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DIELECTROPHORESIS OF CHLOROPLASTS

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SUMMARY

Dielectrophoresis is the migration of neutral particles in a nonuniform electric field (a.c. or d.c.) toward the region of highest field intensity. Dielectrophoresis should be distinguished from electrophoresis which is the migration of charged particles in electric fields. Chloroplasts, isolated from spinach leaves, can be collected on platinum electrodes by dielectrophoresis. Stripped chloroplasts lacking outer envelopes and stroma were prepared from fresh spinach leaves in a 0.5 M sucrose–0.05 M Tris buffer (pH 7.4). The chloroplast preparation was desalted with a mixed anion-cation resin to a resistivity of $3 \cdot 10^4$–$5 \cdot 10^4$ ohm·cm. Dielectrophoresis was conducted in a pin-pin type leucite cell 3.2 mm in diameter and 1.5 mm deep. The 0.425-mm diameter electrodes were 0.85 mm apart and 0.05 mm below the surface of the cell. The collection of chloroplasts with ac current is a function of the frequency. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU)-stabilized chloroplasts had collection maxima at 300, $1 \cdot 10^6$, and $3 \cdot 10^7$ Hz when run at 50 V. The rate of collection is a function of the square root of the time. Both DCMU and darkness tend to stabilize collections. It is suggested that dielectrophoresis may be a useful tool for the study of chloroplast physiology and perhaps, for the preparation and purification of chloroplasts.

Because of their small size and delicate structure and because of their major role in the physiology of plant cells, we were interested to ascertain if chloroplasts could be collected and characterized with the aid of the rapid yet gentle technique of dielectrophoresis. In this study, we report a method for the collection of chloroplasts by dielectrophoresis and indicate the potential of this method for the study of the physiology and biochemistry of chloroplasts.

In a nonuniform electric field (a.c. or d.c.) a neutral body is usually constrained to move toward the region of highest field intensity. Such motion induced by the action of a nonuniform electric field on a polarizable object is termed dielectrophoresis[1,13]. The latter should be distinguished from electrophoresis which is motion caused by the action of an electric field (whether uniform or nonuniform) on a charged object. Dielectrophoresis, acting on the dielectric constant rather than on the charge, has proven useful in making selective separations of either living or inanimate particles and in studying the nature of individual systems[2–9]. The dielectrophoretic response of living cells suspended in aqueous media varies characteristically and considerably with the frequency of the applied field, with the conductivity of the liquid medium,

and with the physiological state of the cell or organism[7,10,11]. Net charge, if any, on the organism is relatively unimportant at the high a.c. frequencies normally used in dielectrophoresis, as long as d.c. is suppressed during the experiments. The technique is considered to be very gentle in that cells collected appear to survive unharmed[6,7,10,11]. It remained to be seen if organelles such as chloroplasts could be handled successfully in this manner.

Chloroplasts were prepared from 50 g of fresh spinach (*Spinacia oleracea* L.) leaves in 100 ml of a 0.5 M sucrose–0.05 M Tris buffer (pH 7.4), containing 1 mM EDTA, 5 mM mercaptoethanol, and 0.01% bovine serum albumin. The leaves were chopped and ground in a Waring Blendor for 5–6 sec. The homogenate containing organelles was filtered through four layers of cheesecloth and the filtrate was centrifuged at 100 × *g* for 1 min. The chloroplasts were precipitated from the supernatant fluid by centrifugation at 600 × *g* for 7 min. Chloroplasts prepared in this manner are largely stripped of their outer membranes and lack much of the stromal protein[12]. Chloroplasts were resuspended from the pellet in 0.25 M sucrose. The chloroplast suspensions were kept on ice prior to dielectrophoresis which was done at room temperature (26°). Stabilization of the chloroplasts was obtained by preparing them in 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Because a low resistivity of the dielectrophoresis solution is critical, preparations were sometimes desalted by shaking with a mixed anion–cation ion exchange resin (one resin volume to two chloroplast preparation volumes).

Dielectrophoresis was done in a pin–pin type lucite (polymethyl methacrylate) electrode chamber. Two rounded platinum electrode tips, 425 μ in diameter and 850 μ apart projected into a small circular chamber 1.5 mm deep and 3.2 mm in diameter. The electrodes were situated about 0.05 mm below the top surface of the chamber. A small drop of the chloroplast suspension placed in the well sufficed for each measurement. The a.c. voltage (50 V) was supplied by a Hewett Packard oscillator, Model 200 CD, amplified by a simple single stage amplifier for the frequency range $10–6 \cdot 10^5$ Hz. For higher frequencies, a Heathkit radio transmitter with a dummy load was used. To minimize the d.c. surge on connecting the a.c. supply to the dielectrophoresis chamber, it was found helpful to run the oscillators in a "standby" condition with 180 kohms shunting the leads just ahead of the final switch connecting the chamber with the a.c. supplies.

The collection of the organelles was observed through a microscope fitted with an ocular micrometer. At magnifications of 100 or 200 ×, it was possible to accurately measure the collection of chloroplasts on the electrodes (Fig. 1). The conductivity of the final chloroplast preparation was determined with an Industrial Research Conductivity Bridge Model No. RC 16B2 provided with platinum-platinum black electrodes and operated at 1000 Hz. Suitable resistivities were between $3 \cdot 10^4$ and $5 \cdot 10^4$ ohm·cm. The optical density at 670 nm of 0.05 ml of chloroplast suspension in 3 ml of 0.5 M sucrose was 0.05–0.1.

The yield spectrum of DCMU stabilized spinach chloroplasts shows three peaks (Fig. 2) indicating the presence of at least three relaxation times. Marked streaming of the liquid was evident at the very low and the very high frequencies. At 20 Hz this streaming was violent and accompanied by visible bubbling at the electrodes. The observed yield spectrum is somewhat remarkable in the extraordinary height of the 300 Hz peak. Single cells such as canine erythrocytes[10] show no peak below 10^5 Hz

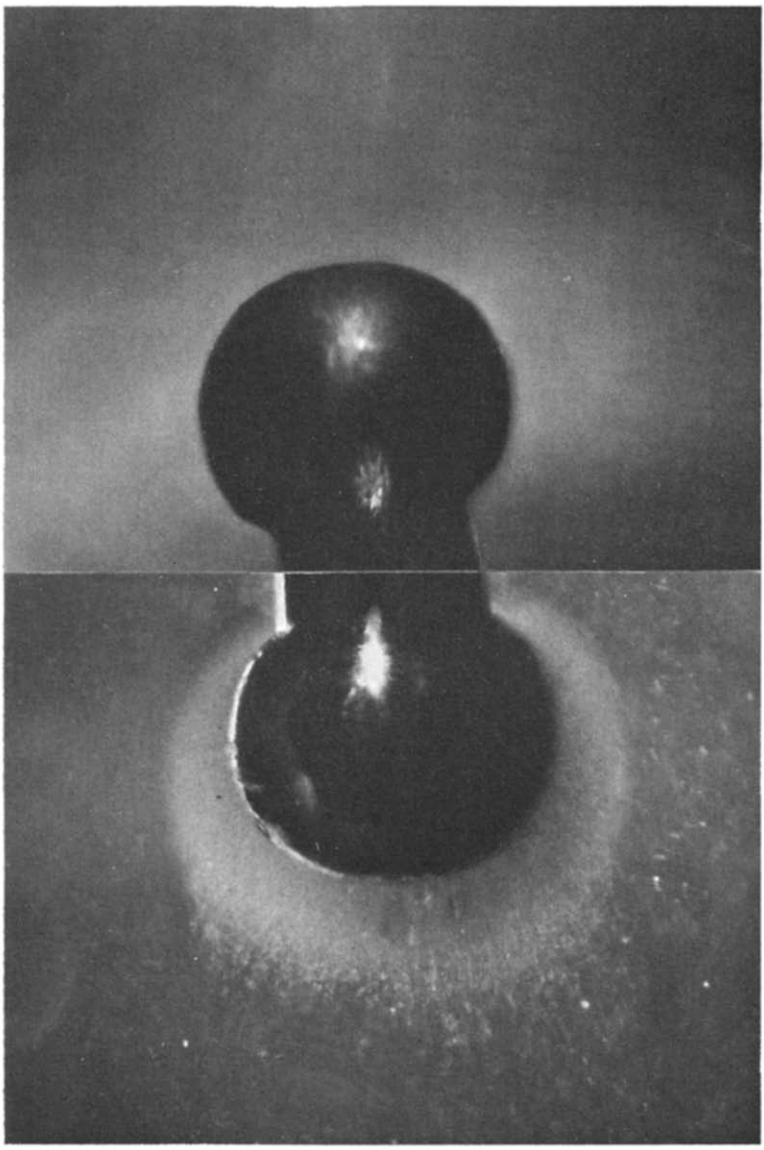

Fig. 1. Photomicrograph of the collection of chloroplasts on the platinum dielectrophoresis electrode. Chloroplasts collect as linear pearl chains. Magnification, 100 ×. Collection was at 300 Hz.

while yeast cells[7,11] and flavobacteria[10] show only mild peaks with little collection at about 10^4 Hz and major peaks in the range of about 10^6 Hz. The yield spectrum is shifted toward lower frequencies when the resistivity is increased.

The rate of collection of chloroplasts is quite rapid and in the early stages follows a rate proportional to the square root of the time in accordance with theory[1,13]. Representative collection rate curves are shown in Fig. 3 for the two frequency peaks of the yield spectrum.

A detailed physical, theoretical analysis of the yield spectrum has not yet been done. For the present, we suggest that the 300 Hz peak is due to a Helmholtz double layer associated with the granal surfaces of the stripped chloroplasts. The extremely large surface area of the grana membranes is consistent with this hypothesis. The 10^6 Hz peak may be associated with material enclosed within the individual thylakoids. The small peak at $3 \cdot 10^7$ Hz may represent chloroplasts which are more intact. Because of the complexity of the analysis, these assignments must remain tentative pending further study.

The dielectrophoresis rate is strongly affected by the physiological state of the chloroplasts. The yield of chloroplasts when suspended in 0.25 M sucrose shows a decline with time. When stabilized by the addition of 10 μM DCMU, the collection

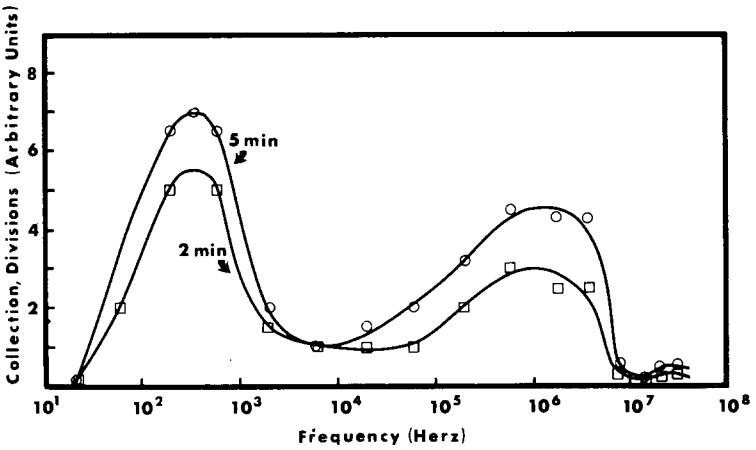

Fig. 2. Yield spectrum for the dielectrophoresis of spinach chloroplasts. Collections were made for 2 or 5 min at 50 V with DCMU stabilized chloroplasts in 0.25 M sucrose. Resistivity, $5.0 \cdot 10^4$ ohm·cm. Collection divisions corresponded to the ocular micrometer scale.

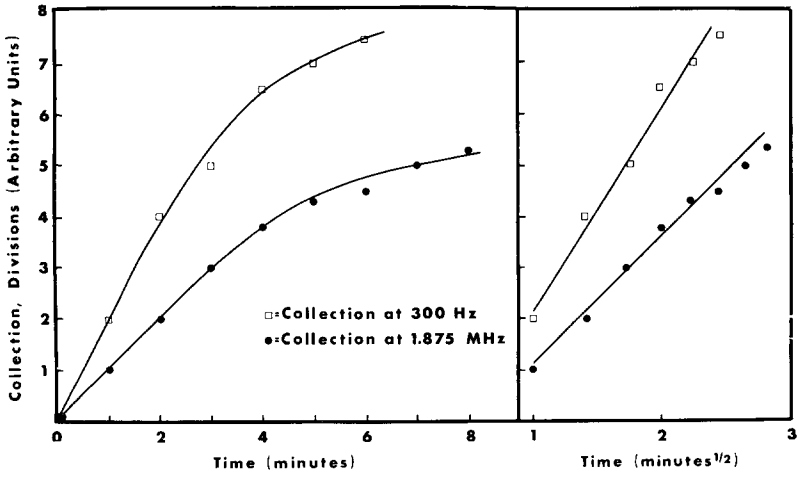

Fig. 3. Time course of chloroplast collection at the two major frequency peaks. Left: Collection rate. Right: Collection rate as a function of the square root of time. 50 V. Resistivity, $5 \cdot 10^4$ ohm·cm.

rates remain higher (Fig. 4). Collection also declines faster if suspensions are stored in the light rather than dark.

In contrast to past observations on the dielectrophoretic collection of cells in which dielectrophoresis appears to cause little change in the cells or organisms, chloroplasts seem to be affected by the field. This is evidenced here at least in the relatively intense fields used to speed the motion of the small chloroplasts (3–5 μ in diameter), and by the ultimate dispersal of the collected mass after some 30 min of field maintenance. The cause of this latter phenomenon is not yet clear, however, it may be related to the loss of potassium or other cations from the thylakoids after collection. Normally in the dielectrophoresis of larger particles (7–10 μ in diameter), about 20 V is applied to suspensions with similar resistivities. Here, the roughly sixfold wattage increase may be of significance.

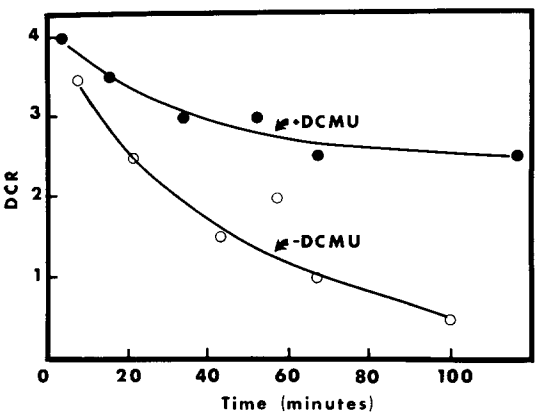

Fig. 4. Stabilization of chloroplast collection in the presence of DCMU. 50 V. Resistivity = $5 \cdot 10^4$ ohm·cm. DCR, dielectrophoresis collection rate.

In summary, the results of these experiments suggest that dielectrophoresis may be a useful tool to study the physiology and biochemistry of chloroplasts. Once the real significance of the peaks of the yield spectrum is understood, it should be possible to study the effect of a variety of materials (uncouplers, inhibitors, *etc.*) on chloroplast functions with a more thorough understanding of the results. Furthermore, because different organelles *e.g.*, mitochondria, microbodies, or intact chloroplasts, would be expected to have different yield spectra, the technique could conceivably be used to separate organelles from each other.

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REFERENCES

1 H. A. POHL, *J. Appl. Phys.*, 22 (1951) 869.
2 H. A. POHL, *J. Electrochem. Soc.*, 107 (1960) 386.
3 H. A. POHL, *Sci. Am.*, 203 (1960) 107.

4 H. A. POHL AND C. E. PLYMALE, *J. Electrochem. Soc.*, 107 (1960) 390.
5 H. A. POHL AND J. P. SCHWAR, *J. Electrochem. Soc.*, 107 (1960) 383.
6 H. A. POHL AND I. HAWK, *Science*, 152 (1966) 647.
7 J. S. CRANE AND H. A. POHL, *J. Electrochem. Soc.*, 115 (1968) 584.
8 H. A. POHL, *J. Electrochem. Soc.*, 115 (1968) 1C.
9 H. A. POHL AND W. F. PICKARD, *Dielectrophoretic and Electrophoretic Deposition*, Electrochemical Society, Inc., New York., 1969
10 K. WILEY, *Dielectrophoretic Studies of Erythrocytes and Flavobacteria*, M. Sc. Thesis, Oklahoma State University, 1970.
11 J. S. CRANE, *The Dielectrophoresis of Cells*, Ph.D. Thesis, Oklahoma State University, 1970.
12 V. ROCHA AND I. P. TING, *Arch. Biochem. Biophys.*, 140 (1970) 398.
13 H. A. POHL, *J. Appl. Phys.*, 29 (1958) 1182.