Rotation of Cells in Nonuniform Alternating Fields

M. MISCHEL and I. LAMPRECHT
Institute of Biophysics, Free University Berlin, Berlin, West Germany

ABSTRACT: Rotation of "lone" cells of the baker's yeast *Saccharomyces cerevisiae* under the influence of nonuniform alternating fields is studied. The spinning rate of the cells shows a quadratic dependence on the applied voltage and no threshold effect when the influence of gravitation is cancelled out by adjusting the density of the buffer to that of the cells. These observations are in agreement with theories established by different authors.

Among the various effects that nonuniform alternating electric fields exhibit on biological material, rotation is one of the most interesting. It was described by several authors for different organisms such as amoebae (Teixeira-Pinto, et al., 1960), erythrocytes (Füredi and Ohad, 1964) and yeasts (Pohl and Crane, 1971). Rotation seemed to be a quite general phenomenon of living and dead cells or inanimate materials that could be observed in a broad range of frequencies. As this effect is closely related to dielectrophoresis (DEP; Pohl, 1978) and to the effective dielectric constant of the particle in the surrounding medium, it may offer an elegant method to determine this parameter and to investigate the physical state of biological material.

Although rotating fields were mentioned briefly (Pohl and Crane, 1971), they were only recently employed to investigate the spinning of single cells (Arnold and Zimmermann, 1982a, 1982b; Mischel, et al., 1982; Mischel and Pohl, 1983). Also recently, theories were developed to explain the occurrence of rotation in cells in the neighborhood of other ones (Holzapfel et al., 1982) and in single cells (Pohl, 1983).

Pohl and Crane (1971) used the description term "cellular spin resonance" (CSR) for this phenomenon, because the spinning of cells appears at a sharply resonant frequency of the external field. If highly homogeneous cell cultures are used nearly all cells spin in the same small frequency interval (Zimmermann et al., 1981). Nevertheless, it is possible to find spinning cells at all applied frequencies, mainly in direct contact with the electrodes.

Although rotation of cells was described long ago, to our knowledge Mischel and Lamprecht (1980) were the first to give quantitative data about spinning rates and their connections with some physical and biologic parameters. These first experiments were performed in a DEP chamber which consisted of a microslide with a central well of 1.0 mm, into which two electrodes dipped at an angle of about 10° . The electrodes were produced from a platinum plate 0.5 mm thick with carefully rounded and polished tops. The minimum distance between the electrodes amounted to 2 mm. The height of the electrode tips above the floor of the well measured 0.3 mm or 35 cell diameters.

A new type of DEP chamber used in recent experiments consisted of a microslide to which two similar platinum electrodes (0.2 mm thick) were glued with epoxy resin with a minimum separation of 0.2 mm or 0.5 mm, resp. Much care was taken in smoothing and polishing the electrode surface and the edges. The surface quality was so high that the reflection of cells could be seen on it, a point which is essential if lone cells are able to spin. Above the electrodes was glued a small Perspex chamber with a volume of 0.35 ml. During experiments the chamber was usually closed with a cover slide, but remained open if a water-immersion objective lens was used.

In both cases rotation was observed under a microscope, type Ortholux (Leitz/Wetzlar) equipped with a television camera (Philips EL 8000) and a video recorder (JVC HR 3660 EG) so that the results could be stored and the spinning rates taken several times by different persons. The alternating field was supplied by a frequency generator (TE 7702; Toellner/Dortmund) and monitored on an oscilloscope (HM 312; HAMEG/Frankfurt/M.)

Baker's yeast, *Saccharomyces cerevisiae* strain "211", (Laskowski, 1960) was used throughout the experiments. The cells were grown in a liquid nutrient broth, harvested in the mid-logarithmic phase, and washed three times in quartz-distilled water to obtain a final conductivity of 2 to 20 μS/cm. If necessary 0.25 m sucrose was added to the suspension to produce an appropriate osmolarity. Sucrose concentrations in this order do not alter the specific density of the solute essentially, so that the yeasts still tend to sediment to the floor of the chamber. The cells were pipetted into the chamber while the ac field was already switched on. Usually, it takes some minutes till spinning of cells is observable.

In these investigations we concentrated on lone cells rotating directly at the electrodes. "Lone" cells in this context means that there are no other cells beside, above, or under the spinning cell and that no inhomogeneities of the field due to imperfect electrodes or particles on them can be detected. Rotation of free-floating lone cells is a very rare event although it was seen from time to time.

In our first set of experiments, looking for a limited range of frequency, we figured out a linear dependence between the spinning rate and the applied field strength (Mischel and Lamprecht, 1980). Typically, a threshold voltage occurred above which the cells started to rotate. This ranged from 1 to 8 V and indicated a weak dependence upon the inertia moment of the cells.

From the theories of Holzapfel, et al. (1982) and Pohl (1983) a quadratic relation between spinning rate and field strength is to be expected. Holzapfel et al. (1982) observed such a behavior of cells lying in chainlike structures on the floor of their chamber. These cells were no longer subjected to the force of gravity. To produce comparable conditions and nevertheless study the spinning at the electrodes we increased the density of the solution by adding 40 weight percent sucrose till the yeast cells floated or filled the chamber up to the level of the electrodes and carefully putting a second layer with the cell suspension of normal density on top. Fig. 1 shows a graph which shows the behavior of floating cells. A logarithmic regression analysis clearly demonstrates the predicted quadratic dependence upon the field strengths and the disappearance of the threshold voltage. The exponent of the parabola amounts to 1.99, the correlation coefficient to 0.9995, and the standard deviation to ± 0.0028. The rotation of the cell was not pursued to lower field strengths because there the spinning becomes less

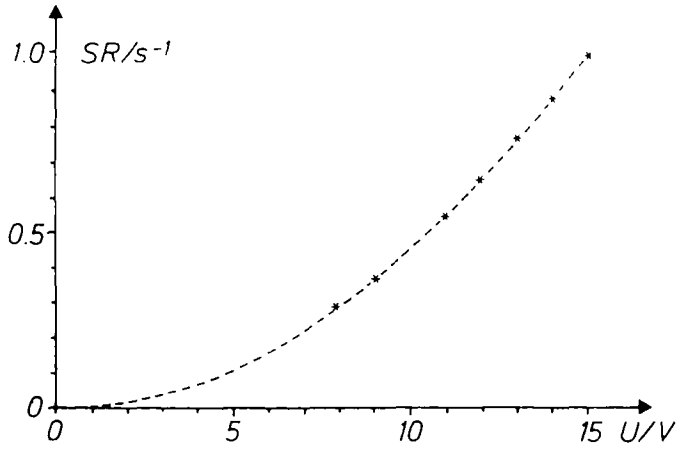

Fig. 1. Spinning rate (given as revolutions per second) for a lone yeast cell *(Saccharomyces cerevisiae*, strain 211) at the electrode as function of the applied voltage (field strengths). Frequency: 250 Hz, conductivity: 6 μS/cm. *: experimental points; ---: least-square fitting curve.

smooth or arrhythmic and large errors are introduced.

If the density of the solution is decreased so that the cells no longer float but slowly sediment in the chamber the shape of the graph is altered. Dependences are represented by a parabolic exponent between 1 and 2 and a plausible threshold voltage. If one calculates the forces acting on the cells by the ac field and by gravitation it becomes obvious that both forces are of equal strength near the threshold voltage. This explains why under special experimental conditions threshold voltages not conforming to the theory are observed (Mischel and Lamprecht, 1980; Zimmermann, et al., 1981).

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