Biochimica et Biophysica Acta, 423 (1976) 548–556

BBA 47057

THE EFFECT OF CATIONS AND MEMBRANE PERMEABILITY MODIFYING AGENTS ON THE DARK KINETICS OF THE PHOTOELECTRIC RESPONSE IN ISOLATED CHLOROPLASTS

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

Centre for Plant Physiological Research, P.O. Box 52, Wageningen (The Netherlands)

(Received August 19th, 1975)

SUMMARY

The kinetics of the photoelectric response induced by saturating light pulses were studied in isolated chloroplasts of *Peperomia metallica* as a function of K^+- and Mg^{2+}-concentrations in the medium in the absence and presence of ionophores for K^+ and divalent cations. The dark decay of the potential generated in the light is found to be accelerated upon an increase in K^+- or Mg^{2+}-concentrations in the presence of valinomycin and A23187. An acceleration of the decay phase in the flash-induced response is also observed immediately after preillumination of the chloroplast. It is concluded that the dark kinetics of the potential decay after short and long light exposures are controlled by two different processes with rate constants of about 20 and 0.2 s^{-1}, respectively.

INTRODUCTION

Electrical potential measurements on chloroplasts in situ, as well as on isolated chloroplasts, have demonstrated the rapid generation of an electrical potential gradient across the chloroplast lamellar membranes after short actinic light flashes [1–7]. The rapid generation of an electrical potential difference is likely to be caused by the primary photochemical charge separation across the thylakoid membrane. The dark decay of the potential change is presumably determined by passive ion fluxes driven by the generated electric field [7, 8]. Evidence for this assumption was obtained mainly from the decay kinetics of the flash-induced spectral changes at 515 nm, which were observed to be accelerated upon additions of valinomycin or gramicidin [8–11]. Electrical potential measurements on isolated chloroplasts in which the membrane permeability as well as the internal ionic composition can be altered are desirable for a closer identification of the passive transport processes at the membrane. So far

* On leave of absence from Biophysics Department, Biological Faculty, Moscow State University, Moscow, U.S.S.R.

there are no experimental indications on the existence of a significant electrical potential difference across the thylakoid membrane in the dark steady state (c.f. refs 10, 2, 4). Therefore, it seems reasonable to assume that the concentrations of the main ions in the chloroplast stroma phase and the thylakoid interior in the dark are about equal. The net flux ϕ_i of an ion species i after the generation of an electrical potential difference V across the thylakoid membrane under constant field assumptions [12] and equal ion concentrations at both sides of the membrane is given by the flux equation (c.f. refs 6, 7):

$$\phi_i = c_i P_i V (F/RT) \quad (1)$$

where c_i is the concentration (in both phases) and P_i the membrane permeability coefficient of the ion i. F, R and T are physical constants with RT/F is 25 mV at 20 °C. According to this equation the efflux of cation i caused by the (positive) electric potential, and hence the rate of potential decay after a flash, should be accelerated concomitantly with an increase in the permeability coefficient and with an increase in the intrathylakoid concentration of the particular cation.

Eqn. 1 is only valid under conditions at which the membrane conductance is independent on the membrane potential. For potential (-changes) of the order of 50 mV this condition seems to be fulfilled [3], according to estimated electrical characteristics of the chloroplasts membranes. Moreover, extensive analyses of the kinetics of the electrochroic absorption changes in broken chloroplasts under a variety of conditions have indicated that the thylakoid membrane behaves linearly with respect to potential and current in single turn-over flashes [11].

In the present work the dark decay of the chloroplast potential change was studied as a function of the ionic composition of the medium in the absence and presence of valinomycin and A23187, which alter the permeability for K^+ [13], and facilitate a neutral exchange of divalent cations against protons [14], respectively. Chloroplasts have been incubated in media of various ionic content in order to obtain different levels of K- and Mg-concentration inside the granum stacks. Our results are interpreted as evidence that the relatively fast potential decay after short flashes reflects the discharge of the membrane capacity, the rate of which is mainly determined by the passive diffusion of K^+ and Mg^{2+} across the membrane. The slow potential decay after prolonged illumination is discussed to reflect the redistribution of ions across the thylakoid membrane between the intrathylakoid space and the stroma phase.

MATERIAL AND METHODS

Measurements were performed on chloroplasts of *Peperomia metallica* isolated and incubated in a medium containing, in addition to a variable concentration of KCl, 0.25 M sucrose, 25 g/l ficoll, 100 mg/l bovine serum albumin and 0.01 M tricine buffer adjusted to pH 7.8 by Tris. The concentration of KCl was varied between 10^{-3} and 10^{-1} M. In some experiments the KCl concentration was 10^{-3} M and $MgCl_2$ was added to the medium at concentrations varying between 10^{-4} and 5.10^{-2} M. Measurements were conducted at 20 °C after incubation of chloroplasts for about 30 min. Isolation procedure and measuring methods were essentially the same as described previously [2, 4]. Potentials were measured by means of micro-capillary

glass electrodes inserted into a single chloroplast, sucked onto a fire-polished tip of a suction micropipette. In order to avoid the possible effect of K^+-leakage from the conventional KCl-filled micro-electrode tips in the chloroplast interior, micro-electrodes were filled with 1 M choline chloride solution. The electrical resistance of these micro-electrodes was approximately 100 mohm and the tip potential about +20 mV with respect to the solution. The electrode signal was fed into a high impedance unity gain amplifier (pass band width 0.5 MHz). The rise time of the electrode response upon passing rectangular current pulses was set at a limit of 0.2 ms after compensation of the electrode capacity. According to criteria discussed elsewhere [2, 6, 7] the measurements reported are regarded as reflecting the response of thylakoids in a granum stack. Chloroplasts were illuminated by saturating white light, either from an electronic flash (half width 80 μs), or from an incandescent lamp.

RESULTS

The time course of the electric potential change after a single flash is shown in Fig. 1. At least three phases can be distinguished; a fast initial rise in potential is followed by a slow increase and finally by a decay of the potential towards the steady state dark level. The second slow phase of the increase in potential after the flash, which is completed in about 20 ms, is usually observed at low salt concentrations in the medium and is less obvious and routinely absent at high salt concentrations. The characteristics of this phase have not been studied in detail so far. Semi-logarithmic plots of the potential change versus time during the decay phase have shown that the potential decay after a flash can be described by a single exponential with time constant τ, where τ is determined from the relation $\ln(V(t+\tau)) = \ln(V(t))-1$ [3]. It has been assumed [3, 7] that this time constant is determined by the rate of discharge of the electric membrane capacity through the passive ion conductance channels

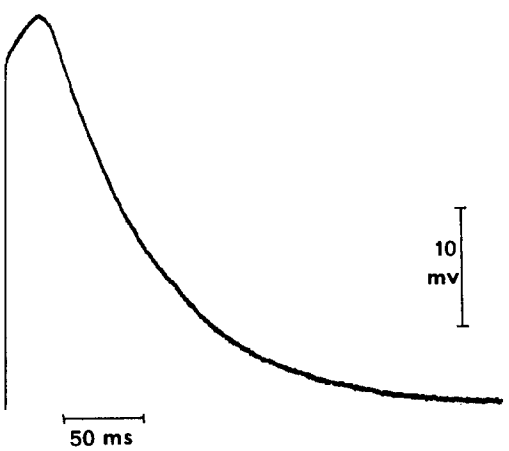

Fig. 1. Time course of the change in the membrane potential after one saturating white flash in a dark-adapted isolated chloroplast of *Peperomia metallica*. Concentration of KCl in the medium 1 mM; rise time of the flash less than 20 μs, half width approx. 80 μs. An upward movement of the trace means an increase in potential.

of the membrane. Accordingly $\tau = C_m/g$, where C_m is the membrane capacity and g the membrane conductance, equal to the sum of the partial ion conductances g_i of the ion species i. Under conditions at which the ion concentrations in the stroma and thylakoid inner phase are equal, the conductance of the membrane for the ion species is given by (e.g. ref. 7)

$$g_i = (F^2/RT)\, P_i\, c_i \tag{2}$$

The membrane conductance is assumed not to change appreciably during and after a short flash. Consequently we obtain:

$$\tau = (C_m/\Sigma P_i c_i)\,(RT/F^2) \tag{3}$$

If the membrane conductance is determined by the conductance of a single ion species, an inverse relation between τ and the internal concentration of this ion is expected. The condition of a predominant conductance for a single ion species is most likely achieved after treatment of chloroplasts with ionophores, but may also exist in untreated chloroplasts under certain physiological conditions.

Effect of K-concentration

In Table I the time constants τ of the dark decay of the potential after a flash are summarized for chloroplasts isolated and incubated in media with different content of potassium in the absence and presence of 0.2 μM valinomycin. The table shows that the time constant is much less sensitive to the outer K^+-concentration in the absence than in the presence of the ionophore. Addition of 0.2 μM valinomycin results in a significant acceleration of the dark decay, i.e. a decrease in τ, at all concentrations of potassium (Table I). Moreover, in the presence of valinomycin chloroplasts exhibited an apparent acceleration of the dark decay with increasing K^+-concentration in the medium. This effect is most likely associated with a change of potassium concentration inside the thylakoids and in the stroma phase due to the passive dark redistribution of K^+ between chloroplast compartments and outer medium under conditions at which the chloroplast membranes have an increased permeability for these ions. At the concentration used valinomycin was found not to affect the magnitude of the flash-induced electric response.

TABLE I

THE TIME CONSTANT OF THE POTENTIAL DECAY IN THE DARK AFTER A SATURATING LIGHT FLASH ESTIMATED IN ISOLATED CHLOROPLASTS OF *PEPEROMIA METALLICA* AT DIFFERENT CONCENTRATIONS OF KCl IN THE SUSPENDING MEDIUM IN THE PRESENCE AND ABSENCE OF VALINOMYCIN

The number between brackets represents the number of experiments, done with different chloroplasts.

[KCl] (mM)	Time constant τ of potential decay (ms)	
	Without additions	+0.2 μM valinomycin
1	88±22(15)	30±6(7)
10	91±32(18)	20±9(6)
50	84±24(6)	14±2(6)
100	78±32(10)	7±3(6)

TABLE II

THE TIME CONSTANT OF THE POTENTIAL DECAY IN THE DARK AFTER A SATURATING LIGHT FLASH ESTIMATED IN ISOLATED CHLOROPLASTS AT DIFFERENT CONCENTRATIONS OF $MgCl_2$ AND KCl IN THE SUSPENDING MEDIUM IN THE PRESENCE OF THE IONOPHORE A23187 (10 μM)

KCl (mM)	Time constant τ of potential decay (ms)		
$MgCl_2$(mM):	0.1	1	10
1	108±31(6)	77±11(5)	42±7(5)
100	–	–	40±23(7)

Effect of Mg-concentration

Ionophore A23187, added to a chloroplast incubation medium containing $MgCl_2$, is expected to cause changes in the internal concentration of Mg^{2+} due to its ionophoretic properties of exchanging di-valent cations for hydrogen ions in biomembranes (c.f. ref. 14). Thus, depending on the Mg-concentration in the outer medium, an increase or decrease of internal Mg-concentration and consequently in the Mg-conductance of the membrane is likely to occur. Changes in the proton concentration in the chloroplast compartments in the dark after the addition of the ionophore are expected to be relatively small, due to the high buffering capacity of the thylakoid and stroma space [15, 16]. For chloroplasts suspended in media containing 1 ml KCl and increasing concentrations of $MgCl_2$, the time constant of the potential decay after a flash was found to decrease progressively in the presence of 10 μM A23187 (Table II). In the absence of the ionophore the time constant was found not be altered

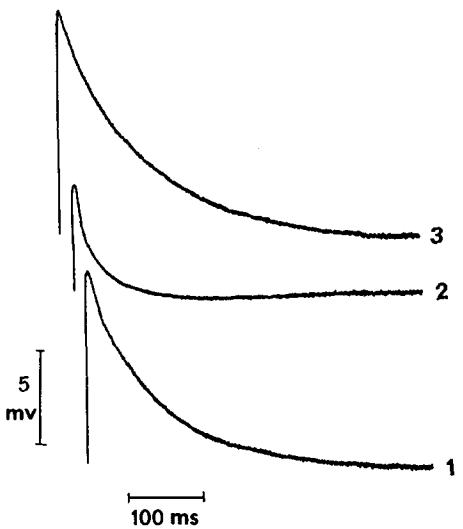

Fig. 2. Time courses of the change in the membrane potential in an isolated chloroplast induced by a short saturating flash, fired after a dark period of 3 min (1), and 100 ms (2) and 10 s (3), respectively, after a 8 s preillumination period with saturating white light. Concentration of KCl in the medium 1mM.

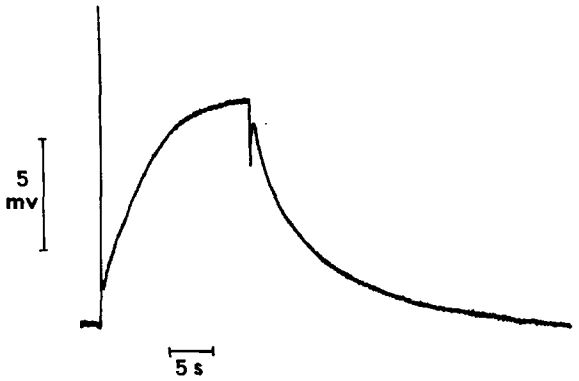

Fig. 3. Time course of the change in the membrane potential during and after prolonged illumination in an isolated chloroplast in the presence of 0.2 μM valinomycin. Concentration of KCl 10 mM.

significantly with increasing Mg-concentration. The ionophore was found not to affect the magnitude of the initial potential rise in the flash.

Effect of pre-illumination

In order to test whether an increase in the internal proton concentration is accompanied by a change in the membrane conductance, the decay kinetics of the photoelectric response were measured on dark-adapted and on pre-illuminated chloroplasts. It is known that a massive uptake of protons into the thylakoid inner space occurs during illumination [15]. As Fig. 2 shows, the dark decay of the flash-induced response is considerably accelerated in a chloroplast which has been pre-illuminated. The time constant of the decay becomes occasionally 2 to 3 times shorter after an 8 to 10 s pre-illumination with respect to the one estimated in dark-adapted chloroplasts. The decrease in τ by pre-illumination was found to be completely reversible. After a 10 s dark period the photoelectric response occurred with the initial slow kinetics.

Potential decay after short and long light exposures

The dark kinetics of the potential changes after short (single flash) and long light exposures (10 s illumination) have been compared in the presence of valinomycin at low concentrations of K in the medium. In the presence of the ionophore the decay after a flash is completed within 10 to 50 ms (Table I). However as Fig. 3 shows, the decay after a 10 s light exposure takes several seconds. Similar results were found in the presence of A23187 and low concentration of $MgCl_2$. These data indicate that the decay after short and long light exposures is controlled by different processes. The initial potential transient upon darkening which is suggested to be caused by the switching-off of the electrogenic charging mechanism [7], has not been studied in its very details and needs further analysis. We presume that the slow dark kinetics after prolonged illumination reflect the redistribution of ions across the thylakoid membrane from a light steady state to the initial dark equilibrium state.

DISCUSSION

The present results show that the decay time of the potential change after a short light flash is affected by the addition of ionophores, by pre-illumination, as well as by variations in the external K^+- and Mg^{2+}-concentrations in the presence of valinomycin and A23187, respectively.

The relative impermeability of the chloroplast envelope for K^+ and Mg^{2+}, as concluded by others [17, 18] would explain the fact that the rate of dark decay is almost insensitive to changes in the external ion concentration in the absence of ionophores (Table I). Nevertheless it is possible to assess whether or not the fluxes are sufficient to account for the experimentally observed decay kinetics. A major role of K^+-ions has been suggested for chloroplasts in situ, since K^+ is the most abundant cation in chloroplasts [6]. If it is assumed that the membrane conductance is exclusively determined by the K^+-fluxes, then, according to Eqn. (3), $\tau = (C_m/P_K c_K)\,(RT/F^2) = 6.5 \cdot 10^3/c_K{}^i\ ms$, with $C_m = 1\ \mu F/cm^2$, $P_K = 4 \cdot 10^{-8}$ cm/s (e.g. refs 6, 7) and $c_K{}^i$ in mM. Values of internal K-concentration, $c_K{}^i$, calculated from the data of Table I are shown in Table III. Qualitatively these results are in agreement with estimates on the K^+-content in spinach chloroplasts as determined by flame photometry [18]. Similar calculations were made for chloroplasts treated with valinomycin. According to Barber [19] the permeability coefficient of the thylakoid membrane towards K^+ increases approximately 10 times after the addition of 0.5 μM valinomycin. Consequently $\tau = 6.5 \cdot 10^2/c_k$ in the presence of this ionophore. Calculated values of c_K are given in the same table. From these approximations it would follow that at high external concentrations (50 to 100 mM) the inside K-concentration is about equal to the external concentration, whereas in low K-media the chloroplasts still contain a significant amount of K^+ in the presence of valinomycin. The latter result is in agreement with conclusions of Gimmler et al. [18], who found that intact chloroplasts retain K^+ in the presence of valinomycin at low external concentrations of potassium. The clear effect of Mg^{2+} on the decay rate in the presence of A23187 suggests that Mg^{2+} are contributing to the membrane conductance, especially under conditions at which the concentration ratio between Mg and K is increased. This conclusion is in line with the results of flux measurements in broken chloroplasts [20], which showed that in continuous light the ratio between Mg- and K-efflux is increased with an increase in the concentration ratio of these ions. For chloroplasts in vivo it has been approximated that this flux ratio is about 0.24 [21].

TABLE III

CALCULATED VALUES OF INTERNAL K^+-CONCENTRATION OF CHLOROPLASTS ISOLATED AND SUSPENDED IN MEDIA WITH DIFFERENT CONCENTRATIONS OF KCl IN THE PRESENCE AND ABSENCE OF 0.2 μM VALINOMYCIN

KCl (mM)	Calculated potassium concentration (mM)	
	Without addition	+0.2 μM valinomycin
1	74±18	22± 4
10	72±24	33±15
50	78±19	47± 7
100	84±34	94±45

The contribution of H^+ to the membrane conductance is expected to be low in the dark [7] due to the low concentration of protons in the thylakoid and stroma phases. However it has been discussed [7], that the proton conductance in the absence of ionophores can become significant with respect to the other conductances due to the rise in internal proton concentration in prolonged illumination. The significant acceleration of the dark decay in pre-illuminated chloroplasts (Fig. 2) qualitatively is consistent with an increased contribution of the proton conductance under these conditions. Similar conclusions were obtained from the kinetics of the 515 nm change in short and prolonged flashes (c.f. ref. 22). The assumption on the constancy of the conductances of the other ions during illumination seems to be justified with respect to the relatively small changes in the internal concentration of these ions which occur in the absence of ionophores [7], but would need justification with respect to the possibility that the membrane permeability coefficients are altered in association with the acidification of the thylakoid interior.

The relatively slow potential changes measured during and after prolonged illumination under conditions of low external K-concentration in the presence of valinomycin (Fig. 3) are suggested to be a reflection of the creation and decay, respectively, of a K-gradient across the thylakoid membrane. As will be shown in a subsequent paper, it was consistently found that the increase in potential in prolonged illumination was much smaller at higher potassium concentrations, a situation comparable to what is occurring in chloroplasts in situ in the presence (e.g. ref. 6) as well as in the absence of valinomycin. The decay of the diffusion potential in the dark will be determined by the rate of passive re-equilibration of K-and H-concentrations between intra-chloroplast space and external medium.

REFERENCES

1 Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1971) Soviet Plant Physiology (Eng. transl.) 18, 204–210
2 Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1972) Nature 236, 175–176
3 Bulychev, A. A., Andrianov, V. K., Kurella, G. A. and Litvin, F. F. (1973) Biophysics of membranes, Proceedings of the Symposium on molecular mechanisms of membrane permeability, Kaunas, pp. 104–113 (in Russian)
4 Vredenberg, W. J., Homann, P. H. and Tonk, W. J. M. (1973) Biochim. Biophys. Acta 314, 261–265
5 Vredenberg, W. J. and Tonk, W. J. M. (1974) FEBS Lett. 42, 236–240
6 Vredenberg, W. J. and Tonk, W. J. M. (1975) Biochim. Biophys. Acta 387, 580–587
7 Vredenberg, W. J. (1975) The intact chloroplast (J. Barber, ed), Elsevier Scientific Publ. Co., Amsterdam, in the press
8 Junge, W. and Witt, H. T. (1968) Z. Naturforsch. 23b, 244–254
9 Junge, W. and Schmid, R. (1971) J. Membr. Biol. 4, 179–192
10 Witt, H. T. (1971) Quart. Rev. Biophys. 4, 365–477
11 Schmid, R. and Junge, W. (1975) Biochim. Biophys. Acta 394, 76–92
12 Goldman D. E. (1943) J. Gen. Physiol. 27, 37–60
13 Lev, A. A. and Buzhinsky, E. P. (1967) Cytology 9, 102–106
14 Reed, P. D. and Lardy, H. A. (1972) J. Biol. Chem. 247, 6970–6977
15 Heldt, H. W., Werdan, K., Milovancev, M. and Geller, G. (1973) Biochim. Biophys. Acta 314, 224–241
16 Walz, D., Goldstein, L. and Avron, M. (1974) Eur. J. Biochem. 47, 403–407
17 Pflüger, R. (1973) Z. Naturforsch. 28c, 779

18 Gimmler, H., Schäfer, G. and Heber, U. (1974) Proc. Third Int. Congr. Photosynthesis Res. (M. Avron, ed.), pp. 1381–1392, Vol. II, Elsevier, Amsterdam
19 Barber, J. (1973) Ion transport in plants (W. P. Anderson, ed.), pp. 191–204, Academic Press, London
20 Hind, G., Nakatani, H. I. and Izawa, S. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1484–1488
21 Nobel, P. S. (1969) Biochim. Biophys. Acta 172, 134–143
22 Boeck, M. and Witt, H. T. (1972) Proc. IInd Int. Congr. Photosynthesis Res. (G. Forti, M. Avron and A. Melandri, eds), pp. 903–911, Junk, The Hague