Influence of Magnesium Ions on the Action of Photosystems I and II at Different Wavelengths

Experiments with Barley Chloroplasts of Different Chlorophyll b Content

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Z. Naturforsch. **35c,** 298–302 (1980); received October 17, 1979/January 23, 1980

Chlorophyll b, Barley, Magnesium Ions, Wavelength Action

Barley leaves grown under a natural light/dark regime have a chlorophyll content of 1300 μg/g fresh weight and a chlorophyll a/b ratio of 2.5–3. When the plants are grown under cycles of 2 min light/118 min dark, the respective values are 50 and 5–9. With chloroplasts with low chlorophyll b content variable fluorescence is depressed by about 30% by $MgCl_2$; with those of high chlorophyll b content a threefold increase is seen instead. Action spectra for variable fluorescence of chloroplasts of high chlorophylll b content show enhancement by Mg^{2+} around 475 and 650 nm; for the system I-mediated methyl viologen reduction, a depression is seen at these wavelengths. These effects are practically absent in chloroplasts with low chlorophyll b content. The data corroborate the hypothesis that a chlorophyll b-containing pigment protein complex is required for regulation of energy transfer to system I and II by magnesium ions.

Introduction

The photosynthetic apparatus of higher plants is thought to have a tripartite organization: Besides photosystem I and II, there is a chlorophyll b-containing pigment protein complex transferring excitation energy to both photosystems [1–3]. Mg^{2+} ions are thought to promote the energy flow to system II [4, 5] by increasing the transfer of light energy from the light harvesting complex to system II and by decreasing the transfer to system I. This has been concluded from the Mg^{2+}-mediated increase in action around 480 nm for system II reactions with a corresponding decrease for system I reactions [3], 480 nm being an absorption maximum of chlorophyll b *in vivo*. If Mg^{2+} acts on the light-harvesting complex and thereby influences chlorophyll b action, these effects should be absent in chloroplasts lacking this complex and chlorophyll b. This is tested here using plants grown in intermittent light which are known to be devoid of the light harvesting pigment protein and to be low in chlorophyll b content [6–8]. Furthermore, the observations are extended into the red part of the spectrum. This should give better information on a specific involvement of chlorophyll b, since in this spectral region an eventual interference from carotenoids is absent.

Abbreviations: Tricine, tris-(hydroxymethyl)-methyl-glycine; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol.

Reprint requests to Dr. E. Loos.

0341-0382/80/0300-0298 $ 01.00/0

Materials and Methods

Barley (*Hordeum vulgare,* var. Firlbecks Union) was grown in a greenhouse for 10–30 days and used for the experiments with chloroplasts of high chlorophyll b content. In order to obtain chlorophyll b-deficient plants, the seedlings were kept in darkness for one week after sowing and then subjected to cycles of 2 min light/118 min dark for two days. In this case, light was provided by white fluorescent tubes (6000 lux), the temperature was maintained at 23 °C.

Chloroplasts were isolated as described previously [3] except that for better yield the initial centrifugation was 3 min at $4000 \times g$. Chloroplasts were finally washed and resuspended in a medium consisting of 10 mM KCl and 20 mM Tricine-KOH (pH 8.0). Chlorophyll concentrations were determined according to Arnon [9].

For excitation spectra variable fluorescence was obtained with the following method: First, dark-adapted chloroplasts were exposed to a series of flashes of the different wavelengths and the fluorescence of each flash was registered. Reference flashes given at the beginning and the end of such a series yielded the same fluorescence. This showed that the intensity of the flashes was weak enough and the dark time between them long enough to avoid accumulation of reduced quencher (Q) which would give rise to an additional fluorescence, the variable fluorescence (F_v; *cf.* [10]); so, the first series of flashes induced prompt fluorescence (F_0) only. Then

a continous background light was turned on, which served to bring Q into a reduced state. With this background light on, the same series of flashes was given which now excited a stronger fluorescence, composed of F_0 and F_v. Variable fluorescence was obtained by subtracting the two sets of measurements.

The flashes (20 ms duration) were obtained by means of an electric shutter (compur M3) in conjunction with a tungsten lamp and a monochromator (Bausch and Lomb). The slits were set for 5 nm half band width. The time between the flashes was about 45 s. The sample (3 ml) was contained in a cuvette of 1 × 1 cm cross section. Chloroplasts were suspended to yield a chlorophyll concentration of 5 µg/ml in a medium containing 10 mM KCl, 20 mM Tricine-KOH, (pH 8) and 16 µM DCMU. Fluorescence was detected at right angles to the exciting beam by a photomultiplier (Hamamatsu R 374) protected by an interference filter and a red glass (Schott DAL 680 nm, 6 mm RG 665). The signals were fed to a storage oscilloscope. Continuous background light was provided from a projector with a heat-reflecting glass, a 5 cm water-filled cuvette, cut-off glasses (Schott GG 420, KIF 560) and a blue green glass (Corning 9782) in the beam. Methyl viologen reduction was monitored spectrophotometrically as absorption increase at 396 nm. The measuring beam fell through the sample cuvette of 1 × 1 cm cross section on a photomultiplier (Hamamatsu R 374) protected from stray light and fluorescence by glass filters (Corning 9782, Schott UG 2). Actinic light from two sides perpendicular to the measuring beam was provided by two slide projectors, each with a heat-reflecting glass, a 5 cm layer of water, an appropriate lens and an interference filter in the beam. Interference filters of 435, 476, 653 and 698 nm were used with half band widths ranging from 11 to 19 nm. The reaction mixture contained 10 mM KCl, 20 mM Tricine-KOH (pH 8), 40 mM cysteine, 20 µM DCIP, 0.2 mM methyl viologen and 10 µM DCMU. To remove oxygen the mixture was bubbled for 1 min with nitrogen, filled into the cuvette and plugged up.

Results

Plants of different chlorophyll b content

When dark grown barley is exposed to cycles of 2 min light/118 min darkness, chlorophyll synthesis proceeds for about two days and then comes to a halt (Fig. 1). The ratio of chlorophyll a/chlorophyll b reached after two days ranged from 5 to 9 in different experiments, the chlorophyll content was about 50 µg/g fresh weight of leaves. Chloroplasts isolated from such plants will be called "chlorophyll b-deficient chloroplasts". Plants grown in the greenhouse had a chlorophyll a/b ratio of 2.5 to 3, contained around 1 300 µg chlorophyll/g fresh weight of leaves and were the source of "normal chloroplasts".

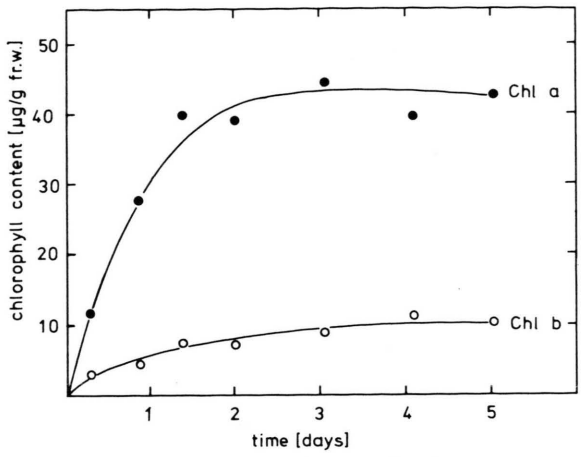

Fig. 1. Kinetics of chlorophyll formation in intermittent light. At time zero, the plants were transferred from dark to cycles of 2 min light/118 min dark.

Prompt and variable fluorescence; effect of Mg^{2+}

In Fig. 2 is plotted the fluorescence excited by 20 ms test flashes superimposed on an increasingly intense background illumination. The fluorescence obtained at zero intensity of background light is considered to be prompt fluorescence (F_0), to which adds at higher intensities fluorescence of variable yield (F_v). A quite weak background light of 0.1 to 0.2 W/m² is sufficient to obtain maximal fluorescence yield. The light intensity employed in action spectra measurements (*cf.* methods) was 0.12 W/m².

With the normal chloroplasts F_v is increased by Mg^{2+} about threefold (Fig. 2A), whereas F_0 is little affected. With chlorophyll b-deficient chloroplasts, however, F_v is decreased in the presence of Mg^{2+} by about 30% (Fig. 2B). Another difference is the relatively lower fraction comprized by F_v from the total fluorescence $F_0 + F_v$.

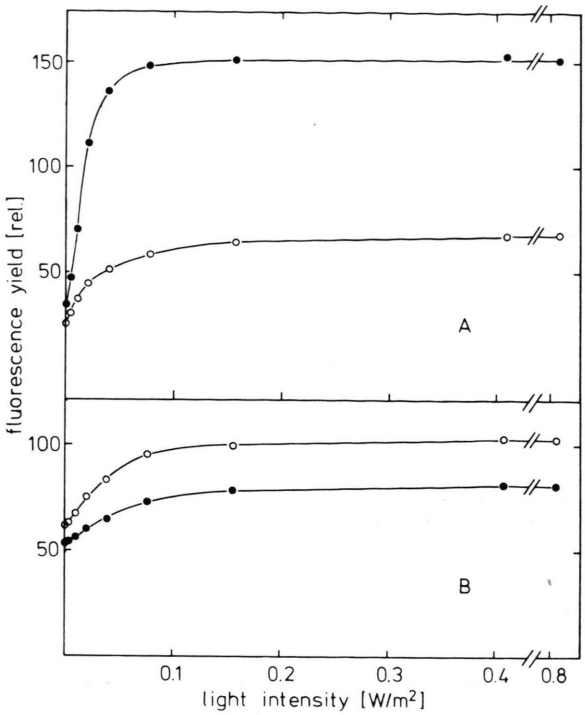

Fig. 2. Fluorescence produced by a test flash (435 nm, 0.08 W/m², 20 ms) *versus* intensity of blue-green background illumination in the presence (●) and absence (○) of 5 mM $MgCl_2$. A: Normal chloroplasts; B: Chlorophyll b-deficient chloroplasts.

Action spectra for variable fluorescence

Variable fluorescence probably originates from system II [10, 11]; action spectra for F_v, therefore, should represent system II action. Fig. 3A shows the result of an experiment with normal chloroplasts. The actions have been normalized at 435 nm. The most pronounced effect of Mg^{2+} is to enhance the relative action of wavelengths around 475 nm. This and the small increase in action around 600 and 650 nm have been observed consistently with two other chloroplast preparations. Action spectra with chloroplasts from intermittent light-grown plants (Fig. 3B) are lacking the peak at 475 nm obviously because of the deficiency in chlorophyll b. Further is lost any significant influence of Mg^{2+} on the shape of the spectra.

Measurements of system I activity

Attempts to use methyl viologen-mediated O_2-uptake in the presence of DCMU with an artificial electron donor system as a test reaction for system I were unsuccessful with chlorophyll b-deficient chloroplasts, because they exhibited a strong light-dependent oxygen uptake even without the donor system. This oxygen uptake may be system I-mediated, but may represent equally well an unspecific photooxidation. System I activity could be measured, however, spectroscopically as methyl viologen reduction under anaerobic conditions with cysteine/DCIP as electron donor. With this reaction the rate of reduction decreased during exposure to light and upon shutting off the light a back reaction of the reduced dye was seen. Instead of measuring slopes it proved to be more practicable to take the absorption increase produced by a 10 s illumination for system I activity.

Plots of this response versus light intensity were slightly curved, indicating the beginning of light saturation. The action (= ratio response/incident light), therefore, varied with the size of the response. To make a valid comparison of the actions of different wavelengths, the intensities of the actinic beams were adjusted to produce equal amounts of reduced dye. This matching was done in the blue

Table I. Effect of 5 mM $MgCl_2$ on ratios of actions of system I – mediated methyl viologen reduction for the wavelength pairs 435/476 nm and 653/698 nm in chloroplasts from normal and intermittent light-grown barley.

	Normal chloroplasts		Chlorophyll b-deficient chloroplasts	
	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$
Action 476 nm / Action 435 nm	0.69	0.54	0.58	0.50
Action 653 nm / Action 698 nm	1.75	1.22	1.47	1.51

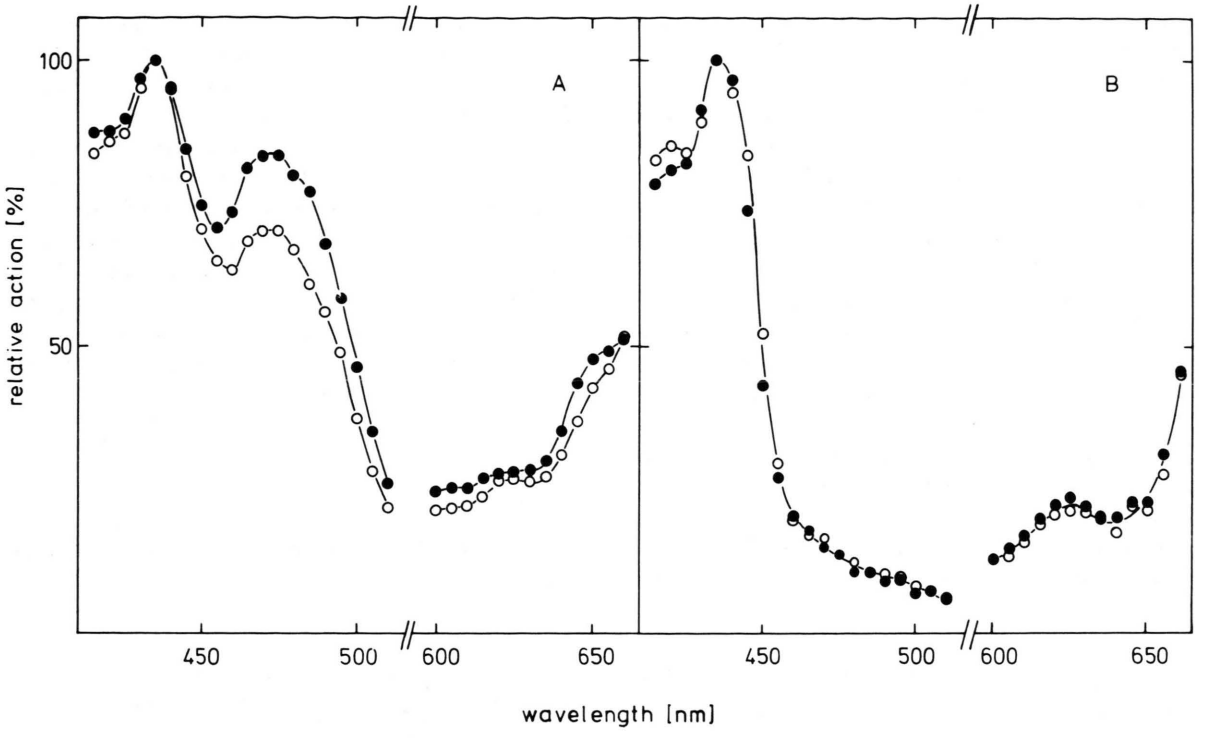

Fig. 3. Action spectra of variable fluorescence in the presence (●) and absence (○) of 5 mM $MgCl_2$. A: Normal chloroplasts; B: Chlorophyll b-deficient chloroplasts.

region for 435 and 476 nm and in the red for 653 and 698 nm. The ratios of actions obtained in this way are listed in Table I. With normal chloroplasts, Mg^{2+} lowers the ratio of action at 476 nm versus that at 435 nm by about 20% and the ratio at 653 nm versus 698 nm by 30%. Chlorophyll b-deficient chloroplasts exhibit a somewhat smaller effect for the blue wavelengths; the ratio for the two red wavelengths is hardly affected by Mg^{2+}. Two independent repetitions of this experiment gave essentially the same results.

Discussion

Barley plants grown in intermittent light show a relatively high chlorophyll a/b ratio between 5 and 9. This agrees well with the figures given by Genge *et al.* [12] for similarly grown barley seedlings. Even higher a/b ratio (> 12) have been found upon cycles of 1 ms light – 15 min dark [13] or with bean and pea [6, 14].

Variable fluorescence of normal chloroplasts is strongly increased by Mg^{2+}, as has been reported by others [4, 5]. With chlorophyll b-deficient chloroplasts, however, Mg^{2+} causes a small depression of F_v. The reason for this is not known. With chlorophyll b-deficient chloroplasts from a barley mutant or intermittent light-grown pea, no or only little stimulation of fluorescence has been observed upon addition of Mg^{2+} [8, 15]. The smaller share of F_v from total fluorescence seen with chloroplasts with low chlorophyll b content (Fig. 2) has also been found in a pea system [14].

With normal chloroplasts, the action spectrum for F_v (Fig. 3A) shows in the presence of Mg^{2+} higher action around 475 nm indicating involvement of chlorophyll b. The smaller effects around 600 and 650 nm may be due to the smaller absorption coefficients of chlorophyll b in this part of the spectrum as compared to those of chlorophyll a. For system I action, however, the wavelengths of predominant chlorophyll b absorption are less effective in the

presence of Mg^{2+} (Table I). This is especially conspicous when comparison is made for the wavelengths 653 and 698 nm, probaly because the ratios of chlorophyll a/chlorophyll b absorption are differing considerably at these wavelengths. This influence of Mg^{2+} on the wavelength dependency of system I and II reactions confirms previous results [3]. It is consistent with the hypothesis that in the presence of Mg^{2+}, transfer from the chlorophyll b-containing light-harvesting complex is increased to system II and decreased to system I. Since the occurence of chlorophyll b seems to be strictly coupled to that of the light-harvesting pigment protein complex [1, 8, 16], this complex seems plausible to undergo interaction with Mg^{2+}. An aggregation of the purified pigment protein has recently been found to be induced by this ion [17].

In chloroplasts with low chlorophyll b content, however, little or no effect of Mg^{2+} is seen on the action of wavelengths absorbed by chlorophyll b (Fig. 3B, Table I). This confirms the evidence that the influence of Mg^{2+} is on the action of chlorphyll b. For the smaller effect persisting on the ratio of System I actions 476/435 nm, the chlorophyll b does not appear to be responsible, since there is no corresponding effect on the ratio of actions 653/698 nm. The "chlorophyll b-deficient" chloroplasts contain still some chlorophyll b; an expected small effect could have been within the limits of experimental error and presumably did not occur due to a not yet fully assembled light harvesting complex, unable to interact with magnesium ions.

Acknowledgement

Thanks are due to Dr. W. Lockau for stimulating discussions.

[1] J. P. Thornber and H. R. Highkin, Eur. J. Biochem. **41,** 109–116 (1974).
[2] W. L. Butler and M. Kitajima, Biochim. Biophys. Acta **396,** 72–85 (1975).
[3] E. Loos, Biochim. Biophys. Acta **440,** 314–321 (1976).
[4] P. Homann, Plant Physiol. **44,** 932–936 (1969).
[5] N. Murata, Biochim. Biophys. Acta **189,** 171–181 (1969).
[6] J. H. Argyroudi-Akoyonoglou and G. Akoyonoglou, Plant Physiol. **46,** 247–249 (1970).
[7] R. G. Hiller, D. Pilger, and S. Genge, Plant Sci. Lett. **1,** 81–88 (1973).
[8] D. J. Davis, P. A. Armond, E. L. Gross, and C. J. Arntzen, Arch. Biochem. Biophys. **175,** 64–70 (1976).
[9] D. J. Arnon, Plant Physiol. **24,** 1–15 (1949).
[10] L. N. M. Duysens and H. E. Sweers, Studies on Microalgae and Photosynthetic Bacteria (J. Ashida, ed.), pp. 353–372, University of Tokyo press, Tokyo 1963.
[11] J. Lavorel, Biochim. Biophys. Acta **88,** 20–36 (1964).
[12] S. Genge, D. Pilger, and R. G. Hiller, Biochim. Biophys. Acta **347,** 22–30 (1973).
[13] G. Akoyonoglou, J. H. Argyroudi-Akoyonoglou, M. R. Michel-Wolwertz, and C. Sironval, Physiol. Plant **19,** 1101–1104 (1966).
[14] P. A. Armond, C. J. Arntzen, J.-M. Briantais, and C. Vernotte, Arch. Biochem. Biophys. **175,** 54–63 (1976).
[15] J. R. Lieberman, S. Bose, and C. J. Arntzen, Biochim. Biophys. Acta **502,** 417–429 (1978).
[16] O. Machold, H. Meister, H. Sagromsky, G. Hoeyer-Hansen, and D. V. Wettstein, Photosynthetica **11,** 200–206 (1977).
[17] J. J. Burke, C. L. Ditto, and C. J. Arntzen, Arch. Biochem. Biophys. **187,** 252–263 (1978).