Studies on the centrifugation of chloroplasts in *Lemna trisulca*

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INTRODUCTION

Northen (1936—1939) demonstrated that to displace chloroplasts in plant cells the centrifugal force must exceed the initial starting acceleration defined as c_0 . The value of c_0 , interpreted as the viscosity or the elasticity of the protoplasm, is considered to be a characteristic trait of the cell as a whole. In cells with one chloroplast it is easy to define the value of c_0 but in cells with numerous chloroplasts some chloroplasts are centrifuged even by weak centrifugal forces while a few remain unaffected even by relatively high accelerations (Virgin 1951). In the latter kind of cells the value of c_0 must be defined according to arbitrarily accepted criteria.

The aim of this investigation has been to find the value of c_0 not for the whole cells but for the particular chloroplasts and to define the statistical variation of this value with regard to light conditions.

MATERIAL AND METHODS

For experiments leaves of the water plant *Lemna trisulca* were used. The plants were collected from a natural habitat when they were in full vegetation (July and August) and were cultivated in an aquarium in stabilized conditions during 2—3 weeks before the beginning of experiments. The observations were carried out on leaves 5—6 mm long in the part consisting of one layer of mesophyll.

An electric centrifuge was used. It was powered through two sliding contact resistances and could be accurately adjusted to the necessary number of revolutions. The speeds in the experiments ranged from 500 to 4500 r.p.m. and thus, since the radius of the centrifuge was 12 cm, the centrifugal accelerations ranged from 33xg to 2700xg. The plants were

placed in slides similar to those used in earlier experiments (Zurzycka and Zurzycki 1951) but made of a plastic plate and provided with a sliding cover glass. The clamps were placed inside the tubes of the centrifuge in tap water. This procedure made possible the orientation of plants with regard to the direction of the centrifugal force.

Since the return of chloroplasts at the end of centrifugation was very rapid (Virgin 1949) and clearly marked in the cells of *Lemna* leaves already two minutes after the centrifuge had stopped the leaves had to be fixed immediately. The time lapse between switching off at maximum speed and placing the leaves in the fixative was 20—30 seconds. Karpachenko's fluid was used as the fixative (I — 4.5 g of chromic acid and 36 ml of glacial acetic acid in 500 ml of water, II — 25 ml of 40 per cent formaline in 500 ml of water; equal parts of the two liquids were mixed directly before fixation). Control tests revealed that slight displacements of chloroplasts occurred even in the first minutes of fixation. To prevent these displacements the fixative was cooled in a refrigerator to 0—3°C and during the first hour of fixing the temperature was maintained at this low level. With this procedure no returns of chloroplasts were observed after centrifuging had stopped.

The leaves selected for the experiments were kept in light of 500 luxes during 4 hours. In these conditions practically all the chloroplasts assumed the flat arrangement. Then after counting the number of chloroplasts on the upper and under face of 10 cells in a chosen part of a leaf it was exposed to light of intensities inducing the desired arrangement of chloroplasts.

The following light intensities and irradiation times were applied:

- darkness 4 hours (apostrophe)
- 500 luxes 4 hours (epistrophe)
- 40 000 luxes 1 hour (parastrophe)
- about 130 000 luxes 2 hours (aggregation)

A low voltage lamp was used as source of light. Light was passed through a heat filter (Schott KG1 5 mm) and reached the objects either directly or through a system of lenses.

After centrifuging and one hour of fixing the undisplaced chloroplasts were again counted in 10 cells of the same part of the leaf as before. In this way it was possible to establish the percentage of chloroplasts which had not been displaced by centrifuging.

RESULTS

The separation of chloroplasts after various times of centrifuging was examined for 3 different centrifuging speeds, i.e. 1500, 3000 and 4000 r.p.m..

corresponding to 300xg, 1200xg and 2140xg. The time of centrifugation ranged from half a minute to 60 minutes. The results illustrated by the curves in fig. 1 indicate that all the chloroplasts were displaced only by the highest centrifugal acceleration. In the case of smaller accelerations the state of equilibrium reached after about 30 minutes remained unchanged by prolonged centrifuging. In the state of equilibrium some chloroplasts were and others were not displaced.

When the value c_0 is regarded as a property of individual chloroplasts and not of the cell as a whole it becomes obvious that the c_0 of the chloroplasts which were displaced was greater than the applied centrifugal acceleration, whereas the chloroplasts that remained undisplaced had

Fig. 1. The displacement of chloroplasts caused by centrifuging plotted against time. x-axis — time of centrifuging, y-axis — the percentage of displaced chloroplasts. The curves illustrate the course of centrifuging for accelerations of 300xg, 1200xg and 2140xg. Each point is the average of 4 measurements

a higher c_0 . Consequently, from the arrangement of chloroplasts after 30–40 minutes of centrifuging and by applying various accelerations it is possible to establish the statistical distribution of the value c_0 for the particular chloroplasts under specified conditions.

The statistical variation of the value c_0 for individual chloroplasts in various light conditions is illustrated by figs. 2–6. After the initial irradiation of 500 luxes (fig. 2) weak centrifugal forces lead to the displacement of only a few chloroplasts. For centrifuging half of the chloroplasts the acceleration must be about 800xg and all chloroplasts are displaced only when the acceleration is 2700xg. The frequency curve of c_0 is more or less symmetrical with the maximum at about 1000xg.

In darkness the chloroplasts assume the apostrophe arrangement and then their behaviour during centrifuging changes considerably (fig. 3). Even when low centrifugal accelerations are applied the proportion of displaced chloroplasts is relatively high (about 50 per cent at 300xg), but

Fig. 2. Variation of the value c_0 of chloroplasts after exposure to light of 500 luxes for 4 hours (epistrophe). x-axis — centrifugal acceleration. Thin line — per cent of displaced chloroplasts after 40 minutes of centrifuging. Each point is the average of 4—5 measurements. Heavy stepped line — frequency distribution of the values of c_0 . Numbers along the y-axis refer to the thin line and numbers in brackets to the frequency curves

Fig. 3. Variation of the value c_0 of chloroplasts in leaves kept in darkness for 4 hours (apostrophe). Details as in fig. 2. Broken line — values for weak light

those that remain are very resistant to acceleration, so much that to displace the last chloroplasts the acceleration must be about 2500xg. The

frequency curve of c_0 is distinctly asymmetrical with one maximum at about 200xg and another faint maximum at about 1400xg.

In strong light of 40 000 luxes the chloroplasts assume the profile arrangement (parastrophe). The displacement of chloroplasts from this arrangement (fig. 4) proceeds similarly as in the case of epistrophe. Low

Fig. 4. Variation of the value c_0 of chloroplasts after exposure to light of 40000 luxes for 1 hour (parastrophe). Details as in fig. 2

Fig. 5. Variation of the value c_0 of chloroplasts after exposure to light of 130000 luxes for 2 hours (aggregation). Details as in fig. 2

accelerations displace only a few chloroplasts. For displacing half of the chloroplasts the acceleration must be 640xg and for displacing all 2500xg. The frequency curve of c_0 is more or less symmetrical but, as compared

to the curve in fig. 2, is somewhat shifted in the direction of lower values with one maximum at about 800xg.

Very strong irradiation (130 000 luxes) causing the aggregation of chloroplasts (Zurzycki 1955) facilitates the displacement of chloroplasts by centrifuging (fig. 5). Half of the chloroplasts are displaced when the acceleration is 360xg and the most resistant when it is 2000xg. A large proportion of chloroplasts is displaced at low accelerations. The frequency curve of c_0 has one peak with the maximum at about 400xg. It is to be stressed, however, that the behaviour of chloroplasts in this last case is not fully comparable to their behaviour in other conditions;

Fig. 6. Variation of the value c_0 of chloroplasts in leaves first exposed to light of 500 luxes for 4 hours then to light of 40,000 luxes for 6—8 minutes. Details as in fig. 2

aggregated chloroplasts do not lie flat against the cell walls as in all the other arrangements and thus their adhesion is here different. Moreover, the grouping of chloroplasts in aggregations probably has some influence on the effects caused by centrifuging.

All the above mentioned arrangements of chloroplasts at the time of centrifugation in a state of equilibrium represent the adaptation of cells to existing light conditions. The question thus arose what would be the behaviour of chloroplasts in the state of disequilibrium proceeding their full adaptation? An answer to this question was sought by examining the susceptibility of chloroplasts to centrifuging in the course of the epistrophe-parastrophe phototactic reaction. Cells with chloroplasts in the flat arrangement (initial irradiation of 500 luxes) were centrifuged immediately after being exposed to strong light (40 000 luxes) for 6—8 minutes. Strong light induces phototactic displacements of chloroplasts so that after about 7 minutes some 20 per cent of them are already arranged

against the lateral walls of cells. When chloroplasts are centrifuged in the course of this reaction their behaviour (fig. 6) differs entirely from what is observed in stabilized conditions of both weak and strong light. A large proportion of chloroplasts (about 35 per cent) is displaced by very weak centrifugal accelerations, i.e. accelerations of less than 100 xg . On the other hand, the ability of other chloroplasts to resist the centrifugal force is very little altered. The frequency curve of c_0 has two well marked peaks with one maximum at about 50 xg and another one at about 700 xg .

DISCUSSION

In cells with numerous chloroplasts there are considerable individual differences between chloroplasts in their tendency to be displaced by centrifuging. When the statistical distribution of these differences are known the state of a cell can be defined more accurately than by defining the value of c_0 for the whole cell with such arbitrarily chosen criteria as e.g. the acceleration necessary to displace after prolonged centrifuging 100 or 90 per cent of chloroplasts.

The results here reported are essentially conformable with Virgin's (1951) results from his experiments on the influence of light on the cells of *Helodea densa*. In *Helodea*, similarly as in *Lemna*, the most difficult to displace by centrifuging are the chloroplasts in cells exposed to weak light, whereas in cells kept in darkness the displacement of chloroplasts is much easier. Some differences in the response of *Lemna* and *Helodea* are apparent in strong light. Virgin states that in *Helodea* leaves exposed to strong light the chloroplasts are easily displaced by centrifuging and that this trait is retained for quite a long time. In *Lemna*, on the other hand, the chloroplasts are easily displaced only during a short time in the course of the epistrophe — parastrophe reaction, but when the chloroplasts assume the profile arrangement they again become resistant to centrifuging. This effect seems to be associated with the fact that in *Lemna* the chloroplasts in the profile arrangement are distributed in those parts of a cell where only weak light penetrates (Senn 1908), i.e. in the same light conditions as those prevailing in weak light on the upper and under walls of cells. In such conditions they again become strongly attached to the external proplasmic layer lining the cell walls. In *Helodea* strong light induces the rotation of the protoplasm and the chloroplasts are kept in constant motion with the rotating protoplasm which makes them very susceptible to centrifugal forces. But even in *Helodea* under certain conditions strong light may cause a temporary decrease and then again an increase of the tendency to resist centrifugal forces (Virgin 1951, fig. 16a).

According to Northern (1937) the value of c_0 defines the elasticity of protoplasm, whereas in Virgin's (1951) interpretation it is a measure of viscosity. This author (Zurzycki 1959) has advanced the suggestion that the tendency of chloroplasts to be displaced by centrifuging (c_0) is essentially a measure of the consistency of that part of protoplasm which connects a chloroplasts with the ectoplasmic layer lining the cell walls. It is therefore a measure of viscosity or of elasticity, but with regard to a definite part of the protoplasm. Already Northern (1938) believed that the definition of c_0 as a measure of the viscosity of protoplasm might be misleading because of the great individual differences between cells. The data here reported on the distribution of the value of c_0 for the particular chloroplasts fully confirm Northern's opinion. It seems that the most correct approach would be to define c_0 as a measure of the consistency of the chloroplast/ectoplasm contact layer determining the force with which the chloroplasts is attached to the motionless protoplasmic layer. The consistency of the chloroplast/ectoplasm layer is greatest when the chloroplasts are exposed to weak light, i.e. on the top and bottom cell walls in weak light and on the lateral walls in strong light. The beginning of phototactic displacements must be preceded by a change of the consistency manifested by a weaker attachment of chloroplasts to the outer protoplasmic layer.

SUMMARY

1. The value of the initial starting acceleration (Northern's c_0) for individual chloroplasts is highly variable in cells of *Lemna trisulca*. The statistical distribution of the value c_0 depends on the prevailing light conditions.

2. The value of c_0 of chloroplasts is highest in weak light (epistrophe) and in strong light (parastrophe) after the arrangement characteristic for the prevailing light conditions is stabilized. In darkness the frequency curve of c_0 is shifted towards lower values.

3. In the course of phototactic displacements of chloroplasts the value of c_0 for a large proportion of chloroplasts is very low.

4. The value of c_0 defines the consistency of that part of the protoplasm which connects the chloroplasts with the ectoplasm and determines the force with which chloroplasts are attached. The consistency of this layer can vary over a wide range when the intensity of incident light changes. The loosening of the consistency marks the beginning of phototactic movements.

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