Biochimica et Biophysica Acta, 449 (1976) 48–58

BBA 47176

EFFECT OF IONOPHORES A23187 AND NIGERICIN ON THE LIGHT-INDUCED REDISTRIBUTION OF Mg^{2+}, K^+ AND H^+ ACROSS THE THYLAKOID MEMBRANE

A. A. BULYCHEV* and W. J. VREDENBERG

Centre for Agrobiological Research, P.O. Box 14, Wageningen (The Netherlands)

(Received March 19th, 1976)

SUMMARY

Passive redistributions of Mg^{2+} and K^+ ions across the thylakoid membranes, occurring in association with the light-driven electrogenic influx of hydrogen ions have been examined in suspensions of broken spinach chloroplasts under a variety of conditions.

(i) In accord with results of Hind et al. (Proc. Natl. Acad. Sci. U.S. (1974) 71, 1484), it was found that at a low K/Mg concentration ratio in the medium, the K-efflux is negligibly small, whereas a substantial Mg-efflux is observed. The converse is true when the K/Mg concentration ratio in the medium is high.

(ii) In the presence of A23187, which was found to cause approximately a 60 % inhibition of the light-induced pH-gradient, a significant influx of Mg^{2+} was observed in the light at a high K/Mg concentration ratio. Conversely the Mg-influx was small in the presence of A23187 when the K/Mg concentration ratio in the medium was low. Under these conditions, the Mg-influx was considerably increased upon the addition of valinomycin. A23187 was found not to affect the K-efflux in the light.

(iii) The light-induced K-influx observed in the presence of nigericin also was found to be dependent on the concentration ratio of the monovalent and divalent cation. Its magnitude increased upon an increase in the K/Mg ratio.

The results are interpreted in terms of a simplified model in which the total passive efflux of cations, driven by the potential set by the electrogenic proton pump, is considered to be a constant fraction of the proton influx. According to this, an increase in the flux of an ion species, induced either by raising its concentration, or by increasing its permeability through the membrane, will cause a decrease in the flux of the other cations. The relevance of the results is discussed with respect to conclusions about the involvement and relative magnitudes of the passive K and Mg effluxes across the thylakoid membrane during energization of intact chloroplasts and chloroplasts in situ.

---

* Permanent address: Biophysics Department, Biological Faculty, Moscow State University, Moscow, U.S.S.R.

INTRODUCTION

Since the proposal of the chemiosmotic hypothesis on energy coupling in energy-conserving membranes [1], a great deal of attention has been given to ion movements across the thylakoid membranes of chloroplasts that occur in association with light-induced proton uptake [2, 3]. The efflux of K^+ and Mg^{2+} [4–7], as well as an uptake of Cl^- [8] has been reported to occur during illumination. With respect to K^+ movement the results obtained so far are somewhat conflicting. Some authors found only a small or even an irreversible efflux of this ion [9]. Hind et al. [6] have shown that the extent of K^+ and Mg^{2+} effluxes from broken chloroplasts is dependent on the relative concentrations of these ions in the suspension medium. The ratio of the K^+ and Mg^{2+} effluxes in intact chloroplasts and in chloroplasts in vivo is not yet known, but it is tentative to assume that this ratio is dependent on the actual concentration ratio of these ions in the chloroplast stroma. Studies on the kinetics of the light-induced changes in the electrical transmembrane potential of thylakoids in intact chloroplasts and in chloroplasts in vivo have suggested evidence that K^+ acts as one of the ions that counterbalance the electrogenic proton influx into the thylakoids [10].

Ionophorous antibiotics have been shown to be a potent tool for the investigation of the ion transport processes associated with chloroplast energization [13–16]. From fluorescence experiments in which ionophores were applied to intact chloroplasts, it has been concluded [13–16] that Mg^{2+} acts as the main exchange ion for proton uptake in chloroplasts in vivo. The suggestion arose from the observation that the ionophore A23187, which is assumed to act as a neutral exchanger of protons against divalent cations in biological membranes [17], produced the reversion of the light-induced fluorescence quenching in intact chloroplasts, whereas nigericin, which facilitates a K^+/H^+ exchange, was inactive, at least in the absence of K^+ in the suspending medium [13, 16].

In this work, the effect of A23187 on the light-induced redistribution of monovalent and divalent cations across the thylakoid membrane was investigated and compared with the effect of nigericin. A tentative scheme is discussed which accounts for the observed effects of these ionophores on H^+, K^+, and Mg^{2+} transport.

MATERIALS AND METHODS

Suspensions of broken chloroplasts were prepared from market spinach. Washed and chilled leaves were homogenised in a medium containing 0.33 M sorbitol, 2 mM $MgCl_2$ and 25 mM HEPES* buffer, adjusted to pH 7.4 with NaOH. The homogenate was filtered through 4 layers of perlon net (pore diameter 50 μm) and centrifuged for 5 min at $3000 \times g$. The sedimented chloroplasts were suspended in a small volume of a medium containing 0.33 M sorbitol and 2 mM HEPES-NaOH buffer, pH 7.4. Chlorophyll content was determined spectrophotometrically [18, 19]. The stock chloroplast suspension was diluted 20 to 50-fold, and KCl and/or $MgCl_2$ were added at final concentrations variable from 0.5 to 50 mM and 0.1 to 3 mM, respectively. The average chlorophyll concentration was 100 $\mu g \cdot ml^{-1}$.

* HEPES: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

Light-induced changes in the activities of H^+, K^+ and Mg^{2+} were determined by means of ion-specific electrodes. The following electrodes were used: Philips liquid membrane electrode IS 560 (K^+) and Orion divalent cation electrode 92-32 (Mg^{2+}). Photosynthetic electron transport was measured as O_2-uptake (Rank Brothers O_2-electrode) in the presence of diquat and 0.2 mM azide, simultaneously with pH-changes. The suspension was continuously stirred and illuminated with white light of approx. $6 \cdot 10^5$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} intensity. Measurements were performed at ambient room temperature.

Light-induced electric potential changes across the chloroplast (thylakoid) membranes were measured with conventional choline chloride-filled micro-electrodes on single isolated chloroplasts of *Peperomia metallica* as previously described [20, 21]. These chloroplasts were suspended in a medium containing 0.25 M sucrose, 25 g/l ficoll, 100 mg/l bovine serum albumin, 1 mM KCl and 0.01 M tricine buffer adjusted to pH 7.8 by Tris. A single chloroplast, sucked onto the polished tip of a suction pipette, was illuminated with saturating white light via a flexible light guide.

RESULTS

The effect of A23187 and nigericin on the light-induced redistribution of Mg^{2+} and K^+

Fig. 1 shows the effect of ionophore A23187 on the light-induced transport of divalent cations in broken chloroplasts suspended in a medium containing 0.5 mM K^+ and 0.1 mM Mg^{2+}. It has been shown that for chloroplasts isolated and suspended in Ca^{2+}-free medium, the flux of divalent ions across the thylakoid membranes is carried mainly by magnesium ions [6]. The upper curve (Fig. 1a) shows a reversible efflux of magnesium ions in the light. The amount of Mg^{2+} released in the light was found to be dependent on the ambient K^+ and Mg^{2+} concentrations in the suspension. Under the conditions of the experiment shown here, this amount was approx. 100 nequiv/mg chlorophyll which corresponds rather well with previously reported data on the Mg^{2+} efflux under similar conditions [6]. In the presence of 10 μM A23187 (Fig. 1b), a net uptake of Mg^{2+} ions in the light is observed. As can also be seen in Fig. 1b, it has consistently been found that the uptake of Mg^{2+} is relatively small and slow for illuminated chloroplasts suspended in media with a low concentration of K^+, but is substantially enhanced by a subsequent addition of 1 μM valinomycin (Fig. 1c).

Fig. 2 shows the effect of A23187 on Mg^{2+} transport in chloroplasts suspended in a medium containing 50 mM KCl and 1 mM $MgCl_2$. There is no noticeable light-induced redistribution of magnesium ions under these conditions in the absence of the ionophore. The same result was found in the presence of 0.1 mM $MgCl_2$ at the same (high) concentration of KCl. In the presence of 10 μM A23187 a massive (900–1300 nequiv/mg chlorophyll) uptake of Mg^{2+} is observed. Subsequent addition of valinomycin up to a concentration of 1 μM was found to have little effect on the Mg^{2+} uptake under these conditions.

Fig. 3 shows the light-induced redistribution of K^+ between chloroplasts and outer medium, containing 0.5 mM K^+ and 0.1 mM Mg^{2+}, in the absence and presence of 10 μM A23187. The extent of the light-induced efflux of K^+ is about 40 nequiv/mg chlorophyll under both conditions. An increase in potassium concentration in the suspending medium up to 5 mM was found to produce a 2.5-fold

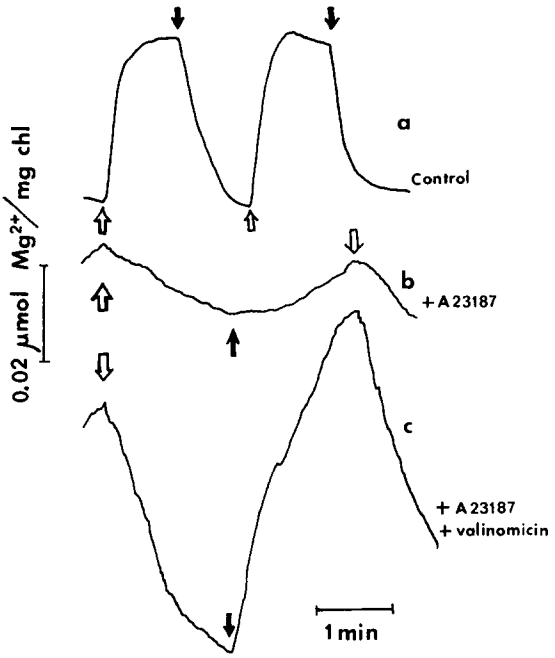

Fig. 1. Light-induced changes in Mg^{2+} activity in a suspension of broken chloroplasts in (a) the absence and (b, c) the presence of 10 μM A23187 (b) before and (c) after the addition of 0.2 μM valinomycin. The chloroplasts were suspended in a medium containing 0.33 M sorbitol, 2 mM HEPES-NaOH buffer, pH 7.4, 0.5 mM KCl, 0.1 mM $MgCl_2$, 20 μM diquat and 0.2 mM NaN_3. Open and closed arrows mark the onset and end, respectively, of an illumination period. An upward movement of the trace means an increase in activity of the ion in the medium. Chlorophyll concentration: approx. 130 μg/ml.

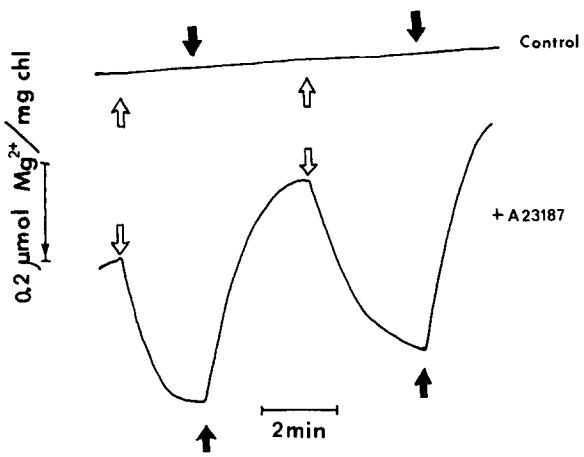

Fig. 2. Light-induced changes in Mg^{2+} activity in a suspension of broken chloroplasts in the absence (control) and presence of 10 μM A23187. Reaction medium and assay were as described in Fig. 1, except for the presence of 50 mM KCl and 1 mM $MgCl_2$.

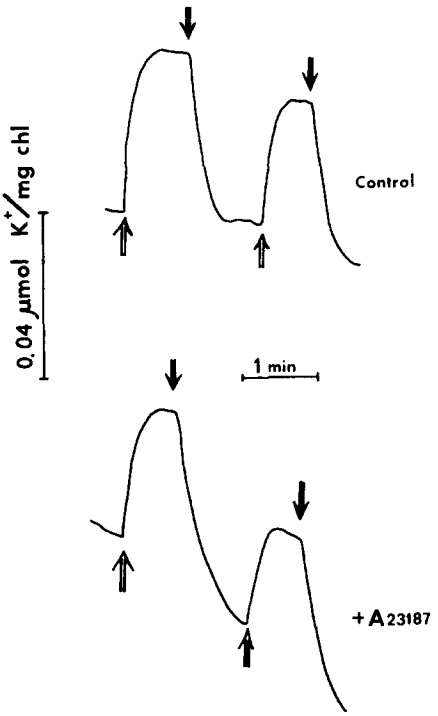

Fig. 3. Light-induced changes in K^+ activity in a suspension of broken chloroplasts in the absence (control) and presence of 50 μM A23187. Reaction medium and assay were as described in Fig. 1 except for a chlorophyll concentration of approx. 260 μg/ml.

stimulation of the light-induced K^+ efflux, both in the presence and absence of the ionophore.

It has been reported [12, 22, 23] that in the presence of nigericin a light-induced uptake of K^+ into chloroplasts occurs. Fig. 4 shows that the extent of the light-induced K^+ uptake at an unaltered K^+ concentration is dependent on the Mg^{2+} concentration in the medium. An increase in Mg^{2+} concentrations from 0.1 to 3 mM results in an approx. 2-fold increase in the extent of light-induced K^+ uptake in the presence of nigericin. Addition of 3 mM EDTA results in a 70 % reduction of the K^+ uptake in the light. Subsequent addition of 1 mM Mg^{2+} eliminated the inhibitory effect.

The effects of A23187 and nigericin on light-induced proton uptake and rate of photosynthetic electron transport

Fig. 5 shows the effect of A23187 and nigericin on the light-induced pH-changes and the oxygen cosumption in a chloroplast suspension containing 50 mM K^+ and 0.5 mM Mg^{2+} and 20 μM diquat as electron acceptor. It can be seen that addition of A23187 has brought about a 50–60 % inhibition of the light-induced pH change and an enhancement of the electron transport rate by a factor of about 2.5. Subsequent addition of nigericin causes a complete inhibition of the light-induced pH change and a further increase in the electron transport rate by a factor of about

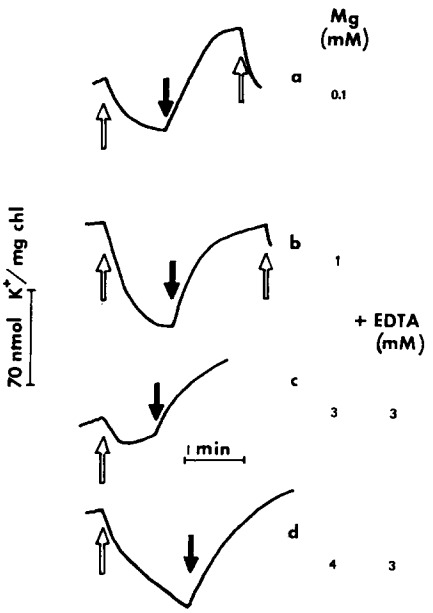

Fig. 4. Light-induced decrease in K^+ activity in a suspension of broken chloroplasts in the presence of 1 μM nigericin at various concentrations of $MgCl_2$ in (a, b) the absence and (c, d) the presence, respectively, of 3 mM EDTA. Reaction mixture and assay were as described in Fig. 1, except for the presence of 3 mM KCl and the following concentrations of $MgCl_2$ (in mM): (a), 0.1; (b), 1.0; (c), 3 and (d), 4. Chlorophyll concentration was approx. 260 μg/ml.

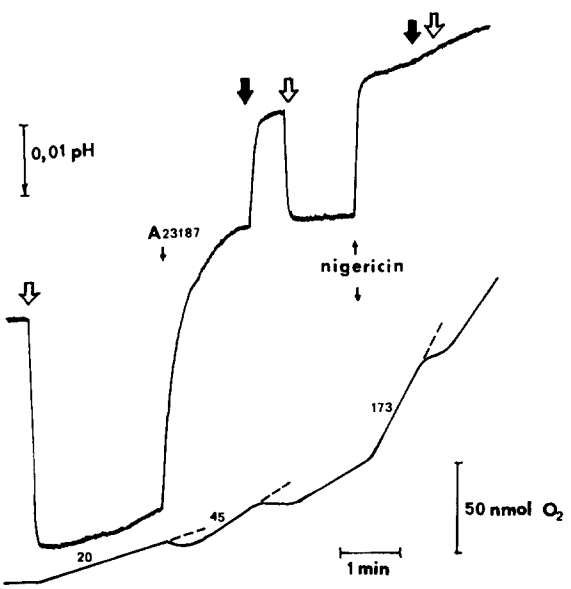

Fig. 5. The effect of A23187 (10 μM) and nigericin (1 μM) on the light-induced pH changes and O_2 consumption in a suspension of broken chloroplasts. The composition of the medium was as described in Fig. 1, except for the presence of 50 mM KCl and a chlorophyll content of approx. 60 μg/ml. The figures along the (lower) oxygen trace are the rates in μatoms · mg^{-1} chlorophyll · h^{-1}. The small closed arrows mark the additions of the ionophores.

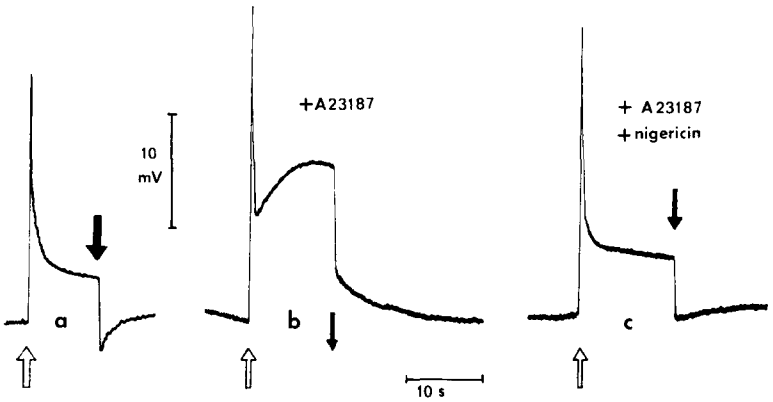

Fig. 6. Light-induced changes in the electrical potential across the thylakoid membranes of an isolated chloroplast of *Peperomia metallica* in the presence of 10 μM A23187 (b) before and (c) after the addition of 1 μM nigericin. A typical control response, measured in the absence of ionophores is shown in (a). The reaction medium was as described in Materials and Methods.

3.5. Nigericin alone was found to produce a complete inhibition of the pH-change and an increase in the rate of electron transport by a factor of about 8.7 under these conditions. Similar results, i.e. a lower uncoupling efficiency of A23187 than of nigericin, were found with chloroplasts suspended in media containing 3 mM Mg^{2+} and 1 mM K^+.

Effect of A23187 and nigericin on light-induced electric potential changes across the chloroplast membrane

The specific ability of A23187 and nigericin to induce a light-dependent uptake of Mg^{2+} and K^+, respectively, makes it possible to assess the involvement of changes in the distribution of K^+ and Mg^{2+} with the light-induced changes in the membrane potential across the thylakoid membranes. Therefore, experiments were conducted in which the photoelectric responses of intact isolated chloroplasts of *Peperomia metallica* were measured in the presence of these ionophores. Fig. 6 shows the potential responses observed after subsequent additions of A23187 and nigericin. In the absence of nigericin, the initial rise (phase 1) and subseqent phase 2 decay, occurring in the first second of illumination, are followed by a slow but substantial increase in potential in the light when A23187 is present. Under these conditions the rapid potential drop in the dark is followed by a slow decay. The slow rise and decay in the light and the dark, observed in the presence of A23187, are inhibited by nigericin. The absence of the transient potential undershoot upon darkening in the presence of A23187 and nigericin is also noteworthy.

DISCUSSION

The present results show that the light-induced release of Mg^{2+} occurring in broken chloroplasts in association with H^+ uptake is specifically reversed in the presence of A23187, with a concomitant decrease in H^+ uptake. This phenomenon, consistent with the ability of A23187 to facilitate a neutral exchange of divalent

cations against protons in biomembranes [17], is similar to the effect of nigericin on light-induced K^+ movement across the thylakoid membrane [12, 22, 24]. In the absence of ionophores, a competitive relation between the effluxes of K^+ and Mg^{2+} has been reported to exist which appears to be determined by the concentration ratio of the two ions in the suspension medium [6].

According to two alternative interpretations for light-induced changes in Mg^{2+} activity [6], the redistribution of Mg^{2+} across the thylakoid membrane is determined either by a reversible binding of this ion to the membrane in response to a conformational change in the membrane, or by transport of the ion across the membrane. The reverse direction of the light-induced change in Mg^{2+} activity in the outer medium in the presence of A23187 (Figs. 1 and 2) is difficult to interprete in terms of the first mechanism, but can be understood for a model which supposes a transport of ions across the membrane (see below). If for such model a passive equilibration of the ion concentrations between the thylakoid inner space and the outer medium is assumed, then the apparent competitive relation between the K^+ and Mg^{2+} fluxes can be qualitatively visualized from two essential requirements for passive ion movement; (1) according to the flux equation (e.g. refs. 10, 25) applied to these particular conditions, the (ef)fluxes of Mg^{2+} and K^+ should be proportional to their respective concentrations when the membrane permeability coefficients of the ions are not altered; (2) in the absence of electrical current through the membrane, the sum of the passive fluxes in the steady state should be equal to the active proton influx. This requirement will cause the relative magnitude of the Mg^{2+} and K^+ fluxes to be determined by the concentration of both ions in the dark steady state and by the membrane permeability coefficients. For instance, an increase in the flux of one ion species, induced either by raising its concentration in the medium, or by ionophoretically increasing its permeation through the membrane, will result in a decrease of the flux of the other ion. According to these requirements, the direction and relative magnitude of the light-induced changes in the Mg^{2+} and K^+ flux at different concentration ratios of these cations in the medium in the presence and absence of A23187 or nigericin, can be qualitatively interpreted on basis of the simplified scheme shown in Fig. 7. Only fluxes of K^+ and Mg^{2+} are considered here, but the model is applicable to the "physiological" situation [26] in which anions are transported as well. The following discussion of the model is restricted to its fundamentals and consequences. This means that the accelerating effect of changes in the cation concentration on the rate of electron transport [27], i.e. on the rate of electrogenic proton pumping, is not dealt with here. The scheme accounts for the fact [6] that in the absence of ionophores the K^+ efflux in the light is in excess of the Mg^{2+} efflux under conditions of a high K/Mg concentration ratio, whereas at a low K/Mg concentration ratio the electrogenic H^+-influx is mainly balanced by an efflux of Mg^{2+} ions. In the presence of nigericin or A23187, a backflow of H^+ from the thylakoids to the outer medium is promoted in conjunction with an influx of K^+ and Mg^{2+}, respectively. This means for example that for control conditions of a comparatively small Mg^{2+} efflux (i.e. at a high K/Mg ratio) a relatively large net influx of Mg^{2+} occurs in the light in the presence of A23187. If, on the contrary, the efflux of Mg^{2+} is comparatively large (i.e. at a low K/Mg concentration ratio), then the addition of A23187 will produce a relatively small net uptake of Mg^{2+}, since the difference between influx and efflux is small. These predictions are consistent

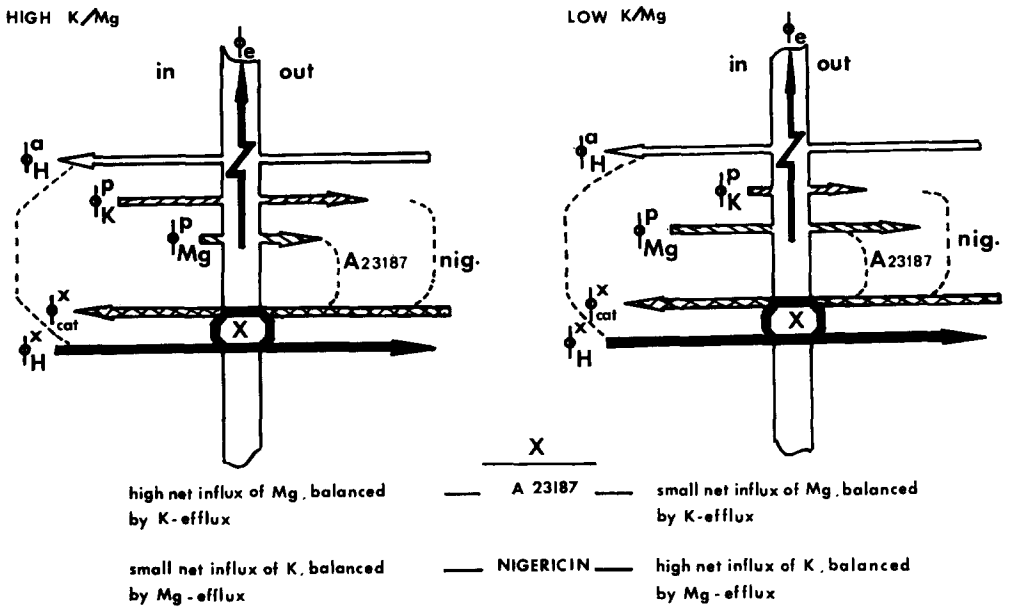

Fig. 7. Tentative scheme for light-dependent cation movements across the thylakoid membrane of chloroplasts, exemplified for a high (left) and low (right) K/Mg concentration ratio of the thylakoid environment, in the absence and presence of an ionophore (X) that facilitates an exchange of protons against divalent cation (X = A23187), or against K^+ (X = nigericin). ϕ_e, light-driven electron flux through the membrane-localized photosynthetic electron transport chain; $\phi_H{}^a$, electrogenic proton (in)flux; $\phi_{Mg}{}^p$, $\phi_K{}^p$, passive potential-driven (ef)flux of Mg^{2+} and K^+, respectively; $\phi_H{}^x$ and $\phi_{cat}{}^x$, passive exchange fluxes of protons and cations, respectively, in the presence of ionophore X. Further explanations are in the text.

with the experiments. Light-induced Mg^{2+} influx in the presence of A23187 was much higher at a high K/Mg concentration ratio (Fig. 2), than under conditions of a low concentration ratio of these ions (Fig. 1). The enhancement of the light-induced Mg^{2+} influx by valinomycin in the presence of A23187 under conditions of a low K/Mg concentration ratio (Fig. 1) is in agreement with the predictions set by the model. A valinomycin-induced increase in the K^+ conductance of the membrane will cause an increase in the light-stimulated K^+ efflux and consequently, as discussed before, the Mg^{2+} efflux will become smaller. As valinomycin is not expected to interfere with the Mg/H-exchange induced by A23187, the net Mg^{2+} influx will increase due to the decrease in the Mg^{2+} efflux. Similarly, the scheme predicts that the net influx (influx minus eflux) of potassium ions in the light in the presence of nigericin increases when the K/Mg-concentration ratio is decreased, and vice versa (Fig. 4). According to the model, a decrease in Mg^{2+} activity is associated with an increase in the K^+ efflux due to the decrease in the light-driven Mg^{2+} efflux, and consequently, the light-driven K^+ influx is decreased. Restoration of the Mg^{2+} efflux by addition of Mg^{2+} leads to a restoration of the K^+ uptake in the light in the presence of nigericin (Fig. 4). It is of interest to note that light-induced K^+ uptake in the presence of nigericin has been reported only for chloroplasts in low

KCl-media (1 mM), at which, as would be expected, the light-induced efflux of the cation in the absence of the ionophore was negligible or even absent [12, 24].

The ability of A23187 to act as an uncoupler, as evidenced by its inhibiting effect on the light-induced pH change and stimulating action of the electron transport rate (Fig. 5), is probably due to the facilitated exchange of protons against Mg^{2+} across the thylakoid membrane [13, 14]. Such uncoupling mechanism is principally similar to that of nigericin in the presence of K^+ [12]. In comparison with nigericin, A23187 was found to be a weaker uncoupler both at high (50 mM) and low (1 mM) concentrations of potassium. Full uncoupling by A23187 has been reported for chloroplasts suspended in a cation-free medium, except for Mg^{2+} [15].

The effects of A23187 and nigericin on the Mg^{2+} and K^+ movements are of relevance with respect to the interpretation of the changes in the electrical potential across the thylakoid membrane measured in prolonged illumination in isolated chloroplasts of *Peperomia metallica.* As discussed more extensively elsewhere [28], the slow rise in potential in the light and the slow decay in the dark in the presence of A23187 (Fig. 6) is suggested to reflect the reversible formation of a K^+ concentration gradient and its associated diffusion potential across the thylakoid membrane. The formation of this gradient, the magnitude of which is not affected by A23187 (Fig. 3), is inhibited in the presence of nigericin [28]. The fact that the slow potential rise (and decay) is not observed in the absence of A23187 is probably due to the fact that under these conditions the contribution of this gradient to the membrane potential is compensated by an (opposing) contribution of the proton gradient [29]. The uncoupling effect of A23187 causes the latter to be substantially suppressed in the presence of the ionophore. With respect to this, the absence of the transient potential undershoot in the light-off reaction in the presence of the ionophores (Fig. 6) is consistent with earlier evidence [10, 29] that this transient is a reflection of the pH-gradient formed in the light across the thylakoid membrane.

The present results are of interest with respect to the involvement and relative magnitudes of the passive K^+ and Mg^{2+}(ef)fluxes across the thylakoid membrane during energization of intact chloroplasts and chloroplasts in situ. Although K^+ and Mg^{2+} concentrations in intact chloroplasts have been reported to be 40 to 140 and 15 to 30 mM, respectively [5, 7, 15, 31], indicating a relatively low concentration ratio of about 5, or even much less [31], we suggest that the experiment (Fig. 2) with broken chloroplasts suspended in 50 mM KCl and 1 mM $MgCl_2$ is more or less representative for the situation in intact chloroplasts. Confirmatory with results of Hind et al. [6], it has been found (A. Schapendonk, unpublished) that the magnitude of the light-driven Mg^{2+} efflux in broken chloroplasts is not increased at Mg^{2+} concentrations above 1 mM both at low and high K^+ concentrations. If this phenomenon was also to occur in intact chloroplasts, this would suggest that the light-induced Mg^{2+} efflux in intact chloroplasts is relatively small in comparison with the K^+ efflux. This suggestion is qualitatively in agreement with conclusions derived from kinetic analyses of the photoelectric membrane responses in isolated chloroplasts under various conditions [10, 28]. On the other hand, a dominant involvement of Mg^{2+} transport in intact chloroplasts has been concluded on basis of the specific differences in the effects of A23187 and nigericin on the fluorescence quenching of chlorophyll *a* in cation-free suspensions of intact chloroplasts [13, 14, 16, 30]. A full discussion of the seeming discrepancy with respect to the conclusions

about the identity of the counter-ion for the electrogenic proton loading of the thylakoids in intact chloroplasts is given elsewhere [32]. Evidence has been obtained [32] that the stromal K^+ concentration in K^+ free suspensions of intact chloroplasts is less than 0.3 mM in the presence of nigericin. At such a low internal K^+ concentration, nigericin is likely not to act as an uncoupler [12], and thus will not cause an inhibition of the fluorescence quenching. This was observed indeed, but has been interpreted differently [13, 15].

REFERENCES

1 Mitchell, P. (1968) Chemi-osmotic Coupling and Energy Transduction, Glynn Research, Bodmin, Cornwall
2 Dilley, R. A. (1971) in Current Topics, Bioenergetics (Sanadi, D. R., ed.), Vol. 4, pp. 237–271, Academic Press, London
3 Walker, D. A. and Crofts, A. R. (1970) Annu. Rev. Biochem. 39, 389–428
4 Dilley, R. A. and Vernon, L. P. (1965) Arch. Biochem. Biophys. 111, 365–375
5 Nobel, P. S. (1969) Biochim. Biophys. Acta 172, 134–143
6 Hind, G., Nakatani, H. Y. and Izawa, S. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1484–1488
7 Pflüger, R. (1974) Ber. Dtsch. Bot. Ges. 87, 383–388
8 Deamer, D. W. and Packer, L. (1969) Biochim. Biophys. Acta 172, 539–545
9 Crofts, A. R., Deamer, D. W. and Packer, L. (1967) Biochim. Biophys. Acta 131, 97–118
10 Vredenberg, W. J. and Tonk, W. J. M. (1975) Biochim. Biophys. Acta 387, 580–587
11 Karlish, S. J. D., Shavit, N. and Avron, M. (1969) Eur. J. Biochem. 9, 291–298
12 Shavit, N., Degani, H. and San Pietro, A. (1970) Biochim. Biophys. Acta 216, 208–219
13 Barber, J., Mills, J. and Nicolson, J. (1974) FEBS Lett. 49, 106–110
14 Barber, J., Telfer, A. and Nicolson, J. (1974) Biochim. Biophys. Acta 357, 161–165
15 Telfer, A., Barber, J. and Nicolson, J. (1975) Biochim. Biophys. Acta 396, 301–309
16 Krause, G. H. (1975) in Proceedings of the 3rd International Congress on Photosynthesis, 1974 (Avron, M., ed.), Vol. II, pp. 1021–1030, North-Holland, Amsterdam
17 Reed, P. N. and Lardy, H. A. (1972) J. Biol. Chem. 247, 6970–6977
18 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
19 Bruinsma, J. (1961) Biochim. Biophys. Acta 52, 576–578
20 Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1972) Nature 236, 175–177
21 Vredenberg, W. J., Homann, P. H. and Tonk, W. J. M. (1973) Biochim. Biophys. Acta 314, 261–265
22 Packer, L. (1976) Biochem. Biophys. Res. Commun. 28, 1022–1028
23 Degani, H. and Shavit, N. (1972) Arch. Biochem. Biophys. 152, 339–346
24 Karlish, S. and Avron, M. (1970) in Electron Transport and Energy conservation (Tager, J. M., Papa, S., Qualiariello, E. and Slater, E. C., eds.), pp. 431–448, Adriatica Editrice, Bari
25 Bulychev, A. A. and Vredenberg, W. J. (1976) Biochim. Biophys. Acta 423, 548–556
26 Deamer, D. W., Crofts, A. R. and Packer, L. (1967) Biochim. Biophys. Acta 131, 81–96
27 Gross, E., Dilley, R. A. and San Pietro, A. (1969) Arch. Biochem. Biophys. 134, 450–462
28 Vredenberg, W. J. and Bulychev, A. A. (1976) Plant Sci. Lett. 7, in the press
29 Vredenberg, W. J. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 53–88, Elsevier, Amsterdam
30 Krause, G. H. (1974) Biochim. Biophys. Acta 333, 301–313
31 Gimmler, H., Schäfer, G. and Heber, U. (1975) in Proceedings of the IIIrd International Congress on Photosynthesis, 1974 (Avron, M., ed.), Vol. II, pp. 1381–1392, North-Holland, Amsterdam
32 Vredenberg, W. J. (1976) in Proceedings of the C.N.R.S. International Workshop on Transmembrane Ionic Exchanges in Plants, Rouen/Paris, Editions du C.N.R.S., Paris, in the press