Rotation of Cells and Ion Exchange Beads in the MHz-Frequency Range

G. Küppers, B. Wendt, and U. Zimmermann
Arbeitsgruppe Membranforschung am Institut für Medizin der Kernforschungsanlage Jülich, Postfach 1913,
D-5170 Jülich, Bundesrepublik Deutschland

Z. Naturforsch. **38 c,** 505 – 507 (1983);
received February 18, 1983

Rotation, Polarisation, Ion Exchange Beads, Protoplasts

Whereas human red blood cells and protoplasts of leaves of *Avena sativa* show rotation only at discrete frequencies in the kHz-range of a linear alternating electric field, rotation of these cells is observed at practically every frequency in the MHz range (15 to 200 MHz).

The cell rotation in the MHz-range can be explained in terms of field induced polarisation and orientation of permanent dipoles within the membrane and the cell.

This interpretation is supported by the finding that Chelex beads (polystyrene cross-linked with divinylbenzene and coupled to iminodiacetic acid) do exhibit rotation in the MHz-range, but not in the kHz-range. It is most interesting that the Cu^{2+}- and Ca^{2+}-forms of the Chelex beads show a shift in the rotation frequency spectrum in addition to an increase in the magnitude of the rotation speed with respect to the Na^+-form. At frequencies in the MHz-range at which rotation of the Chelex beads is not observed, formation of chains of beads from the electrodes occurs instead. This is due to positive polarisation of the beads leading to positive dielectrophoresis. The results support the view that rotation needs negative polarisability which is determined by the functional groups.

Introduction

Cells can rotate in a linear alternating field [1 – 4]. Under appropriate experimental conditions all cells in a suspension rotate at a particular characteristic frequency of the alternating electrical field [3]. Zimmermann and colleagues [4, 5] have shown that the rotation of cells is generally attributable to a dipole-dipole interaction between at least two neighbouring cells (so-called multi-cell rotation). The dipole-dipole interaction leads to the creation of a "local" rotational field which itself results in a torque. This torque is different from zero in its temporal mean and leads to the rotation of cells by hydrodynamic interactions.

In accordance with the theory [4], rotation of a single cell is thus only observed when the cell is exposed to a rotational electrical field [5, 6]. The direction of rotation of the single cell depends on the direction of the vector of the rotating field [5, 7].

The characteristic frequencies studied for various cell species are in the kHz range. In this frequency range the dipoles are generated by charge separation across the membrane (Maxwell-Wagner dispersion). The characteristic frequency at which rotation of cells of a given species is observed is determined by the relaxation time of the dipole generation. Since the relaxation time of charge separation is a function of the external conductivity, the characteristic frequency of rotation shifts towards higher values if the external conductivity is increased [5, 7]. From a plot of the characteristic frequency versus the external conductivity the specific capacity of the membrane can be calculated [5, 7].

Charge separation is not the only mechanism for dipole generation. Polarisation of material and the orientation of dipoles arising from proteins or lipid molecules within the membrane are alternative mechanisms for dipole generation which should be observed at much higher frequencies.

In this communication we report on multi-cell rotation experiments on cells and on the rotation of ion exchange beads in the MHz-range.

Material and Methods

Two parallel platinum electrodes (diameter 200 μm) were glued on to a microslide with a gap of 300 μm between them and connected to a high frequency generator [8]. Human blood was withdrawn from apparently healthy donors and the red blood cells prepared in the usual way [3]. Mesophyll protoplasts were obtained from leaves of *Avena sativa* by digesting the cell walls enzymatically with cellulysin (Calbiochem. San Diego, USA) [9]. For rotation the cells were suspended in isotonic sugar solutions. The Chelex beads (Bio-Rad Laboratories, München, FRG) had an average diameter of 50 μm.

Results and Discussion

Human red blood cells and plant protoplasts from leaves of *Avena sativa* exhibit rotation over a broad frequency range between 5 MHz and 200 MHz. In particular, rotation was observed in the range be-

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Reprint requests to Prof. Dr. U. Zimmermann.

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tween 30 MHz and 170 MHz for these cell species. In this frequency range, rotation was observed at practically every frequency. The occurrence of the broad frequency range for rotation in the MHz-range can be explained in terms of field induced polarisation and orientation of permanent dipoles within the membrane and the cell. In order to support this interpretation, ion exchange beads with different functional groups were investigated for their ability to rotate in an alternating electrical field. Most of the experiments were performed on Chelex beads (diameter about 50 μm). Chelex beads are made up of polystyrene cross-linked with divinylbenzene and coupled to iminodiacetic acid. The sodium-form (which is strongly dissociated) as well as the Ca^{2+}- and Cu^{2+}-forms were studied. The complex-forming constants for Na^+, Ca^{2+} and Cu^{2+} are $K_1 \approx 40*$, $K_1 \approx 2.5 \times 10^3*$ and $K_1 \approx 4 \times 10^{10}* \ 1 \cdot mol^{-1}$, respectively [10]. Because of the high specific density of the Chelex beads, the beads were incubated in distilled water containing 45% Percoll (pH = 7). At this Percoll concentration the beads float between the two electrodes so that their rotation is not influenced by gravitational forces [3]. As expected, the beads do not rotate in the kHz-range, since charge separation requires an intact envelope (membrane). This negative finding provides further support for the above assumption that charging processes at the membrane are responsible for the creation of dipoles in the cell in the kHz-range [4]. However, the Chelex beads do rotate in the MHz-range. The rotation of Chelex beads is particularly marked when the functional groups are complexed by Ca^{2+} or Cu^{2+}. Strong rotation at low field strengths is observed in the 20 – 100 MHz-range. (The exact values of the field strength cannot be given because of problems with standing waves in the set up [8].) On the other hand, the Na^+-form exhibits only weak rotation in the 100 – 300 MHz-range and slightly increased rotation in the range above 200 MHz.

Conversely, in the frequency ranges in which rotation is not observed pearl chain formation occurs [11]. The pearl chains begin to form from the electrodes (Fig. 1). If the frequency at which pearl chain formation is observed is switched to the rotation frequencies, the chains flip into a 45° orientation (Fig. 2). When the beads are oriented at 45° with respect to each other, maximum rotation is observed. This is as predicted by the theory and also in agreement with the results of earlier experiments in the kHz-range [4]. The 45° orientation of the pearl chains at the characteristic frequency for rotation can be attributed to a negative polarisability of the particles [4]. The negative polarisation causes the beads to be repelled by the electrodes, and by tipping into a 45° orientation they are taking up the state of minimum energy. Pearl chain formation, on the other hand, is observed in the case of positive polarisability [11]. Under these conditions the chains are attracted to the electrodes.

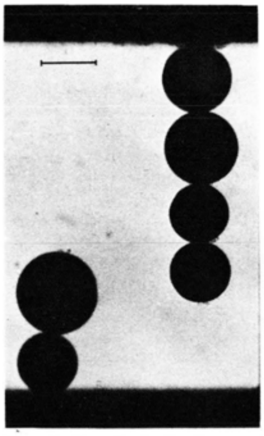

Fig. 1. Pearl chain formation of Chelex beads complexed with Cu^{2+} (blue = dark) in an alternating electric field. (E = approximately 50 $V cm^{-1}$, ν = 10 MHz). Bar = 50 μm.

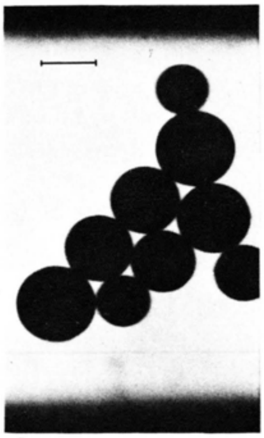

Fig. 2. 45° orientation of pearl chains of Cu^{2+} Chelex beads after switching on the optimum frequency (40 MHz) for rotation. (E = approximately 50 $V cm^{-1}$). Bar = 50 μm.

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* Values for free iminodiacetic acid.

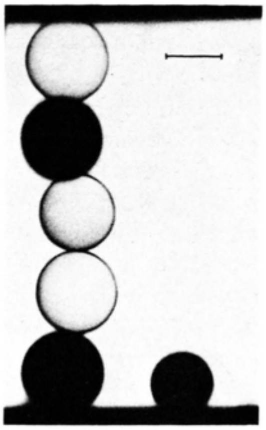
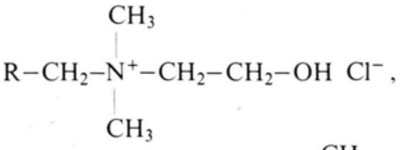


Fig. 3. Formation of a mixed pearl chain of Na^+ (white) and Cu^{2+} (blue, dark) Chelex beads in an alternating electric field. (E = approximately 50 $V\,cm^{-1}$, ν = 10 MHz). Bar = 50 µm.

The different polarisation of the Chelex beads for either rotation or pearl chain formation can be easily demonstrated by using a mixture of Chelex beads of the Na^+- and the Cu^{2+}-form (or Ca^{2+}-form). Both types of Chelex beads can be distinguished by their colour (Na^+-form: white; Cu^{2+}-form: blue). In the 20 − 100 MHz range where rotation of the Cu^{2+}-Chelex beads is observed, only chains emanating from the electrodes are observed for Na^+-Chelex beads. There are no mixed chains. In the 1 − 20 MHz range, in which both Na^+- and Cu^{2+}-Chelex beads form chains emanating from the electrodes mixed chains do occur (Fig. 3). In the 100 − 250 MHz range where both Chelex bead types exhibit rotation, mixed chains with a 45° orientation also occur in the electrode gap. Rotation is observed under these conditions even if a white bead is adjacent to a blue bead. The change of polarisability of the cells with frequency probably arise from the frequency dependence of the complex dielectric constant of the cells or beads [7]. Dissociated groups apparently lead to a positive polarisation of the cells and, in turn, to pearl chain formation.

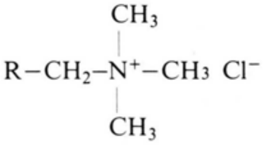
This conclusion is supported by studies of other polystyrene beads with different dissociated groups.

Beads with strongly dissociated groups such as



$$R{-}CH_2{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{N^+}}{-}CH_2{-}CH_2{-}OH\ \ Cl^-,$$

$R{-}SO_3^-H^+$ and



$$R{-}CH_2{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{N^+}}{-}CH_3\ \ Cl^-$$

exhibit pearl chain formation but no rotation over the entire frequency range up to 250 MHz.

The results presented here show that rotation observed in cells in the MHz-range is attributable to a dielectric polarisation of material or to an alignment of functional groups with permanent dipoles in response to the field.

We believe that this opens up interesting aspects for the future application of rotation studies to the investigation of cells and cell membranes.

Acknowledgement

We are very grateful to W. M. Arnold for helpful discussions. This work is supported by a grant from the BMFT (03 7266) to U.Z.

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