ORIGINAL ARTICLE

Can molecular properties of human red blood cells be accessed by electrorotation?

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Abstract

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Electrorotation (ER) is a single cell dielectric method which can be used to characterize the structural and electrical properties of biological cells at a high parameter resolution. The method has been applied to measure ER spectra of human red blood cells (HRBC) in rotating electric fields ranging from 100 kHz to 250 MHz and at external conductivities ranging from about 1 mS/m to 3 S/m. With increasing medium conductivity the anti- and the co-field peak of the spectra shifted towards higher frequencies. At external conductivities higher than 1 S/m only anti-field rotation has been observed. At these conductivities the peaks show distortions which could not be explained by the common single shell model normally applied to extract dielectric cell parameters. In this paper, we interpret the spectra by frequency dependent parameters for the cytoplasm. Nevertheless, the results suggest that also the membrane parameters are frequency dependent.

Key words : high ionic strength, dielectric dispersion, characteristic frequency, single cells

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For characterizing dielectric properties of single biological cells, techniques like ER and dielectrophoresis have been used (Arnold and Zimmermann, 1998; Gimsa *et al.*, 1991). In ER, cells are practically suspended in a medium of low ionic strength and exposed to a rotating electric field ($\vec{E}$). As a result, an electric dipole moment ($\vec{m}^*$) is induced in the cell, which is, at given time, spatially out of phase with respect to the rotating field:

$$\vec{m}^* = \frac{4\pi\, abc}{3} \varepsilon_0 \varepsilon_e \vec{E} K^* \qquad (1)$$

where ε_0, ε_e, a, b, c and $\vec{E}$ stand for permittivity of vacuum, the dielectric constant of the suspending medium, the axes a, b, and c of an ellipsoidal cell, and the external field strength, respectively. Complex parameters are marked by an asterisk. K^* is the Clausius-Mossotti factor which is the frequency dependent part of the induced dipole moment. The interaction between the rotating field and the induced dipole moment results in a torque ($\vec{T}^*$), causing an oblate spheroidal cell (a=b=r) to rotate:

$$\vec{T}^* = \frac{4\pi\, r^2 c}{3} \varepsilon_0 \varepsilon_e \vec{E}^2\ \mathrm{Im}(K^*) \qquad (2)$$

Please note that the factor of 3 in the denominator of the volume term of Eq. 2 is often cancelled for a factor of 3 introduced by the depolarizing coefficient of spherical objects (for discussion please see Gimsa and Wachner, 1999). For modelling the induced dipole moment of cells, frequency independent cell parameters, i.e. frequency independent permittivities and conductivities, are usually assumed. The observed dispersions are then explained by the compartmentalized structure of the cells (Gimsa *et al.*, 1991; Asami *et al.*, 1989). Nevertheless, in reality cell compartments are not homogeneous. The plasma membranes of biological cells such as HRCBs are composed of various proportions of lipid and protein contents. The cytoplasm of HRBCs is extremely rich in proteins, especially hemoglobin, and cytoplasmic dielectric dispersions have already been described for the cytoplasm of these cells (Gimsa *et al.*, 1996). For this, a frequency dependent permittivity and conductivity have been taken into account. In this paper, we used HRBCs since their cytoplasm is not compartmentalized and should therefore allow for a simple theoretical description. The ER spectra measured at high external conductivities exhibit deviations from a Lorentzian peak behavior that most probably reflect frequency dependent molecular properties. For estimation of the frequency dependent parameters, we applied our spheroidal single shell model introducing frequency dependent cytoplasmic properties (Gimsa and Wachner, 1999).

Materials and Methods

Two solutions, a 300 mOsm solution of sucrose and a 300 mOsm NaCl solution, both containing 1 mM phosphate buffer (pH 6), were appropriately mixed to adjust the medium conductivity in the range from 0.02 S/m to 3 S/m. For each experiment 15 µl of whole blood has been suspended into 15 ml of this solution and samples of 5 µl of the suspension were introduced into a 4-electrode micro-chip chamber fabricated by semiconductor technology (Gimsa *et al.*, 1996). For measurements the cells were exposed to a rotating electric field in the frequency range from 100 kHz to 250 MHz applied by a radio frequency generator HP 8131A (Hewlett Packard, USA). The generator applied 4 progressively 90°-phase shifted signals of 5 Vpp to the 4 perpendicularly arranged ultra-micro electrodes. The distance between two opposing electrode tips was about 300 µm. At every conductivity the ER spectra of at least five different cells were recorded via a video system. All measurements were finished within 5 min after suspending the cells in the measuring solution. At the end of each experiment the conductivity of the suspension was measured by a conductometer. For determining the characteristic frequencies of the ER peaks in the spectra a non-linear least error-square fit procedure was used to fit a function of two

Lorentzian peaks to the measuring points.

Results

Figure 1 shows ER spectra for eight selected external conductivities. Co- and anti-field rotation peaks are clearly visible at low (Figures 1A and B) and medium (Figures 1C-E) conductivities, respectively. The two ER rotation peaks in each spectrum characterize the membrane capacity and the cytoplasmic conductivity dispersions, respectively (for details see Gimsa *et al.*, 1991).

At high conductivities (Figures 1F-H) two negative peaks have been observed. With increasing conductivity both peaks are shifted towards higher frequencies (see also Figure 2). Around 1 S/m the second peak is changing its sign. Please note that the frequency of peak rotation is only equivalent to the characteristic frequency of an underlying process for well separated peaks. Thus, the superposition of different peaks may mimic peak frequencies different from the actual characteristic frequencies. In Figures 2 and 3 the results of the fits have been plotted over the external conductivity. Two characteristic frequencies (Figures 2A and B) and the two respective peak heights (Figures 3A and 3B) are given. Each point represents the results of at least five fitted cell spectra.

Measured data have been plotted together with those already published Gimsa *et al.* (1996). To calculate the theoretical curves in Figure 2 we assumed an oblate cell model (Gimsa and Wachner, 1999) with long half-axis, axis ratio, relative external permittivity, membrane conductance and membrane capacitance of 3.6 µm, 1:2, 78, 125 S/m^2, and 0.997 10^{-2} F/m^2, respectively. For the conductivity and the relative permittivity of the cytoplasm frequency independent values of 0.535 S/m and 50, respectively, have been

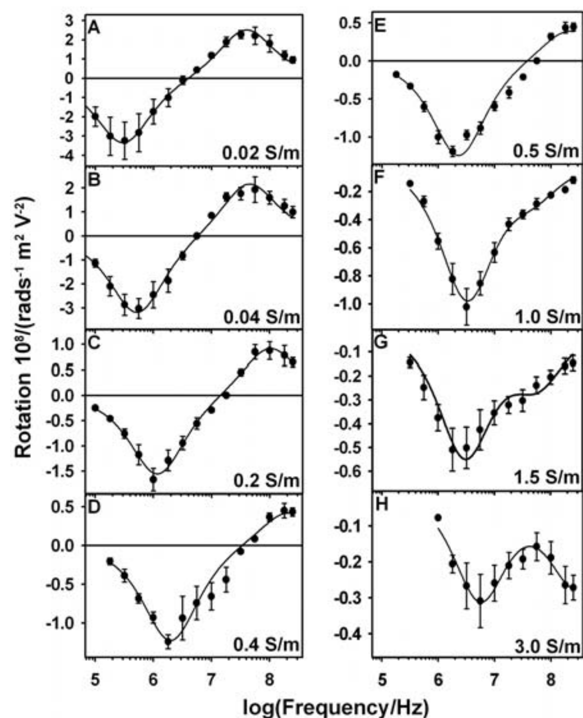

Figure 1. ER spectra of HRBC at different external conductivities. Two superimposed Lorentzian peaks each of them given in the form of $2 \cdot Rpeak \cdot f \cdot fc/(f^2 + fc^2)$ have been fitted to the measuring points, with Rpeak, f, and fc standing for peak rotation, field frequency, and characteristic frequency, respectively.

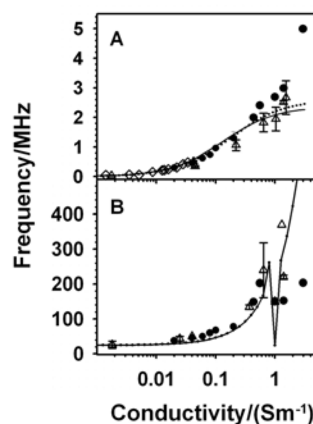

Figure 2. Fitted characteristic frequencies of the first (fc_1, A) and second peak (fc_2, B) over external conductivity (circles). Open rhombi and triangles are representing data already published in Gimsa *et al.* (1996), respectively. For curves see text.

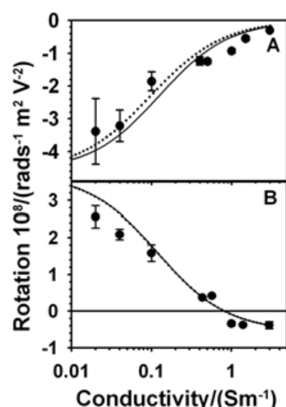

Figure 3. Rotation peak amplitudes of the first (A) and second peak (B) obtained from the spectra fits. The curve describing the rotation peak behavior has been obtained from the model used in Figure 2 by multiplying the peak amplitudes of the Clausius-Mossotti function with a scaling factor (see text).

assumed (solid line). The dotted line represents frequency dependent parameters with a conductivity of 0.4 S/m and a relative permittivity of 212 at low frequencies, dispersing around 15 MHz to become 0.535 S/m and 50 at high frequencies, respectively (compare to parameters in Gimsa *et al.* 1996). For conductivities lower than 0.5 S/m the second peak was positive, switching to negative at conductivities higher than 1.0 S/m (Figure 3B). This switching led to the discontinuity in the f_{c2}-function in Figure 2B. At high conductivity values the rotation speed was very low (Figures 3A and B). For model calculations of the rotation amplitude we used the imaginary part of K^* (see Eqs.1 and 2) arbitrarily scaled to account for the complex friction conditions of a cell rotating in close vicinity of the surface of the electrode chip. At all conductivities the same scaling factor has been used.

Discussion and Conclusion

The ER spectra of biological cells usually exhibit two strong, conductivity dependent rotation peaks. The two peaks can be attributed to two distinct relaxation processes. In our experiments the measured rotation spectra of HRBCs closely agree with the superposition of two Lorentzian peaks at low conductivities but deviate from this shape at conductivities above 0.4 S/m. This deviation cannot be explained by the structural dispersions of a single shell model with frequency independent parameters. We believe that it is due to the molecular membrane and cytoplasmic properties. For the first time frequency dependent parameters for the cytoplasm of HRBCs have been introduced by Gimsa *et al.* (1996). Nevertheless, in this paper the spectrum of an oblate spheroidal Laplace model with a confocal membrane thickness has been fitted to the measuring points. In the present paper we increased the number of measuring points and apply our new dielectric cell model (Gimsa and Wachner, 1999). In Figures 2A and B the parameters published in Gimsa *et al.* (1996) were introduced into the new model, leading to slightly different theoretical curves. A thorough investigation of the reason for this deviation is still open. Nevertheless, these differences in the two models cannot explain the strong deviations of the curves from the measuring points above 0.6 S/m. Introduction of frequency dependent cytoplasmic properties like in Gimsa *et al.* (1996) only lead to a minor improvement. The generation of curves describing the theoretical peak behaviour (characteristic frequency and peak height) of the model is a complex problem and a thorough interpretation of our new data will need some more time. Some uncertainty in the data may arise from a temperature increase in the measuring chamber especially at higher external conductivities. Experiments to consider this problem more thoroughly are under way. Nevertheless, the present data suggest a membrane conductivity increase and a capacitance decrease at high external conductivities. Alas, frequency dependent parameters must also be assumed for the membrane. These will reflect molecular dispersions and lead to a more realistic dielectric HRBC model. Most probably the findings on

HRBCs will be of a more general importance and may change our notion of the electric properties of biological cells at high frequencies.

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