Dielectrophoretic Rotation in Budding Yeast Cells

Maja Mischel and Ingolf Lamprecht

Institut für Biophysik der Freien Universität Berlin,
Thielallee 63−67, D-1000 Berlin 33

Rotation of budding yeast cells in an alternating non-uniform electric field of low frequency was investigated. Rotation frequency was found to be proportional to field strength above a threshold, and varied from cell to cell. The threshold is inversely correlated with the moment of inertia of the cells, while the slope of rotation frequency versus field strength increases with the moment. Rotation frequencies varied between 1 and 10 cycles per second. Clear differences between the dielectrophoretic behaviour of living and heat-inactivated yeast cells were observed.

Introduction

Dielectrophoresis − the action of alternating non-uniform electric fields on neutral particles − has recently been receiving increased attention [1−4]. The most obvious effect is the pearl-chain formation of cells along the field lines in the space between and at the surface of the electrodes [3] and the dielectrophoretic collection of neutral particles which distinguishes between dead and living biological cells.

Rotation of spheres near the surface of the electrodes was described briefly by Pohl [1] without further detailed investigations. This was perhaps due to the difficulties in measuring rotations of homogeneous or unstructed spheres. We overcame the problem by using cells of the budding yeast *Saccharomyces cerevisiae*. Buds or scars on the surface of the budding yeast are suitable markers to facilitate the observation of the rotation and the determination of its frequency. Moreover, the utilization of a video recording system enables the repeated investigation of the same cell and the evaluation of its shape.

Experiments on dielectrophoretic rotation are always performed on a single cell. Therefore, biologic

variance plays a more important role than in the bulk pearl-chain formation and collection of cells. Investigations are time consuming as the state of rotation is very labile and rotating cells suddenly tend to stick at the electrode without rotation, to aggregate with other cells or to disappear into the inter-electrode space.

As with pearl-chain formation and collection, dielectrophoretic rotation distinguishes between living and dead cells. In the present investigation the differences in the correlation between the rotation frequency and the field strength and not the frequency spectrum of dead and living cells [1, 3] were of interest.

Method and Material

Cells of the budding yeast *Saccharomyces cerevisiae*, strain 211 [5] were harvested in the mid logarithmic phase, washed and fractionally centrifuged to obtain a suspension with a high percentage of budding cells. The cells were resuspended in distilled water to get a very low conductivity which is essential for the dielectrophoretic effects. If necessary for correct osmolarity, 0.25 m sucrose was added to the suspension. In this way, conductivities of $10^{-4} \div 10^{-3}$ S/m were obtained which remained constant during the time of the experiment. Yeast cells showed no abnormal shape or metabolic behaviour in these suspensions. Cell densities of 2.1×10^6 cells per ml proved to be optimal for rotational experiments.

Dielectrophoretic rotation was observed under a microscope type Ortholux (Leitz/Wetzlar) equipped with a television camera (Philips EL 8000), a monitor (SABA T 240 F) and a video recorder (JVC HR 3660 EG). The time for 10 cycles was measured directly, and shape and dimension of the cells measured on the monitor during replay from the video recorder at low speed. The dielectrophoretic chamber consisted of a flat micro slide with a cavity into which two platinum electrodes with rounded ends dipped with a separation distance of 2 mm. Alternating voltage of up to 30 Vss with frequencies up to 5 MHz was supplied by a frequency generator type TE 7702 (Toellner/Dortmund).

If necessary cells were killed by heating them to 70 °C for 3 min. After this treatment no respiration was observable by means of an oxygen electrode.

Results and Discussion

Yeast cells form prolonged ellipsoids, the buds often appearing along the axis of the ellipsoid. Rotation in the dielectrophoretic field is possible around any axis except that coincident with the field. Frequently one observes rotations around the figure axis or the connection line between the mother cell and the bud, often around an axis perpendicular to this. In a few cases, a movement over the poles of the cell and the bud occurs so that a nonuniform, bumpy motion results. Sometimes, the orientation is suddenly changed during one experiment. The inertia moments of the cell are quite different for the various orientations. For calculation purposes the yeast cells are considered as prolonged ellipsoid with a dielectrophoretic rotation around one of the two axis of the ellipse.

The observed rotational frequencies of budding yeast cells lie between 0.2 and 3 Hz. Far higher frequencies are possible with small spherical particles but their values are hard to determine accurately. Rotations of up to 20 cycles per second could be estimated approximately.

While pearl-chain formation is mainly found at high frequencies between 500 kHz and 5 MHz, rotation is favoured by low frequencies. If the frequency of the electric field is decreased further, electrolytic phenomena become relevant and disturb dielectrophoresis; at high frequencies the cells stick to the electrodes without rotation as a consequence of the collecting mechanism.

Rotation can only be obtained at moderate field strengths. In electric fields below a special threshold the cells adhere to the electrode, at high values they tend to leave the electrodes in a "repulsion" manner. Fig. 1 exhibits the linear relationship between the applied voltage and the spinning of the cell. If one assumes a homogeneous field, this voltage transforms to a field strength of 3750 V/m maximum.

For synthetic spherical particles, Pohl [1] proposed a direct proportionality between the field and the spinning frequency, which cannot be applied for yeast cells. The behaviour found in the present experiments is described by

$$\omega = K\,(U - U')\,,$$

where U is the voltage, U' the threshold of onset of rotation and K a constant differing from cell to cell.
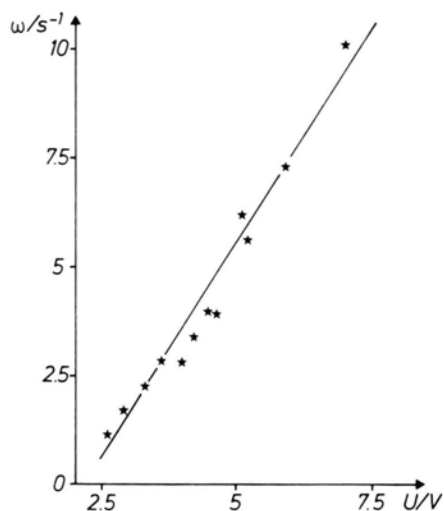

Fig. 1. Rotational frequency ω of a yeast cell as function of the applied voltage U. The distance between the electrodes was 2 mm, the conductivity of the suspension 4.35×10^{-4} S/m.
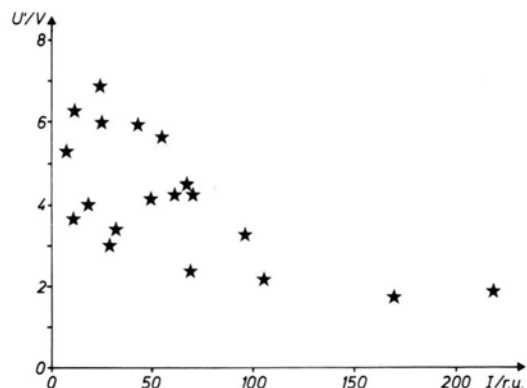

Fig. 2. Threshold voltage U' as function of the moment of inertia I (in relative units). The experimental parameters are as in Fig. 1.

It seems plausible that K is not only depending on the inertia moment (see below), but on other biological parameters (age, shape, metabolic activity) and physical ones, too (frequency of the field, viscosity, dielectric constants of medium and cells, conductivity). As only single cells are observed for short periods, these parameters are hard to investigate.

The threshold U' is not a function of the physical behaviour of the electrode systems but of the biological properties of the cells, being inversely correlated with the moment of inertia of the cells
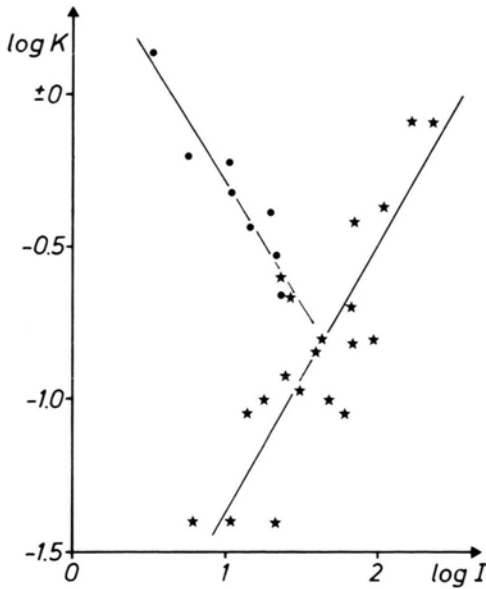
Fig. 3. Proportionality factor K as function of the moment of inertia I for living (*) and for heat inactivated (●) yeast cells.

(Fig. 2). Cells with a small moment start to rotate only in high fields, while large moment cells rotate even in low fields. In the present investigations U' varies between 750 and 5000 V/m. The U'-values are essentially lower for cells inactivated by heat treatment than for living ones.

The factor K is positively correlated with the moment in living cells, but negatively in heat-treated ones. It varies by a factor of 25 for cells of different inertia moments. Fig. 3 shows the logarithmic dependence of this proportionality factor K on the moment I for living and heat inactivated cells.

The spinning of the yeast cells follows the linear relationship to the field as predicted by Pohl [1]. But inserting the experimental parameters of the present investigations into his formula renders rotational frequencies larger than the observed values by a factor of 10 to 50. This demonstrates that his simple expression, valid for synthetic spheres, does not hold for living or dead yeast cells. There is insufficient understanding of the theoretical basis of this phenomen.

As with pearl-chain formation and cell collection, a discrimination between living and dead cells is possible by means of dielectrophoretic spinning. Further investigations are necessary to test whether this method may be used to study the influences of chemicals, such as poisons or antibiotics, or physical effects like irradiation on the viability and metabolic performance of microbial cells. More experiments in this direction seem to be justified.

[1] H. A. Pohl, Dielectrophoresis, Cambridge University Press, Cambridge 1978.
[2] V. M. Fomcenkov and B. K. Gavriljuk, Studia Biophys. 65, 35 (1977).

[3] R. Glaser, C. Pescheck, G. Krause, K. Schmidt, and L. Teuschner, Z. allg. Mikrobiol. 19, 601 (1979).
[4] K. P. Schmidt, R. Glaser, C. Pescheck, and G. Krause, Studia Biophys. 75, 81 (1979).
[5] W. Laskowski, Z. Naturforsch. 15 b, 495 (1960).