could not be determined, as its relaxation peak merges with the main positive one. Permeability measurements [27,28] as well as electrorotation experiments carried out by Geier et al. and by Aussieker [15,16] show a similar dependence. Since both Geier et al. and Aussieker presume a cell wall thickness of 0.3 μm, their values of the wall conductivity should be divided by about 2 for direct comparison. From Geier et al. [15] a cell wall conductivity of only 17 μS/cm occurs at a medium conductivity of 10 μS/cm. This is possibly due to the use of a different yeast strain and different

TABLE I

Physical properties of different cellular compartments of the yeast S. cerevisiae

Values were determined by comparing the electrorotation spectra with a modified two-shell model [4]. Errors reflect corresponding standard deviations. bc: budding cells, sc: single cells.

Parameter	Living cells		Dead cells [a]
	this work	other authors	
Cytoplasmic conductivity (μS/cm)	5500 ± 500	2300 [20] 2200–3500 [10]	100–800
Membrane conductivity (μS/cm)	$\leq 2.5 \cdot 10^{-4}$–4.5		> 0.1
Cell wall conductivity (μS/cm)	100– > 500	17 [15]; 130 [16]	300
Spec. membrane capacitance (μF/cm^2)	1.1	1.1 [10;16]	
Wall thickness (μm)	0.11 ± 0.02 (sc) 0.21 ± 0.03 (bc)	0.12 [26]; 0.16 [27;28]; 0.13 [29]	
Scaling factor	0.81 ± 0.05 (sc) 0.5–0.7 (bc) [b]	0.6 [15]; 0.9 [16] (sc)	0.6–0.8 (sc) 0.4–0.6 (bc) [b]

[a] At a medium conductivity of 25 μS/cm.

[b] Decreasing with increasing size of bud.

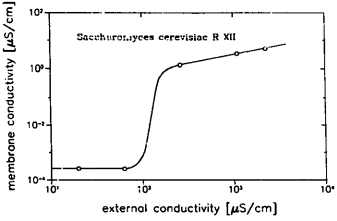

Fig. 5. Influence of the conductivity of the suspension on the membrane conductivity of cells of *S. cerevisiae* R XII in a log-log plot.

growth conditions. The measurements of Aussieker [16], which were carried out using the same yeast as in these experiments and under the same growth conditions, confirm our results, yielding a cell wall conductivity of 130 μS/cm at a medium conductivity of 10 μS/cm. The uncertainty of the determined cell wall conductivity and thickness are supposed to be about 50%, since the properties of the wall can be deduced only from the small asymmetry of the main positive rotation peak, nevertheless, the standard deviations are less than 20%.

The specific membrane capacitance is given mainly by the characteristic frequency of the anti-field rotation and was calculated to be 1.1 μF/cm^2 in accordance with other electrorotation experiments [16] as well as impedance measurements of yeast suspensions [10].

The size of the anti-field relaxation peak decreased with increasing medium conductivity. In the scope of the two-shell model, this can be interpreted as an increase of the conductivity of the membrane (Fig. 5).

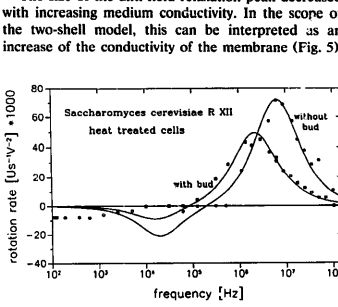

Fig. 6. Rotational spectra of heat treated yeast cells of *S. cerevisiae* R XII. The peak frequency depends mainly on the inside conductivity, which is time dependent due to an efflux of cytoplasm through the permeabilized membrane.

For medium conductivities less than about 100 μS/cm the membrane appeared 'closed' with an upper conductivity limit of 0.3 nS/cm, while for medium conductivities higher than 200 μS/cm the membrane became increasingly permeable. It follows that, at low ion concentrations the cell protects itself against loss of electrolytes by closing membrane channels, whereas in a more physiological surrounding enhanced exchange of molecules is possible.

Simulating the electrorotational spectra of heat treated cells revealed a high dependence on the wall properties. The increase in membrane conductivity to at least 0.1 μS/cm suggests some damage to the membrane. A similar effect has been observed for Ag^+-treated yeasts and explained by a linkage of silver ions to the thio-groups of proteins [12]. Hence, the elevated temperature might have led to a denaturation of membrane spanning proteins, although a phase transition of lipids is also possible. By heating the cells for a relatively short and well defined period and measuring two rotation spectra in rapid succession it was possible to observe a decrease of the cytoplasmic conductivity to 4% of the initial value during the first 30 min and a further reduction by about half in the next 30 min after heating. This temporal dependence explains the spread of the calculated conductivities of the interior of heat treated cells. The main structure of the cell wall appears to remain uninfluenced by the heat treatment, since the calculated cell wall conductivity of 0.3 mS/cm at suspension conductivities of 25 μS/cm equals that of intact cells, taking into account a slight conductivity increase in the environment of the cell caused by the efflux of cytoplasmic ions. Rotational spectra of yeast cells exposed to a detergent or ultra-sound [12], are almost identical to those of heat treated cells. Therefore, in all cases the behaviour can be assumed to reflect merely the rotation of the cell wall. The use of a slightly smaller scaling factor for heated cells compared to untreated cells suggests a change in the surface structure of the cell wall, possibly due to receptor molecules becoming more rigid [30].

The anti-field rotation below 10 kHz could not be simulated using the two-shell model of Fuhr [4], but can be interpreted as a consequence of surface charges [5] or ionic diffusion [21] (α dispersion).

Analysing the influence of experimental errors (e.g. in field strength, conductivity or viscosity) on the determined cell properties requires an extensive Monte Carlo simulation. So the deviations mentioned in Table I merely reflect the statistical standard deviations of the experiments and are probably caused mainly by the variability of the biological subjects. Since the cells were drifting during an experiment over distances of about 10 to 30 μm due to dielectrophoretic forces, the effective electric field strength varied slightly. This is probably the main cause for errors, since the electroro-

tational effect is proportional to the square of the field strength.

Conclusion

The development of both frequency generators and a suitable measuring chamber has made it possible to compare the most detailed models for electrorotation with experimental results over nearly the complete frequency range covered by the theories. A good agreement was found with these theories, and the inconsistencies of the cytoplasmic conductivity have been explained by molecular relaxations omitted in the theories. The two-shell model was used for the determination of the properties of various cellular compartments and to monitor their temporal behaviour. It has also been possible to measure the cell wall thickness for individual cells. Previously this was only possible for larger collectives or for specially prepared and therefore dead cells. The measurement of electrorotational spectra from about 100 Hz to 1 GHz offers a convenient method to simultaneously investigate the properties of distinct components of single cells in vivo under variable environmental conditions.

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