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INTERPRETATION OF ELECTROROTATION OF PROTOPLASTS
II. Interpretation of experiments

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This paper deals with the electrorotational behaviour of protoplasts. It is related to the theoretical discussion in /5/. Protoplasts of different species (Avena sativa, Brassica oleracea, Kalanchoe daigremontiana, Daucus carota, and Solanum tuberosum) were investigated. There is evidence that both membrane systems, tonoplast and plasmalemma, respectively, influence the protoplast rotation spectrum in the range of the first resonance frequency. Types of protoplasts were classified, using theoretical and experimental data. Emphasis is put on the necessity to consider the electrorotational spectra of protoplasts as those of multi-shell spheres.

1. Introduction

Cells can be made to spin in continuously and discontinuously rotating fields /1,7,9/. In our investigations we used both kinds of fields. The rotating fields were created in chambers with four electrodes, which were driven by sinusoidal or square-topped pulses with a key ratio of 1:1 having progressive phase shifts of 90° to each other. Differences in the electrorotational spectra measured with both kinds of fields were negligible.

The reasons for the electrorotation, interactions between time-dependent polarizations of all constituents of the cell and the external electric field were already discussed in /5,7/. As demonstrated in previous papers at least a single-shell model must be used to understand the contra- and co-field spin of cells /6,8/. For such particles two resonances were found, one in the kHz- and the other in the MHz-range. In earlier publications both peaks were investigated experimentally in different objects /4,8/. When a rotating electric field near the first resonance is applied to living cells a contra field spin occurs. At frequencies in the range of the second resonance a co-field spin

can be observed. These results are in agreement with our theoretical predictions. Detailed theoretical descriptions and experimental results have already been published /4/.

Previously we reported that the electrorotational spectrum of nearly 70% of Avena sativa mesophyll protoplasts in the frequency range of the first peak can be well fitted, using the formula described in /9/:

$$R = R_{max} \frac{2kf}{1+(kf)^2} \qquad (1)$$

(k - time constant in s; f - frequency of external field; R - rotation; R_{max} - rotation of the characteristic frequency)

In most of the relevánt cases this equation can be used for the description of a single-shell sphere considering only one resonance. From theoretical considerations we have seen that in fact in most cases even curves of three-shell models are similar to a curve derived from formula (1). Whether the interpretation of the measured points as those of a single-shell model is possible or not depends on some preconditions.

As we discussed in /5/ preconditions for the possibility of a sufficient fit may be the following:

A. Protoplasts in fact consist only of an electric homogeneous internal solution covered by a single shell.
B. Protoplasts have a thick and highly conductive cytoplasma, or the existing vacuoles etc. are very small at least.
C. Plasmalemma and tonoplast have nearly the same electrical properties or the conductivity of both is very low ($< 10^{-6}$ S/m).
D. Deviations from a curve shape of formula (1) type were too small to be measurable with our current possibilities.

Case A and B may be discussed as single-shell spheres considering the protoplast as a single-shell sphere consisting of a homogeneous internal solution and of the plasmalemma. In case C very good fits are possible, but the membrane capacity can only be described by a series connection of two membranes if the cytoplasma is very thin and not too conductive. In case D great residues in the interpretation may occur.

2.Method

Measuring chamber:

A four electrode arrangement was used driven by sinusoidal or square-topped pulses as described in /7/. In the case of sinusoidal fields we used field strengths between 2700 and 4200 V/m, for the measurements with square topped pulses those of about 3000 or 3800 V/m. Observations were confined to the centre of the chamber. Protoplasts were held at the boundary layer between layered isotonic solutions of equal conductivities, or were measured after sedimentation at a gelatine surface. Protoplast diameter and the cell rotation speed in dependence of the field frequency were measured by using microscopic observation.

Material:

Kalanchoe daigremontiana and Avena sativa mesophyll protoplasts were prepared as described previously /1,10/. Brassica oleracea protoplasts were prepared as described in /2/. Daucus carota protoplasts were made from cells cultivated in a cell-suspension culture as described in /3/. The protoplasts were produced by treatment with an enzyme solution for 19 hours at 22°C and pH 5.8. We used cellulase HUPC, produced by the Institut fur technische Mikrobiologie, Adlershof. 100 ml of the enzyme solution contained 100 mg $CaCl_2*2H_2O$, 3.37 g mannitol, 3.37 g sorbit, 58.5 mg MES-buffer and 10 mg NaH_2PO_4 as well as 1% cellulase (MES=Morpholinoethane sufonic acid). After this treatment protoplasts were washed in a 0.37 M mannitol solution two times. Solanum tuberosum protoplasts were prepared by treatment of sliced leafs with 0.5% cellulase/0.6 M mannitol solution at 25°C for 3 hours. After this treatment protoplasts were washed in the measuring solution, consisting of 0.6 M mannit, 0.25 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM phosphate buffer (PB) (pH 5.8), and 1 mM KCl, 4 times. Vacuoles of Avena sativa were produced by a short decrease of the osmotic pressure in the external medium.

3. Results and Discussion

We were able to produce continuously rotating electric fields up to 10 MHz. Although this is a large part of the useful frequency range, we were only able to investigate the described deviations

from a single-shell sphere resonance type in the frequency range of the first resonance (1 kHz-1MHz). In this range the influence of both membranes should be measurable. In order to get comparable values we normalized the rotation speed on the square of the field strength /1,9/ and plotted it against the frequency of the external field. Then equation (1) was fitted to these points. For the analysis we selected the protoplast results in curves where measured points are deviated near the fitted curve without showing a tendency (well fitted curves) and curves where the deviation of the measured points indicates a tendency (badly fitted curves)(see Fig.2). From our considerations we saw that this relationship cannot be expressed by the regression coefficient.

TAB. 1:
Proportions of well fitted protoplasts and their specific membrane capacities

OBJECT	Number of measured objects	Well fitted data in %	$c_m * 10^{-2}$ in F/m^2 from well fitted objects
Solanum tuberosum	15	60	0.51±0.16
Daucus carota	23	39	0.51±0.17
Brassica oleracea var. capitata	67	19	0.41±0.16
Kalachoe daigremontiana	51	98	0.28±0.08
Avena satíva	101	66	0.56±0.17
Vacuoles (Avena sativa)	27	100	0.46±0.09

The proportion of protoplasts which could not be fitted in a reasonable way was species dependent. As expected all vacuoles could be well fitted. Table 1 contains values of the specific membrane capacity (c_m) of protoplasts that could be well fitted.

c_m was calculated using an equation described in /1/:

$$c_m = Ge/(\P * f_{c1} * R) \qquad (2)$$

(Ge-external conductivity; f_{c1}-resonance frequency; R-radius of the cell)

f_{c1} was calculated from measured rotations in the range from 1 kHz to 1 MHz by non-linear regression using formula (1). Of course equation (2) can only give an estimation, because it is exclusively applicable to single-shell models with negligible membrane conductivity and comparatively high internal conductivity.

In Fig. (1) a well fitted (A) and another significant derived protoplast (B) rotation spectrum is shown.

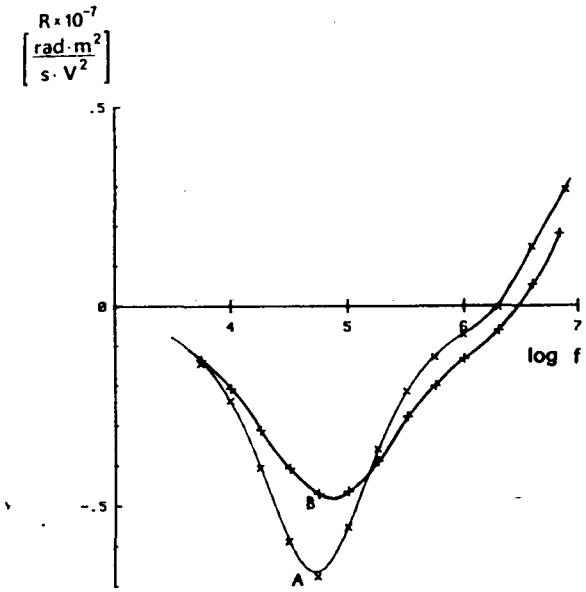

FIG. 1

Measured rotations of Avena sativa protoplasts; External conductivity 7 mS/m
A. Diameter 30 µm; Up to 1 MHz this curve was fitted numerically
B. Diameter 36 µm

Measured points of some typical protoplasts and corresponding curves fitted by non-linear regression as described above are illustrated in Fig. (2) A-D:

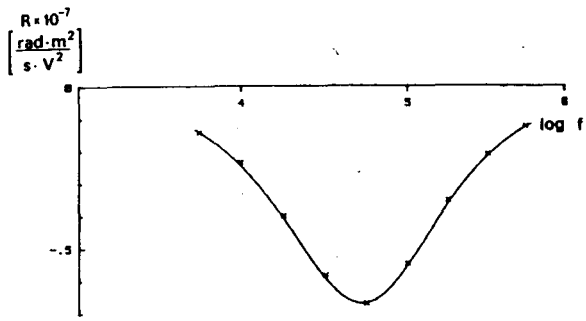

FIG. 2 A

Avena sativa protoplast;
Diameter 30 µm;
External conductivity 7 mS/m

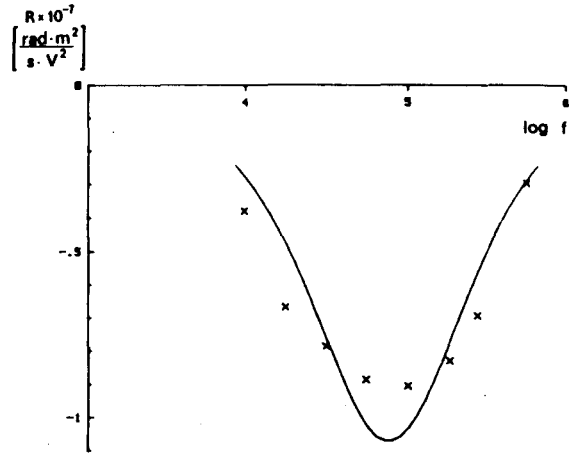

FIG. 2 B

Mesophyll protoplast of Brassica oleracea;
Diameter 22 µm;
External conductivity 8.6 mS/m

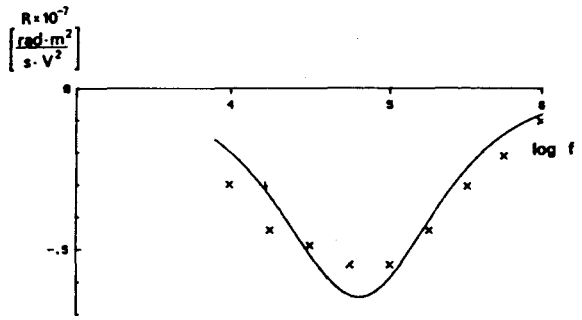

FIG. 2 C

Avena sativa protoplast;
Diameter 36 µm;
External conductivity 5.3 mS/m

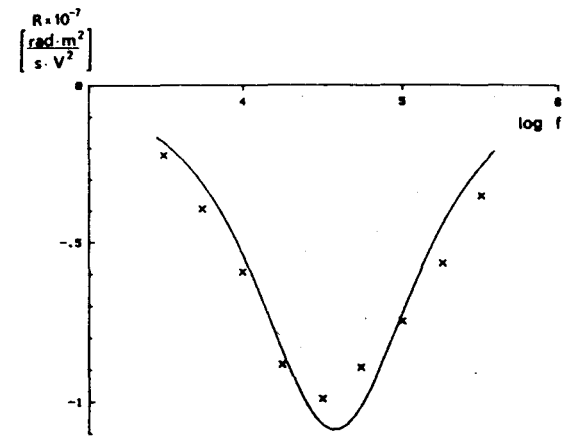

FIG. 2 D

Daucus carota protoplast;
Diameter 97 µm;
External conductivity 11 mS/m

In Fig.2 A the curve for a single-shell model can be fitted very well to measured rotations. In Fig.2 B-D the measured points are broadened in comparison to the fitted curve. In case C and D you can suppose a second but smaller peak in the left and the right part of the curve, respectively. All these cases can also be found in our theoretical discussion /5/.

A more detailed interpretation of Tab.1 and Fig.2 A-D requires a

geometrical description of the protoplasts used in the experiments. The following protoplast types can be observed:

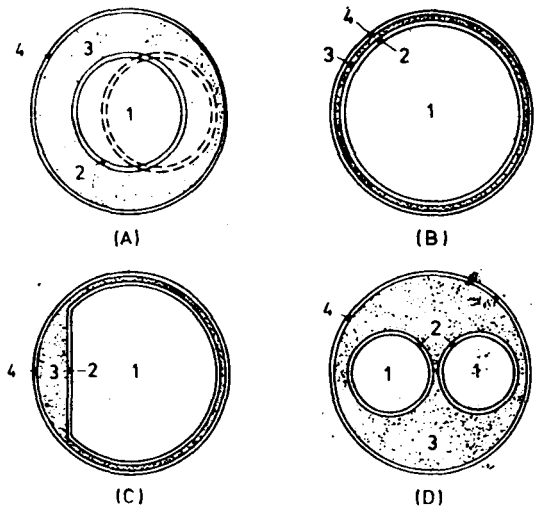

FIG. 3
Protoplast types visible in our protoplast suspensions (1-vacuole space, 2-tonoplast, 3-cytoplasma, 4-plasmalemma)

Nearly all Avena protoplasts were of type A. If we provide a high cytoplasma conductivity f_{c1} will be determined by the plasmalemma and the dielectric properties of the external medium. If the presumption of low membrane conductivities (less than 10^{-6} S/m) was right we found specific plasmalemma capacities of about $(0.56\pm0.17)*10^{-2}$ F/m^2.

The Kalanchoe daigremontiana protoplasts correspond to type B. If both membranes have identical electric properties and are tightly connected (small cytoplasma layer) ARNOLD and ZIMMERMANN's /1/ interpretation of two capacitors in series connection may become applicable at a first approximation. f_{c1} is determined by both membrane systems. From this simple consideration half the value of the specific membrane capacity has to be expected (see Tab.1).

From Brassica oleracea two protoplast types were investigated: protoplasts made from suspension culture having only very small and degenerated plastides and protoplasts of mesophyll cells with normal chloroplasts. For our experiments we variated the conductivity of the external solution (8,16 and 32 mS/m) by calibration with KCl. Within this conductivity range and with a

number of 67 investigated cells, however, we were not able to classify the changes of the curve shape according to our theoretical predictions. This is probably caused by the large number of parameters which influence the rotation spectrum. In no case was it possible to differenciate between the two cell species. Most cells could not be well fitted.

In the case of Solanum tuberosum we found that a higher percentage of cells could be well fitted (see Tab.1). On an average the cells with a sufficiently good fit had a higher apparent membrane capacity. We found $(0.51\pm0.16)*10^{-2}$ F/m^2 for the well fitted protoplasts and $(0.31\pm0.13)*10^{-2}$ F/m^2 for the badly fitted protoplasts. We think that the main reason for this fact is that cells which could be fitted better had smaller vacuoles.

All investigated vacuoles could be well fitted. This corresponds to the discussion in /5/. The calculated apparent membrane capacities are comparatively small. Estimations show that this cannot only be an effect of the not considered membrane conductivity.

The rotation spectra from protoplasts of type C and D (see Fig.3) are difficult to interpret. Protoplasts of type C, cells with polar-located cytoplasm, we frequently found in Kalanchoe protoplast suspensions.

In accordance with the theoretical part /5/ we found a large number of different curve shapes. For an exact interpretation of the measured rotation spectra a large number of parameters must be known. In this paper we restricted ourselves to the indication of deviations of protoplast rotation spectra from those of single-shell models.

Altogether the calculated specific membrane capacities are comparable with the value stated in /11/ but too small in comparison with data from other literature /13/. Reasons for these deviations could be the following:

- Membrane conductivities are higher than expected (10^{-6} S/m). This would shift the resonance frequency towards higher values, but the calculated apparent membrane capacity will only be influenced slightly /8/.
- Even if the protoplasts can be fitted well by a single-shell model, the capacity calculated in this way is an "apparent" value which can be derived from the the real one /5/.

- Cell organelles and other internal membranous structures may influence the rotational spectrum.
- The specific membrane capacities of protoplasts in fact differ from species to species and from cell to cell.

In general, the experimental results confirm the theoretical prediction that the electrorotational behaviour of protoplasts is much more complex than that of single-shell spheres.
Summarizing the results it can be stated that:

1. Most of the protoplasts show an electrorotation spectrum similar to that of single-shell spheres. The correctness of the reduction to such a model, however, depends on some preconditions.
2. In all cases of bad fits we found that the maximum of the regression curve is higher than the measured rotations. This corresponds to the theoretical curves /5/. The influence of a second membrane causes a broadening of the rotation curves.
3. This deformation of the resonance curve can be satisfactorily measured and has to be considered for the interpretation of protoplast experiments. In principle each rotation curve includes information about all dielectrics (the vacuole space, the membrane systems and the cytoplasma).
4. In most protoplast experiments plasmalemma properties mainly determine the first peak. Calculated specific membrane capacities of vacuoles are as low as those of protoplasts.
5. In our theoretical and practical considerations we never found the case of two separated maxima in the range of the first peak. This is in contradiction to /12/.
6. For a more correct interpretation of protoplast experiments measurements have also to be carried out in the frequency range of the second peak and measurements of all relevant geometric data have to be performed in addition. It would be a great advantage if measurements could be continued with the same cell after changing the external conductivity etc..

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References

/1/ ARNOLD, W.M., ZIMMERMANN, U., Ztschr. f. Naturk. 37C (1982) 908
/2/ BAUER, R., RYSCHKA, U., LEIKE, H., Olomouc, 24.-29.Sept. (1984) 407
/3/ DUDITS, D., KAO, K.N., CONSTABEL, F., GAMBORG, O.C., Can. J. Bot. 54 (1976) 1063
/4/ FUHR, G., (1985) Dissertation, Humboldt-University, Berlin, GDR
/5/ FUHR, G., GIMSA, J., GLASER, R., studia biophysica 108 (1985) 149
/6/ FUHR, G., GLASER, R., HAGEDORN, R., Biophys. J. 48 (1985) in press
/7/ FUHR, G., HAGEDORN, R., GORING, H., studia biophysica 102 (1984) 221
/8/ GLASER, R., FUHR, G., in: BLANK, M., (Ed.) Electric Double Layers in Biology, Plenum Press New York, 1985
/9/ GLASER, R., FUHR, G., GIMSA, J., studia biophysica 96 (1983) 11
/10/ HAMPP, R., ZIEGLER, H., Planta, 147 (1980) 485
/11/ LOVELACE, R.V.E., STOUT, D.G., STEPONKUS, P.L., J. Membrane Biol. 82 (1984) 157
/12/ MISCHEL, M., POHL, H.A., J. Biol. Physics 11 (1983) 98
/13/ ZIMMERMANN, U., Biochim. Biophys. Acta 694 (1982) 227

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