Journal of Bioenergetics and Biomembranes, Vol. 15, No. 6, 1983

On the Correlation Between the Activity of ATP-hydrolase and the Kinetics of the Flash-Induced P515 Electrochromic Bandshift in Spinach Chloroplasts

R. L. A. Peters,[1] M. Bossen,[1] O. van Kooten,[1] and W. J. Vredenberg[1]

Received April 5, 1983; revised June 3, 1983

Abstract

The P515 absorbance change upon single-turnover light flashes has been studied in intact leaves and isolated chloroplasts from spinach. A comparative study of the effects of preillumination on the kinetics of the P515 response and on the activity of the chloroplast ATPase has been made. The slow component (reaction 2) in the flash-induced P515 response normally present in dark-adapted chloroplasts is reduced or even absent under conditions in which the ATPase is activated by preillumination. This suppression of reaction 2 appeared to be temporary in leaves and chloroplasts; its duration in chloroplasts is shown to be dependent on the amount of ATP present. Tentoxin inhibits the preillumination-dependent suppression of reaction 2.

Key Words: P515; electrochromic bandshift; ATPase; ATP hydrolysis; spinach chloroplasts.

Introduction

The P515 electrochromic bandshift (for a review see Witt, 1979) following a single saturating light flash in dark-adapted and well-preserved chloroplasts shows multiphasic rise and decay kinetics (Horváth *et al.*, 1978; Schapendonk *et al.*, 1979). These kinetics cannot be explained in terms of generation and decay of a transmembrane electric field. Such a field would be expected to follow a single exponential dark decay with a rate constant determined by the membrane capacitance and the membrane conductance, provided these are field-independent (Bulychev and Vredenberg, 1976). The decay rate, however, seems to be dependent on the membrane integrity rather than on the membrane conductance (Schapendonk *et al.*, 1979). According to Schapen-

[1]Laboratory of Plant Physiological Research, Agricultural University, Wageningen, Gen. Foulkesweg 72, 6703 BW Wageningen, The Netherlands.

0145-479X/83/1200-0335$03.00/0

donk (Schapendonk *et al.*, 1979) the decay of the P515 absorbance change in intact chloroplasts is almost exclusively determined by the decay of a special type of electrochromic change (called reaction 2) that is not directly linked to a transmembrane potential but rather to an intramembranal local field in the vicinity of the P515 complex. It has been suggested (Vredenberg and Schapendonk, 1981, Schuurmans *et al.*, 1981a) that this local, intramembranal field may involve changes in surface charges on the CF1 part of the ATPase complex. In spite of the fact that the origin of the slow component (reaction 2) is still unclear, it must be stated that the occurrence of multiphasic rise and decay kinetics of the P515 bandshift obviously depends on the state of activation of the chloroplast ATPase (Joliot and Delosme, 1974; Girault and Galmiche, 1978; Junge *et al.*, 1970; Morita *et al.*, 1981). Recently, a P515 electrochromic shift has been shown to occur in the dark following the addition of ATP to a suspension of light-activated chloroplasts (Schreiber and Rienits, 1982; Schuurmans *et al.*, 1981b). Moreover, there seems to be a strict complementarity of the ATP-induced and the light-induced P515 shift in chloroplasts with respect to the slow phase (Schreiber and Rienits, 1982).

In this communication we present results that support the idea that the activity of the chloroplast ATPase is reflected in an alteration in the kinetics of the P515 bandshift. Activation of the chloroplast ATPase by short periods of illumination in intact leaves or intact chloroplasts results in a complete suppression of reaction 2 from the P515 shift induced by a subsequent light flash. The same results are obtained with broken chloroplasts after short periods of illumination in the presence of DTE and ATP. In both cases, a single saturating light flash following a period of preillumination results in an electrochromic shift characterised by a fast rise and a subsequent single exponential dark decay with a half-time of approximately 75 msec. The suppression of reaction 2 caused by preillumination appears to be reversible. The duration of the suppression is dependent on the amount of ATP present in the sample. Broken chloroplasts in the absence of DTE and ATP appear to be completely insensitive to preillumination. Inhibition of the ATPase by the addition of 2.5 μmol/liter tentoxin completely prevents the suppression of reaction 2 by preillumination in intact chloroplasts as well as in broken chloroplasts in the presence of ATP and DTE.

Material and Methods

Freshly grown spinach (*Spinacia Oleracea*) was used for all experiments. The plants were grown under high-pressure mercury lamps (Philips HPLR 400) at an intensity of approximately 80 W/m^2 with a light period of 8 hours per day. Provisions were made to keep the temperature at the leaf and soil

surface at 18–20°C. The relative humidity of the atmosphere was minimal (70%). Intact chloroplasts were routinely isolated according to a modified method of Walker (Cockburn and Walker, 1968) as described by Schapendonk (1980). This procedure routinely yielded preparations with 90–95% intact chloroplasts as determined by ferricyanide reduction (Heber and Santarius, 1970). Broken chloroplasts were obtained by a 60-sec osmotic shock on ice, in a medium containing 5 mmol/liter $MgCl_2$ and 10 mmol/liter Tricine adjusted to pH 7.8 with NaOH, and subsequent addition of double-strength assay medium which contained 660 mmol/liter sorbitol, 2 mmol/liter $MgCl_2$, 2 mmol/liter $MnCl_2$, 4 mmol/liter EDTA, and 100 mmol/liter Hepes, adjusted to pH 7.8 with NaOH. The Hill reaction rate in freshly broken and uncoupled chloroplasts was determined to be minimal 200 μmol O_2/mg chl./h. The ratio of electron-transport activities of NH_4Cl-uncoupled and coupled chloroplasts was determined to be minimal 5. Chlorophyll content was determined spectrophotometrically (Bruinsma, 1961). Absorbance changes at 518 nm induced by single-turnover flashes in either intact leaves or isolated chloroplasts were measured in a modified Aminco chance absorption difference spectrophotometer as described by Schapendonk (1980). Leaf and chloroplast measurements were performed at 3°C. Samples were dark-adapted at 3°C for at least 1 h before measuring. Saturating single-turnover actinic flashes with a half-width of 8 μsec were transmitted to the sample via light guides. The output voltage of the photomultiplier, linear with the intensity of the 518-nm measuring beam transmitted by the sample, was fed into a differential amplifier (Tektronix 5A22). After compensation, the amplified output signal was sampled and averaged on a Minc-11 minicomputer (Digital Equipment Co.) with a limiting time constant of 0.3 msec. The photomultiplier was shielded from actinic light by an appropriate filter combination. Preillumination with red light came from a 250-W tungsten lamp and was transmitted to the sample via light guides. ATP hydrolysis in chloroplasts was determined with the bioluminescent firefly luciferin–luciferase assay method. Chloroplasts were illuminated with light of wavelengths above 665 nm. Immediately after cessation of the illumination, 200 μl luciferin–luciferase (LKB Wallac) was added to the sample. The light emission associated with the ATP-hydrolyzing luciferin–luciferase reaction was determined by a photomultiplier. Calibration was carried out by titrating known amounts of ATP. This assay method was linear up to 10^{-6} mol/liter ATP with a lower detection limit of 10^{-11} mol/liter ATP.

Results and Interpretation

A representative example of the time course of the absorbance change at 518 nm (ΔA518) upon a single-turnover light flash in dark-adapted intact

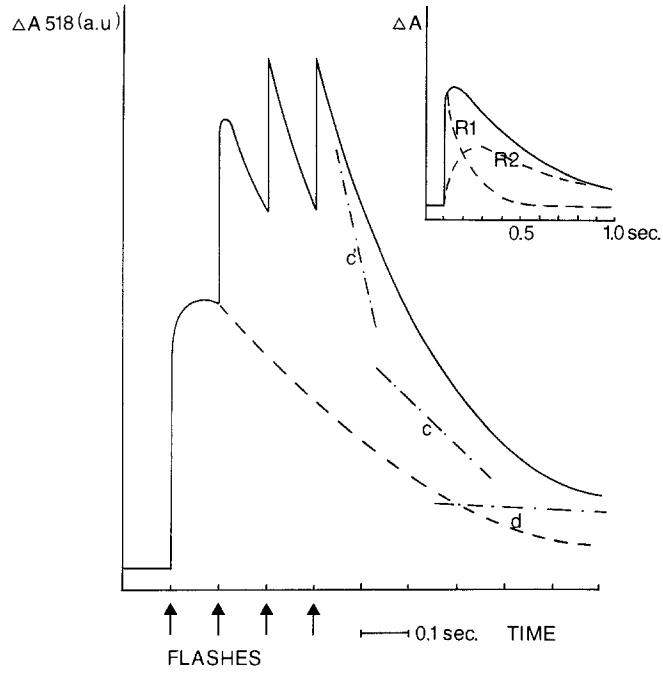

Fig. 1. Absorbance changes at 518 nm in dark-adapted intact chloroplasts induced by a single flash (dashed curve) and by a series of four flashes fired at 100-msec time intervals (solid curve). Average of 10 series of flashes fired at a rate of 0.10 Hz. The decay kinetics of the third and subsequent flashes are identical and consist of three single exponentials, representative for phases c', c, and d (dot-dash curve). The insert shows the deconvolution of the overall signal induced by a single flash into reaction 1 and reaction 2. The maximal ΔA518 ($\Delta I/I$) reached in a single saturating light flash was approximately 8×10^{-3}.

chloroplasts is illustrated in Fig. 1 (dashed curve). From this figure it can be seen that ΔA518 occurs with complex multiphasic rise and decay kinetics. Analysis of the semilogarithmic plots of these changes (Schapendonk, *et al.*, 1979) has revealed five different phases in the kinetics. An initial fast absorbance increase, called phase a, completed within a time shorter than the resolution time of the measuring system (0.3 msec), is followed by a relatively slow increase in absorbance in the first 20–70 msec. According to this analysis, the subsequent decrease in absorbance appears to be composed of three different phases. A major slow phase c with a half-life of about 400 msec is preceded by a fast phase c' with a half-life of about 75 msec and followed by a small phase d decaying with a half-life of about 1500 msec. These data have been interpreted as an indication that the phase c' decay is associated with the initial fast rise (Schapendonk, 1980). This interpretation is corroborated by

data of the absorbance response in a series of four consecutive flashes, fired at time intervals of 100 msec as shown in Fig. 1. The decay kinetics of the response after the third and subsequent flashes were found to be identical, and could be characterized by three single exponential decay phases equal to phases c', c, and d. According to Schapendonk and Vredenberg (1979) phases a and c' have been ascribed to a reaction called reaction 1. Thus, with the determination of phase a (1st flash) and phase c' (4th flash), the response of reaction 1 could be determined (inset, Fig. 1). The response obtained by subtracting reaction 1 from the overall P515 response upon a single flash has been attributed to that of reaction 2 (inset, Fig. 1). The rise kinetics of reaction 2 show a slow absorbance increase within 150 msec. The decay of reaction 2 after the flash is biphasic, with rate constants determined by the characteristic relaxation times of phases c and d. The addition of 5 μmol/liter gramicidin to a sample of intact chloroplasts was found to abolish reaction 2 and, as expected, to enhance the decay rate of reaction 1 (phase c'), as can be seen from Fig. 2. Surprisingly, the addition of gramicidin did not affect phase d (inset, Fig. 2). This result, in combination with the observation (Schapen-

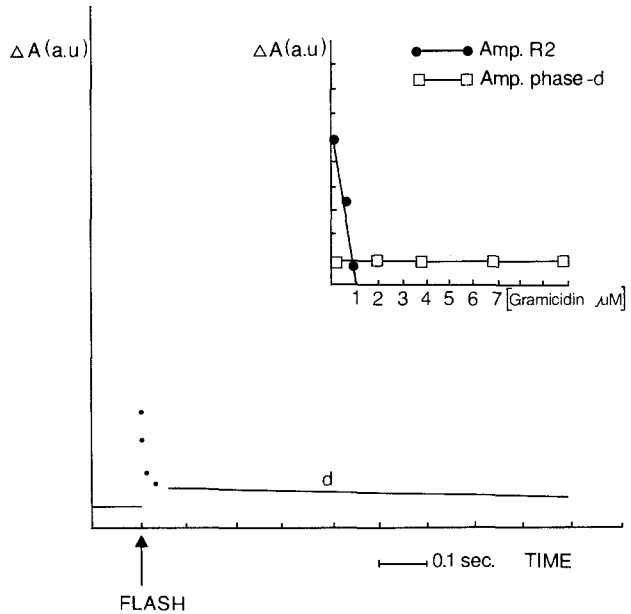

Fig. 2. Absorbance changes at 518 nm in dark-adapted intact chloroplasts induced by a single flash in the presence of 3 μmol/liter gramicidin. The magnitude of the initial rise is virtual because of the rapidity of the decay and the 5-msec time resolution at which it was measured. Average of 10 single flashes fired at a rate of 0.10 Hz. The insert shows the effect of gramicidin on the amplitudes of reaction 2 and phase d.

donk *et al.*, 1979) regarding the dissimilarity of the spectrum of phase d compared to the spectrum of P515, has strengthened the conclusion that phase d is not due to an electrochromic response of the P515 pigment complex. Phase d may be due to a change in light scattering. The contribution of phase d to the overall signal of P515 is about 8% in intact chloroplasts and intact leaves, and is negligibly small in broken chloroplasts. Therefore, reaction 2 in intact chloroplasts and intact leaves needs a small correction for the virtual absorbance change at 518 nm associated with phase d. After this correction is made, the kinetics of the P515 response in intact leaves, intact chloroplasts, and broken chloroplasts are found to be similar. Figure 3 shows the effect of preillumination on the amplitude of the reaction 2 component in the P515 response, induced by a light flash given 5 sec after an illumination period in intact leaves, intact chloroplasts, and broken chloroplasts. It shows that the

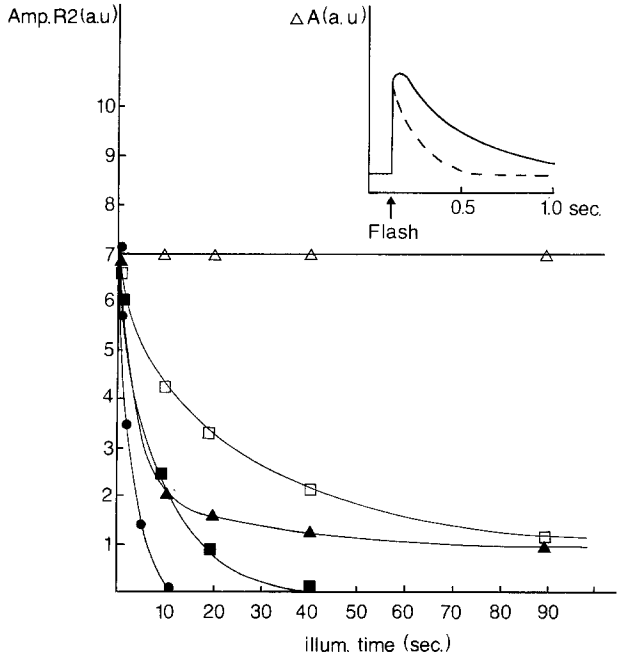

Fig. 3. The effect of preillumination on the amplitude of reaction 2 of the flash-induced P515 response in intact leaves (●), intact chloroplasts (■), and broken chloroplasts in the absence (△) and presence of 300 μmol/liter ATP (□). The effect of preillumination in broken chloroplasts in the presence of 300 μmol/liter ATP and 1 mmol/liter DTE is also shown (▲). The inset shows the P515 response induced by a single light flash in intact chloroplasts before (solid curve) and after (dashed curve) a preillumination period of 40 sec. 1 a.u. corresponds to approximately 8×10^{-4} ($\Delta I/I$).

illumination of intact leaves for 10 sec results in the complete suppression of reaction 2 from the flash-induced P515 response. The light flash causes only a reaction 1 type electrochromic shift characterized by a fast rise and a single exponential dark decay (inset, Fig. 3). The remaining reaction 1 type electrochromic shift is insensitive to more prolonged periods of illumination (data not shown). An identical effect of preillumination is found for a suspension of intact chloroplasts, although the duration of the light period required for a complete suppression of reaction 2 appears to be longer (40 sec). The suppression of reaction 2 from the overall signal appears to be temporary and can be completely overcome by a period of dark adaption preceding the light flash. From Fig. 4 it can be seen that after a dark period of 20 min following a period of 60 sec illumination, complete recovery of reaction 2 in a subsequent light flash occurred in leaves and intact chloroplasts. Surprisingly, broken chloroplasts in the absence of DTE and ATP are insensitive to preillumination (see Fig. 3). In these samples, the P515 response induced by a single saturating light flash cannot be altered significantly by a period of preillumination, neither with respect to reaction 1 nor with respect to reaction

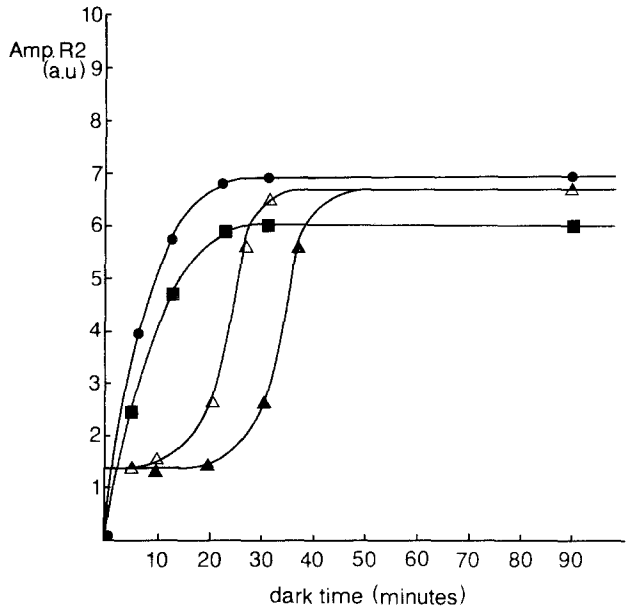

Fig. 4. The effect of dark adaptation after a 60-sec preillumination on the recovery of the amplitude of reaction 2 in intact leaves (●), intact chloroplasts (■), and broken chloroplasts in the presence of 1 mmol/liter DTE, with 100 μmol/liter ATP (△) or with 300 μmol/liter ATP (▲), respectively. 1 a.u. corresponds to approximately 8×10^{-4} ($\Delta I/I$).

2. The addition of ATP and DTE to these samples preceding the period of illumination, however, resulted in a significant (70%) suppression of reaction 2 in a subsequent light flash. In accordance with the results mentioned earlier for intact leaves and intact chloroplasts, this suppression of reaction 2 from the overall signal is reversible. The duration of the period of dark adaption following the period of illumination and required for a full recovery of reaction 2 in a subsequent light flash appears to be dependent on the amount of ATP present in the sample (Fig. 4). As can be seen from Table I, illumination of intact chloroplasts, as well as broken chloroplasts in the presence of ATP and DTE, results in an activation of the latent ATPase, whereas the ATPase in broken chloroplasts in the absence of DTE and ATP cannot be activated. These results suggest evidence for a close connection between the kinetics of the P515 shift and the state of activation of the chloroplast ATPase. Under conditions in which the chloroplast ATPase is activated and ATP hydrolysis can be measured, the slow component of the P515 shift induced by a single light flash is largely if not completely suppressed. This suppression is demonstrated to be reversible, and the duration of the suppression period

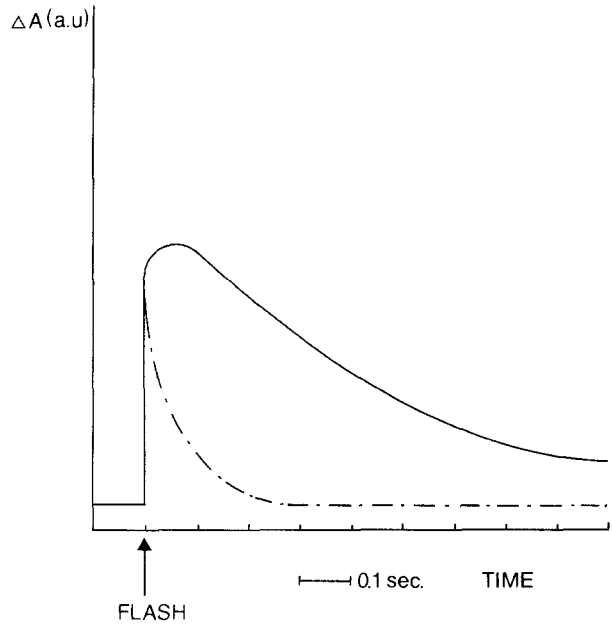

Fig. 5. The effect of a preillumination period of 120 sec on the kinetics of the flash-induced P515 response in intact chloroplasts in the presence (solid curve) and absence (dot-dash curve) of 2.5 μmol/liter tentoxin. The maximal ΔA518 ($\Delta I/I$) reached in a single saturating light flash was approximately 8×10^{-3}.

appears to be dependent on the amount of ATP present in the sample. Figure 5 shows the effect of preillumination on the kinetics of the P515 response in intact chloroplasts in the presence and absence of 2.5 μmol/liter tentoxin. In the presence of tentoxin, preillumination of the sample has no effect on the kinetics of the P515 response induced by a subsequent light flash. Tentoxin is known to be a strong inhibitor of the CF1 part of the ATPase (Arntzen, 1972). Accordingly no ATP hydrolysis was measured in the samples in the presence of tentoxin (see Table I).

Discussion

According to Petrack (Petrack and Lipmann, 1961) illumination of chloroplasts with strong light in the presence of thiol reagents results in the activation of the latent ATPase. In our experiments, we demonstrate a partial need for artificial thiol reagents in suspensions of chloroplasts broken in the absence of Mg^{2+}. From Table I it can be seen that the chloroplast ATPase in intact chloroplasts can be fully activated by illumination alone. In accordance with Mills and McKinney (Mills *et al.*, 1980; McKinney *et al.*, 1978) we suggest that activation of the chloroplast ATPase in intact chloroplasts is modulated by the physiological dithiol protein thioredoxin which is reduced by ferredoxin via Fd-thioredoxin reductase in the light. Recently it has been suggested (Shahak, 1982) that the thiol modulator of the ATPase is loosely bound to the membrane in a Mg^{2+}-dependent mode. In accordance with this we find that the chloroplast ATPase in chloroplasts, broken in the presence of 4 mmol/liter $MgCl_2$, can be activated by illumination in the absence of DTE. In our experiments excess amounts of ATP were present in the sample during the illumination period (see Table I). We suggest that under these conditions activation of the chloroplast ATPase still occurs by modulation of the CF1 part of the ATPase by the physiological thiol modulator. The efficiency of the system is somewhat less than in intact chloroplasts. This loss might be caused by a partial removal of intermediary compounds from the thylakoid membrane during the breaking procedure. Apparently the lower efficiency is partly compensated by the relatively high concentration of ATP. The addition of DTE to a suspension of chloroplasts, broken in the presence of 4 mmol/liter $MgCl_2$, did not further increase the rate of ATP hydrolysis (Table I). On the contrary, pronounced effects of the addition of DTE on the capacity to hydrolyze added amounts of ATP were found in chloroplasts, broken in the absence of $MgCl_2$. The ATPase present in these chloroplasts, which could not be activated by illumination alone, became activated by illumination in the presence of 1 mmol/liter DTE (data not shown).

From Figs. 3 and 4 and Table I, it can be seen that activation of the

Table I. The Rate of ATP Hydrolysis (mol ATP/mg chl./min) in Dark-Adapted and Light-Activated Intact and Broken Chloroplasts[a]

		After 60-sec illumination in the presence of					
Chloroplast	Dark-adapted (no addition)	No addition	100 μmol/liter ATP	1 mmol/liter DTE	1 mmol/liter DTE + 2.5 μmol/liter tentoxin	100 μmol/liter ATP + 1 mmol/liter DTE	100 μmol/liter ATP + 1 mmol/liter DTE + 2.5 μmol/liter tentoxin
Intact	n.h.d.[b]	7×10^{-8}		6×10^{-8}	n.h.d.		
Broken	n.h.d.	n.h.d.	4×10^{-8}			4×10^{-8}	n.h.d.

[a]Light-activation was performed under conditions mentioned in the table. Hydrolysis was measured in the presence of 100 μmol/liter ATP which, if not present during preillumination, was added before measurement. The rate of hydrolysis in intact chloroplasts refers to the rate measured after breaking the chloroplasts immediately after the pretreatment.

[b]No hydrolysis detectable, i.e., less than 10^{-11} mol ATP/mg chl./min.

chloroplast ATPase coincides with a temporary suppression of the slow component (reaction 2) of the P515 shift induced by a light flash. Under conditions in which the chloroplast ATPase is activated and ATP hydrolysis takes place, no reaction 2 can be measured, neither in intact nor in broken chloroplasts. This is in conformation with results reported by Schreiber and Rienits (1982). Moreover, from Fig. 4 it can be seen that the duration of the suppression of reaction 2 after activation of the ATPase depends on the amount of ATP present in the sample. Further evidence that activation of the ATPase is involved in this phenomenon stems from the fact (see Fig. 5) that an inhibition of the ATPase by tentoxin results in prevention of the suppression of reaction 2. This seems true for both intact and broken chloroplasts. It was found that in the presence of tentoxin no ATP hydrolysis could be induced (Table I). Ample evidence has been provided that upon ATP hydrolysis reverse coupling reactions are induced (for a review, see Shahak and Avron, 1980). Reverse coupling has been demonstrated to be accompanied by the generation of a membrane potential, as suggested by an electrochromic response of P515 (Schreiber and Rienits, 1982) and by the response of the extrinsic-field-indicating probe oxonol VI (Avron *et al.*, 1982; Galmiche and Girault, 1980). There seems to be a strict complementarity of the ATP-induced and light-induced absorbance changes around 515 nm with respect to reaction 2 (Schreiber and Rienits, 1982). These results, in combination with the results described in this communication, give strong support for the proposal (Schapendonk *et al.* 1979) that the P515 bandshift is composed of two separate reactions called reaction 1 and reaction 2. In this interpretation reaction 1 is associated with a light-induced charge separation across the thylakoid membrane and a consequent single exponential dark decay of the created trans-membrane electric field, presumably by ion-charge diffusion. This reaction 1 type electrochromic shift is independent of a preestablished energetic state of the membrane created by either a preceding light flash or a period of ATP hydrolysis. On the contrary, the reaction 2 type electrochromic shift proves to be extremely sensitive toward a preestablished energetic state of the membrane. As shown in Fig. 1, reaction 2, i.e., the second slow rise, is largely absent in the P515 response of a single flash following two preceding flashes separated in time for 100 msec. Induction of ATP hydrolysis in the dark also causes a complete suppression of reaction 2 in a light flash during a period dependent on the amount of ATP present in the sample (Fig. 4). In accordance with Schapendonk and Vredenberg (Schapendonk *et al.*, 1979) and with Schreiber and Rienits (1982), we suggest that reaction 2 finds its origin in intramembranal local fields in the vicinity of the P515 pigment complex. These fields obviously can be created either by light-driven electron transport or by ATP hydrolysis. The energetic requirement to generate these fields apparently is low, because of the small number of single saturating light

flashes required to saturate reaction 2, and the long periods at which it can be sustained by an activated ATPase. A tentative model describing the origin of reaction 2 in terms of such local intramembranous fields has recently been published (Westerhoff *et al.*, 1983).

Acknowledgments

This research was partly supported by the Netherlands Foundation for Chemical Research (Stichting Scheikundig Onderzoek in Nederland), financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). The authors would like to thank Dr. J. J. S. van Rensen and Dr. J. F. H. Snel for fruitful discussions.

References

Arntzen, C. J. (1972). *Biochim. Biophys. Acta* **283,** 539–542.
Avron, M., Admon, A., Vinkler, C., and Korenstein, R. (1982). In Photosynthese, Deutsche Forschungsgemeinschaft, Würzburg, pp. 93–106.
Bruinsma, J. (1961). *Biochim. Biophys. Acta* **52,** 576–578.
Bulychev, A. A., and Vredenberg, W. J. (1976). *Biochim. Biophys. Acta* **449,** 48–58.
Cockburn, W., and Walker, D. A. (1968). *Plant Physiol.* **43,** 1415–1418.
Galmiche, J. N., and Girault, G. (1980). *FEBS Lett.* **118,** 72–76.
Girault, G., and Galmiche, J. N. (1978). *Biochim. Biophys. Acta* **502,** 430–444.
Heber, U., and Santarius, K. A. (1970). *Z. Naturforsch. Teil B* **25,** 718–728.
Horváth, G., Droppa, M., Mústardy, L., and Faludi-Daniel, A. (1978). *Planta* **141,** 239–244.
Joliot, P., and Delosme, R. (1974). *Biochim. Biophys. Acta* **357,** 267–284.
Junge, W., Rumberg, B., and Schröder, H. (1970). *Eur. J. Biochem.* **14,** 576–581.
McKinney, D. W., Buchanan, B. B., and Wolosiuk, R. A. (1978). *Phytochemistry* **17,** 794–795.
Mills, J. D., Mitchell, P., and Schürmann, P. (1980). *FEBS Lett.* **112,** 173–177.
Morita, S., Itoh, S., and Nishimura, M. (1981). *Plant Cell Physiol.* **22,** 205–214.
Petrack, B., and Lipmann, F. (1961). In *Light and Life* (McElroy, W. D., and Glass, B., eds.), Johns Hopkins, Baltimore, pp. 621–630.
Schapendonk, A. H. C. M., Vredenberg, W. J., and Tonk, W. J. M. (1979). *FEBS Lett.* **100,** 325–330.
Schapendonk, A. H. C. M. (1980). Doctoral Thesis, Agricultural University Wageningen.
Schreiber, U., and Rienits, K. G. (1982). *Biochim. Biophys. Acta* **682,** 115–123.
Schuurmans, J. J., Peters, A. L. J., Leeuwerik, F. J., and Kraayenhof, R. (1981a). In *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F., Quagliariello, E., Siliprandi, N., and Slater, E. C., eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 359–370.
Schuurmans, J. J., Leeuwerik, F. J., Sin Oen, B., and Kraayenhof, R. (1981b). In *Proceedings of the 5th International Congress on Photosynthesis* (Akoyunoglou, G., ed.), Vol. 1, Balabon International Science Services, Philadelphia, pp. 543–552.
Shahak, Y., and Avron, M. (1980). *Methods Enzymol.* **69c,** 630–641.
Shahak, Y. (1982). *FEBS Lett.* **145,** 223–229.
Vredenberg, W. J., and Schapendonk, A. H. C. M. (1981). In *Proceedings of the 5th International Congress on Photosynthesis* (Akoyunoglou, G., ed.), Vol. 1, Balabon International Science Services, Philadelphia, pp. 489–498.
Westerhoff, H. V., Helgerson, S. L., Theg, S. M., van Kooten, O., Wikström, M. K. F., Skulachev, V. P., and Dancshazy, Z. (1983). *Acta Biol. Acad. Hung.*, in press.
Witt, H. T. (1979). *Biochim. Biophys. Acta* **505,** 355–427.