QUENCHING OF CHLOROPHYLL FLUORESCENCE IN CHLOROPLAST PHOTOSYSTEM II PARTICLES BY MAGNESIUM IONS

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1. Introduction

The fluorescence yield of chlorophyll pigments in chloroplast membranes is determined not only by the trapping ability of photosystem II reaction centers (as defined by the redox state of the primary acceptor) but also by the molecular organization of the membrane itself [1–3]. The latter phenomenon is thought to be the basis for regulating the relative degree of excitation of PS II and PS I and is influenced by the presence of cations [4–8]. The observed effects of addition of Mg^{2+} to Mg^{2+}-depleted chloroplasts is an elevated fluorescence yield [3–6]. In actively photosynthesizing chloroplasts, the Mg^{2+}-induced fluorescence rise is followed by a slow quenching process which has been interpreted as being the result of H^+ gradient-induced Mg^{2+} efflux from the chloroplast [3,7,9] or more generally, in terms of quenching due to the 'high-energy' state [10,11]. These observations are complicated by the diversity of ionic effects on whole chloroplasts, which may be osmotic or related to energy coupling (see review, ref. [12]) or be involved in PS I [13] as well as PS II interactions. It would seem necessary to define these effects of ions in simpler systems (e.g., see ref. [13]). In this paper a Mg^{2+}-induced decrease in fluorescence yield of PS II particles is described, which is accompanied by an inhibition of their photochemical activity.

Abbreviations: DPC, diphenyl carbazide; DPIP, 2,6-dichlorophenolindophenol; PS II, photosystem II; PS I, photosystem I

2. Materials and methods

Triton photosystem II fragments were isolated from pea chloroplasts by the method of Vernon [14]. The absence of PS I components was ascertained by the failure to detect either cyt. *f* and cyt. *b*-563 in oxidized-minus-reduced spectra at 77°K and by the absence of PS I activity. Chlorophyll fluorescence at room temperature was excited using a tungsten–halogen lamp blocked by Corning 4-96, 5-58 and 1-75 glass filters giving an intensity of 12 $J.m^{-2}.s^{-1}$. Emission at 90°K was detected with an S-20 photomultiplier blocked by a 694 nm Balzars interference filter. The photomultiplier current was amplified by a Keithley current amplifier and passed either to a strip chart recorder or a Tracor Northern NS 570 Signal Averager. Excitation was initiated by the opening of Uniblitz electronic shutter. The chlorophyll concentration was 5 mg/ml and the reaction medium contained 0.25 M sucrose and 7 mM Mops (adjusted to pH 6.8 with NaOH). Photochemical activity was measured using DPC as donor and DPIP as acceptor [15].

3. Results

Photosystem II particles, when excited in the absence of diuron, show a slow rise in the variable fluorescence yield which approaches ∿70% of F_{max}, as defined by the yield on reduction with dithionite (fig.1A). Addition of diuron increases the

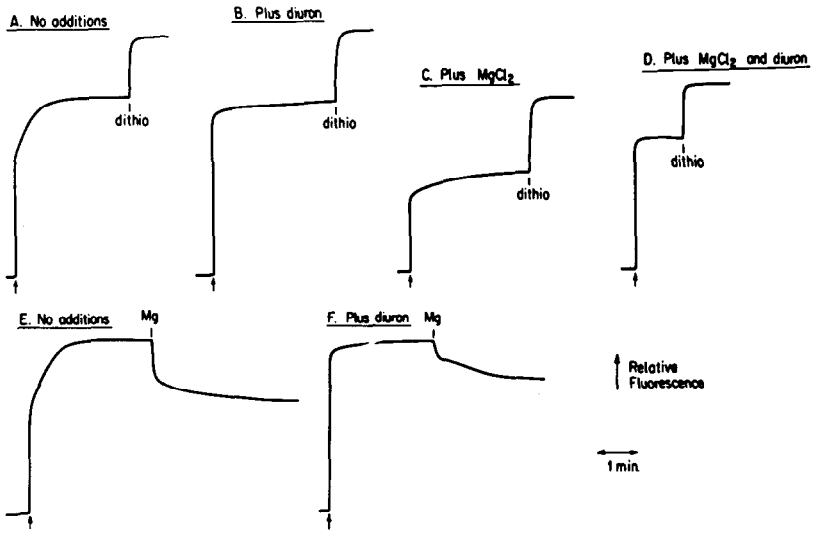

Fig.1. Effects of $MgCl_2$ on chlorophyll fluorescence of photosystem II particles. (A) Control, (B) plus 2 μM diuron, (C) plus 5 mM $MgCl_2$, (D) plus 5 mM $MgCl_2$ + 2 μM diuron, (E) $MgCl_2$ (5 mM) addition in the light, (F) as (E) except plus 2 μM diuron. Upward arrows indicate shutter opening.

rate of this rise in yield (fig.1B), presumably by slowing the rate of reoxidation of the primary acceptor.

Addition of Mg ions prior to illumination decreases the rise in F_v and the amplitude of F_{max} (fig.1C, D). When Mg^{2+} is added to particles excited in the absence of Mg^{2+}, a quenching of fluorescence is seen (fig.1E, F). F_{max} decreases by approx. 25–30% upon addition of 5 mM $MgCl_2$. The Mg^{2+}-induced quenching can be duplicated by high concentrations of NaCl (approx. 150 mM), but can be induced equally well by Ca^{2+}. The extent of the Mg effect as well as the value of control F_{max} of PS II particles is very sensitive to their condition, with overnight storage of particles at –20°C being sufficient to cause greater than 50% decrease in these responses. All the data described here were obtained using freshly prepared particles.

It is of interest, in terms of understanding the mechanism of this effect to determine whether F_o, F_v or both are being quenched by Mg^{2+}. Fluorescence rise curves are shown in fig.2. On this time scale the slow F_v increase is only partially seen. Mg^{2+} quenches F_o by approx. 10%. This compares to quenching of F_{max} by 25–30% (see fig.1).

The quenching of PS II fluorescence by Mg^{2+} is accompanied by a decreased photochemical activity. Rates of DPIP reduction are inhibited by up to 90% of the rate obtained without Mg^{2+} (fig.3A). The inhibition by Mg^{2+} is highly dependent on light intensity; at high (approx. X 5 saturation intensity) the Mg inhibition is reduced to only 20% (fig.1B). The intensity dependence of the inhibition is clearly shown in fig.4. Mg^{2+}-Induced inhibition occurs over the same concentration range as the quenching of fluorescence with both effects saturating at approx.

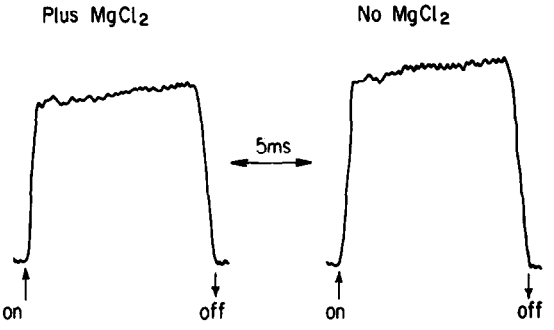

Fig.2. Effect of $MgCl_2$ on the induction of chlorophyll fluorescence. Measurements were made in the presence of 2 μM diuron ± 5 mM $MgCl_2$.

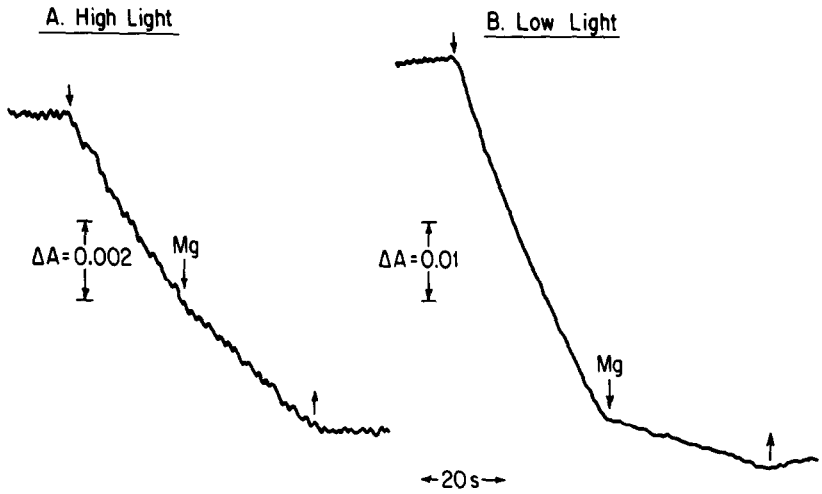

Fig.3. Inhibition of reduction of DPIP by $MgCl_2$. (A) At a chlorophyll concentration of 1.5 μg/ml and 20 μM DPIP. (B) At a chlorophyll concentration of 15 μg/ml and 40 μM DPIP. Light filtered though Corning 2-62. DPC concentration was 0.4 mM. Reduction of DPIP was measured at 550–500 nm using an Aminco DW-2 spectrophotometer, the photomultiplier being blocked by a Balzars DT Gruen and Corning 4-96 filters.

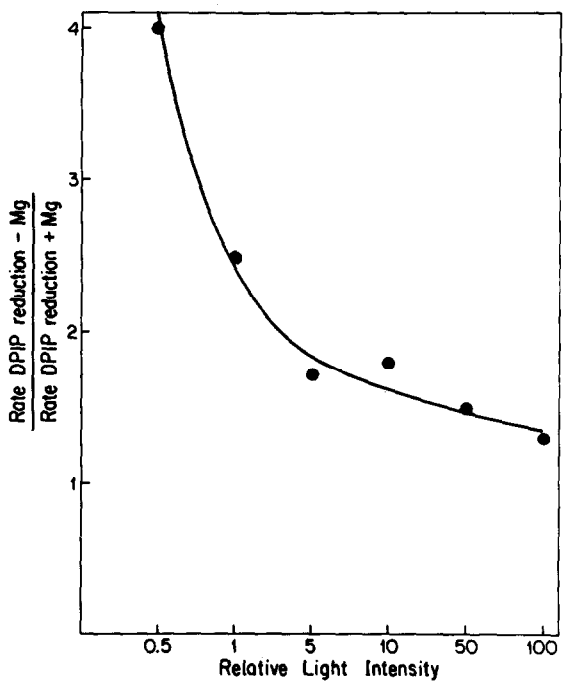

Fig.4. Effect of light intensity on Mg inhibition of DPIP reduction. Conditions as in fig.3A. Light intensity was reduced by means of Balzars neutral density filters, with a 100% value of 1050 $J.m^{-2}.s^{-1}$.

3–4 mM (fig.5). The exact concentration necessary for half maximum inhibition of DPIP reduction varies also as a function of light intensity, explaining the slight differences between the curves.

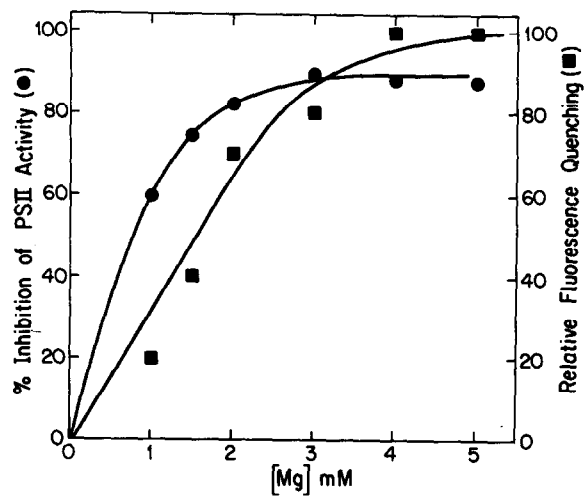

Fig.5. Quenching of chlorophyll fluorescence and inhibition of DPIP reduction at different $MgCl_2$ concentrations. Chlorophyll fluorescence quenching was measured as in fig.1E and DPIP reduction as in fig.3B.

4. Discussion

The quenching of chlorophyll fluorescence in PS II particles occurs in the presence or absence of diuron, being seen as a decrease in F_{max} and is therefore not related to a change in the redox state of Q. The quenching process can decrease F_{max} by approx. 25–30%, but is accompanied by a 90% inhibition of photochemical activity. Both F_o and F_v are quenched, but to differing extents, with F_o being quenched by approx. 10%. Differential quenching of F_v and F_o has been described after ultraviolet [16] and Tris [17] treatment of chloroplasts, although a larger difference was found in these situations. In addition, the Mg-induced increase in [4] and decrease [11] in fluorescence yield in chloroplasts both involve F_v rather than F_o. Interpretation of changes in fluorescence yield has been greatly aided by the model of Butler and Kitajima [18]. In PS II particles there exists a situation similar to the early version of this model where complication due to energy transfer to PS I is avoided. It was shown that differential sensitivity of F_o and F_v will depend on whether reaction center chlorophyll or bulk chlorophyll is being quenched. Both types of quenching can lead to inhibition of PS II activity. Inhibition of PS II activity by quenching of chlorophyll excitation would be expected to be overcome if sufficiently high light intensity was used, so that adequate utilization of excitons by photochemistry could still occur despite a decrease in the quantum yield; this is clearly seen in fig.3 and 4. The Mg-induced quenching in PS II particles may be due to quenching at reaction center, i.e., an elevated non-radiative decay (kd) of excited reaction center chlorophyll. However, the quenching of F_o is substantial and the photochemical activity very sensitive to the Mg-induced effects for such a small decrease in F_{max}. This would imply that different values have to be given to the various rate constants for dissipation of excited reaction center chlorophyll than were assumed for whole chloroplasts studies, or that quenching at the level of bulk chlorophyll is occurring. A subsequent paper will analyze these alternatives quantitatively. Clearly, studies of fluorescence in PS II particles may help to set more precise limits on the decay processes associated with PS II in the absence of the additional complexity of energy transfer to PS I.

The quenching of chlorophyll fluorescence in PS II particles by Mg^{2+} may be related to the effects of cations on whole chloroplasts. Mg^{2+}-Induced quenching has been reported in whole chloroplasts and is proposed to be a separate process from Mg-enhanced fluorescence [11]. Alternatively, the Mg effects on fluorescence and PS II activity, which are essentially opposite to those usually seen in whole chloroplasts, may relate to an altered sidedness of the particle membrane. A recent electrical explanation of cationic effects on PS II has been proposed [8,19]; in such a theory, membrane sidedness with respect to binding sites may be of prime importance in determining the kind of effects seen in the presence and absence of cations [9,20]. The PS II particle membrane may have a changed orientation or accessibility of cation binding sites. The observed inhibition of PS II, which occurs at physiological Mg^{2+} concentrations, may also represent part of a regulatory system to control the rate of non-cyclic electron flow.

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References

[1] Papageorgiou, G. and Govindjee (1968) Biophys. J. 8, 1316–1328.
[2] Mohanty, P., Braun, B. Z. and Govindjee (1973) Biochim. Biophys. Acta 292, 459–476.
[3] Krause, G. H. (1974) Biochim. Biophys. Acta 333, 301–313.
[4] Murata, N. (1969) Biochim. Biophys. Acta 189, 171–181.
[5] Homann, P. H. (1969) Plant Physiol. 44, 932–936.
[6] Gross, E. L. and Hess, S. C. (1973) Arch. Biochem. Biophys. 159, 832–836.
[7] Barber, J., Telfer, A. and Nicolson, J. (1974) Biochim. Biophys. Acta 357, 161–165.
[8] Barber, J. and Mills, J. (1976) FEBS Lett. 68, 288–292.
[9] Mills, J. and Barber, J. (1975) Arch. Biochem. Biophys. 170, 306–314.
[10] Murata, N. and Sugahara (1969) Biochim. Biophys. Acta 189, 182–192.
[11] Jennings, R. C., Garlaschi, F. M. and Forti, G. (1976) Biochim. Biophys. Acta 423, 264–274.
[12] Barber, J. (1976) Trends Biochem. Sci. 1, 33–36.

[13] Gross, E. L. and Davis, D. J. (1976) Brookhaven Sym. Biol. No. 28, p. 362.
[14] Vernon, L. P., Ke, B. and Shaw, E. R. (1967) J. Biol. Chem. 241, 4101–4109.
[15] Vernon, L. P. and Shaw, E. R. (1969) Biochem. Biophys. Res. Commun. 36, 878–882.
[16] Yamashita, T. and Butler, W. L. (1968) Plant Physiol. 43, 2037–2040.
[17] Yamashita, T. and Butler, W. L. (1969) Plant Physiol. 44, 1342–1346.
[18] Butler, W. L. and Kitajima, M. (1975) Biochim. Biophys. Acta 376, 116–125.
[19] Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174–181.
[20] Mills, J. D., Telfer, A. and Barber, S. (1976) Biochim. Biophys. Acta 440, 495–505.