

Conformational changes in spinach (*Spinacia oleracea*) leaves chloroplasts *in vivo*

JANINA GODZIEMBA-CZYŻ

Department of Plant Physiology, Institute of Molecular Biology,
Jagiellonian University, Cracow

(Received: November 30, 1974)

Abstract

Changes in the surface area of chloroplasts from intact cells of spinach leaves (*Spinacia oleracea*) induced by blue (370—500 nm) and red (600—850 nm) light of various intensity (10^2 — 5×10^5 erg cm⁻²s⁻¹) were investigated. The changes are described in terms of mean surface area in μm^2 and frequency of occurrence of surface size classes.

Low intensity blue light caused enlargement of the chloroplast surface (as compared with that in darkness), whereas high intensity light markedly reduced it.

Exposure of chloroplasts to red light produces an increase of the surface in proportion to the intensity of the light and irradiation time.

INTRODUCTION

One of the many reactions of chloroplasts to changes in external conditions, and above all light conditions, are modifications of their shape, surface area and volume. The first pertinent observations were reported as early as the 19th and the beginning of the 20th century (Stahl, 1880; Senn, 1908). The most convenient plant material for this kind of study were algae in view of their large chloroplasts (Gicklhorn, 1933; Peteler, 1939). The above quoted papers, however, were purely descriptive and gave no numerical data.

Extensive quantitative investigations were performed for moss chloroplasts on *Mnium undulatum* as model (Zurzycki, 1964). The results obtained for bryophyte chloroplasts constitute an important comparative material, because their structure and shape resemble those of higher plants.

Changes in the shape and volume of chloroplasts are induced by light and may be revealed by various methods: surface measurement (Zurzycki, 1964), measurement of diameter and thickness (Hilgenheger, Menke, 1965; Zurzycki, 1974) and the electron microscope (Kushida, Itoh, Izawa, Shibata, 1964; Miller, Nobel, 1972).

The finding that isolated chloroplasts also react to light conditions by changes in shape and structure (Itoh, Izawa, Shibata, 1963; Packer, 1963) made possible the follow-up of these changes by simple methods giving more exact results. To these methods belong measurement of the degree of light dispersion in a suspension (Itoh, Izawa, Shibata, 1963; Packer, 1963), measurement of the compact mass after centrifugation (Belsky, Siegenthaler, Packer, 1963; Izawa, Good, 1966), of chloroplast volume by means of a Coulter counter (Itoh, Izawa, Shibata, 1963) and examination in an electron microscope (Izawa, Itoh, Shibata, 1963; Sundquist, Burris, 1970).

At present the trend of studies runs towards the establishment of changes in the fine structure of chloroplasts caused by irradiation (Sundquist, Burris, 1970; Miller, Nobel, 1972), detection of the pigments, mediating the conformation changes (Izawa, Itoh, Shibata, 1963; Zurzycki, 1964) and elucidation of the complicated mechanism of these transformations (Packer, 1963; Itoh, Izawa, Shibata, 1963; Dilley, Vernon, 1965; Krause, 1973; De Filippis, Pallagby, 1973).

Since there are few reports up to date in the literature concerning chromatic light, the present study was aimed at investigation of the influence of blue and red light on the conformation of chloroplasts in intact cells of *Spinacia* leaves. It was endeavoured to express these changes numerically and compare them with analogous changes noted in mosses on the example of *Mnium undulatum* (Zurzycki, 1964) and *Funaria hygrometrica* (Zurzycki, 1974).

MATERIAL AND METHODS

The experiments were performed with spinach (*Spinacia oleracea*) leaves from a stable source. In this material the surface area of the chloroplasts used for the experiments ranged from 3.1 to 28.7 μm^2 (notwithstanding the light conditions); the mean surface area was 13.5 μm^2 . This range is much smaller than that reported by Honda (1971 — 6.2–102.9 μm^2 , mean surface area 27.7 μm^2). Thus the plant material was rather uniform.

From even-sized leaves 2×2 cm squares were cut, infiltrated with water and placed on moist filter paper in Petri dishes. The preparations

were then placed in the chamber of the irradiation apparatus (Lechowski, 1972). As source of light a projection lamp (750 W, 110 V) was used. The light passed through a suitably built optical system which focussed the light and directed its beam into the chamber with the preparation. In this way a light spot of 2 cm diameter was obtained. The optic system was provided with a heat radiation-absorbing water filter and two glass filter combinations: for red light RG2+KG1 (range 600—850 nm) and for blue light BG1+GG13 (range 370—500 nm). The irradiation chamber was surrounded by a water jacket maintaining a constant temperature around 25°C.

Light intensity was regulated by neutral filters and changing the lamp voltage by means an autotransformer. Measurements at the level of the preparation were taken with a Zeiss V Th-8EN thermocouple.

Preparations were made of leaves kept for 12 h in darkness. After infiltration with water the preparations were again transferred to darkness (control) or illuminated with blue or red light of the following intensities: 10^2 , 10^5 and 5×10^5 erg cm⁻²s⁻¹. The time of illumination or keeping in darkness was 0.5, 1, 1.5 and 2 h, respectively.

Chloroplast observations in the illuminated preparations were made under a PZO microscope, ocular $\times 15$, 100 immersion objective, and the contours were delineated with the use of a drawing ocular PZO MNR-1. The enlargement of the drawing was $\times 4,000$. The drawings were planimetric and by suitable conversion the surface area of the chloroplasts was obtained in μm^2 .

Each series of experiments was performed on 4 preparations from which a total of 200 chloroplast drawings were obtained. After determining their surface area the mean was calculated.

RESULTS

The mean surface area of spinach leaves kept for 12 h in darkness was 11.9 μm^2 .

The dependence of the chloroplast surface on the intensity and time of exposure to blue light is shown in the Table and Fig. 1. Analysis of the numerical data shows that at low light intensities of about 10^2 erg cm⁻²s⁻¹ the chloroplast surface enlarges (from 11.9 μm^2 in darkness to 14.4 μm^2 after 0.5 h). The longer the time of exposure the smaller was the increment in surface area. On the other hand, at higher intensities of blue light (10^5 — 5×10^5 erg cm⁻²s⁻¹) the surface of chloroplasts distinctly decreased (to 7.5 μm^2 at intensity 5×10^5 erg cm⁻²s⁻¹, exposure time 1.5—2 h). The decrease in the chloroplast surface is also dependent on the time of exposure, but after about 1.5 h of action of blue light, the surface stabilizes at a constant value.

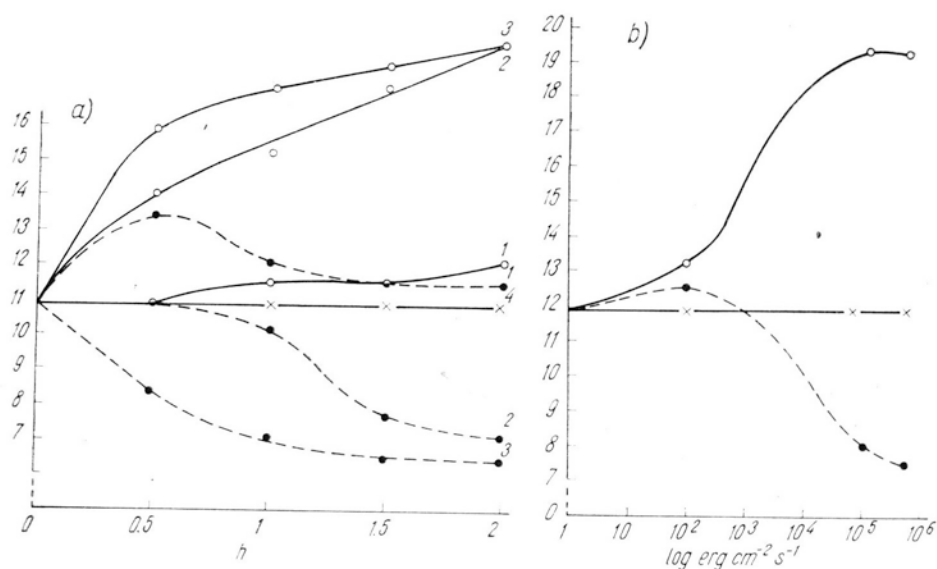


Fig. 1. Effect of blue and red light on the surface areas of spinach chloroplasts, depending on light intensity and the time of illumination

a — x axis — time of illumination (h), y axis — surface area of chloroplasts (μm^2). Continuous line — effect of red light and darkness, interrupted line — effect of blue light. Curves: 1 — light intensity $10^2 \text{ erg cm}^{-2} \text{s}^{-1}$, 2 — light intensity $10^5 \text{ erg cm}^{-2} \text{s}^{-1}$, 3 — light intensity $5 \times 10^5 \text{ erg cm}^{-2} \text{s}^{-1}$. b — x axis — logarithm of light intensity in $\text{erg cm}^{-2} \text{s}^{-1}$, y axis — as in Fig. 1a. Continuous line denotes effect of red light (points denoted by clear circles) and darkness, interrupted line — effect of blue light. Time of illumination constant, 2 h.

Table 1

Effect of blue and red light on the surface area (μm^2) of spinach chloroplasts

| Light intensity | | Blue light 370—500 nm | | | | Red light 600—850 nm | | | |
|--|-----------|--------------------------|------|------|------|-------------------------|------|------|------|
| intens. in $\text{erg cm}^{-2} \text{s}^{-1}$ | time in h | 0.5 | 1 | 1.5 | 2 | 0.5 | 1 | 1.5 | 2 |
| | | | | | | | | | |
| 0 | | 11.9 | 11.9 | 11.9 | 11.9 | 11.9 | 11.9 | 11.9 | 11.9 |
| 10^2 | | 14.4 | 13.1 | 12.5 | 12.5 | 11.9 | 12.9 | 12.5 | 13.1 |
| 10^5 | | 11.9 | 11.2 | 8.7 | 8.1 | 15.0 | 16.2 | 18.1 | 19.4 |
| 5×10^5 | | 9.4 | 8.1 | 7.5 | 7.5 | 16.9 | 18.1 | 18.7 | 19.4 |

The Table and Fig. 1 also show the relation between the chloroplast surface area, light intensity and the time of exposure to red light. Spinach chloroplasts exposed to red light increase their surface area, except when the lowest light intensity $10^2 \text{ erg cm}^{-2} \text{s}^{-1}$ and the shortest time of exposure (0.5 h) are applied and the surface area remains of the same size as in darkness. At higher red light intensities a consider-

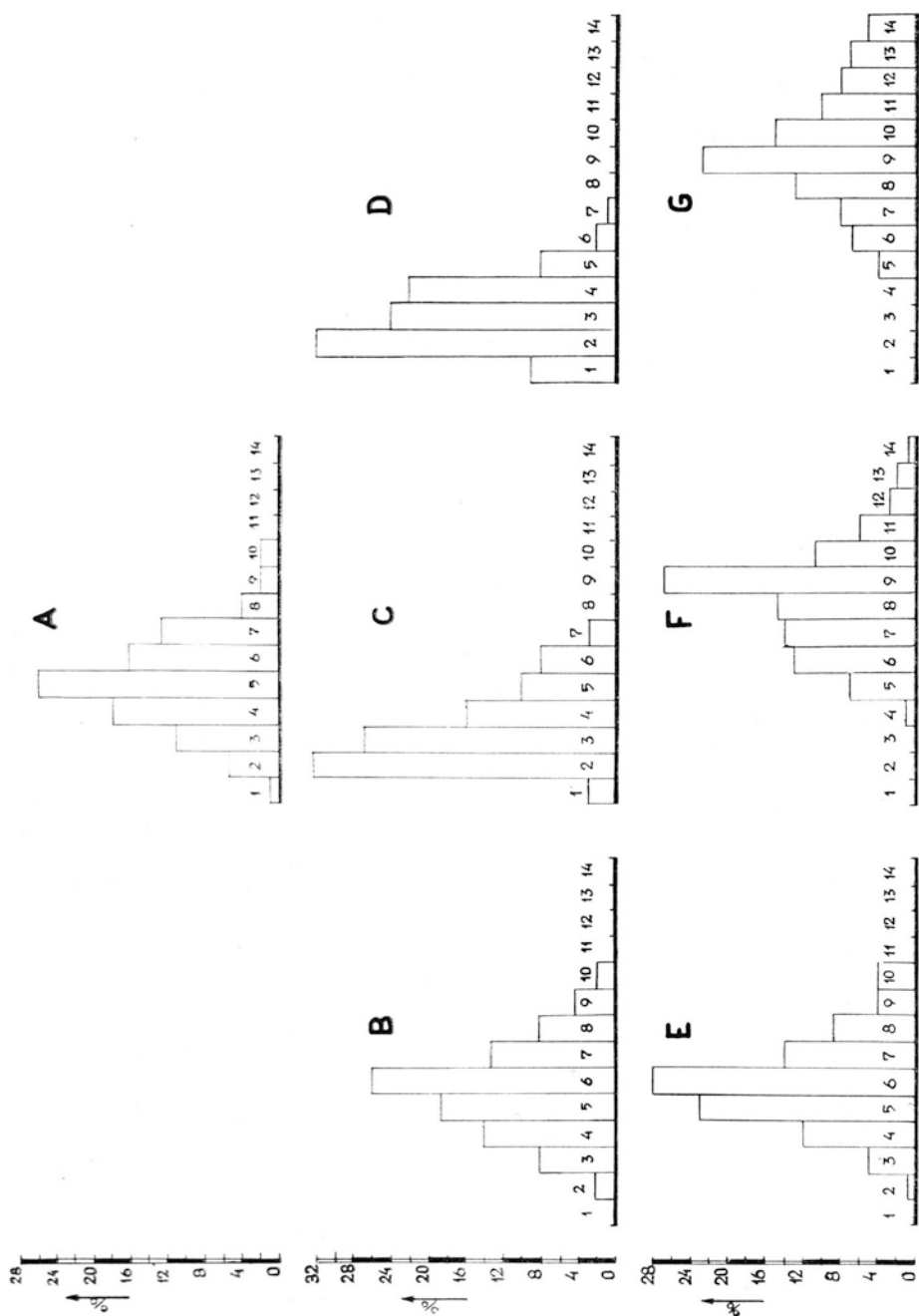


Fig. 2. Distribution of chloroplast surface area (%) in dependence on kind and intensity of light. Time of illumination constant, 1.5 h. *A* — darkness, *B* — *D* blue light, *E* — *G* — red light. Light intensities: *B* and *E* — $10^3 \text{ erg cm}^{-2} \text{ s}^{-1}$, *C* and *F* — $10^5 \text{ erg cm}^{-2} \text{ s}^{-1}$, *D* and *G* — $5 \times 10^5 \text{ erg cm}^{-2} \text{ s}^{-1}$. Size classes of surface area: 1 — $3.7 \mu\text{m}^2$, 2 — $5.6 \mu\text{m}^2$, 3 — $7.5 \mu\text{m}^2$, 4 — $9.4 \mu\text{m}^2$, 5 — $11.2 \mu\text{m}^2$, 6 — $13.1 \mu\text{m}^2$, 7 — $15 \mu\text{m}^2$, 8 — $16.9 \mu\text{m}^2$, 9 — $18.7 \mu\text{m}^2$, 10 — $20.6 \mu\text{m}^2$, 11 — $22.5 \mu\text{m}^2$, 12 — $24.4 \mu\text{m}^2$, 13 — $26.2 \mu\text{m}^2$, 14 — $28.1 \mu\text{m}^2$.

able increase in surface area is observed (from $11.9 \mu\text{m}^2$ in darkness to $19.4 \mu\text{m}^2$ at a $10^5 \text{ erg cm}^{-2}\text{s}^{-1}$ intensity, time 2 h). This process is saturated at a light intensity of $10^5 \text{ erg cm}^{-2}\text{s}^{-1}$ and further increase of light intensity does not change the chloroplast surface.

The distribution of chloroplast surface size is shown in Fig. 2. The surface areas are arranged in classes according to their mean sizes, e.g. $7.5 \mu\text{m}^2$ includes the range $6.9\text{--}8.1 \mu\text{m}^2$. All the diagrams refer to the same exposure time of 1.5 h.

The diagrams show still more distinctly the changes in spinach chloroplast surface described. If we analyse the dispersion of chloroplasts with different surface areas in dependence on the kind and intensity of light, it is readily seen that under weak blue light 26.5 per cent of chloroplast surface area lies within the range $12.5\text{--}13.7 \mu\text{m}^2$, whereas at higher intensities 32 per cent are found within the range of small surfaces of $5.0\text{--}6.2 \mu\text{m}^2$.

Under red light, particularly of high intensity, the highest per cent of even-sized chloroplasts (23—27) is in the class with a range of $18.1\text{--}19.4 \mu\text{m}^2$, whereas in darkness 26 per cent exhibit a surface ranging from 10.6 to $11.9 \mu\text{m}^2$. Thus a distinct shift towards a larger chloroplast surface area is observed.

The mean error for all the surface areas listed is $\pm 0.1\text{--}0.3 \mu\text{m}^2$.

When analysing the frequency of the size classes of spinach chloroplasts, it is seen that the mean surface sizes in the given light conditions (listed in the Table) are as a rule in agreement with the size of the surface areas occurring most frequently. Only at higher blue light intensities are the largest chloroplasts difficultly reduced in size. In such a population the most frequently found chloroplasts have a surface smaller than the mean.

Hongladarom, Honda and Wildman (1968) affirm that it is not possible to decide whether the conformation changes concern above all a certain group of chloroplasts in the population or the whole population. On the basis of the results obtained at present it appears that the changes in the chloroplast surface induced by light involve the entire population.

DISCUSSION

Senn (1908) and Zurzycki (1964, 1966, 1974) claim that blue light (range 370—500 nm) is the only range of visible light which causes shrinkage of chloroplasts, manifested by their assuming a rounded shape and a reduction of their surface.

The investigations performed for mosses chloroplasts on the example of *Mnium undulatum* (Zurzycki, 1964) found confirmation as regards

the chloroplasts of a representative of higher plants — spinach.

Spinach chloroplasts react similarly as do those of *Mnium undulatum* on exposure to blue light of various intensity, the conformation changes are, however, more pronounced and occur within a shorter time. For instance at an intensity of 5×10^5 erg cm⁻²s⁻¹ the moss chloroplasts surface, first increases and then continues to decrease during the whole time of exposure (6 h).

Spinach chloroplasts, on the other hand, show from the beginning of illumination a distinct decrease of the surface area which stabilises after 1.5 h of exposure.

Red light (600—850 nm) causes a reaction opposite to that under blue light of high intensity. The reaction consists in an increase of the chloroplast surface (as compared with its size in darkness) and is similar in the chloroplasts of moss and spinach. At an intensity of 5×10^5 erg cm⁻²s⁻¹ the surface area increment is most pronounced during the first two hours of exposure, whereas at an intensity of 10^5 erg cm⁻²s⁻¹ this increase is slower, especially in the case of moss. At an intensity of 10^4 erg cm⁻²s⁻¹ the surface area of moss chloroplasts is similar to that in darkness, while spinach chloroplasts still exhibit a slight increment of surface area at 10^2 erg cm⁻²s⁻¹.

The investigations on the moss *Funaria hygrometrica* (Zurzycki, 1974) demonstrated that red light induces an increase of the chloroplast surface area with at the same time a decrease of their thickness. This finds expression in a reduced volume (shrinkage) by about 10 per cent, independently of light intensity. Blue light has a similar action, but only at low intensity. At high intensities it causes a considerable reduction of the surface area and decrease of volume (up to 35% as compared with the chloroplasts kept in darkness).

Notwithstanding the slight quantitative differences, moss and spinach chloroplasts only increase their surface under red light. Under blue light an increase of the surface area may be observed at low or medium intensities. Strong blue light causes a considerable reduction of the chloroplast surface and volume (*Funaria hygrometrica*).

The reaction of isolated moss chloroplasts is about the same as that in intact cells. The differences are only quantitative as regards the rate of changes and their absolute values (Zurzycki, 1966).

Nobel et al. (1972) illuminating chloroplasts in the leaves of *Pisum sativum* with white light noted a slight decrease of the surface area (18.7 ± 0.3 μm^2), as compared with the chloroplasts kept in darkness (19.0 ± 0.2 μm^2). The cause of such minute changes within the limits of error was probably the short time of the experiment (10 min) and low light intensity (4 000 lux).

The structural changes in chloroplasts are controlled by light by way of two reactions (Zurzycki, 1966, 1967): Reaction I is connected

with the system of photosynthetic absorption and leads to stretching of the lamellar system, this giving as effect an increase in surface area (flattening) and a volume reduction (shrinkage). Light even of low intensity of the entire visible spectrum is active here (maximum at 435 and 680 nm). This reaction is arrested by such photophosphorylation inhibitors as NH^+ ions, o-phenathroline, hydroxylamine, DCMU, CCP (Itoh, Izawa, Shibata, 1963; Packer, 1963; Zurzycki, 1966), it is not sensitive, however, to cyanides (Itoh, Izawa, Shibata, 1963). Reaction II produces a contraction of the lamellar system (reduction of chloroplast surface area). It occurs under strong shortwave light, and is not susceptible to photophosphorylation inhibitors, but may be blocked by JKK. The action spectrum lies within the limits of 350—500 nm (maximum at 450 and 360—380 nm), this indicating possibly a participation of flavin pigments (Zurzycki, 1966, 1967).

Light-induced shrinkage of chloroplasts may be interpreted as an indice of electron transport coupled with photophosphorylation (Izawa, Good, 1966; Krause, 1973). The contraction of the lamellar system of chloroplasts may be elicited under light by the hydrogen ions gradient through the thylakoid membrane (Heber, 1969) until the gradient is dispersed by way of photophosphorylation. The shrinkage of chloroplasts indicates a high photophosphorylation potential or a high energetic state of this system. The phenomenon of fluorescence is associated with the energetic state of thylakoids (Krause, 1973). The process of fluorescence inhibition is accompanied by chloroplast contraction and is the reflection of a high energetic state associated with active proton transport.

On the other hand, factors promoting fluorescence inhibit chloroplast contraction. Krause (1973) suggests that light-induced proton translocation may cause certain structural changes in the thylakoid membranes system, responsible for the enhanced contraction and fluorescence inhibition.

Chloroplast shrinkage cannot be the reflection of the energetic state under all conditions. The effect of light changes in dependence on the ion environment of the chloroplasts. Shrinkage occurs when the chloroplasts are exposed in phosphate buffer in the presence of salts of weak organic acids, the absence of these compounds in the chloroplast suspension causes osmotic disturbances (Devlin, 1971; Krause, 1973).

For eliciting chloroplast contraction the presence during illumination of an appropriate electron acceptor is necessary. At the same time there occurs a flow of magnesium and potassium ions maintaining electric neutrality, and water outflow for maintaining osmotic equilibrium (Dilley, Vernon, 1965). This is manifest in the rise of pH of the chloroplast suspension (Jagendorf, Hind, 1963). These changes are reversible in darkness. Magnesium, potassium and hydrogen ions

transport may act as a control mechanism activating various enzymes (Dilley, Vernon, 1965). Since the first phase of chloroplast flattening associated with volume shrinkage to about 30 per cent (as compared with that in darkness) occurs as early as the first second, it cannot be an osmotic reaction resulting from the loss of potassium, chlorine and sodium ions, since the absolute value of the osmotic potential in the chloroplasts increases. During the next hour of illumination the chloroplast volume was only reduced by 5 per cent, but they lost about 50 per cent of their specific ion content (De Filippis, Pallaghy, 1973). This initial chloroplast flattening may be due to conformational changes of the chloroplast protein owing to rapid ATP and hydrogen ion changes (Miller, Nobel, 1972; De Filippis, Pallaghy, 1973).

The mechanism giving rise to photostructural changes is thus not explained as yet. The relation between photostructural reaction and photophosphorylation, and the fact that similar structural changes as those caused by light can be achieved by the action of ATP (Itoh, Izawa, Shibata, 1963; Packer, 1963) seem to suggest a role played by photophosphorylation in the mechanism of contraction of the lamellae.

The author is deeply indebted to Professor J. Zurzycki for suggesting the line of investigations and valuable advice in the course of this work.

REFERENCES

- Belsky M. M., Siegenthaler P. A., Packer L., 1965, Evidence for conformational changes in *Euglena* chloroplasts. *Plant Physiol.* 40: 290—293.
- Devlin R. M., Barker A. V., 1971, Photosynthesis. Van Nostrand Reinhold Comp. 159—179.
- Dilley R. A., Vernon L. P., 1965, Ion and water transport processes related to the light-dependent shrinkage of spinach chloroplasts. *Arch. of Biochem. and Biophys.* 111: 365—375.
- De Filippis L. F., Pallaghy C. K., 1973, Effect of light on the volume and ion relations of chloroplasts in detached leaves of *Elodea densa*. *Aust. J. biol. Sci.* 26: 1251—1265.
- Gicklhorn J., 1933, Über aktive Chloroplasten Kontraktion bei *Spirogyra* und den Aggregatzustand der Spiralbänder. *Protoplasma* 17: 571—592.
- Heber U., 1969, Conformational changes of chloroplasts induced by illumination of leaves in vivo. *Biochim. Biophys. Acta* 180: 302—319.
- Hilgenheger H., Menke W., 1965, Lichtabhängige anisotrope Veränderungen des Chloroplastenvolumens in lebenden Zellen. *Z. Naturforsch.* 20b: 699—701.
- Honda S. I., 1971, Interpretations on chloroplast reproduction derived from correlations between cells and chloroplasts. *Planta* 97: 1—15.
- Hongladarom T., Honda S. I., Wildman S. G., 1968, Swelling of spinach chloroplasts induced by HCHO and KMnO_4 an effect partially hidden by the wide size range of chloroplasts. *Plant and Cell Physiol.* 9: 159—168.

- Itoh M., Izawa S., Shibata K., 1963, Shrinkage of whole chloroplasts upon illumination. *Biochim. Biophys. Acta* 66: 319—327.
- Izawa S., Good N. E., 1966, Effects of salts and electron transport on the conformation of isolated chloroplasts. I. Light scattering and volume changes. *Plant Physiol.* 41: 533.
- Izawa S., Itoh M., Shibata K., 1963, Action spectrum for the shrinkage of chloroplasts. *Biochim. Biophys. Acta* 75: 349—354.
- Jagendorf A. T., Hind G., 1963, Photosynthetic Mechanismus of Green Plants. National Academy of Science, National Research Council, Washington, Publication 1145: 599.
- Krause G. H., 1973, The high-energy state of the thylakoid system as indicated by chlorophyll fluorescence and chloroplast shrinkage. *Biochim. Biophys. Acta* 292: 715—728.
- Kushida H., Itoh M., Izawa S., Shibata K., 1964, Deformation of chloroplasts on illumination in intact spinach leaves. *Biochim. Biophys. Acta* 79: 201—203.
- Lechowski Z., 1972, Action spectrum of chloroplast displacements in the leaves of land plants. *Acta Protozoologica* 11: 201—209.
- Miller M. M., Nobel P. S., 1972, Light-induced changes in the ultrastructure of pea chloroplasts in vivo. *Plant. Physiol.* 49: 535—541.
- Nobel P. S., Chang D. T., Wang C., Smith S. S., Barcus D. E., 1969, Initial ATP formation, NADP reduction, CO₂ fixation and chloroplast flattening upon illumination pea leaves. *Plant Physiol.* 44: 655—661.
- Packer L., 1963, Structural changes correlated with photochemical phosphorylation in chloroplast membranes. *Biochim. Biophys. Acta* 75: 12—22.
- Peteler K., 1939, Amöboide Formänderung der Diatomeen Plastiden. *Protoplasma* 32: 9—19.
- Seen G., 1908, Die Gestalts- und Lagerveränderungen der Pflanzen chromatophoren. Engelmann, Leipzig.
- Stahl E., 1880, Über den Einfluss von Richtung und Stärke der Beleuchtung auf einige Bewegungserscheinungen im Pflanzenreiche. *Bot. Ztg.* 38: 229.
- Sundquist J. E., Burris R. H., 1970, Light-dependent structural changes in the lamellar membranes of isolated spinach chloroplasts: measurement by electron microscopy. *Biochim. Biophys. Acta* 223: 115—121.
- Zurzycki J., 1964, Investigations on the contraction of chloroplasts in *Mnium undulatum* (L.) Weis. *Protoplasma* 58: 458—482.
- Zurzycki J., 1966, Investigations on the contraction of chloroplasts in *Mnium undulatum* (L.) Weis. II. Studies on isolated chloroplasts. *Acta Soc. Bot. Pol.* 35: 281—291.
- Zurzycki J., 1967, Wpływ światła na strukturę aparatu fotosyntetycznego. *Postępy Biochemii* 13: 233—260.
- Zurzycki J., 1974, The effect of blue and red light on the volume changes of *Funaria* chloroplasts in vivo. *Photosynthetica* 8: 338—343.

Author's address

Dr Janina Godziemba-Czyż
Institute of Molecular Biology,
Jagiellonian University,
Grodzka Str. 53; 31-001 Kraków, Poland

*Zmiany konformacyjne chloroplastów liści szpinaku
(Spinacia oleracea) in vivo*

Streszczenie

1. Badano zmiany w wielkości powierzchni chloroplastów nieuszkodzonych komórek liści szpinaku (*Spinacia oleracea*) indukowane światłem niebieskim (370—500 nm) i czerwonym (600—850 nm) o różnych intensywnościach w zakresie 10^2 — 5×10^5 erg cm⁻²s⁻¹. Zmiany były definiowane poprzez średnią powierzchnię w μm^2 i frekwencję klas wielkości powierzchni.

2. Niskie intensywności światła niebieskiego wywołują powiększanie się powierzchni chloroplastów (w porównaniu z chloroplastami z ciemności), natomiast w wysokiej intensywności chloroplasty wyraźnie zmniejszają powierzchnię.

3. Naświetlanie chloroplastów światłem czerwonym powoduje powiększanie się powierzchni proporcjonalnie do intensywności i czasu naświetlania.