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THE EFFECT OF MAGNESIUM IONS ON ACTION SPECTRA FOR REACTIONS MEDIATED BY PHOTOSYSTEMS I AND II IN SPINACH CHLOROPLASTS

ECKHARD LOOS

Fachbereich Biologie der Universität, 84 Regensburg, Universitätsstr. 31 (G.F.R.)

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SUMMARY

Action spectra were measured for positive changes in variable fluorescence (emission > 665 nm) excited by a beam of 485 nm chopped at 75 Hz. The action of two further beams was compared, one being variable, the other (reference) constant with respect to wavelength and intensity. Comparison was achieved by alternating the reference and the variable wavelength beams at 0.3 Hz and adjusting the intensity of the latter such as to cancel out any 0.3 Hz component in the 75 Hz fluorescence signal. The relative action then was obtained as the reciprocal of the intensity of the variable wavelength beam. Similarly, action spectra were measured for O_2 evolution with ferricyanide/*p*-phenylenediamine as electron acceptor, and for O_2 uptake mediated by methyl viologen with ascorbate 3-(*p*-chlorophenyl)-1,1-dimethylurea as electron donor in the presence of 2,6-dichlorophenolindophenol.

Addition of 5 mM $MgCl_2$ increases the relative action around 480 nm for the change in variable fluorescence and *p*-phenylenediamine-dependent O_2 evolution, and decreases it for methyl viologen-mediated O_2 uptake with 2,6-dichlorophenolindophenol/ascorbate as electron donor in the presence of 3-(*p*-chlorophenyl)-1,1-dimethylurea. The change in variable fluorescence and O_2 evolution are stimulated by $MgCl_2$, whereas O_2 uptake is inhibited by it.

The results are discussed in terms of a model assuming a tripartite organization. of the photosynthetic pigments (Thornber, J. P. and Highkin, H. R. (1974) Eur. J. Biochem. 41, 109–116; Butler, W. L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72–85). $MgCl_2$ is thought to promote energy transfer to Photosystem II from a light-harvesting pigment complex serving both photosystems.

INTRODUCTION

Effects of Mg^{2+} on energy transfer between the two photoreactions have been deduced from the action of this ion on fluorescence and Hill reactions [1–3]. By

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; CMU, 3-(*p*-chlorophenyl)-1,1-dimethylurea; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v, fluorescence of variable yield.

several authors, Mg^{2+} and other divalent cations have been found to stimulate System II-dependent phenomena, for instance to increase the variable fluorescence (F_v) [4, 5], the peak ratio of the 685 nm/735 nm fluorescence emission [1] and to accelerate the Hill reaction with DCIP or ferricyanide as electron acceptor [1, 2, 6]; on the other hand, the reduction of NADP or methyl viologen with an artificial electron donor system in the presence of DCMU, a reaction driven by System I, was inhibited by Mg^{2+} [1, 2]. To explain these observations, the hypothesis has been proposed [1] that Mg^{2+} is interrupting a spill-over of energy from Photosystem II into Photosystem I, thus keeping more of the light energy in Photosystem II. Alternatively, the action of Mg^{2+} has been discussed in terms of a promotion of energy flow from System I into System II [3]; an energy transfer in this direction has recently been claimed to occur in chloroplasts from *Euglena* [7]. To decide between these two hypotheses, an experimental approach was chosen which has been similarly followed by Vernotte and coworkers [8]: when action spectra are measured with and without Mg^{2+} for reactions mediated by Photosystem I or Photosystem II, a change in the shape of one of the spectra should indicate an energy transfer from the other, differently absorbing photosystem; this in turn serving as an energy donor, should show no change due to Mg^{2+} in the wavelength dependence of its respective reaction. According to these considerations action spectra in the presence and absence of Mg^{2+} were determined for changes in F_v (ΔF_v) and for *p*-phenylenediamine reduction, both being System II-sensitized reactions [9–11]; further, for methyl viologen-mediated O_2 uptake in the presence of an artificial electron donor plus CMU, a reaction used to measure System I activity [2, 8]. The results presented in this work are not consistent with either of the two models but support the recently emerged concept of a tripartite organization of the photosynthetic pigments [12, 13].

MATERIALS AND METHODS

Spinach was grown in the green-house; for isolation of chloroplasts 30 g of washed, cooled leaves were homogenized for 4 s in 120 ml of a medium containing 0.4 M sucrose, 10 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 3.5 mM $Na_4P_2O_7$ and 20 mM Tricine/KOH (pH 8.0). The slurry was filtered through two layers of thick cotton cloth and chloroplasts were collected from the green juice by centrifugation at $4000 \times g$ for 40 s. The pellet was washed with a medium containing 0.4 M sucrose, 10 mM KCl, 20 mM Tricine/KOH (pH 8.0) and finally resuspended in the basic medium of 10 mM KCl, 20 mM Tricine/KOH (pH 8.0) to yield a stock suspension of 250 μg chlorophyll/ml.

Changes in F_v were measured as the increase in fluorescence yield of a chopped 485 nm light beam (75 Hz) when light of other frequency was added; this corresponds to a reduction of the quencher Q [9]. Since only the action of Photosystem II in reducing Q should be measured, CMU was included in the reaction mixture to prevent an oxidation of Q by Photosystem I (cf. ref. 9). However, Q was reoxidized otherwise, probably by oxygen, upon shutting off the additional light. This made it possible to obtain action spectra for ΔF_v in the following way (Fig. 1): additional light of constant intensity is chopped at 0.3 Hz, producing in the 75 Hz fluorescence signal a 0.3 Hz modulation representing ΔF_v .This modulation is compensated by adjusting the intensity of an alternating beam of variable wavelength; the relative action then is given

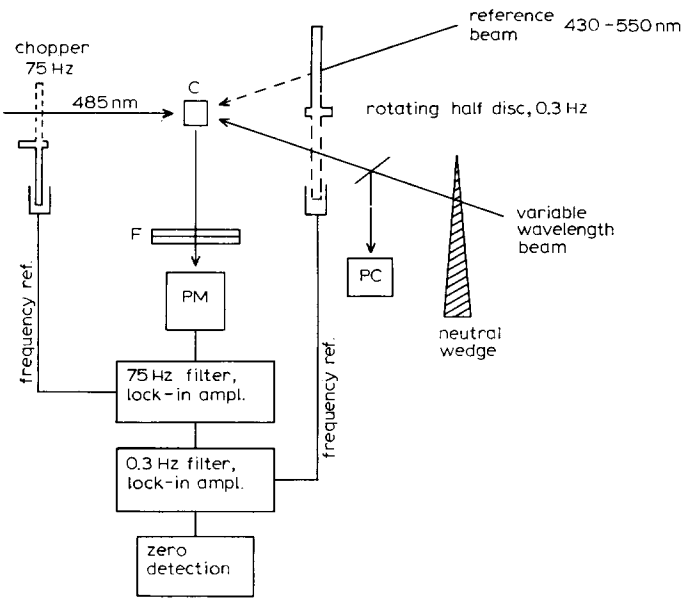

Fig. 1. Diagram of set-up for measuring action spectra for ΔF_v. C, sample cuvette of 1 by 1 cm cross section; F, blocking filters Filtraflex DT (Balzers) and RG 665, 6 mm (Schott); PM, photomultiplier (Hamamatsu R 374); PC, calibrated vacuum photocell (Hamamatsu R 310).

by the inverse of the intensity of the variable wavelength beam. The sensitivity of the method depends mainly on the size of the signal caused by any single 0.3 Hz beam and on the noise at the output of the 0.3 Hz lock-in amplifier (cf. ref. 14). Under the conditions used here the relative actions for ΔF_v were measured with an accuracy of approx. $\pm 2\,\%$. Measurements within the course of an experiment were reproducible within the same limits. The method provides action spectra under steady-state conditions and eliminates errors due to aging of the chloroplasts or to eventual non-linear intensity vs. rate characteristics (cf. ref. 14). The beam chopped at 75 Hz and the one of variable wavelength were obtained from two 500-mm grating monochromators (Bausch and Lomb) with slits set for 6 nm half-band width and with filters KIF 560 and BG 38 (both Schott), respectively, to cut down stray light. The reference beam presented a broad blue band defined by filters BG 23 plus BG 28 (Schott). The intensity of the variable wavelength beam (unchopped) was relatively low, e.g. 100 ergs/cm^2 per s at 485 nm, which fell in a range of a linear relationship between light intensity and ΔF_v. The time-averaged intensity of the 75 Hz, 485 nm beam was 280 ergs/cm^2 per s. This intensity proved to be saturating for ΔF_v when the intensity of the 75 Hz beam was varied and ΔF_v was caused by only one 0.3 Hz beam. Absorption spectra were measured using the variable wavelength beam with the photomultiplier 3.5 cm behind the cuvette. An opal glass was inserted between them, as well as a KIF 560 filter (Schott) to cut off fluorescence.

Relative rates of O_2 uptake or O_2 evolution were measured with a rate electrode similar to that described by Pickett and French [15]. Action spectra were obtained analogous to those for ΔF_v by matching the reference beam with the variable wavelength beam. At the light intensities to be used, there was a linear relationship between

intensity and O_2 evolution, whereas with the O_2 uptake reaction a non-linearity occurred, indicating beginning light saturation; action spectra for O_2 uptake were measured at rates which on a light vs. intensity curve were 10–30 % below linearity.

RESULTS

Action spectra for changes in variable fluorescence (ΔF_v)

In Fig. 2A are shown action spectra for ΔF_v in the presence and absence of Mg^{2+}. The ion caused approx. a 5-fold increase of ΔF_v; for better comparison, therefore, the spectra have been normalized at 435 nm. With Mg^{2+}, a relatively stronger contribution of pigments absorbing around 480 nm, obviously chlorophyll *b* and/or carotenoids, is seen. The ratio $+Mg^{2+}/-Mg^{2+}$ of the peaks at 475 nm is 1.17 in Fig. 2A; in three other sets of spectra with different chloroplast preparations, it varied between 1.11 and 1.21. There is a flattening effect of Mg^{2+} on absorption spectra of chloroplasts [16]; to exclude the possibility that the difference in the action spectra was due to such an effect, absorption spectra were measured with precautions taken to avoid distortions from scattering (see Materials and Methods). Mg^{2+} causes a weak flattening (Fig. 2B); the difference in absorption, however, cannot account for the difference between the action spectra, neither in size nor in its wavelength dependence (Fig. 2, bottom). A comparison of Figs. 2A and 2B shows further a relatively high efficiency of chlorophyll *b* and/or carotenoids, in sensitizing ΔF_v even without Mg^{2+}. The same has previously been observed [17], probably with regard to steady-state fluorescence. Variable and steady-state fluorescence seem to emanate mainly from the same pigment system [9, 10]. Action spectra for steady-state fluorescence in fact were affected by Mg^{2+} similarly to those for ΔF_v, though to a somewhat less pronounced degree (unpublished results).

The hypothesis of Mg^{2+} suppressing spill-over from Photosystem II to Photosystem I is not supported by the results obtained, since the shape of the action spectrum for ΔF_v should be invariant in that case. A Mg^{2+}-induced energy transfer from

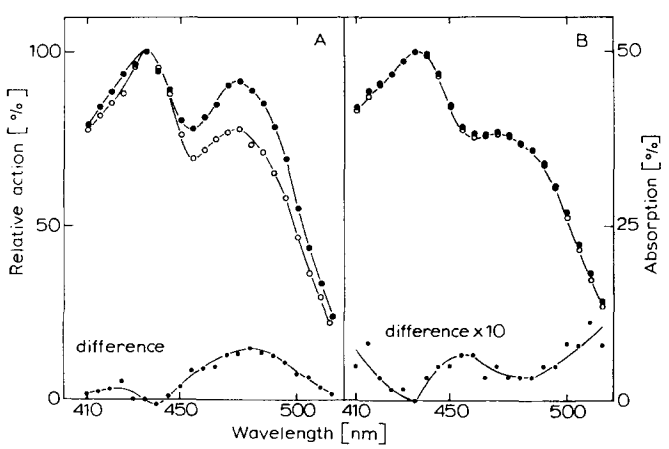

Fig. 2. Action spectra for ΔF_v (A) and absorption spectra (B) in the absence (circles) and presence (dots) of 5 mM $MgCl_2$. The basic medium contained 5 μg chlorophyll/ml and 15 μM CMU.

Photosystem I to Photosystem II does not seem plausible either, since the pigments brought into action by the ion have a relatively high chlorophyll *b* and/or carotenoid content, a property which is not only exhibited by Photosystem I [18].

Action spectra for O_2 evolution with p-phenylenediamine as electron acceptor

Reduction of *p*-phenylenediamine is supposed to be driven by Photosystem II [11]; it was followed here by detecting O_2 evolution. Action spectra for this reaction were measured in addition to those for ΔF_v in order to substantiate the expectation of Mg^{2+} acting equally on the spectral dependence of all reactions sensitized by Photosystem II. The rate of O_2 evolution was increased by Mg^{2+} (Table I); this agrees with observations of others [19], especially on the analogous DCIP reduction [1, 2].

TABLE I

RELATIVE RATES OF O_2 EVOLUTION AND O_2 UPTAKE IN THE PRESENCE AND ABSENCE OF Mg^{2+}

For O_2 evolution the basic medium in the flow system contained 0.15 mM ferricyanide and 50 μM *p*-phenylenediamine; for O_2 uptake the additions were instead 5 μM DCIP, 2 mM ascorbate, 15 μM CMU, 1 μM methyl viologen and 1 mM NaN_3. The concentration of $MgCl_2$ was 5 mM if present. Exposures of 1 min were given with light of 410 nm as used for action spectra.

Experiment	$-Mg^{2+}$	$+Mg^{2+}$	$+Mg^{2+}/-Mg^{2+}$
O_2 evolution (relative)			
1	30	47	1.56
2	28	42	1.50
O_2 uptake (relative)			
3	360	270	0.75
4	255	112	0.44

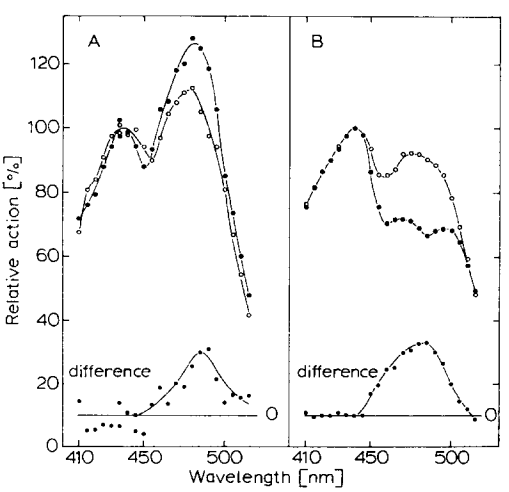

Fig. 3. Action spectra for O_2 evolution (A) and O_2 uptake (B) in the absence (circles) and presence (dots) of 5 mM $MgCl_2$. The reaction mixtures used are given in the legend to Table I.

An example of the action spectra obtained is depicted in Fig. 3A. Clearly, Mg^{2+} promotes the efficiency in the region around 480 nm, as was the case with ΔF_v. The ratio $+Mg^{2+}/-Mg^{2+}$ of the peaks at 480 nm, which is 1.12 in Fig. 3A, was 1.09 in another experiment; with chloroplasts from field-grown spinach, it was as high as 1.65. The spectra are, in shape, different from those for ΔF_v, e.g. the ratio of the 480 nm/435 nm peaks is higher; this may be caused by enhanced mutual shading in the denser chloroplast suspension required for the O_2 measurements (cf. ref. 20). Since, however, the effect of Mg^{2+} is qualitatively the same, identical conclusions are drawn as from the spectra for ΔF_v.

Action spectra for O_2 uptake mediated by System I

To obtain action spectra for a System I-dependent reaction, O_2 uptake was measured with an artificial electron donor system in the presence of CMU to block electron transport from System II. The effect of Mg^{2+} on the rate of this reaction was to slow it down (Table I) which generally is in line with the literature [2, 21]; (however, see ref. 19). Action spectra in the presence and absence of Mg^{2+} are presented in Fig. 3B. In contrast to the spectra for System II activity (Figs. 2A and 3A) a diminished action of blue-green light is seen in the presence of Mg^{2+}; this is in accordance with a lowered action of chlorophyll *b* for System I-mediated O_2 uptake in the red wavelength range [8]. Chlorophyll *b* seems to be more affected than carotenoids, since with Mg^{2+} a carotenoid peak emerges which otherwise is hidden in the chlorophyll *b* action. The difference spectrum $(-Mg^{2+})-(+Mg^{2+})$ peaks around 480 nm, as does the corresponding spectrum $(+Mg^{2+})-(-Mg^{2+})$ for *p*-phenylenediamine reduction (Fig. 3, bottom). The spectra for O_2 uptake are more precise, due to the relatively larger rates of O_2 exchange available for measurements (cf. Table I). With three different chloroplast preparations the ratio $+Mg^{2+}/-Mg^{2+}$ of action at 480 nm was 0.71, 0.72 and 0.78.

The decrease of O_2 uptake rates by Mg^{2+} with a concomitant loss in chlorophyll *b* action would suggest an interruption of energy transfer from Photosystem II to Photosystem I, a conclusion reached previously on the basis of action spectra work [8]. This, however, cannot be reconciled with other results of this study (see above).

DISCUSSION

The hypothesis of Mg^{2+} suppressing spill-over from Photosystem II to Photosystem I is consistent with the observed increase in Photosystem II activity (ΔF_v and *p*-phenylenediamine-dependent O_2 evolution). The spectral dependence, however, of a Photosystem II-driven reaction should not be influenced by such a regulation, since the pigment composition of Photosystem II should not be altered by Mg^{2+}. The Mg^{2+}-induced changes of the action spectra for Photosystem II-sensitized reactions (Figs. 2A and 3A), therefore, do not support the above hypothesis. By analogous reasoning, a control of spill-over from Photosystem I to Photosystem II is not compatible with a changing wavelength dependency of a reaction mediated by Photosystem I (Fig. 3A). Instead, the phenomena can be explained satisfactorily in the framework of a tripartite organization of the photosynthetic apparatus as put forward recently [12, 13]. According to this model, a light-harvesting pigment protein complex

is able to transfer light energy into the two photosystems proper. This antenna pigment complex is supposed to have a relatively high chlorophyll *b* content, whereas less chlorophyll *b* is ascribed to Photosystem II and essentially none of this pigment to Photosystem I. If it is realized that Photosystem II-driven reactions are generally increased and Photosystem I-mediated ones inhibited by Mg^{2+} (Table I; refs. 1, 2 and 6), and this is correlated with the appearance, or disappearance, respectively, of chlorophyll *b* action (Figs. 2A and 3), the following seems to be plausible: the energy transfer from the antenna pigment complex into both photosystems is shifted by Mg^{2+} such as to feed a greater portion into Photosystem II, a gain for Photosystem II which is a loss for Photosystem I.

There are relatively smaller changes caused by Mg^{2+} in the action spectrum for *p*-phenylenediamine reduction than in that for O_2 uptake, e.g. the percentual changes are +9 % and −28 %, respectively, at 480 nm (Fig. 3). This would suggest for the absorption spectra a greater similarity of the light-harvesting complex with Photosystem II than with Photosystem I. This agrees with the above model which predicts the greater similarity between the chlorophyll *b*-rich light-harvesting complex and Photosystem II to be due to the occurrence of chlorophyll *b* in Photosystem II and lack of it in Photosystem I.

It is difficult to give a quantitative estimate of the Mg^{2+}-induced shift of energy transfer. One would have to consider secondary effects of Mg^{2+} on reaction rates [8] and would have to make assumptions, e.g. on the efficiency of energy transfer into Photosystems I and II. Another challenging problem would be to demonstrate that Mg^{2+} affects energy transfer also in vivo, and if so, how it comes into action.

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