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SLOW 514 nm ABSORPTION PHASES AND OXYGEN EXCHANGE TRANSIENTS IN *ULVA*

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SUMMARY

1. The slow 514-nm spectral changes in *Ulva* were studied using bright continuous 650-nm light. Transient and steady-state absorption changes were compared with changes in net rate of O_2 exchange in a system designed to measure both parameters simultaneously.

2. Time courses of the 514-nm absorption change show three phases following the onset of light: one rapid increase and two slower ($\geqq 1$ s) transient increases. Upon cessation of the light three transient absorption phases also follow: a rapid decrease and two slower (>1 s) transient increases. Parallel transient phases (but opposite in sign) were found at 480 nm.

3. The kinetics of the slow 514-nm absorption transients correlate with the characteristic induction transients in net O_2 exchange.

4. Similar difference spectra and the restoration kinetics of the light-on and light -off transient phases indicate that the slow 514-nm absorption changes reflect the same component(s) and process(es).

5. The experimental results are discussed in terms of the electrochromic hypothesis for the 515-nm absorption shift. We interpret the slow 514-nm absorption changes in *Ulva* as a reflection of relatively slow ionic readjustments across the photosynthetic membranes.

INTRODUCTION

Light-induced absorption changes around 515 nm were first described in *Chlorella* by Duysens [1]. Subsequent studies in numerous laboratories have shown that spectral shifts in this region of the spectrum are complex and composed of several transient phases.

The concensus is that rapid (<1 μs), laser-induced 515-nm absorption changes are due to electrochromic shifts in absorption of chlorophyll and carotenoid pigments embedded in the photosynthetic membranes [2–4]. These absorption shifts are thought to occur in response to the potential set-up across the thylakoid or chromatophore membrane by the primary photochemical charge separation. With longer flashes or

continuous light, slower (ms → s) 515-nm absorption phases follow the rapid phase [2, 3, 5–9]. The physical or biochemical basis for the slower 515-nm absorption phases has not been resolved [3, 9, 10].

Previous attempts to relate the different phases of the 515-nm spectral shift with electron flow transients have been only partially successful. For example, similar transient patterns were noted for the 515-nm absorption and oxygen transients although the kinetics of the two processes were quite different [6, 7, 11]. In these studies both the rapid and slow 515-nm absorption phases were compared with the induction phases in O_2 evolution. Subsequent measurements of the kinetics of the initial rapid 515-nm absorption phase ($\leqq$20 ns) [12, 13], however, preclude the possibility of any correlation of this phase with secondary electron transport events. Comparisons between the slower absorption and O_2 induction phases, measured independently, are further complicated by the wide spectrum of conditions which influence the transient phases.

In this study the kinetics of the 515-nm spectral shift were investigated by comparing transient and steady-state absorption changes with net rates of O_2 exchange in a system designed to measure both parameters simultaneously. Our results show that moderate to saturating intensities of 650-nm light generate a sequence of slow 515-nm absorption transients and parallel O_2 induction transients.

MATERIALS AND METHODS

Experiments were done with *Ulva curvata* collected on the Atlantic seashore. Thalli were maintained in filtered sea water supplemented (20 ml/l) with Provasoli's ES medium as modified by West (see McLachlan [14]). Cultures were kept at 15 °C and illuminated 14 h/day with fluorescent and incandescant light (approx. 120 ft candles).

Absorption changes were measured with an Aminco-Chance dual-wavelength difference spectrophotometer (Aminco Model 4-8461) and relative rates of O_2 exchange with a modified Haxo and Blinks [15] rate electrode. Absorption changes were recorded with either a strip-chart recorder (Esterline Angus Model E1101S) or an oscilloscope. O_2 exchange was monitored using an electrical circuit similar to that described by Myers and Graham [16] except that a cathode potential of −0.6 V was used. Current changes were recorded with a strip-chart recorder (Honeywell Model 153).

The standard Aminco cell holder was replaced by an electrode assembly which enabled simultaneous recording of both absorption changes and relative oxygen exchange. The electrode assembly (Fig. 1) consisted of two lucite components: one containing the stationary platinum electrode (5mm × 8 mm), algae and a retaining dialysis membrane; the other, a reference electrode (agar/salt bridge connected to a saturated calomel half-cell) and connections for circulating solutions.

Instead of the customary solid platinum electrode, a fine-mesh platinum screen was used as the cathode (80-mesh, platinum - 10 % rhodium gauze, Englehard Industries, Newark, N.J.). The platinum screen was embedded in lucite and the surface filed and polished to expose a smooth longitudinal section of the embedded screen. As shown in Fig. 1, the assembled electrode formed a small chamber through which filtered sea water was pumped. The sea water was temperature-regulated

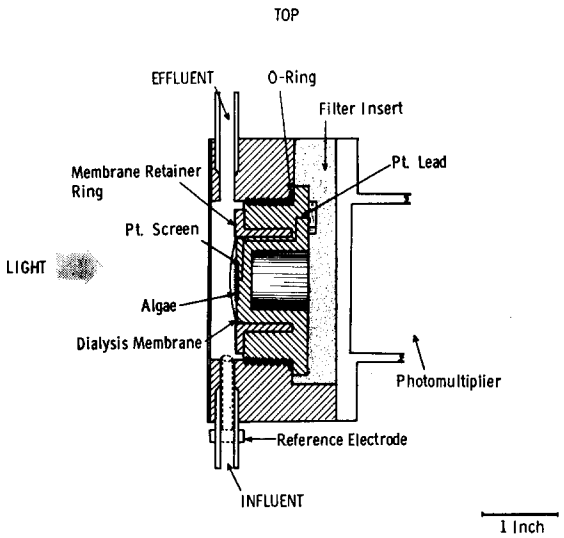

Fig. 1. Longitudinal section of electrode assembly.

(23 °C), equilibrated with 5 % CO_2 in air and circulated at a rate of approx. 500 ml/min.

Actinic light, from a 1000-W microscope projection lamp, was passed through 16 cm of water, a Corning cut-off filter (2-58) and Baird Atomic interference filter (BA 1-6500). Corning (4-76) and Kodak Wratten (64) filters were used to screen actinic light from the photomultiplier. The actinic beam was focused through the side of the spectrophotometer and reflected onto the tissue with a front-surface mirror. Incident light intensities were measured with an Epply thermopile and Keithley microvoltammeter (Model 150A).

A single piece of thallus was placed directly on the platinum electrode and kept appressed to the surface with a stretched dialysis membrane. Normally, the electrode was assembled and the tissue illuminated with weak 650-nm light for 2–3 h. This relatively long period of adaptation allowed the tissue to equilibrate with the circulating solution and tended to eliminate slow signal drifts. Although these conditions caused no apparent damage to the tissue (up to several days), a fresh thallus was used each day.

RESULTS

Light-induced O_2 and 514-nm absorption transients

Illumination of algae with moderate to saturating intensities of 650 nm light gives rise to a complex series of O_2 and 514-nm absorption transients. Fig. 2 shows a typical sequence of induction transients. As described previously [17–20], O_2 transients are often observed before attaining a steady-state rate of O_2 exchange. Vidaver [19] designated the rapid ($\leqq$1 s) burst of O_2 the "pre-a" transient and the slower wave (approx. 15–20 s) the "a" transient. The magnitude of these positive transients and the appearance of subsequent negative spikes, one of which is apparent in Fig. 2, following the "pre-a" transient, vary considerably with experimen-

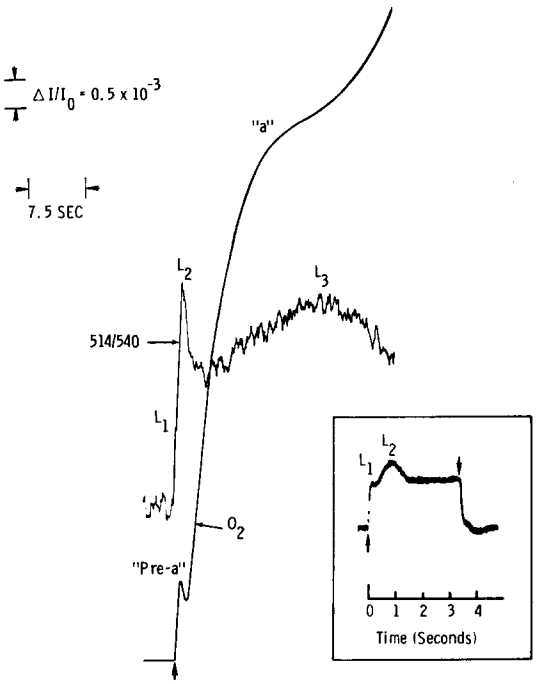

Fig. 2. Time course of the 514-nm absorption change and relative rate of O_2 exchange. Actinic intensity $2.3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}; dark adapted 10 min; $\uparrow$ light on. Insert shows oscilloscope recording of 514-nm absorption change for different sample. Actinic intensity $3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}; dark adapted 3 min; $\uparrow$ light on, $\downarrow$ light off.

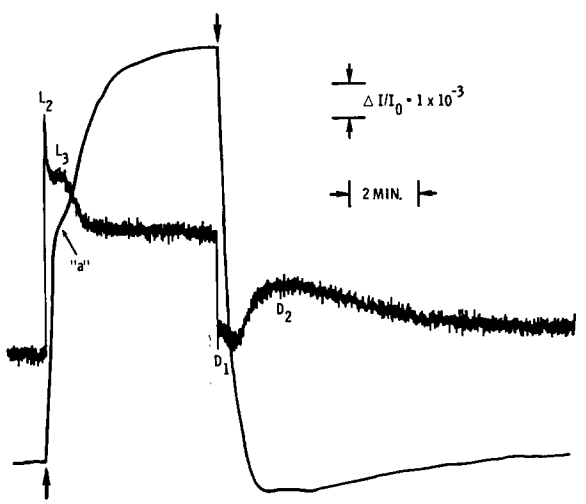

Fig. 3. Time course of the 514-nm absorption change and relative rate of oxygen evolution. Actinic intensity $1.5 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}; dark adapted 9 min; $\uparrow$ light on, $\downarrow$ light off.

tal conditions, e.g. dark adaptation, light intensity and quality, etc.

A complete rise curve for O_2 evolution in 650-nm light and the negative transient observed in a subsequent dark period are shown in Fig. 3. In this experiment the lower sensitivity and slower time base precluded resolution of the "pre-a" transient, however, an "a" apike is still evident. Fig. 3 does illustrate the pronounced net increase in the rate of O_2 consumption following 650-nm illumination. The apparent increase in O_2 consumption reaches a maximum after about 80 s of darkness then slowly diminishes to the original dark base level.

The light-induced absorption changes occurring at 514 nm are also shown in Figs 2 and 3. Three transient increases in absorption are observed upon addition of 650-nm light to a dark-adapted sample (Fig. 2). A very rapid transient ($<$150 ms), designated L_1, is followed by a second transient (approx. 1 s), designated L_2. The two early transients are clearly resolved with faster recording times (see insert Fig. 2). The kinetics and properties of the L_1 and L_2 transients indicate that they are comparable to the Type 2, Phase 1 and Phase 2, absorption changes originally described in *Chlorella* by Witt and Moraw [6]. As shown by the main recording in Fig. 2, the absorption at 514 nm does not decline simply to a steady-state level following the L_2 transient [6, 7]. Rather, the absorption level decreases then subsequently increases (approx. 20–25 s) to a new peak designated L_3. The L_3 transient and subsequent decrease in absorption to a true steady-state level in the light are shown in Fig. 3. Interestingly, the absorption kinetics observed upon removal of the 650-nm beam were equally complex. The initial rapid decrease in absorption is followed by two transient increases in absorption. These transients have not been previously described. The small transient rising and decaying in the first 20–25 s of darkness, is designated D_1. The large, slower-developing transient, designated D_2, requires approx. 9–10 min of darkness to decay.

It should be noted that prolonged ($\geqq$10–20 s) pre-illumination was required for development of the large D_2 transient phase. Short periods (few seconds) of pre-illumination caused temporary absorption shifts below the steady-state dark level as previously reported. The relative magnitudes of the 514-nm absorption transients, like the various O_2 transients described, were also quite variable. Such factors as light quality, intensity, dark adaptation and culture conditions for the algae affected the magnitudes and the kinetics of the different absorption phases. Consequently, comparisons between relative rates of O_2 exchange and absorption changes occurring at 514 nm are simplified by measuring both parameters simultaneously.

Kinetic correlations between O_2 and 514-nm absorption transients

Comparison of the slow, light-induced 514-nm absorption changes and net rates of O_2 exchange reveals a definite kinetic correlation between the two phenomena. As shown in Figs 2 and 3, the "pre-a" and "a" O_2 transients and subsequent approach to a steady-state rate of O_2 evolution have similar kinetics, respectively, to the L_2, L_3 and subsequent steady-state 514-nm absorption phases. Two qualifications should be noted about the transients in the light. We found no transient phase in O_2 exchange corresponding kinetically to the rapid 514-nm L_1 transient. Secondly, we generally observed the absorption transients to be slightly out of phase (slower) with the corresponding O_2 transients. This feature was particularly apparent when the

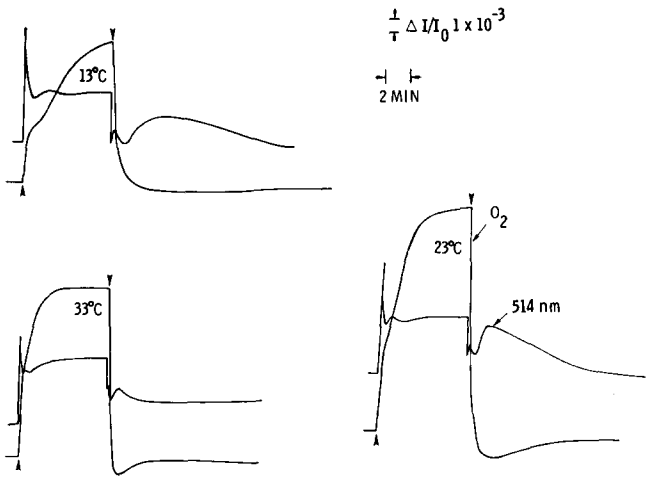

Fig. 4. Effect of temperature on the kinetics of the slow 514-nm absorption and O_2 induction transients. Actinic intensity $2.1 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}; dark adapted 20 min at 13 °C and 14 min at 23 °C and 33 °C; ↑ light on, ↓ light off.

positive O_2 transients were followed by net decreases in rate of O_2 evolution (negative spikes). As shown in Fig. 3, the kinetics of 514-nm D_2 transient phase parallel the transitory increase in the rate of O_2 consumption following 650-nm illumination. The high steady-state rate of O_2 evolution in continuous light precluded the observation of an O_2 transient corresponding to the small 514-nm D_1 transient. With shorter light exposures ($\leqq$35 s), higher sensitivities, and faster recording times, a more rapid O_2 transient was observed having kinetics approximating the D_1 transient phase. Reid [21] likewise found a complex series of O_2 induction transients in *Chlorella* following short light exposures.

The experiments of Fig. 4 show the effects of temperature on the slow 514-nm absorption and corresponding O_2 transient phases. The traces, recorded from the same piece of tissue, suggest a similar temperature dependency. The rate of appearance and recovery of the L_3 and "a" transients in the light and the D_1, D_2 and O_2 uptake transients in the dark were increased approx. 4-fold for a 20 °C increase in temperature. Lower temperatures similarily prolonged the L_2 and "pre-a" induction phases while the rapid L_1 transient appeared relatively insensitive to temperature as previously reported [6, 18].

The magnitude of the O_2 and slow absorption transients was also affected by temperature. In general, increasing temperatures resulted in a marked damping of the slow induction phases found with both the onset and offset of 650-nm illumination.

Spectra of 514-nm absorption transients

Fork [22] has recently reported a light-dark difference spectrum for *Ulva* using 1-s saturating red light exposure. We found similar maxima and minima difference peaks in the blue and green region of the spectrum. The major positive peak at 514 nm is associated with an equally prominent negative peak at 480 nm. As shown in Fig. 5, identical light and dark slow transient phases are observed at

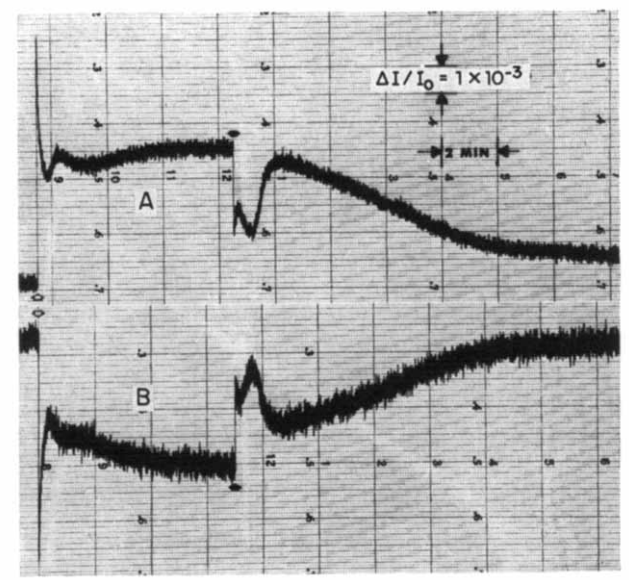

Fig. 5. Time course of the 514-nm absorption change (A) and the 480-nm absorption change (B). Actinic intensity $2.1 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}; dark adapted 14 min; $\uparrow$ light on, $\downarrow$ light off.

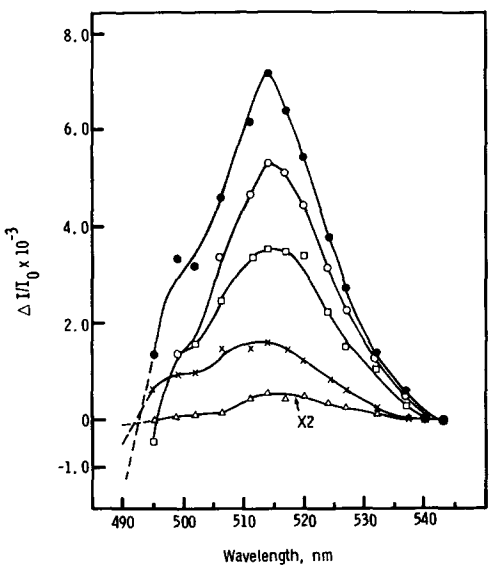

Fig. 6. Difference spectra for various phases of 514 nm absorption change. (●), L_2; (○), L_3; (□), steady-state; (×), D_2; (△), D_1; constant regimen of 5 min light, 20 min dark for each wavelength; Actinic intensity $3.1 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}.

514 nm and 480 nm of approximately equal magnitude but opposite in sign.

The question arises whether the multiple 514-nm transient phases represent absorption changes of a single pigment form or, in part, light-induced scattering changes. In Fig. 6, we plotted the difference spectrum for both transient and steady-state absorption changes between 495 and 543 nm. All spectra had peak absorptions around 514 nm and a shoulder around 500 nm. The relatively slow time-base used in the experiment of Fig. 6 precluded the observation of the rapid L_1 transient. In separate experiments, however, we found it to have a similar difference spectrum.

Dependence of slow 514-nm transient phases on a single reacting pool

The similar difference spectra for the 514-nm transients suggested that the same pigment(s) were involved in both the fast and slow phases of the absorption change. Subsequent experiments have shown that a single "pool" of the pigment(s) giving rise to the 514-nm absorption shift is probably involved in both the light and dark transient phases. As indicated previously, the magnitude of the different transient phases vary depending upon dark adaptation of the cells. In the experiment of Fig. 7, continuous 650-nm light was given to reach a steady-state 514-nm absorption change. The red light, which was saturating for the initial spike height, was subsequently interrupted by dark periods of varying duration. In Fig. 8A, we plotted the magnitude of the initial spike (L_1 plus L_2 transient) as a function of the dark spacing between red light exposures. The spike height was determined in two ways. First, the dark absorption level (b—a_0, b′—a′, etc., in Fig. 7) attained just preceeding the red light was used. As reported by Fork and DeKouchkovsky [7] the size of the rapid 514-nm spike (L_1 transient), measured in this fashion, shows a complex dependency on the dark spacing between light exposures (solid curve, Fig 8A). However, we have shown that the absorption at 514 nm varies considerably in the dark period following bright 650-nm light requiring approx. 10 min of darkness to reach a steady-state level at 23 °C. For this reason, we calculated spike height relative to a constant level of absorption at 514 nm, i.e. the steady-state attained in continuous 650-nm light (b—c, b′—c, etc. in Fig. 7). As shown in Fig. 8A (dashed line), regeneration of the spike plotted in this fashion appeared complete after approx. 1 min of darkness.

We inferred that, if the initial spike (L_1+L_2 transient) and the D_1 and D_2

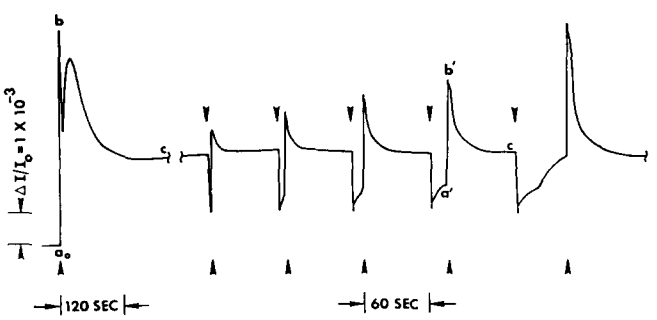

Fig. 7. Time course of the 514-nm absorption change with dark interruptions of increasing duration. Initial dark period 14 min, followed successively by 2-, 5-, 10-, 15- and 45-s dark periods where indicated; ↑ light on, ↓ light off; actinic intensity $2.9 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Note that the time base was changed during the steady-state of first light exposure from 120 s/division to 60 s/division.

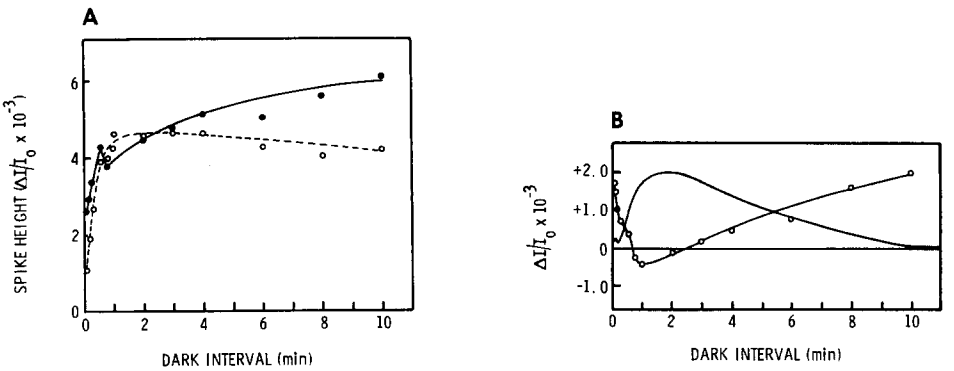

Fig. 8. Effect of dark adaptation on the magnitude of the 514-nm absorption transients. Results taken from experiment in Fig. 7. (A). Initial spike height (L_1+L_2 transients) as a function of dark adaptation measured relative to absorption level just preceeding light exposure (solid markers) and relative to the steady-state absorption level attained during 650-nm light (open markers). (B) Difference curve (open markers) for initial spike height measured relative to dark and steady-state absorption levels as a function of preceeding dark period (from (A)). Smooth curve represents the time course of 514-nm absorption change following 650-nm light. Note: the initial rapid decrease in absorption following removal of the actinic beam is not included. The minimum absorption obtained after removing the light was arbitrarily taken as zero.

transient phases involved separate fractions of the pigment(s) responsible for the 514-nm absorption shift, the regeneration curves plotted in Fig. 8A should have been parallel. On the contrary, with a single-reacting 514-nm component, one would expect dissimilar curves in which the difference between the two regeneration curves would reflect the kinetics and magnitude of the long-lived transitory changes in absorption at 514-nm seen in the dark. In Fig. 8B we plotted the difference between the two regeneration curves (open circles) and the time course of the 514-nm absorption (solid line) following removal of the 650-nm beam. Except for a small discrepancy with short dark intervals (<2 min) there is a good inverse correlation between the difference curve and actual time course curve of the dark transient in Fig. 8B. Thus, transient changes in the state of the 514-nm pigment in the dark period following bright 650-nm light have a direct effect on the magnitude of the initial transient phases observed in subsequent red light.

Two other features are apparent in the time course of Fig. 7. The large L_3 transient phase observed in the initial trace after a long dark period (14 min) was absent with short dark interruptions ($\leqq 1$ min) but re-appeared following longer dark periods ($\geqq 2$ min). Secondly, in this experiment, the large D_2 transient phase was actually slightly greater than the steady-state 514-nm absorption change in red light. This is the reason for the small negative portion of the difference curve in Fig. 8B.

DISCUSSION

We have observed three distinct 514-nm transient phases in *Ulva* following the onset of bright System II light; an L_1 transient with kinetics faster than the time resolution of our instrument followed by two, slower L_2 and L_3 transient phases.

We assume that our L_1 transient is comparable to the rapid transient phase originally described by Witt, Moraw [5, 6] and others [7–9, 23]. Junge and Witt [24] propose that this rapid absorption shift represents the initial electric field generated across the thylakoid membrane by the photoacts. The main focus of this paper has been on the slower ($\geqq$ 1 s) 514-nm transient phases and the corresponding O_2 induction transients.

Witt [3, 25] has suggested that slow 515-nm changes ($\geqq$ 1 s) may be due to changes in light-scattering, de-epoxidation of carotenoids or changes in membrane potential. The similar difference spectra for the various transient phases (Fig. 5) and the corresponding absorption changes at 480 nm (Fig. 6) indicate that non-specific light scattering changes are not the cause of the slow transient phases in *Ulva*. The present experiments do not exclude the possibility that a carotenoid de-epoxidation cycle may be involved. However, these carotenoid changes have generally been obtained with high light intensities (above saturation) and are very slow or in some cases are irreversible [26–28].

Our results are consistent with the hypothesis that the 515-nm shift reflects an electrochemical potential across the thylakoid membrane. Reinwald et al. [29] reported a linear relationship between magnitude of the 515-nm shift and the amount of plastoquinone reduced by flashes of increasing duration. We assume that the initial O_2 burst (pre-a-transient) in bright System II light reflects the reduction of the plastoquinone pool [30, 31]. As predicted, a large absorption increase (L_2 transient) does coincide with the initial O_2 burst (pre-a-transient). The subsequent decrease in absorption at 514 nm and corresponding increase in rate of O_2 evolution presumably reflect a partial decay in membrane potential and partial oxidation of the plastoquinone pool due to the initiation of ATP consumption by CO_2 fixation reactions. With longer dark adaptation a second induction wave is observed. Again we find an increase in absorption at 514 nm (L_3 transient) paralleling the momentary net decrease in rate of O_2 evolution ("a" transient). The cause of this second slow induction phase is not known, although similar O_2 transients have been observed in *Chlorella* with both the concentration and modulated rate electrodes, suggesting that it represents a true decrease in O_2 evolution rate [18, 32].

The slow, pronounced, post-illumination 514-nm transient phases (D_1 and D_2) were surprising. The experiments of Fig. 7 and 8 indicate that both the light and dark 514-nm transient phases involve the same complement of pigment(s) responsible for the 514-nm absorption change. This would suggest that slow but large increases in membrane potential can occur in the absence of light. Our experiments with whole cells preclude a definitive description of the processes generating the D_1 and D_2 transient phases. It is interesting to note, however, that "dark" absorption shifts around 515 nm similar to those induced by light, have been generated in chloroplasts and chromatophores upon addition of KCl, ATP or pyrophosphate [33–35]. Thus, the dark transient phases in *Ulva* may reflect slow ion equilibration and the development of diffusion potentials, or to the formation of an ion gradient by reversed ATPase activity [33, 36].

The reason for the net increase in rate of O_2 consumption corresponding to the D_2 absorption phase is unclear. For example, we have observed small oxygen uptake transients upon the addition and removal of far-red light without concommitant 514-nm absorption shifts (see also [17, 18, 20, 21, 31, 37]). Nevertheless, this O_2

uptake transient may, in part, reflect the autooxidation of the large plastoquinone pool since the kinetics of this transient following bright red light parallel the dark restoration of the initial O_2 burst (unpublished). A similar backreaction of O_2 with reduced intermediate electron transport carriers has been suggested for *Chlorella* [38, 39].

Initial attempts to find similar 515-nm absorption increases following bright pre-illumination in isolated chloroplasts (Class II type) have been unsuccessful. This may simply reflect membrane permeability changes resulting from the isolation procedure and/or the loss of the distinct compartmentalization found in the whole cell. Larkum and Bonner's report on the 515-nm shift with Class I chloroplasts, however, does show a comparable D_2 transient phase, albeit smaller, following bright 650-nm illumination (see Fig. 4 of ref. 40).

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