Biochimica et Biophysica Acta, 368 (1974) 141–147

BBA 46816

CORRELATION BETWEEN STATES I AND II IN ALGAE AND THE EFFECT OF MAGNESIUM ON CHLOROPLASTS

PIERRE BENNOUN

Equipe de Recherche No. 16, Centre National de la Recherche Scientifique, Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75231 Paris, Cedex 05 (France)

(Received March 6th, 1974)
(Revised manuscript received June 26th, 1974)

SUMMARY

Various experimental data are presented showing the identity between the effects induced by prolonged illumination of algae and those induced by Mg^{2+} in isolated chloroplasts. State I of algae corresponds to the state with magnesium in chloroplasts and State II to the state without magnesium. These effects are closely related to the presence of active System I centers and independent of the presence of active System II centers.

INTRODUCTION

Bonaventura and Myers [1] and Murata [2] have reported a slow adaptation of algae to intensity and wavelength of illumination. When algae are exposed to an illumination exciting preferentially System II (light II) for several minutes, adaptation to State II is achieved. Prolonged illumination with light I (i.e. far red light) induces adaptation to State I. When in State II, algae display a lower fluorescence yield of chlorophyll compared to State I.

According to Bonaventura and Myers [1], a variation of the distribution of absorbed quanta between the two photosystems is responsible for this effect. Delrieu [3] proposed an alternative hypothesis involving a variation of the apparent equilibrium constant between the two photoreactions.

The magnesium effect in isolated chloroplasts was reported by Homann [4] and analyzed in more detail by Murata [5]. The latter author showed that for a given non-saturating light intensity Mg^{2+} increase the rate of Photoreaction II and the fluorescence yield and decrease the rate of Photoreaction I. In collaboration with Vernotte et al. [6], I have shown that large modifications of the action spectrum of methyl viologen reduction were induced by Mg^{2+}. The Emerson effect [7] in isolated chloroplasts was shown by Sun and Sauer [8, 9] and by Sinclair [10] to be magnesium

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

dependent. Murata [5] proposed that Mg^{2+} suppress the spillover of excitation energy from pigment System II to pigment System I. This interpretation was also put forward by Sun and Sauer [8, 9] and by Briantais et al. [11].

I have been interested in determining whether a correlation exists between light-induced and magnesium-induced phenomena. This paper attempts to confirm and extend previous results concerning both effects and to establish a correlation between State I and the state with magnesium and between State II and the state without magnesium.

MATERIALS AND METHODS

(a) Studies on the magnesium effect in chloroplasts are limited by the ability to prepare suitable experimental materials. These require a high degree of chloroplast integrity, a factor highly dependent upon the quality of the starting material. Lettuce from the local markets while capable of a sizable Mg^{2+} effect gives results too variable to permit reproducible experiments. For this reason, I developed a method for isolating cell-free preparations from *Chlamydomonas reinhardi* displaying large reproducible magnesium effects: 150 ml of a log phase culture are spun down and resuspended in 15 ml of 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) buffer, pH 7.5, containing 0.5 M sorbitol, 10 mM NaCl, 5 % dextran and 2.5 % ficoll. The cell suspension is broken in a Yeda press operated at 90 kg/cm^2, then diluted by addition of 120 ml of 20 mM HEPES buffer, pH 7.5, containing 0.2 M sorbitol and 10 mM NaCl. After centrifugation, the pellet is resuspended in 3 ml of 20 mM HEPES buffer, pH 7.5, containing 0.4 M sorbitol, 10 mM NaCl, 10 mM KCl and 0.5 % bovine serum albumin. The mutant strains of *Chlamydomonas* used in this work were screened as described previously [12].

(b) Oxygen measurements were performed with a modulated polarograph [13]. The experiments comparing v/V to E/E_{max} were carried out as described by Joliot et al. [14]. A stroboslave type 1539-A was used for the experiments of oxygen flash yield. Fluorescence measurements were performed with an apparatus described previously [15].

RESULTS

Fig. 1 shows fluorescence induction in the presence of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) for algae in States I and II and for chloroplasts with and without magnesium. A lower fluorescence yield for both the constant and variable parts is observed either in State II or without magnesium. Also, the half time of the fluorescence rise is longer in State II or without magnesium. The inflection point is less pronounced in State II or without magnesium. These characteristics of the fluorescence induction with and without magnesium are in agreement with those of previous authors [5, 11]. However, parallel experiments in States I and II are new. With regard to the fluorescence induction in the presence of DCMU, transition from State II to State I is qualitatively equivalent to the addition of magnesium. However, the latter induces larger effects.

Fig. 2 shows the relation between the stationary rate (v) of oxygen evolution and the amount of oxygen (E) evolved after a short flash of saturating intensity

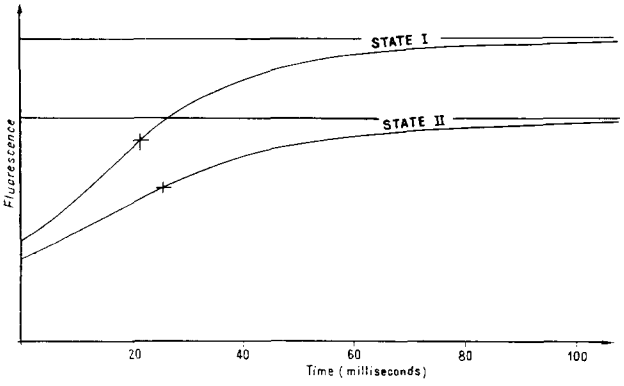

Fig. 1. (A) Fluorescence induction in the presence of 10 μM DCMU. Cells are preilluminated for 15 min in either light I (700 nm) or light II (650 nm), then kept in the dark for 45 s before addition of DCMU and beginning of the experiment. Intensity of light I and II is such that 10 quanta are absorbed per System II center per second. The area over the fluorescence rise curve in State I is 1.35 times larger than in State II. The same ratio is observed between the maximum fluorescence yield in States I and II. The half rise of the fluorescence induction is indicated by a cross. *Chlorella* at 20 °C.

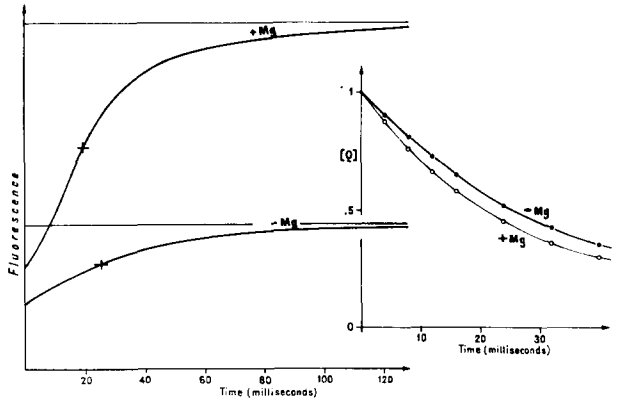

Fig. 1. (B) Fluorescence induction in the presence of 10 μM DCMU. $MgCl_2$ is added in the dark 10 min before DCMU addition and beginning of the experiment. The half rise of the fluorescence induction is indicated by a cross. The area over the fluorescence rise curve is 2.5 times larger in the presence than in the absence of Mg^{2+}. The same ratio is observed between the maximum fluorescence yields with and without magnesium. In the right part of the figure, we compute the kinetics of disappearance of the quencher Q in the light from the fluorescence rise curves: the remaining area over the fluorescence curve was measured at various points during the time course. The amplitude of both curves were normalized to 1. Cell-free preparation of *C. reinhardi* at 20 °C.

which measures the concentration of active System II centers. The plot of v versus E shows that experimental points obtained with and without magnesium belong to different curves. Such discontinuity is also observed between States I and II. The ratio v/E in far red light is smaller in State II than in State I, and also smaller without than with magnesium. This is consistent with recent results by Wang and Myers [16] concerning States I and II in algae. The parameter of energy transfer (a) is smaller in State II than in State I and smaller without than with magnesium. This effect of Mg^{2+}

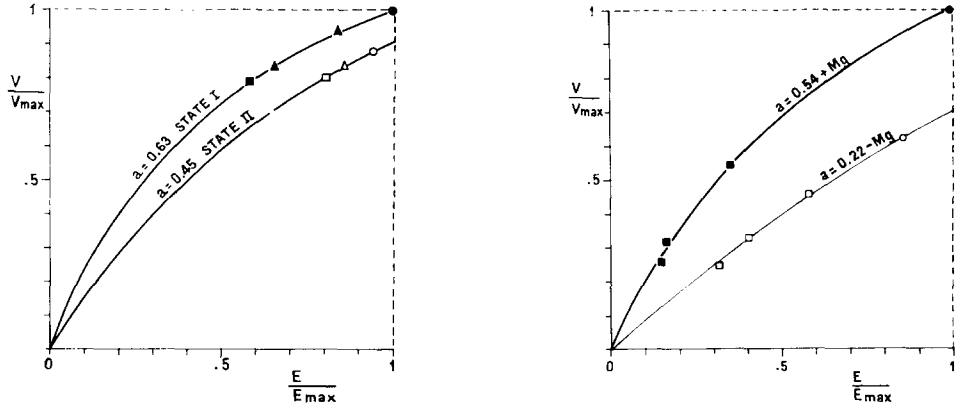

Fig. 2. (A) Stationary rate of oxygen evolution measured by a weak modulated detecting beam in the presence of various background illumination versus saturating flash yield of oxygen evolution in the presence of the same background light: squares, background 650 nm; triangles, white background; circles, background 710 nm. States I and II are developed as described in Fig. 1A. Measurements are performed 15 s after the beginning of illumination with the background light following 15 min of preillumination with either light I or II. The curves are drawn from Joliot's equation of energy transfer [14] with suitable parameter fitting. *Chlorella* at 20 °C. (B) Same plot as A for cell-free preparations of *Chlamydomonas* with or without 8 mM $MgCl_2$: circles, 710 nm background; squares, 650 nm background of various intensity. Theoretical curves from Joliot's equation of energy transfer [14] are drawn with suitable parameter fitting.

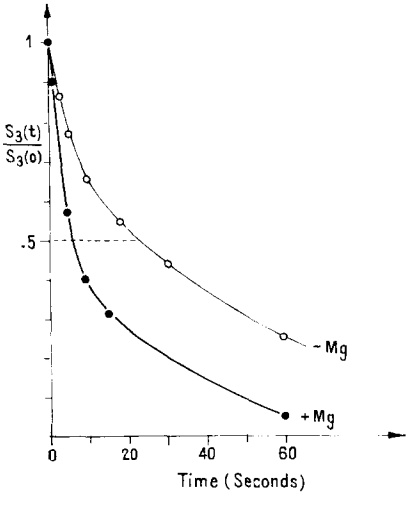

Fig. 3. Kinetics of deactivation of oxygen precursor S_3 in the dark following 10 s of illumination at 650 nm. A short saturating flash is fired at various times (t) following the end of the preillumination. The amount of oxygen evolved after each flash measures the concentration of S_3. Intensity of the preillumination is such that each System II center absorbs 5 photons/s. Cell-free preparations of *Chlamydomonas* with and without 8 mM $MgCl_2$.

on the apparent efficiency of energy transfer between System II units is in agreement with recent results by Marsho and Kok [17]. One can notice that either in State II or in the absence of magnesium, the oxygen flash yield measured in the presence of a far red background is smaller than that measured in State I or with magnesium. This experiment shows again the equivalence between State I and the state with magnesium and between State II and the state without magnesium. Quantitatively, the transition induced by magnesium is more pronounced than that induced by light.

Fig. 3 shows the deactivation pattern of the oxygen precursor S_3 (as defined by Kok et al. [18]) following illumination at 650 nm (light II). Lemasson and Barbieri [19] have shown that this reaction is faster in State I than State II. Similarly, this deactivation is faster in the presence than in the absence of magnesium. The addition of magnesium induces an acceleration of deactivation more pronounced than that observed on going from State II to State I.

TABLE I

MAXIMUM FLUORESCENCE YIELD IN THE PRESENCE OF 10 μM DCMU AND WITH AND WITHOUT 8 mM $MgCl_2$

Magnesium is added in the dark 10 min before addition of DCMU. No DCMU is added for strain F34. Data are normalized with regard to the yield obtained in the presence of magnesium. Cell-free preparations of *Chlamydomonas* at 20 °C.

Strain	$+Mg^{2+}$	$-Mg^{2+}$
WT	100	40
F34	100	46
F14	100	105

TABLE II

MAXIMUM FLUORESCENCE YIELD IN THE PRESENCE OF 10 μM DCMU

Cells are either dark adapted or preilluminated for 15 min in light I or II as in Fig. 1A. Data are normalized with regard to the yield obtained after light I preillumination. No DCMU is added for strain F34. *Chlamydomonas* at 20 °C.

Strain	Light I	Dark	Light II
WT	100	89	75
F34	100	91	100
F14	100	102	100

Table I shows the maximum fluorescence yield of cell-free preparation of wild type and two previously screened mutant strains of *Chlamydomonas* [12]. F34 shows no active System II centers but normal System I activity. The inactive centers of this mutant do not quench fluorescence [15]. F14 shows normal System II activity, but no System I activity (there is no detectable P_{700}) [15]. In the absence of active System II centers (F34) the variation of fluorescence induced by magnesium is just as large as in the wild type. By contrast, magnesium is almost without effect when System I centers are inactive (F14). This observation was checked with four indepen-

dent strains devoid of System I activity. It is important to notice that besides the modification of the fluorescence yield, magnesium induces a modification of the action spectrum of methyl viologen reduction in the strain F34 devoid of active System II centers as it does in the wild type. In the presence of reduced diaminodurene as electron donor the rate of methyl viologen reduction was measured under low light intensity at 650 and 700 nm. The same incident light intensity was used at both wavelengths (same number of photons/s). I observed that the ratio of the rates of methyl viologen reduction at 650 and 700 nm was 30 % lower in the presence than in absence of magnesium.

Table II shows the maximum fluorescence yields for algae preilluminated with either light I or II or dark adapted. With regard to the maximum fluorescence yield, dark-adapted wild-type cells are in an intermediary state between State I and II. When the mutant with inactive System II centers (F34) is preilluminated with light I or II an increase of fluorescence is observed which corresponds to a transition towards State I. Illumination of the mutant with inactive System I centers (F14) is essentially without effect on the maximum fluorescence yield. The same results were obtained with four independent strains devoid of System I activity.

This study of the mutant strains give an additional argument of the identity of the light-induced and magnesium-induced phenomena: both are independent of the presence of active System II centers and highly dependent upon the presence of active System I centers.

DISCUSSION

The above experiments demonstrate the identity between the effects observed in vivo by Bonaventura and Myers [1] and in vitro by Murata [2]. These two kinds of effects differ only quantitatively: for all the measurements reported here the effects induced by magnesium on good chloroplast preparations are larger than that induced by prolonged illumination on whole cells.

The interpretation of Bonaventura and Myers [1] implying a variation of the fraction α of total absorbed photons delivered to System II is not sufficient to account for the data presented here. In this hypothesis, one would predict no change in the area over the fluorescence rise curve (Fig. 1) between the two states and a variation of the half time of the fluorescence rise proportional to the variation of the fluorescence yield. Contrary to this, the experiment shows a large variation of the area over the fluorescence rise curve and a large variation of the fluorescence yield correlated to a small change in the half time of fluorescence rises. This pattern is consistent with the concept of an additional quenching process occurring in State II and without magnesium. One possible way to represent this is to assume with Murata [5] a controlled spillover of excitation energy from pigment System II to pigment System I. Another possibility suggested by our results is that fewer active System II centers are present in State II and that the inactive centers still quench fluorescence. These two possibilities are not mutually exclusive and further investigation will be necessary to decide their respective relevance to this problem.

In State I and in the presence of magnesium, enhancement is maximum and a large fraction of pool A is in the reduced state under light II illumination[17]: deactivation is then fast. In State II and in the absence of magnesium, a smaller part of the

pool is reduced under light II as indicated by the higher concentration of active System II centers and deactivation is slower. This is in agreement with a mechanism of deactivation implying a back electron flow from A^- to Q as discussed previously [20].

The study of mutant strains leads to a clear cut conclusion concerning the lack of dependence towards the presence on active System II centers for both light and magnesium effects. The absence of an effect when active System I centers are missing may be less easily interpretable. Though the mutant strains used in this work are affected by a single gene mutation it is possible that the inactivity of System I centers is associated with some kind of modification of the thylakoid membranes affecting a site of action of magnesium. However, as identical results were obtained with four independent mutant strains, it is likely that magnesium is acting in close relation with System I photocenters.

In relation with this result is the observation by MacRobbie [22] of a light-driven cation flux in intact *Nitella* cells displaying an action spectrum of the System I type.

The problem of the regulation of magnesium concentration in vivo is open. I observed that NH_4Cl did not inhibit the effect of magnesium. Also this effect is observable in mutant strain F54 that I screened previously [12]. This strain has an impaired ATPase and cannot phosphorylate in the light [21]. This regulation is therefore independent of the proton gradient and the ATP level.

ACKNOWLEDGEMENT

I thank Dr Bruce A. Diner for critical reading of the manuscript.

REFERENCES

1 Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–383
2 Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251
3 Delrieu, M. J. (1972) Biochim. Biophys. Acta 256, 293–299
4 Homann, P. (1969) Plant. Physiol. 44, 932–934
5 Murata, N. (1969) Biochim. Biophys. Acta 189, 171–181
6 Vernotte, C., Briantais, J. M. and Bennoun, P. (1973) C. R. Acad. Sci. Paris, 277 D, 1695–1698
7 Emerson, R., Chalmers, R. and Cedestrand, C. (1957) Proc. Natl. Acad. Sci. U.S. 43, 133
8 Sun, A. S. K. and Sauer, K. (1971) Biochim. Biophys. Acta 234, 399–414
9 Sun, A. S. K. and Sauer, K. (1972) Biochim. Biophys. Acta 256, 409–427
10 Sinclair, J. (1972) Plant. Physiol. 50, 778–783
11 Briantais, J. M., Vernotte, C. and Moya, I. (1973) Biochim. Biophys. Acta 325, 530–538
12 Bennoun, P. and Levine, R. P. (1967) Plant. Physiol. 42, 1284–1287
13 Joliot, P. and Joliot, A. (1968) Biochim. Biophys. Acta 153, 625–634
14 Joliot, P., Joliot, A. and Kok, B. (1968) Biochim. Biophys. Acta 153, 635–652
15 Bennoun, P. (1971) Thèse de Doctorat d'Etat, Paris
16 Wang, R. T. and Myers, J. (1974) Biochim. Biophys. Acta 347, 134–140
17 Marsho, V. and Kok, B. (1974) Biochim. Biophys. Acta 333, 353–365
18 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
19 Lemasson, C. and Barbieri, G. (1971) Biochim. Biophys. Acta 245, 386–397
20 Bouges-Bocquet, B., Bennoun, P. and Taboury, J. (1973) Biochim. Biophys. Acta 325, 247–254
21 Sato, V. L., Levine, R. P. and Neumann, J. (1971) Biochim. Biophys. Acta 253, 437–448
22 McRobbie, E. A. C. (1965) Biochim. Biophys. Acta 94, 64–73