Cellular Spin Resonance of Yeast in a Frequency Range up to 140 MHz

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Rotation of living and dead yeast cells with and without buds was examined in rotating electric fields through a frequency range of 90 Hz to 140 MHz. For living yeast maximum rotation was observed in co-field direction at 30 MHz as predicted from model calculations, but with an unexpected sharp second resonance at 80 MHz for nonbudding cells. Also in the high frequency range the rotation rate was found to be proportional to the square of the applied field strength. The resonance of dead cells lies at 4 MHz; at lower frequencies these cells spin against the field analog to living cells, but without any resonance.

Introduction

In rotating electric fields cells can be observed to spin with or against the field dependent on the applied frequency, a phenomenon called electrorotation or cellular spin resonance (CSR) [1, 2]. Most objects investigated so far show a resonance like behaviour at frequencies of about 100 kHz in contra-field direction, whilst above 1 MHz they spin with the field [2–4]. Heat killed yeast cells were observed to spin in co-field direction around 40 kHz [3]. Ag^+-treated or sonificated yeast cells show maximum rotation in co-field and contra-field direction below 2 kHz and around 600 kHz, respectively [5]. Liposomes exhibit a similar behaviour [7].

A resonance of rotation of protoplasts at about 70 MHz was predicted by Fuhr *et al.* [1] and for a model cell by Sauer and Schlögel [6]. For technical reasons, no suited frequency generators and four-pole circuits were available in this frequency range up to now. The development of such circuits [8] enabled us to pursue electrorotation up to 140 MHz.

At frequencies below 1 MHz the rotation rate has been shown to be proportional to the square of the applied field strength [3, 9, 10] as predicted by theory. It was interesting to prove this proportionality also for high frequencies.

Materials and Methods

Cells of the baker's yeast *Saccharomyces cerevisiae* were grown for one day in a complete medium at 30 °C, harvested and diluted with double distilled water to a titer of 5000 cells per ml resulting in a conductivity of 2 to 5×10^{-4} S/m. Dead cells were obtained by heating to 70 °C for 4 min.

Drops of 0.3 µl were placed on 12 platinum rings (0.4 mm diameter) allowing the cells to sediment. Drops with exactly one cell were chosen and transfered into the measuring chamber with the help of a micropipette.

Before each experiment the chamber was washed with double distilled water at least three times and was subsequently dried by an air jet.

The rotation chamber consisted of four radially arranged platinum wires (0.1 mm diameter) with a distance of 0.4 mm glued onto a microscopic slide in such a way that the electrodes were 1 mm above the surface. They were surrounded by a plastic ring of several millimeters diameter supporting the cover slip with the hanging drop. Thus the cell could rotate without friction between the electrodes on the bottom of the drop. By gravity cells were always positioned at the deepest point, so that lateral forces caused by field inhomogeneities could be compensated to a certain extent.

The electrodes were connected to ground as shortly as possible by means of 50 Ω resistors to avoid reflections on the wires due to the high frequencies used. Amplitude, frequency and phase relation of the AC voltages were monitored at sockets soldered directly at two electrodes. For that purpose an oscilloscope (Philips PM 3266) was used with 1:10 attenuation probes (PM 8935/40). The oscilloscope has a 3 dB bandwidth of only 100 MHz. Therefore it was calibrated against a high frequency millivoltmeter (Rohde & Schwarz). The four sinusoidal signals, with 90° phase shift each, were delivered by three specially developed generators: one for frequencies from 90 Hz to 3.5 MHz, a second one for 2.5 to 140 MHz described elsewhere [8]. A third generator rendering fixed frequencies was used to establish the voltage dependence, as it allowed the voltage to be varied without any phase shifts. To find out the signal's harmonic content a spectrum analyzer (Tek-

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tronix A 7704, plug in unit 7L12) was used on loan. The 3rd and 5th harmonic were determined to be 30 and 35 dB lower than the fundamental oscillation, respectively.

Rotation was observed by means of a microscope (Ortholux, Leitz/Wetzlar) in connection with a TV-camera (Philips EL 8000) and a monitor (Contec).

Results and Discussion

Four representative CSR spectra of living and dead cells with and without buds are shown in Fig. 1. Below 10 MHz the living cells exhibit the known behaviour with a contra-field resonance (negative rotation rates) at about 100 kHz [2–4]. Above 1 MHz the cells rotate in co-field direction with a broad maximum around 40 MHz. This is in good agreement with the theories [1, 6] predicting for protoplasts and model cells a positive resonance around 70 MHz with a similar shape. A sharp second peak at 80 MHz occurs with such an intensity only for cells without buds. It seems to contradict the mentioned computations for protoplasts, but may be explained, if the calculations take into account more details of the cell's structure. For budding cells the peak is missing or at least suppressed almost entirely.

Dead cells show a rotation in co-field direction with a maximum at 2 to 4 MHz about 3 times slower than the high frequency resonance of living yeast. Below 100 kHz contra-field rotation can be observed without any resonance down to less than 100 Hz with spinning rates more than one order of magnitude slower than for living cells. Arnold *et al.* [5] report a similar behaviour for Ag^+-treated and for sonificated yeasts. There is rough agreement with experiments of Mischel and Pohl [3], who also observed co-field rotation of dead yeast cells, but at frequencies two orders of magnitude lower than in these investigations.

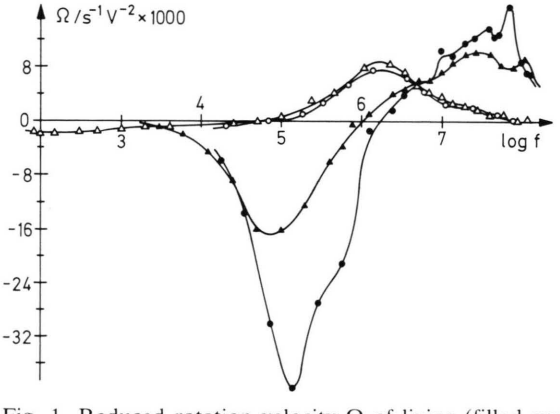

Fig. 1. Reduced rotation velocity Ω of living (filled symbols) and dead cells (open symbols) with (triangles) and without buds (circles) as function of the frequency f of the external rotating field.

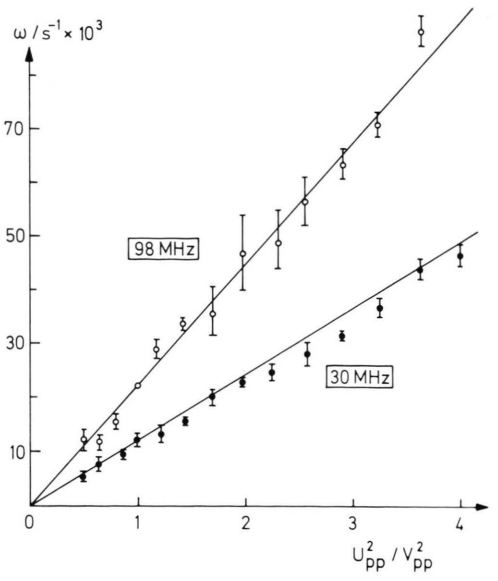

Fig. 2. Dependence of rotation speed ω on the square of the applied voltage U at two selected frequencies of 30 MHz and 98 MHz.

The electrorotation's dependence on the square of the applied voltage is shown in Fig. 2 for two selected frequencies of 30 MHz and 98 MHz. A logarithmic regression analysis delivers an exponent of 1.97 and 2.04, respectively; thus the dependence can be supposed to be quadratic with a correlation coefficient of $r = 0.997$ for each frequency.

Our investigations extend the range of electrorotation for the first time above 10 MHz and show good agreements with the theoretical predictions [1, 6]. Future experiments are specially aimed for the additional peak around 80 MHz and its connection to the appearing of the bud and the position of the cell in the cell cycle. Further attention shall be paid to the frequencies, where the spinning direction changes its sign.

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