Eur Biophys J (1987) 14:253–256

European
Biophysics Journal

# Chloroplast dielectrophoresis

## Effects of frequency and surface charge

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Received October 28, 1985/Accepted in revised form June 16, 1986

**Abstract.** Dielectrophoretic coefficients of chloroplasts, untreated and treated with cationized ferritin, have been measured in axisymmetric ac electric fields at different frequencies. The treated chloroplasts have surface charge density 2.4 times smaller than the untreated ones.

The dielectrophoretic coefficients are in the range $10^{-25}$ F · m$^2$ to $7 \times 10^{-25}$ F · m$^2$ for frequencies from 6,000 Hz to 1 MHz. Dielectrophoretic effects have not been observed for frequencies from 1 to 6,000 Hz and from 1 MHz and 10 MHz. The surface charge decrease leads to an increase of 2–3 times in the dielectrophoretic coefficients and slight shift of the dielectrophoretic mobility of lower frequencies.

These results may be qualitatively explained on the basis of the existing theories for cell and vesicle dielectrophoresis.

**Key words:** Chloroplasts, dielectrophoresis, surface charge

## Introduction

Motion of polarizable particles in a nonhomogeneous electrical field, which was termed dielectrophoresis in 1951 (Pohl 1978) or dipolophoresis (Petkanchin and Stoylov 1975), is becoming an important tool in both fundamental research and applications to biotechnology, in particular to cell fusion (Zimmermann 1982).

We have developed a device for measuring the dielectrophoretic behavior of single cells and cell organelles (Dimitrov et al. 1984). In this device the electrical field distribution has cylindrical symmetry and therefore can be easily represented by an analytical formula.

We have measured the dielectrophoretic coefficients, i.e. the coefficients of proportionality between the dielectrophoretic force and the electrical field (see Eq. (1)), for red blood cells (Tsoneva et al. 1985), protoplasts (Stoicheva et al. 1985; Stoicheva and Dimitrov 1986b), lymphocytes, myeloma cells and hybridomas (Stoicheva and Dimitrov 1986a; Dimitrov et al. 1985). The values of the dielectrophoretic coefficients depend on the cell type and frequency, and to a smaller extent on the type of medium surrounding the cell. There are no experimental data on surface charge effects in dielectrophoresis of cells and cell organelles.

The electric properties of chloroplasts have been intensively investigated mainly in relation to their function in photosynthesis. Usually their surface charge in different conditions has been measured. There is only one paper (Ting et al. 1971) that deals with chloroplast dielectrophoresis. These authors have measured the collection rates at spherical electrodes as a function of the ac field frequency. They have not investigated the effect of surface charge and have not measured the dielectrophoretic coefficients of single chloroplasts.

This work studies dielectrophoresis of single chloroplasts of decreased surface charge at different frequencies.

## Materials and methods

### Preparation of chloroplasts

The chloroplasts were isolated by the method of Jensen and Bassham (1966), modfied by Heber

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*Abbreviations:* DCMU – 3-(3,4-Dichlorophenyl)-1,1-Dymethylurea; EDTA – Ethylenediaminetetraacetic acid; HEPES – (N-2 Hydroxyethylpiperozine-N′-2-ethanesulphonic acid); MES – (2[N-morpholino]ethane sulfonic acid); TRICINE – (N-tris[Hydroxymethyl]methyl glycine); DMPA – (N,N-dimethyl-1,3-propanediamine)

(1972) as follows. Leaves from pea were homogenized for 5 s at high speed in an isolation medium containing 0.33 *M* sucrose, 0.002 *M* EDTA, 0.001 *M* $MgCl_2$, 0.001 *M* $MnCl_2$, 0.02 *M* NaCl, 0.004 *M* cysteine, 0.0005 *M* $K_2HPO_4$, 0.002 *M* ascorbic acid and 0.05 *M* MES buffer (pH 6.1). The chopped material was filtered through a cheese cloth and centrifuged at 600 × *g* for 20 s. The supernatant was again centrifuged at 2000 × *g* for 2 min. The pellet was resuspended in a buffer containing 0.33 *M* sucrose, 0.002 *M* EDTA, 0.001 *M* $MgCl_2$, 0.001 *M* $MnCl_2$, 0.02 *M* NaCl, 0.0005 *M* $K_2HPO_4$ and 0.05 *M* HEPES buffer (pH 6.7) and the centrifugations were repeated. The chloroplasts were resuspended in a solution containing 0.33 *M* sucrose, 0.005 *M* $MgCl_2$, 0.01 *M* TRICINE buffer (pH 8.0) at a concentration of 4 mg of chloroplasts per ml. More than 80% of the isolated chloroplasts were intact according to the test of Lilley et al. (1975). The chlorophyll concentration in a probe was determined by the method of Arnon (1949). Finally, the intact chloroplasts were resuspended in a solution of 0.33 *M* sucrose and used for dielectrophoretic measurements.

### *Cationized ferritin treatment*

In order to change the surface charge density Cationized Ferritin was used to label negatively charged chloroplast membranes. The chloroplast suspension was incubated for 15 min at 20 °C with 10 µl cationized ferritin (SIGMA, 10 mg/ml prepared by coupling horse spleen ferritin with DMPA) in 0.01 *M* TRICINE (pH 8.0) buffer as above. After the labelling procedure the chloroplasts were washed twice and then were resuspended in appropriate medium for dielectrophoresis of surface charge density measurements.

### *Dielectrophoretic measurements*

The dielectrophoresis was performed in a device where the nonuniform ac electric field was created between two concentric electrodes (Dimitrov et al. 1984; Stoicheva et al. 1985). Briefly, a platinium wire of radius $R_i = 0.24$ mm is placed in a hollow metal cylinder of inner radius 1 mm, outer radius 2 cm and height 5 mm. The remaining part of the device is made from plexiglas. We have used a sinusoidal ac electric field with a voltage from 0 V to 19 V and frequency from 1 Hz to 10 MHz.

The dielectrophoretic coefficients were calculated from the measured dielectrophoretic velocities of single chloroplasts following the method developed by Tsoneva et al. (1986) and recently modified by Stoicheva et al. (1985) on the basis of equation

$$F = -1/2\, \alpha\, E \nabla E = 6 \pi \mu R_c V, \tag{1}$$

where $F$ is the dielectrophoretic force, $R_c$ is cell radius, $\mu$ is viscosity, $V$ – dielectrophoretic viscosity, $\alpha$ – dielectrophoretic coefficient.

The organelles were resuspended in 0.33 *M* sucrose. The conductivity of the solution was $5.2 \cdot 10^{-6}$ S/cm. The frequency effects were investigated for a population of 10 organelles resuspended in isotonic medium. All the measurements were done at 18 °C.

### *Surface charge measurements*

The surface charge density was measured by the method of Chow and Barber (1980a, b) using the fluorescence of a 9-aminoacridine probe. This molecule carries a single positive charge at pH 7.0 and its fluorescence intensity decreases when it is concentrated near negatively charged surfaces (Chow and Barber 1980a and b). The measurements were performed in a medium containing 0.33 *M* sucrose, 50 µ*M* EDTA, 0.00085 *M* KOH, 10 µ*M* DCMU and HEPES buffer 0.001 *M* (pH 7.5 with HCl). The chlorophyll concentration in a probe was 7 µg chlorophyll per ml. Fluorescence from 9-aminoacridine (20 µ*M* in the probe) was excited at $\lambda = 390$ nm and measured at $\lambda = 450$ nm using a Jobin Yvon 3 JD spectrofluorimeter (slit width 4 mm).

The presence of 50 µ*M* EDTA in the medium removes all bound divalent cations and brings the fluorescence to minimum levels. The fluorescence was brought back up by titrating with small aliquots from a stock solution of either KCl or $MgCl_2$. The relative fluorescence intensity at each salt level was normalized according to $F_{\max}$ (the maximum fluorescence obtained on adding 20 m*M* $MgCl_2$) and plotted (see Fig. 1). The concentrations of KCl and $MgCl_2$, which gave the relative fluorescence, were calculated by linear interpolation from the data of Fig. 1. The surface charge density ($\sigma$) was calculated from these values by using the equation

$$\sigma = -\left[2 RT \varepsilon_r \varepsilon_0 (C'^2 - 4 C' C'')/C''\right]^{1/2},$$

where $R$ is the gas constant, $T$ is the absolute temperature, $\varepsilon_r = 78.5$, $\varepsilon_0 = 8.8 \times 10^{-12}\ \mathrm{F \cdot m^{-1}}$, $C'$ – concentration of monovalent ions in solution, $C''$ – concentration of divalent ions in solution.

It has been shown that low and high values of $F/F_{\max}$ are not suitable for the calculation of $\sigma$ (Møller et al. 1984). For this reason $\sigma$ obtained at $F/F_{\max} = 0.88$ was used for comparisons.

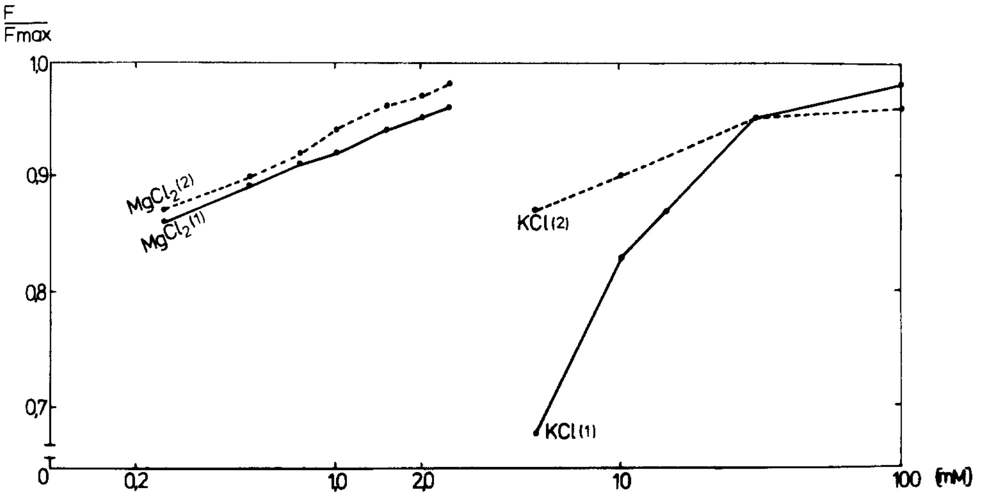

**Fig. 1.** Dependence of 9-aminoacridine fluorescence on the concentration of mono- and divalent cations in chloroplast suspensions. (1) denotes the curves for untreated chloroplasts, (2) denotes the curves for chloroplasts treated with Cationized Ferritin

## Results and discussion

Table 1 shows that the dielectrophoretic coefficients $\alpha$ do not vary significantly with the frequency in the frequency range from 6,000 Hz to 1 MHz. There is slight maximum at 300 kHz in this region. Below 6,000 Hz and above 5 MHz we have not observed significant dielectrophoretic effects, except for repulsion from the central electrode. We have not observed any rotation at all frequencies investigated. It is interesting to note that we have not observed the low frequency maximum in the chloroplast dielectrophoretic mobility as seen by Ting et al. (1971) when measuring the collection rates. This may be due to the lower voltage we have used or to other reasons.

Table 2 presents the dielectrophoretic coefficients $\alpha$ for cationized ferritin treated chloroplasts. The chloroplast surface charge was calculated from the dependence of 9-aminoacridine fluorescence on the cation concentration as shown in Fig. 1. The values for untreated and treated chloroplasts are: $\sigma_{(1)} = -0.045\ \mathrm{C/m^2}$ and $\sigma_{(2)} = -0.019\ \mathrm{C/m^2}$, respectively, at $F/F_{\max} = 0.88$.

It should be noted that the solution used for measurement of the surface charge differs slightly from that used for the dielectrophoretic measurements. We believe that the surface charge density, at least the ratio of the values for treated and untreated chloroplasts, does not differ significantly for the two solutions.

There are two major differences between treated and untreated chloroplasts 1) the maximum values of $\alpha$ are higher, $(7.0 \pm 2.4) \times 10^{-25}\ \mathrm{F \cdot m^2}$ compared to $(2.0 \pm 0.4) \times 10^{-25}\ \mathrm{F \cdot m^2}$, $R_c = (2.5 \pm 0.3) \times 10^{-6}\ \mathrm{m}$, for untreated chloroplasts and 2) the smallest mea-

**Table 1.** Dielectrophoretic coefficients $\alpha \times 10^{25}\ \mathrm{F \cdot m^2}$ of untreated chloroplasts of radii $R_c \times 10^6$ m at different frequencies in 0.33 *M* sucrose solutions. The applied voltage was 18 V, 10 V, 18 V, 15 V and 18 V for frequencies 6 kHz, 10 kHz, 300 kHz, 500 kHz and 1,000 kHz, respectively

| 6 kHz | | 10 kHz | | 300 kHz | | 500 kHz | | 1,000 kHz | |
|---|---|---|---|---|---|---|---|---|---|
| $R_c$ | $\alpha$ | $R_c$ | $\alpha$ | $R_c$ | $\alpha$ | $R_c$ | $\alpha$ | $R_c$ | $\alpha$ |
| 2.5 | 1.1 | 2.0 | 1.7 | 2.5 | 2.4 | 2.5 | 2.2 | 2.5 | 1.3 |
| 2.5 | 1.1 | 2.5 | 2.1 | 2.5 | 2.2 | 2.5 | 2.1 | 2.0 | 2.3 |
| 2.0 | 0.8 | 2.0 | 1.7 | 2.0 | 1.2 | 2.5 | 2.1 | 2.0 | 1.4 |
| 2.5 | 1.1 | 3.0 | 2.5 | 3.0 | 2.6 | 3.0 | 2.3 | 3.0 | 1.5 |
| 3.0 | 1.3 | 2.5 | 2.1 | 3.0 | 2.5 | 2.0 | 1.9 | 2.5 | 1.2 |
| 2.0 | 0.8 | 2.5 | 2.1 | 2.5 | 2.1 | 2.5 | 2.0 | 3.0 | 1.3 |
| 2.0 | 0.8 | 2.5 | 2.1 | 2.5 | 2.4 | 2.0 | 1.7 | 2.0 | 2.2 |
| 2.5 | 1.1 | 3.0 | 2.6 | 2.0 | 1.7 | 2.5 | 2.2 | 3.0 | 1.3 |
| 2.5 | 1.1 | 2.0 | 1.7 | 3.0 | 2.6 | 3.0 | 2.6 | 2.0 | 2.3 |
| 2.0 | 0.8 | 2.5 | 2.2 | 2.5 | 2.2 | 2.5 | 2.2 | 2.5 | 1.3 |

**Table 2.** Dielectrophoretic coefficients $\alpha \times 10^{25}\ \mathrm{F \cdot m^2}$ of chloroplasts of radii $R_c \times 10^6$ m, treated with cationized ferritin, in 0.33 *M* sucrose solutions. The applied voltage was 18 V, 10 V, 10 V and 18 V for frequencies 8 kHz, 5 kHz, 500 kHz and 900 kHz, respectively

| 8 kHz | | 5 kHz | | 500 kHz | | 900 kHz | |
|---|---|---|---|---|---|---|---|
| $R_c$ | $\alpha$ | $R_c$ | $\alpha$ | $R_c$ | $\alpha$ | $R_c$ | $\alpha$ |
| 2.5 | 1.4 | 2.0 | 5.2 | 3.0 | 8.2 | 2.0 | 0.8 |
| 2.0 | 1.7 | 2.5 | 8.5 | 2.5 | 6.1 | 2.5 | 0.9 |
| 2.5 | 1.3 | 2.5 | 6.4 | 3.0 | 13.1 | 2.0 | 0.8 |
| 2.0 | 1.6 | 2.0 | 6.7 | 2.0 | 9.9 | 2.0 | 0.8 |
| 2.5 | 1.1 | 2.5 | 8.3 | 3.0 | 5.3 | 2.5 | 0.9 |
| 2.5 | 1.4 | 2.5 | 6.9 | 2.5 | 5.3 | 2.5 | 0.9 |
| 2.0 | 1.2 | 3.0 | 9.7 | 2.5 | 6.1 | 2.5 | 0.9 |
| 3.0 | 2.6 | 3.0 | 6.8 | 2.0 | 7.2 | 2.5 | 0.9 |
| 3.0 | 1.3 | 2.5 | 8.1 | 3.0 | 6.2 | 2.0 | 0.7 |
| 2.5 | 1.1 | 2.0 | 5.3 | 3.0 | 7.3 | 2.0 | 0.8 |

sured values are at 900 kHz:

$$(\alpha = (0.80 \pm 0.08) \times 10^{-25}\,\mathrm{F \cdot m^2}$$

$$R_c = (2.3 \pm 0.3) \times 10^{-6}\,\mathrm{m})$$

and an $\alpha$ these are smaller than those for untreated chloroplasts. Consequently, the dielectrophoretic effect for the treated chloroplasts (which have smaller surface charge) is more significant and slightly shifted to lower frequencies compared to the case of untreated chloroplasts.

For treated chloroplasts we have not observed positive dielectrophoresis above 1 MHz and below 800 Hz. Very slow motion of these organelles toward the outer electrode (negative dielectrophoresis) was noted in the frequency range from 1 to 10 MHz. Repulsion from the central electrode was observed at frequencies below 800 Hz.

At the present stage in the development of the theory of cell dielectrophoresis (Pohl and Crane 1972; Crane and Pohl 1972; Shilov and Estrela-L'opis 1975; Sauer 1983; Chizmadzhev et al. 1985) we are not able to explain all our experimental data.

The recent theory of Sauer (1983) takes into account the dielectric losses for spherical dielectric particles, which can be important for high frequencies. However, there are no data for the complex dielectric constants of the chloroplasts. The theory of Chizmadzhev et al. (1985) emphasizes the contribution of the surface conductivity due to the electric double layer, because of surface charges. For example, they obtained a dielectrophoretic coefficient of the order of $10^{-24}\,\mathrm{F \cdot m^2}$ at 1 MHz and surface charge 0.02 $\mathrm{C/m^2}$ (see their Fig. 6), which coincides with our observations. We observed an increase of the dielectrophoretic coefficients with increasing charge at high frequencies (> 900 kHz) as predicted by their theory, but at low frequencies we have observed the opposite behavior as can be seen in Table 1 and 2. This can be due to the complex structure of the chloroplasts and the simplifying assumptions used in the theory of Chizmadzhev et al. (1985).

More detailed comparison of our results with available theories is difficult, because of lack of data for polarizability and conductivity of the chloroplasts.

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