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COMPLEMENTARITY OF ATP-INDUCED AND LIGHT-INDUCED ABSORBANCE CHANGES AROUND 515 nm

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A comparative study of the light-induced and the ATP-induced changes of P-515 absorbance gave the following results: (1) Following light activation of the latent ATP-hydrolase, ATP can induce a $\Delta A(515)$ of about the same size as that observed either in continuous light or by a saturating light flash. The ATP-induced $\Delta A(515)$ is stable in the dark as long as ATP is hydrolysed. (2) Any preceding ATP-induced $\Delta A(515)$ reduces the size of a consequent light-induced $\Delta A(515)$, and vice versa. The total P-515 absorbance change which can be induced by ATP and light is constant; there is strict complementarity of ATP- and light-induced $\Delta A(515)$. (3) The suppression of the flash-induced $\Delta A(515)$ by a preceding ATP-induced $\Delta A(515)$ is accompanied by an about 15-fold acceleration of the overall dark-decay rate, which is not further accelerated by addition of 0.2 μM valinomycin. (4) Adopting the kinetic model of Schapendonk (Doctoral Thesis, Wageningen, 1980) it is concluded that the apparent acceleration of the overall dark-decay rate results from a specific elimination of the slowly decaying 'Reaction II' component. ATP hydrolysis is suggested to produce and to maintain the Reaction II-type electrochromic pigment shift in the dark. (5) The data offer an alternative explanation to the prevailing notion that increased proton conductance via the activated ATPase is the main cause for the apparent acceleration of the overall decay rate of the flash-induced $\Delta A(515)$ following preillumination or under 'phosphorylating conditions'. (6) On the basis of the presented data it is argued that the total number of available sites which can produce a Reaction II-type electrochromic pigment shift is strictly limited. Consequently, the notion of a localized ATP- or light-induced field is favored. The properties of this localized field would suggest a close link to energy-dependent changes at the coupling factor complex and to the electrogenic reactions coupled with cyclic photophosphorylation.

Introduction

The light-induced absorbance change around 515 nm is widely accepted as being the result of an electrochromic response of the P-515 pigment complex to an electric field generated by the primary light-induced charge separation in the thylakoid membrane (for a review, see Ref. 1). Following a saturating, short light flash, P-515 absorbance in well preserved chloroplasts displays a biphasic rise followed by a complex decay [2–4]. By preillumination the amplitude of $\Delta A(515)$ is decreased and the overall decay rate is substantially accelerated [2,5,6]. There is evidence from a number of laboratories that the preillumination effect on the kinetics of the P-515 absorbance change is related to the functioning of the thylakoid ATPase [2,6–9]. Recently, Morita et al. [9] demonstrated a close correlation between the activity of the ATP-hydrolase and the apparent $\Delta A(515)$ de-

Abbreviations: PS, photosystem; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Chl, chlorophyll.

cay rate. These authors concluded that the decay rate is mainly determined by the H^+ flux through the ATPase, in agreement with the conclusions by other investigators, who determined the dependence of the decay rate under phosphorylating or nonphosphorylating conditions [2,7,8]. On the other hand, according to an analysis by Schapendonk [10], there is some doubt as to whether protons contribute significantly to the membrane conductance, whether or not under phosphorylating conditions. According to Schapendonk et al. [4], the decay of the P-515 absorbance change in intact chloroplasts is almost exclusively determined by the decay of a special type of electrochromic change (called Reaction II) which is not directly linked to a transmembrane potential but rather to an intramembrane, local field in the vicinity of the P-515 pigment complex. Vredenberg and Schapendonk [11] as well as Schuurmans et al. [12] suggested that this local, intramembrane field may involve surface charges on the CF_1 part of the ATPase complex.

When chloroplasts are illuminated with strong light in the presence of thiol reagents, the latent ATPase is activated [13] and upon ATP hydrolysis reverse coupling reactions are induced (for a review, see Ref. 14). Reverse coupling has been demonstrated by monitoring reduction of the primary PS II acceptor Q [15–18], formation of a transthylakoidal proton gradient [16–18], stimulation of chlorophyll luminescence [18–20] and formation of a membrane potential, as suggested by the response of the extrinsic field-indicating probe oxonol VI [21,22].

Recently, we devised a measuring system with which it became possible to monitor ATP-induced absorbance changes following light activation of the latent ATP-hydrolase [23]. Upon ATP hydrolysis, a pronounced absorbance increase around 515 nm is induced, the difference spectrum of which is very similar to that induced by light. In the present study, we applied the same measuring system for a comparative study of ATP- and light-induced P-515 absorbance changes, monitored in the same chloroplast samples. The results suggest complementary between ATP-induced and light-induced P-515 absorbance changes, in particular, with respect to the Reaction II-component of the flash-induced change. This finding has consequences for the interpretation of the apparent acceleration of the $\Delta A(515)$ decay by preillumination in the presence of an active ATPase. The data support the notion of Reaction II being intimately linked to a local field in the vicinity of the ATPase [10–12].

Materials ands Methods

Intact chloroplasts were isolated from freshly harvested spinach leaves according to the method of Jensen and Bassham [24]. Intactness was routinely between 75 and 90%, as estimated by the ferricyanide method [25]. If not stated otherwise, 30-μl aliquots of intact chloroplasts (at about 1.5 mg Chl/ml in buffer B were suspended in 60 μl of buffer C (as buffer B but at pH 7.8) and illuminated for 1 min with intense white, heat-filtered light (300 W/m^2). The chloroplasts were then exposed for 30 s to hypotonic conditions (dilution by a factor 4 in 5 mM $MgCl_2$, 2.5 mM Hepes-KOH, pH 7.8) and finally resuspended in half-strength buffer C (pH 7.8; modified to give a final Mg^{2+} concentration of 7.5 mM). The final reaction mixture was about half isotonic strength, containing chlorophyll at about 60 μg/ml. Light activation, hypotonic treatment and measurement of absorbance changes were carried out at 10°C with continuous stirring in a specially designed cuvette, which was linked to a laboratory-built single-beam spectrophotometer by trifurcated fiber optics. The fiber optics cuvette assembly was essentially similar to that descibed previously for fluorescence measurements [20] except that the measuring-light fiber bundle was connected to the exit slit of a monochromator, and that the stirring was with a disc mirror mounted on a disc-shaped magnet. The depth of the cuvette (from the lower surface of the sealing perspex cone to the mirror surface) was 0.5 cm, resulting in an effective path length for the measuring light of 1 cm. Calibration of absorbance changes was by comparison with equivalent changes brought about by the addition of known volumes of water. According to the Lambert-Beer law, $\Delta A(H_2O) = Ax\Delta v/v$. Actinic light was applied either as continuous illumination with a fiber bundle connected to a tungsten-halogen lamp via an electronic shutter (Compur eletronic-m) or by single turnover light flashes

from a General Electrics FT 230 xenon flash tube. As estimated from the flash-induced change of chlorophyll fluorescence in the presence of DCMU and NH_2OH, the flash was about 80% saturating. Actinic light was filtered through 6 mm Schott RG 665 glass filters. The photomultiplier was protected by 8 mm Corning CS 4-96 glass filters. Signals were recorded on a digital oscilloscope (Nicolet Explorer III), linked to a Hewlett-Packard 9825 computer. Generally, the quality of the flash-induced absorbance changes was such that averaging of several signals was not required. For quantitative evaluation of the changes the signal-to-noise ratio was improved by a computer-assisted curve-smoothing routine.

Results and Interpretation

Published data on P-515 absorbance changes are generally presented as transient curves (mostly the average of a great number of repetitively measured single curves) without any indication of the absolute level of P-515 absorbance at the time a transient change is induced. With such an approach, the assumption is made that the electrical field reflected by P-515 absorbance will decay to zero after a few seconds of darkness. This assumption appears justified, as indeed the flash-induced $\Delta A(515)$ shows a rapid decay to the original baseline [1–12]. When applying longer periods of continuous illumination, however, it becomes more difficult to decide whether the signal returns to the original dark level, because of slow, nonspecific changes in apparent absorbance which overlap the actual P-515 absorbance changes. These nonspecific changes (including changing light scattering, sample settling, temperature equilibration, formation of minute gas bubbles, etc.) can be minimized by applying a continuously stirred, temperature-controlled, 'Ulbricht-sphere'-type absorbance cuvette which was recently developed in our laboratory [23]. With this type of cuvette assembly, even in the single-beam mode nonspecific absorbance changes are sufficiently low to detect light-induced and chemically induced P-515 absorbance changes in rapidly stirred samples.

Fig. 1 gives an example of the system performance, demonstrating the correlation between ATP-induced and light-induced P-515 absorbance

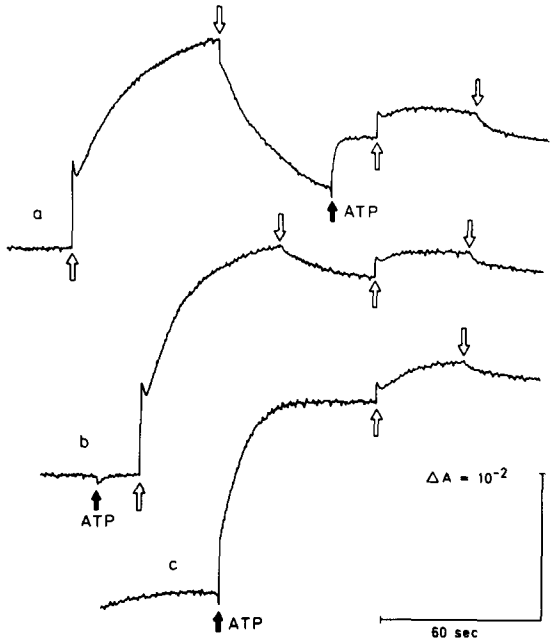


Fig. 1. Comparison of light-induced and ATP-induced absorbance changes at 515 nm. Samples were rapidly stirred and thermostatically maintained at 10°C. Light was switched on and off at the open arrows. ATP was injected as 1-μl aliquots of a 0.33 M solution (pH 7.8) resulting in a final concentration of 0.5 mM. Absorbance at 515 nm was 0.65. Curve a: light activated (see Materials and Methods), with a 3 min dark time to the first recorded transient. Curve b: dark adapted (for 3 h following chloroplast isolation). Curve c: light activated, with a 3 min dark time to ATP addition.

changes. Continuous illumination of a dark-adapted sample induces the well known biphasic rise of 515 nm absorbance (Fig. 1a). Upon darkening, absorbance decays with biphasic kinetics. When ATP is added to this preilluminated sample, 515 nm absorbance is increased again, although not to the same high level as reached during the previous illumination. A second illumination given following ATP addition results in only a small further increase of absorbance. Upon redarkening, only a minor decay of absorbance is observed. In Fig. 1b the sequence of illumination and ATP addition is reversed, i.e., ATP is added to a dark-adapted sample and then two consecutive light-dark cycles are given. Without preceding illumination, ATP addition induces practically no absorbance increase. Upon ensuing illumination, $\Delta A(515)$ rise kinetics very similar to those in Fig.

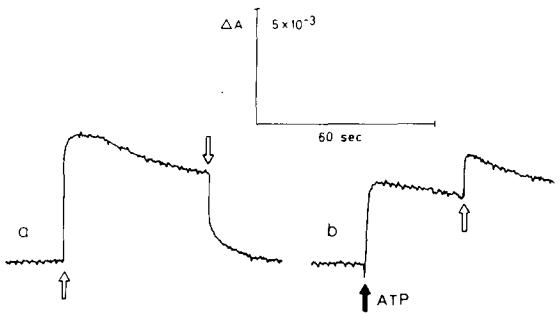


Fig. 2. Comparison of light-induced and ATP-induced P-515 absorbance changes in the presence of 5 mM NH_2OH. Samples were light activated and given a 3 min dark time before the recording of light- or ATP-induced changes. NH_2OH was added immediately following light activation. ATP was added to give a final concentration of 0.5 mM.

1a are observed, i.e., the mere presence of ATP in a nonactivated sample does not affect the light-induced response. However, there is a large effect on the decay kinetics upon darkening. The presence of ATP prevents most of the dark decay, and in a second illumination only a small light-induced $\Delta A(515)$ is observed. Apparently, during the first illumination period the latent ATP-hydrolase was activated and ATP hydrolysis maintained most of the $\Delta A(515)$ built up in the light. (It should be noted that these data suggest light activation of the ATPase in the absence of added cofactors, such as dithiothreitol or phenazine methosulfate, in hypotonically treated chloroplasts. Although dark adapted for at least 1 h, chloroplasts might be still in some way preactivated from the intense illumination the spinach leaves received shortly before chloroplast isolation. A reactivation of the ATPase in shocked chloroplasts in the absence of dithiothreitol was reported before by Bakker-Grunwald and Van Dam [26]). Fig. 1c shows the ATP-induced $\Delta A(515)$ in a sample in which the chloroplasts were preilluminated while still intact (see Materials and Methods), and sufficient dark time was given for the light-induced absorbance changes to decay. There is a biphasic ATP-induced rise to a high stationary 515 nm absorbance level. Illumination gives only a small further $\Delta A(515)$, which upon darkening only partially decays. When a sample is preactivated as in Fig. 1c and illuminated before ATP addition, the 515 nm induction kinetics are practically identical to those displayed in Fig. 1a and b (data not shown).

The results of Fig. 1 suggest that ATP hydrolysis produces a 515 nm absorbance change which is stable over an extended period of darkness, and which places a limitation on the maximal amplitude of an additional light-induced $\Delta A(515)$. Closer analysis of the data reveals that as far as the rapid components of the ATP-induced and of the light-induced $\Delta A(515)$ are concerned, there is a surprising complementarity between the two absorbance changes. Under the differing conditions of Fig. 1a–c, the sum of ATP-induced and light-induced rapid $\Delta A(515)$ components appears to be constant.

It was shown before that the slow part of the ATP-induced absorbance change around 515 nm is not specific for P-515 [23]. A similar conclusion concerning the slow phase of the light-induced $\Delta A(515)$ was drawn before by Gimmler [27]. The relative contribution of the slow, ATP- or light-induced responses is substantially increased around 535 nm, suggesting the interference of ATP- or light-induced changes in light scattering. It is known that light-scattering changes depend on a functional PS II reaction center [27,28]. As shown before, addition of the PS II inhibitor NH_2OH will cause a preferential suppression of the slow ATP-induced absorbance change [23] allowing an accurate determination of the ATP-induced specific P-515 absorbance change. Without suppression of the slow rise component a quantitative determination of the rapid component can be complicated, when, e.g., at low concentrations of added ATP the overall response is slower and smaller as compared to the situation with saturating amounts of ATP (see Fig. 1c).

In Fig. 2, the light-induced as well as the ATP-induced 515 nm absorbance changes in the presence of 5 mM NH_2OH are displayed. It is apparent that this PS II inhibitor not only eliminates most of the slow ATP-induced change, but also the slow rise component of the light-induced change. Also the rapid, transient decay following the initial rise phase is suppressed by NH_2OH. It was checked that NH_2OH would not affect ATPase activity nor dissipate a transthylakoidal proton gradient (data not shown). This system appeared well suited for a quantitative correlation of

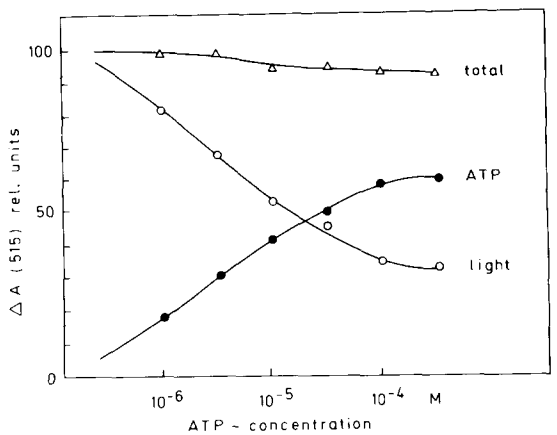
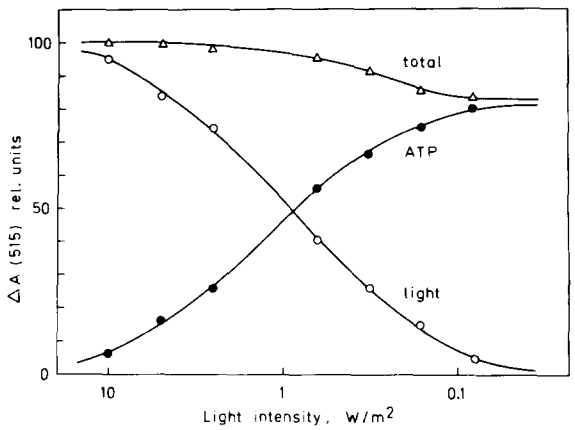


Fig. 3. Complementarity between the P-515 absorbance changes induced consecutively by ATP addition and illumination. The parameter being varied is ATP concentration. 5 mM NH_2OH was present as in the experiment of Fig. 2. ATP was injected 3 min after activation as 1-μl aliquots to give the indicated final concentrations. The onset of actinic illumination (10 W/cm^2) was 15 s after ATP addition. When water was added instead of ATP, the light-induced response was 100 relative units, which correspond to an absolute absorbance change of $6 \cdot 10^{-3}$. For the determination of the ATP-induced responses the absorbance change produced by the dilution effect (1 μl in 700 μl) was taken into account.



Fig, 4. Complementarity between the P-515 absorbance changes induced consecutively by actinic illumination and ATP addition. The parameter being changed is actinic light intensity. The light-induced changes were recorded 2.5 min after activation and ATP addition was 30 s thereafter. Final ATP concentration, 0.5 mM. Other conditions as in Fig. 3.

light-induced and ATP-induced specific P-515 absorbance changes.

By the addition of different amounts of ATP to preactivated chloroplasts, varying levels of an ATP-induced $\Delta A(515)$ can be established and the $\Delta A(515)$ during consecutive illumination (as shown in Fig. 2b for a saturating ATP concentration) can be monitored. In Fig. 3, the result of a series of such measurements is shown. With increasing ATP concentration the ATP-induced $\Delta A(515)$ is increased and concomitantly the light-induced $\Delta A(515)$ is decreased. There is complementarity between the two signals, as evidenced by the constancy of the total $\Delta A(515)$.

The question arises as to whether complementary between ATP- and light-induced $\Delta A(515)$ will also hold when the light-induced $\Delta A(515)$ is produced first and then the ATP-induced $\Delta A(515)$ is measured. To establish different levels of light-induced $\Delta A(515)$, the intensity of the actinic beam was varied. In Fig. 4, the ATP-induced $\Delta A(515)$ (at saturating ATP concentration) as well as the previously established light-induced $\Delta A(515)$ are plotted as a function of light intensity. All samples were equally light activated during a preceding illumination with strong white light (see Materials and Methods). It is apparent that the $\Delta A(515)$ which can be induced by ATP becomes smaller, the more $\Delta A(515)$ has already been built up in the light. At the highest light intensity applied (which is the same as that used in the experiments of Figs. 1–3), the light-induced $\Delta A(515)$ is almost saturated, and only a very small ATP-induced $\Delta A(515)$ is produced. As already apparent from the previous figures, the maximal ATP-induced response is somewhat smaller (by about 20%) than the maximal light-induced $\Delta A(515)$. This is not surprising, as one may expect part of the ATPase molecules not to be active at the moment of ATP addition. Presumably, this part is primarily determined by the 10–25% of broken chloroplasts present in the preparation, which may not become activated in the absence of artificial cofactors. This aspect explains the decay of the total $\Delta A(515)$ with decreasing actinic light intensity shown in Fig. 4.

The results presented so far give evidence that the total $\Delta A(515)$ is limited which can be induced by either light or ATP hydrolysis. There is complementarity between the light-induced and the ATP-induced P-515 absorbance changes. This finding offers a straightforward explanation for

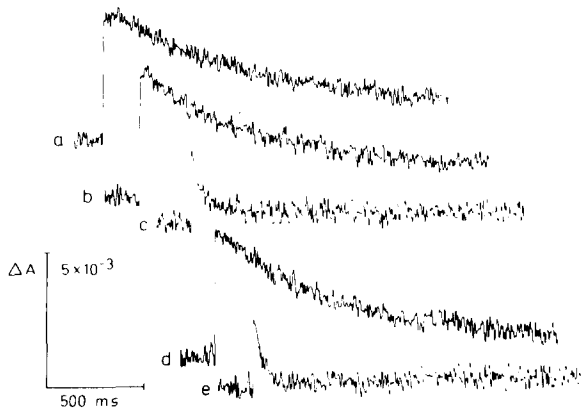


Fig. 5. Flash-induced changes of P-515 absorbance. Curve a: sample dark adapted for 3 h; no addition, Curve b: sample light activated, with 4 min dark time to flash; no addition. Curve c: light activated, 3 min dark time to addition of ATP (final concentration 0.5 mM). Curve d: same sample as that of curve c, hydrolyzing added ATP for 5 min, at which time tri(*n*-butyl)tin was added (final concentration 8 μM). The flash was given 30 s following addition of the inhibitor. Curve e: dark-adapted sample in the presence of 0.2 μM valinomycin. Flash-induced responses very similar to that shown in curve e were measured in light-activated samples with or without ATP addition in the presence of 0.2 μM valinomycin.

the observed suppression of the light-induced $\Delta A(515)$ under conditions of active ATP hydrolysis [2,7,9]. However, so far the data do not explain the reported acceleration of the overall decay rate of the flash-induced $\Delta A(515)$ caused by preillumination [2,5,6] which has been correlated with the activity of the ATP-hydrolase [7,9]. To approach this aspect, the correlation between the ATP-induced $\Delta A(515)$ and the flash-induced $\Delta A(515)$ was investigated.

In Fig. 5, flash-induced P-515 absorbance changes are presented for various conditions of ATP-hydrolase activity. Comparison of curves a and b demonstrates that preillumination as such, which leads to activation of the ATP-hydrolase [13,20,26], does not yet cause a substantial change of the flash-induced response. When ATP is added to a preactivated sample, the amplitude of the flash-induced change is suppressed and the overall decay rate is accelerated by about a factor of 15 (curve c). A flash-induced response practically identical to that in Fig. 5c was measured after 20 min of darkness following ATP addition (data not shown). When tri(*n*-butyl)tin, a specific ATPase inhibitor [29,30], was added to the same sample, a response similar to that before ATP addition is observed (curve d). It may be noted that upon tri(*n*-butyl)tin injection a rapid 515 nm absorbance decrease was observed (data not shown), the size of which was about the same as that of the P-515 absorbance increase induced by ATP and that of the resulting suppression of the flash-induced response. Curve e shows the flash-induced P-515 absorbance change in the presence of 0.2 μM valinomycin, with a dark-adapted, ATP-free sample. Practically the same response was found when valinomycin was present in a sample hydrolyzing ATP (showing the response of curve c).

By analogy with the experiment displayed in Fig. 3 for continuous illumination, the flash-induced $\Delta A(515)$ was measured at different levels of ATP-induced $\Delta A(515)$. As shown in Fig. 6, also for the amplitude of the flash-induced $\Delta A(515)$ there is complementarity with the previously established ATP-induced $\Delta A(515)$. As with continuous illumination, there is a part of the light-induced P-515 response which is not suppressed by ATP hydrolysis. The figure also shows the ATP-

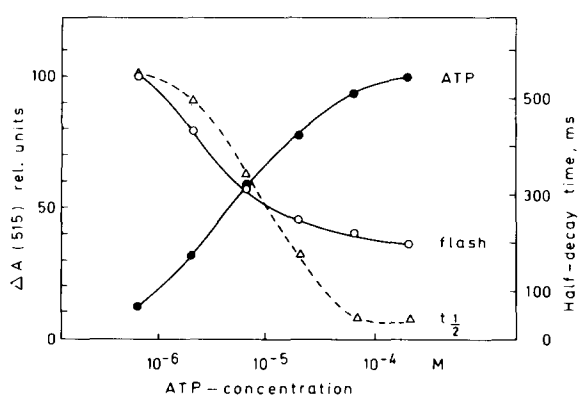


Fig. 6. Correlation between the P-515 absorbance changes induced consecutively by ATP addition and flash illumination. The parameter being varied is ATP concentration. Conditions as in Fig. 3. Maximal ATP- and flash-induced responses were normalized to 100 relative units which correspond to $4.3 \cdot 10^{-3}$ absolute absorbance units in the case of ATP addition and to $4.0 \cdot 10^{-3}$ absolute units with flashes. A comparative evaluation of the absolute ATP- and flash-induced changes should take into account that the flashes were only about 80% saturating. 'Half-decay times' were measured from the moment of flash triggering to the time the transient absorbance increase was half decayed.

induced dependency of the relative acceleration of the overall decay rate. Half-times of the decay curves are plotted vs. ATP concentration. The observed dependency is very similar to that shown for the amplitude of the flash-induced $\Delta A(515)$. Hence, amplitude suppression and decay acceleration go along with a complementary ATP-induced increase in P-515 absorbance.

A kinetic analysis of the flash-induced P-515 absorbance change provided by Schapendonk [10] suggests that the overall response is composed of two main components. Reaction I (rise time 0.5 ms; half-decay time about 50 ms) and Reaction II (half-rise time about 50 ms; half-decay time about 500 ms), with the decay of Reaction II determining the overall decay rate. According to Schapendonk [10], moderate concentrations of valinomycin will selectively inhibit Reaction II. In view of this analysis, one may suspect that the flash-induced response observed at saturating ATP concentration (Fig. 5c) with a half-decay time of about 30 ms (as compared to a half-decay time of about 450 ms in an ATP-free, nonactivated sample) may be equivalent to the isolated Reaction I component. This conjecture is supported by the observation that valinomycin at 0.2 μM, which when added to a control sample will accelerate the decay rate about 15-fold (see Fig. 5e), did not further affect the flash-induced P-515 response in a sample with maximal ATP hydrolysis (not shown in the figures). It may be concluded that ATP hydrolysis preferentially induces a Reaction II type of electrochromic pigment shift, and by doing so preferentially eliminates the Reaction II component of the flash-induced P-515 absorbance change.

Discussion and Conclusions

The above data demonstrate complementarity between the ATP-induced and the light-induced P-515 absorbance changes. This finding is difficult to reconcile with a simple model of P-515 absorbance being governed exclusively by a bulk transthylakoidal membrane potential. There is no reason why such a bulk potential should be limited to a fixed value. Indeed, larger transmembrane potentials and correspondingly larger 515 nm absorbance changes than those reported here can be induced by salt injection (rapid creation of a diffusion potential upon K^+ influx in the presence of valinomycin) [31,32]. The observed complementarity suggests a limited number of available sites which can accumulate a limited number of electrical charges either by ATP hydrolysis or by illumination. This complementarity appears to apply only for the Reaction II component of the light-induced P-515 absorbance change. No complementarity is apparent for the Reaction I component of the flash-induced P-515 change, and our data give no indication of any appreciable ATP-induced change of the Reaction I type. In agreement with the ideas put forward by Schuurmans et al. [12] and by Vredenberg and Schapendonk [11], we tend to conclude that Reaction II is closely linked with a localized field in the vicinity of the ATPase. During ATP hydrolysis, the buildup of this field may be directly monitored via the P-515 absorbance change. The limited number of sites available for a Reaction II-type field could be directly related to the number of CF_1 molecules at the surface of the thylakoid membrane. The existence of fixed charges on CF_1, and energy-dependent charge reorientation in the vicinity of CF_1 may be inferred from work of Kraayenhof's laboratory [12,33].

With the assumption that reverse coupling involves the same intermediates as forward coupling, the presented results may be considered relevant also for the understanding of the mechanism of ATP synthesis. Our results tend to suggest that a Reaction II-type localized field could be a relatively late, if not the last, high-energy intermediate during the coupling reaction. It might constitute an important element of a hypothetical 'microchemiosmotic' type of coupling mechanism [34]. So far, it is not clear how the magnitude of such a localized potential could be calibrated to determine it's contribution to the total proton-motive force. Estimates of the membrane potential maintained in continuous light were generally for bulk potentials [35,36], the amplitude of which was found to be too low to contribute significantly to the total proton-motive force. In our opinion, the existence of a 'Reaction II-type localized potential', maintained during steady-state light conditions as well as by ATP hydrolysis in the dark, could explain most of the reported apparent discrepancies concerning equilibration between the proton-

motive force and the phosphorylation potential [37,38]. To substantiate this point, correlative measurements of phosphorylation potentials and of the amplitude of absolute P-515 absorbance under a variety of conditions are required. With the development of the measuring system used in the present study, such quantitative correlation may be possible.

An obvious consequence of the observed complementarity between the ATP-induced $\Delta A(515)$ and the Reaction II component of the flash-induced P-515 change is the apparent acceleration of the overall P-515 dark-decay rate under conditions when ATP hydrolysis has induced a $\Delta A(515)$. With this concept, various observations from other laboratories find a straightforward explanation [1,2,6–10] without the need of invoking changes of membrane proton conductance upon ATP hydrolysis. Our conclusions are essentially in agreement with previous suggestions by Schapendonk [10] and Schuurmans et al. [12].

A number of publications from Hind's laboratory [3,39,40] suggest that there is close correlation between the slow, flash-induced $\Delta A(515)$ component and cyclic photophosphorylation. It appears likely that Schapendonk's Reaction II and Hind's $P\text{-}518_S$ reflect the same process. According to Hind et al. [40] cyclic electron flow plays an important role in the regulation of ATP/NADPH and of overall photosynthesis. The notion of a direct influence of ATP hydrolysis on Reaction II introduces a new aspect into the discussion of the factors regulating cyclic electron transport.

Note added in proof (Received July 8th, 1982)

In a very recent publication (which was brought to our attention only after submission of the present report), Schuurmans et al. [41] propose a model which may account for most of the findings of the present report. These authors localize the Reaction II-type field, which is also sensed by the oxonol VI probe [12], in the region of the Rieske iron-sulphur protein complex. They propose that the 'slow field' is communicating with a localized high proton concentration which is fed by the protons dislocated (not necessarily translocated across the bulk membrane) by the redox systems in forward as well as in reverse electron flow. Such a domain of a localized intramembrane proton buffer, the capacity of which is limited, should slowly equilibrate with the much larger proton buffer of the thylakoid interior space (see also Ref. 42). This model, advanced by Schuurmans et al. [41], appears to offer an excellent working hypothesis for further elucidation of the energy-transducing events in the thylakoid membrane.

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