ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXXVII — nr 1
1968

Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*

V. Studies on plasmolized cells

J. ZURZYCKI

INTRODUCTION

As it was shown in the previous parts of this study the photoreceptor active in the cells of *Funaria hygrometrica* is regularly arranged in respect to the cell walls. The molecules of the photoreceptor are oriented so that their vector responsible for absorption of blue light is situated parallel to the cell surface (Zurzycki 1967 b). Electron microscopic studies of the Funaria cells suggest that only two structural components i.e. cell walls and outer plasma membranes offer enough great stability and orientation to be the site of oriented photoreceptor molecules. (Zurzycki 1967 d). The aim of the present study is to decide which from the above mentioned elements plays the role of the basal structure for the photoreceptor. The way of solving this problem was found in the studies of plasmolized cells where plasmalemma and cell walls are spaciously separated.

METHOD

The leaves of *Funaria,* after cutting off from the stem, were kept 24 hours in sucrose solution in 0,1 M phosphate buffer pH 6,9. While remaining in the plasmolyticum the material was illuminated with low light intensity. For microscopic examination leaves were mounted on the slide in the same medium. Cover glasses were sealed with hot vaseline to prevent any changes in concentration of the medium due to evaporation.

Registration of the position of chloroplasts was made by counting the number of chloroplasts having a flat position on the upper and lower cell face. Countings were made in time intervals in the same 10 chosen cells. Illumination of material was performed according to the previously described method (Zurzycki 1967 b).

RESULTS

The osmotic value of the *Funaria* leaf cells lies between 0,35 and 0,4 M/l sucrrose. In 0,35 M/l usually no signs of plasmolysis are visible, in 0,4 M/l the protoplasts are detached from the cell walls at two edges. This is true of the cells of the middle part of the leaf. The gradient of osmotic value makes that the cells lying near the basis of the leaf show the beginning of plasmolysis in some lower concentrations of the plasmolyticum, the cells in the apical region — in higher ones. The shape of a plasmolized protoplast depends on the sucrose concentration. Till about 0,6 M/l the detached parts of a protoplast have round outlines after 24 hours of plasmolysis. In the concentrations higher than 0,75 M/l the protoplast is shrunken irregulary and shows a strong adhearance to the cell walls (fig. 1).

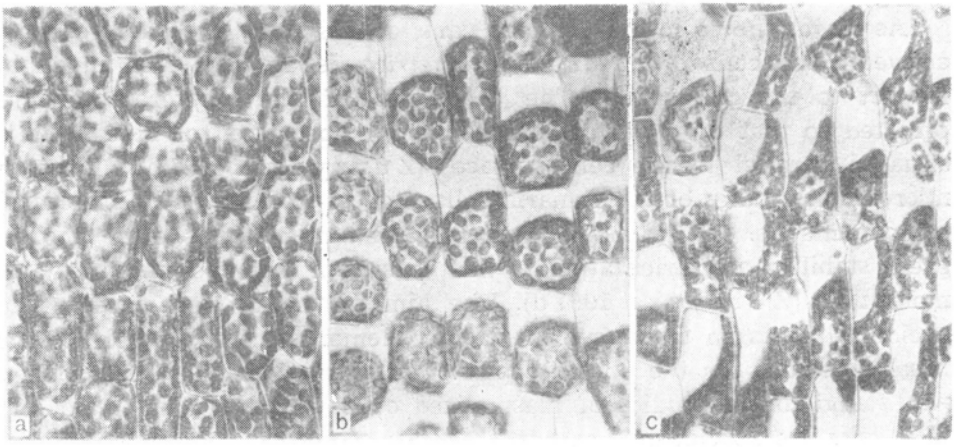


Fig. 1. Shapes of plasmolized protoplasts after 24 hours treatment with buffered sucrose solutions of different concentration: a — 0,4 M/l, b — 0,5 M/l, c — 0,8 M/l.

The problem which should be solved was to answer the question if the border parts of a protoplast detached from the cell walls are still the site of regularly arranged photoreceptor molecules. Because the cytochemical detection of a photoreceptor failed (Zurzycki 1967 c), the only criterion of its existence is the photoreaction of chloroplasts. At first it was therefore necessary to find if plasmolysis does not stop the displacements at all. Only in the case when the secondary mechanism of displacements is not quite disturbed due to plasmolysis the rearrangements of chloroplasts may be the sign of light reception in the cell.

The ability of chloroplasts to photo-displacements was studied for strong light reaction. Leaves with plasmolized cells were irradiated with blue light of the intensity of 180 000 erg/cm²sec. The results are shown in fig. 2. In plasmolized cells an exact determination of the total

number of chloroplasts was impossible. Therefore the criterion of rearrangements of chloroplasts was based on the absolute number of chloroplasts being in a given moment in flat position as determined in 10 chosen cells. This is the case why the initial number differs from case to case and is the lower the bigger the degree of shrinkage of protoplast. As it can be seen in fig 2 the ability to displacements is the

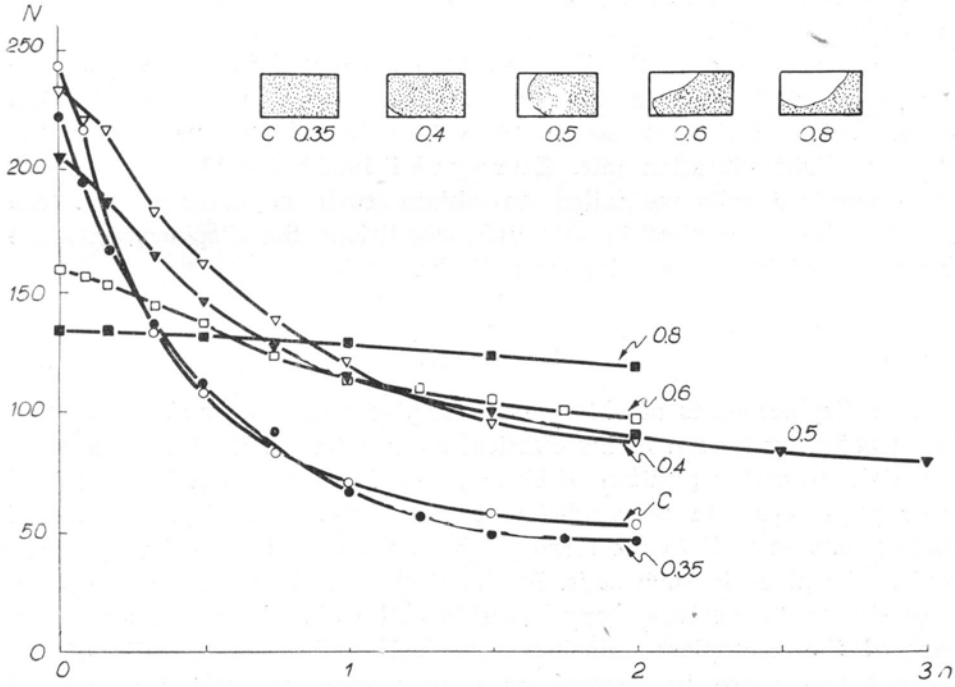


Fig. 2. Displacements of chloroplasts in high intensity (180 000 erg/cm² sec.) of blue light in dependence on the concentration of plasmolyticum. X axis — time in hours, Y axis — number of chloroplasts in flat position in 10 chosen cells. Figures by the curves show the molar concentration of sucrose.

same in phosphate buffer (C) and in sucrose solutions which do not induce plasmolysis (till 0,35 M/l). The higher the concentration of plasmolyticum (above 0,35 M/l) the slower and more limited the displacements. In 0,8 M/l only very slight displacements could be stated.

For further experiments the 0,5 M/l concentration of sucrose was chosen as a matter of compromise. In this concentration the protoplast is distinctly detached from one of the side cell walls and displacements induced by high light intensity are still not very disturbed.

Cells plasmolized in 0,5 M/l sucrose were irradiated with blue polarized light of the intensity 130 000 erg/cm²sec. directed either perpendicular or parallel to the longer cell axis. The position of chloroplasts taken after 4 hours of irradiation is shown in fig. 3 and 4

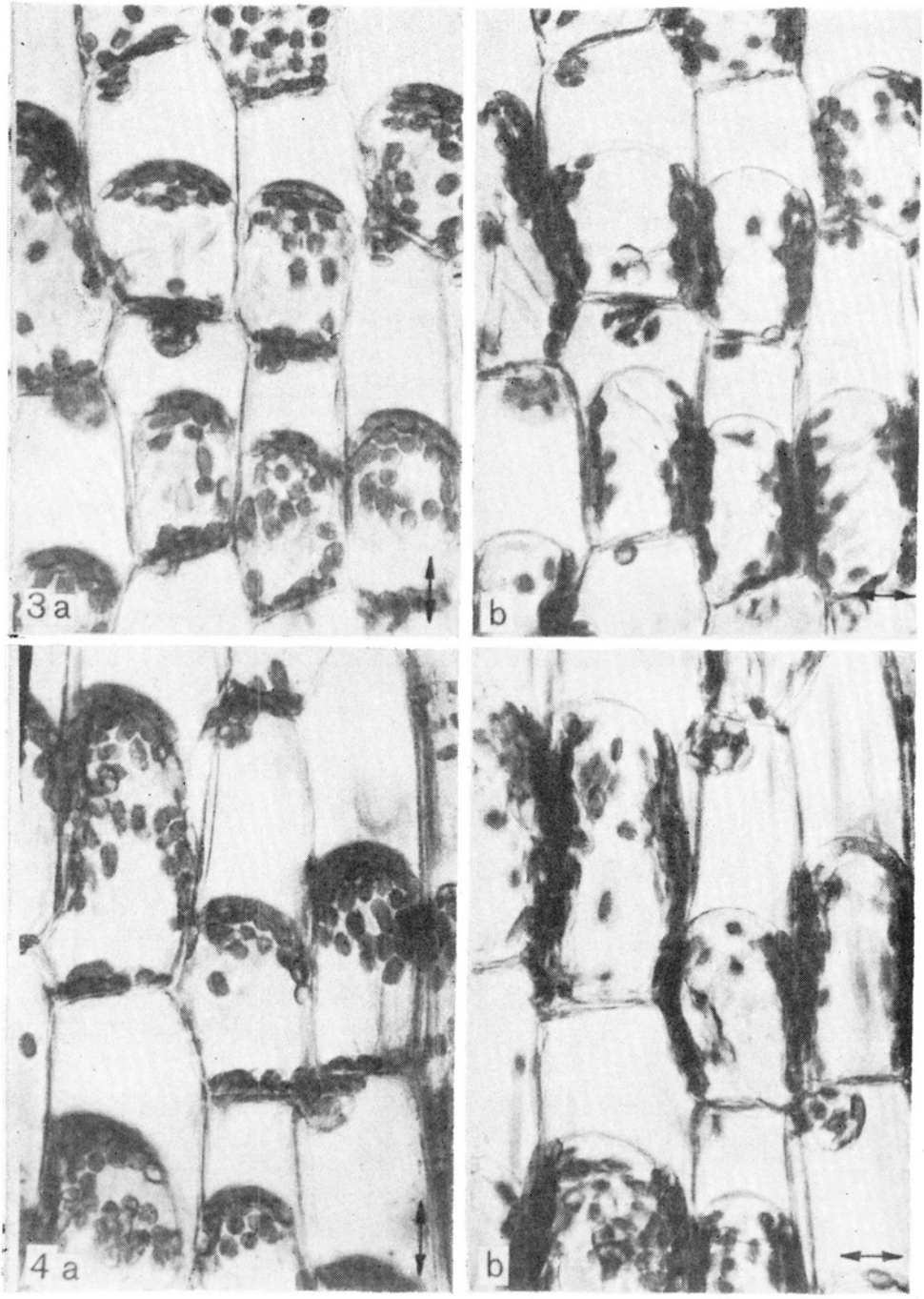
(Plate I). The reaction of chloroplasts is clearly the same as in normal unplasmolized cells (Zurzycki 1967 b) i.e. they show the accumulation on these side parts of protoplasts which are perpendicular to the vibration plane of light. The parts of a protoplast detached from cell walls react in the same manner as the parts which are in contact with cell walls. The conclusion may be drawn that a regular arranging of photoreceptor molecules is retained in the protoplast after its disjunction from the cell walls.

In the normal cells the illumination with low light intensity (300—1000 erg/cm²sec. of blue light) induces the inverse arrangement i.e. accumulation of chloroplasts by the cell walls oriented parallel to the plane of light vibration (cfr. Zurzycki 1967 b fig 11). In the case of plasmolized cells we failed to obtain such an arrangement. The probable reason is that in this light conditions the displacements are extremely slight, almost stopped entirely.

DISCUSSION

The displacements of chloroplasts in plasmolized *Funaria* cells were just treated by Senn in his classical studies (1908). He found that the low light intensity position of chloroplasts is at least partialy retained after plasmolysis in favorable light conditions. The displacements of chloroplasts as well to the strong light position as to the dark position could take place in such cells. In the first case the chloroplasts do not only situate themselves along the side cell walls but also at the side part of the protoplast detached from cell walls. The results of the present study are in general agreement with the above mentioned observations of Senn. The quantitative treatment of the displacements show only that the speed and range of the displacements depend on the degree of dehydration of the protoplast. According to Senn the chloroplasts of *Funaria* accumulate in sucrose solution considerable amounts of starch and in that condition the displacements are retarded and a tendency to dark arrangement takes place. This may be the reason why we did not obtain clear displacements in low light intensity of polarized light.

In plasmolized cells the structure and properties of the outer layer of a protoplast does not undergo essential changes. This may be deduced from the general osmotic behaviour of the protoplast and was recently confirmed by Sitte (1963) in his electron microscopy studies of plasmolized cells. Sitte found that the plasmolized protoplast is surrounded by a plasmalemma membrane of the same structural character as the unplasmolized one. The "normal" structure of the outer plasma membrane was especially clearly visible for plasmolysis in sucrose solutions.



Figs 3 and 4. Position of chloroplasts after 4 hours irradiation with polarized blue light of intensity 130 000 erg/cm² sec. Cells plasmolized in 0,5 M/l sucrose solution. a — plane of light vibration parallel to the longer cell axis, b — the same cells after irradiation with light vibrating perpendicular to the longer cell axis.

In the former part of this study (Zurzycki 1967 d) a supposition was made that photoreceptor molecules are situated either in the cell wall or in the outer plasma membrane. The reaction to strong light of a plasmolized cell which results in the accumulation of chloroplasts at the side parts of protoplast is not a sufficient proof for the existence of photoreceptor in the plasma membranes detached from the cell walls. The localization of chloroplasts in that case may result in flowing out of them from the upper and lower faces of the cell which are most

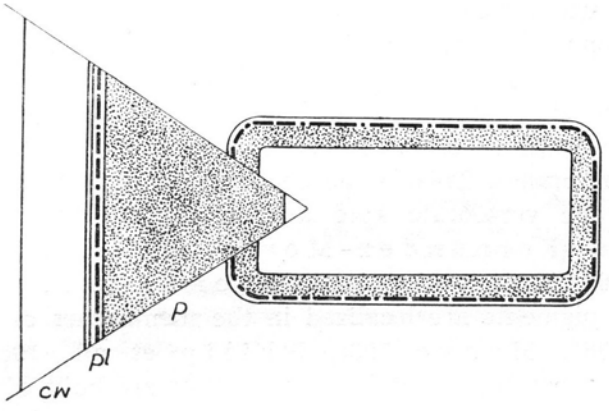


Fig. 5. Scheme of localization of the photoreceptor molecules. *cw* — cell wall, *pl* — outer plasma membrane, *p* — protoplasm.

intensivelly illuminated and the protoplast is usually in contact with cell walls along these faces. On the other hand the results obtained with polarized light show that the detached parts of a protoplast react "normaly" i.e. they are the site of attraction for chloroplasts when light vibrates perpendicularly to the surface of the protoplast or the chloroplasts avoide these places in the case of parallel vibration. The conclusion may be drawn that even after detaching from the cell walls the molecules of the photoreceptor are still regularly arranged in the protoplast. It means in comparision with the results of previous studies that the molecules of the photoreceptor are situated in the outer plasma membrane, as it is schematically shown in fig. 5.

This conclusion may explain the doubtful interpretation of the results obtained by densitometric studies made in polarized light. We may take as the quantitative characteristic of dichroism the value $k\parallel/k\perp$ where $k\parallel$ and $k\perp$ are the absorption coeficients of the structure in question by parallel and perpendicular plane of light vibration in respect to a given plane or axis of the structure (in this case to the surface plane of the protoplast). Judging from the behaviour of chloroplast the ratio $k\parallel/k\perp$ should be of order 5—10 (Zurzycki 1967 b) and in densitometric studies we obtained only 0—0,4 (Zu-

r z y c k i 1967 c). If one takes into consideration that the arranged pigment molecules are localized in the plasma membrane of thickness of the order 100Å it is comprehensible that either none or only some part of the dichroism can be detected by the densitometric method in a light microscope.

The situation of riboflavin in the lipoproteid membrane may have the important influence on the absorption properties of this pigment. As it was mentioned by T h o m a s, the position and mutual relation of the absorption peaks of riboflavin differ considerably in lipid solvent in comparision to water solvent (T h o m a s 1965 fig 70). This phenomenon may explain some disagreements between the action spectrum and the absorption spectrum of riboflavin in water (Z u r z y c k i 1967 a).

It should be stressed that very often the photoreceptors active in many photobiological reactions in plants and animals are situated in the plasma membranes. This is the case with visual pigments in rods and cones of the vertebrate eyes and in rhabdomers of the insect compound eyes (F e r n á n d e z - M o r á n 1959, H a r t l i n e 1959, W o l k e n 1960, K e n n e d y 1964, T h o m a s 1965). Similarly the photosynthetic pigments are localized in the membranes of chloroplasts (T h o m a s 1960, M e n k e 1966, P h i l i p et all. 1966). In the mentioned cases special photoreceptor organelles are built of a composed lamellar system. In the case of *Funaria* cell we have to do with a very simple system in which the photoreceptor is localized in one layer of the outer plasma membrane. One may suppose that this primitive system is characteristic of plant cells and is connected with many kinds of photoreactions. Displacements of chloroplasts are only one of the easily observed kinds of these reactions.

SUMMARY

Photo-displacements of chloroplast in plasmolized *Funaria* cells were studied. It was found that a strong light rearrangement of chloroplasts takes place after plasmolysis in sucrose solutions when the degree of dehydration of protoplast is not too great. Arrangements taken by chloroplasts in plasmolized cells under illumination with polarized light show that the parts of protoplast detached from cell walls are still the site of a regularly arranged photoreceptor. The conclusion was drawn that the photoreceptor molecules are localized in the outer plasma membrane.

Department of Plant Physiology
Jagellonian University, Cracow, Grodzka 53.

(Entered: January 19, 1967)

LITERATURE

F e r n á n d e z - M o r á n H., 1959, Fine structure of biological lamellar systems. in: Biophysical Science — A study program. ed. by J. B. Oncley, New York. J. Wiley. 319—330.

Hartline H. K., 1959, Receptor mechanisms and the integration of sensory information in the eye. in: Biophysical Science — A study program. ed. by J. L. Oncley. New York. J. Wiley. 515—523.

Kennedy D., 1964, The photoreceptor process in lower animals. in: Photophysiology ed. by A. C. Giese, Acad. Press. New York. 2:79—121.

Menke W., 1966, The structure of the chloroplasts. in: Biochemistry of chloroplasts. ed. by T. W. Goodwin. Acad. Press. London 1:3—18.

Philip M., Litilhac W. and Park R. B., 1966, Localisation of chlorophyll in spinach chloroplast lamellae by fluorescence microscopy. J. cell. Biol. 28: 582—585.

Senn G., 1908, Gestalts- und Lageveränderung der Pflanzen-Chromatophoren. W. Engelmann, Leipzig.

Sitte P., 1963, Zellfeinbau bei Plasmolyse. II. Der Feinbau der *Elodea*-Blattzellen bei Zucker- und Ionenplasmolyse. Protoplasma 57:304—333.

Thomas J. B., 1960, Chloroplast structure. in: Encyclopedia of plant physiology ed. by W. Ruhland. Springer Verlag, Berlin 5(1):511—565.

Thomas J. B., 1965, Primary photoprocesses in biology. North-Holland Publ. Co. Amsterdam.

Wolken J. J., 1960, Photoreceptors: comparative studies. in: Comparative biochemistry of photoreactive systems. ed. by M. B. Allen. Acad. Press. New York. 145—167.

Zurzycki J., 1967 a, Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*. I. Action spectrum. Acta Soc. Bot. Polon. 36:133—142.

Zurzycki J., 1967 b, Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*. II. Studies in polarised light. Acta Soc. Bot. Polon. 36:143—152.

Zurzycki J., 1967 c, Properties and localisation of the photoreceptor active in displacement of chloroplasts in *Funaria hygrometrica*. III. Cytochemical studies. Acta Soc. Bot. Polon. 36. (in press).

Zurzycki J., 1967 d, Properties and localisation of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*. IV. Electron microscopy. Acta Soc. Bot. Polon. 36. (in press).

Własności i lokalizacja fotoreceptora czynnego w ruchach chloroplastów u Fanaria hygrometrica.

V. Badania na komórkach poddanych plazmolizie

Streszczenie

Badano przemieszczenia chloroplastów indukowane światłem w splazmolizowanych komórkach liścia *Funaria hygrometrica*. Stwierdzono że plazmoliza nie jest czynnikiem blokującym mechanizm ruchów — przemieszczenia są tylko zwolnione i zmniejszony jest ich zakres w zależności od stopnia odwodnienia protoplastu. Reakcja chloroplastów w splazmolizowanych komórkach na światło spolaryzowane wykazuje że części protoplastu oddzielone od ścian komórkowych zawierają regularnie ustawione drobiny fotoreceptora. Wyniki powyższe pozwalają wnosić że drobiny fotoreceptora są zlokalizowane w zewnętrznej błonie plazmatycznej (plazmalemie).