Oxygen Exchange in *Ulva* Using a Bare Platinum Electrode with 4 Microsecond Saturating Light Flashes

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**ABSTRACT**

Illuminating algae (*Ulva* species) on a bare platinum electrode with 4 microseconds of saturating light flashes leads to the familiar four-step oscillatory pattern of O$_2$ exchange. However, it appears that this O$_2$ exchange is a composite of O$_2$ evolution and endogenous O$_2$ uptake in the algae. We have added the O$_2$ uptake component to the O$_2$ exchange curve in order to obtain the corrected O$_2$ evolution flash sequence which shows a similar four-step oscillation as reported previously, but with negligible damping over the first three oscillations.

O$_2$ exchange measurements using a bare platinum electrode with saturating light flashes yield information on the kinetics of both O$_2$ evolution and O$_2$ uptake in green plants, algae, and isolated chloroplasts.

An O$_2$ uptake has been observed during flash illumination of algae and chloroplasts (14, 15, 25, 28), but the sites of this uptake are still uncertain. O$_2$ uptake in the light may occur via the Mehler reaction, which results in photoreduction of O$_2$ (19, 22, 23) or the oxygenase reaction of ribulose bisphosphate carboxylase-oxygenase (2, 4, 11). Mitochondrial respiration in the light (11) does not vary on a time scale of $s$, and for this reason, it most likely does not contribute to a flash-induced O$_2$ uptake. Recently, Beck et al. (3) have shown that a biphasic O$_2$ consumption occurs in untreated photosystem II (PSII) membranes after continuous illumination. The fast phase of O$_2$ consumption has been attributed to dissipation of reactive intermediates formed during illumination, while the slow phase appears to be a property of the PSII O$_2$-evolving complex. Reduced endogenous plastochlorine has been suggested by Beck et al. as the electron donor for the slow consumption of O$_2$, which is catalyzed by the O$_2$-evolving complex.

O$_2$ evolution results from PSII water-splitting activity. The currently accepted model of O$_2$ evolution, the $S$-state hypothesis (10, 17) requires the generation and cooperation of four photochemically formed oxidizing equivalents. The major evidence for this model involves a linear four-step oscillation in O$_2$ evolution observed during a series of saturating light flashes (13, 17). The data are compatible with chemical intermediates successively cycling through five different oxidation states, $S_0$ through $S_4$. Each $S_n$ state differs from the preceding state, $S_{n-1}$, by the loss of an electron. When the intermediates reach the most oxidized state, $S_4$, O$_2$ is released in the final step as $S_0$ returns to $S_4$ in the dark.

The periodicity of four in the flash yield sequence of O$_2$ evolution is consistent with this model, but the damping to a steady-state value after a number of flashes is still the subject of debate (8, 10, 16, 18, 26). This damping has been explained by Forbush et al. (10) as a phase loss due to double hits or misses at the reaction center. However, since light-induced O$_2$ uptake occurs in green plants and algae, endogenous O$_2$ uptake during a flash sequence may influence the single flash yield to the extent that measured yields are not equivalent to actual water-splitting yields.

Most of the recent O$_2$ evolution experiments were done using the modulated polarograph technique described by Joliot and Joliot (12), which selects the 'fast' light-induced response, i.e. O$_2$ evolution, from any slower light-induced response, i.e. O$_2$ uptake. With this system, a decrease in the rate change of O$_2$ consumption at the cathode simply indicates less O$_2$ is reaching the electrode. Any information pertinent to the O$_2$ uptake will be lost, while not necessarily eliminating the O$_2$ uptake occurring within the chloroplast. With a bare Pt electrode, simultaneous O$_2$ consuming and evolving reactions are recorded as a net rate of O$_2$ exchange, which may be negative or positive depending on the magnitude of the competing processes. Thus, if a transient O$_2$ uptake occurs in response to a light flash in the alga, it would be present in the measured O$_2$ exchange.

Schmid and Thibault (25) have shown that in tobacco chloroplasts, O$_2$ uptake affects the O$_2$ yield in a sequence of flashes, but have not shown the ramifications of the O$_2$ uptake on the flash yield sequence. To determine the effect of this uptake, O$_2$ exchange (evolution and/or uptake) was measured with a bare platinum electrode for the marine alga, *Ulva* species, during a series of saturating light flashes.

**MATERIALS AND METHODS**

O$_2$ evolution and uptake measurements were made *in vivo* using the stationary platinum polarographic electrode described by Chandler and Vidaver (5). The slow rate of diffusion of O$_2$ in water produces a time lag in the measurement of a change in the O$_2$ reaching the electrode; to minimize this diffusion-limited response of the electrode system, the aequous phase of the sample was kept as small as possible, and thin (one or two cell layers) algal samples were used. Discs, 6.5 mm in diameter, were cut from algae with a cork borer and samples were tightly held on the bare Pt electrode with a stretched cellophane membrane cut from thin dialysis tubing and soaked in seawater. The membrane was stretched to cover the Ag/AgCl counter electrode and secured with an O-ring. Seawater served as the electrolyte providing the electrical conductivity between the Pt and Ag/AgCl electrodes. In studies using the electron transport inhibitor, the algal discs were kept in seawater containing 5 $\mu$M DCMU for 1 h in the dark prior to measurement.

The electrode assembly was housed in a stainless steel pressure cell (5, 20) with a Lucite window at one end. Excitation light was lead to the sample through fiber optic bundles from an EG&G FX224 xenon flash lamp operated at 0.7 kV with a 10 $\mu$F
discharge capacitor resulting in a pulse width at half height of 4 μs. The flash intensity was measured with a Schottky photodiode detector (United Detector Technology, Inc.). Using 450 nm as the average wavelength of the xenon flash, the emission was calculated to be $2 \times 10^{15}$ photons per pulse. For all O₂ measurements, the Pt electrode was at a potential of −0.7 V with respect to the Ag/AgCl electrode. For each experiment, the electrodes were polarized after the sample had dark-adapted for 4 to 5 min. Generally, 2 to 3 min more were required to obtain a nearly constant baseline on the signal averager. The baseline reached in the dark with an applied potential of −0.7 V represents the current flow due to O₂ reduction at the Pt electrode (21). O₂ reduction current was measured as the voltage across a 1 kΩ series resistor and recorded on a Tracer Northern Model TN 1710 signal averager and a Moseley 7001 AR X-Y recorder. The signal averager was triggered simultaneously with the first flash by a Hewlett Packard 3301B function generator and 20 to 30 flashes were given to the sample at 3.3 Hz. The most distinct four-step O₂ pattern was observed at this frequency.

Fresh samples of Ulva sp. were collected from Brockton Point in Stanley Park, Vancouver, B.C., and maintained in an aerated seawater tank at 15°C with a 16 h photoperiod, using artificial light provided by cool-white fluorescent tubes with intensity of 10 W m⁻². All experiments were carried out at room temperature. If a sample was used for a second measurement, it was allowed to dark-adapt for 5 min, which was sufficient for good reproducibility.

Total Chl content per algal sample was measured in 80% acetone using the method of Arnon (1).

RESULTS AND DISCUSSION

The diffusion of O₂ through the electrolyte, the dialysis membrane, and the respiring algal sample to the Pt electrode occurs in response to an O₂ concentration gradient. O₂ produced by the sample in the light can diffuse through the dialysis membrane into the sample holder, but mainly diffuses toward the Pt electrode, where the O₂ concentration is small due to its continuous reduction. The change in the current due to O₂ reduction following each light pulse, and the constant maximum slope of the rising O₂ signal, are both measures of the change in O₂ exchange by the alga. Plotted as a function of flash number, both of these yield a damped four-step oscillatory pattern of O₂ exchange.

Evolution of O₂ by algae in response to a flash occurs on a time scale comparable to the duration of the flash. Dekker et al. (6) have shown by means of UV flash-induced absorbance changes that the half-time for the $S₁ → (S₀ → S₁)$ transition is 1300 μs, thus within a few ms after the onset of the flash, no more O₂ is evolved by the plant. The resultant O₂ curve for each flash simply indicates the rate at which O₂ is reduced at the Pt cathode from the onset of a flash to the beginning of the next flash. This O₂ reduction is dependent on the ambient O₂ in the sample holder. If no successive flash is given, the O₂ curve will decay back to the original baseline. However, with a 3.3 Hz flash frequency, the rate of O₂ reduction at the cathode does not stabilize after each flash and the baseline steadily increases due to a "pile-up" of the individual O₂ pulses. To eliminate the effect of the increasing ambient O₂ concentration on the O₂ reduction rate at the cathode, each O₂ pulse was corrected for the O₂ evolved from the previous flash. However, this correction does not account for O₂ evolved by the plant which is consumed in a PSI or PSII reaction without diffusing to the Pt electrode.

O₂ exchange (evolution and uptake) in Ulva as a function of flash number at 3.3 Hz flash frequency is shown in Figure 1. The linear four-step flash yield sequence may be deduced by plotting the change in O₂ current due to each light pulse. For example, point C denotes the rate of O₂ being reduced at the cathode at the beginning of the fourth flash, whereas point D denotes the maximum rate of O₂ reduction at the cathode for this flash. The difference $D - C = 0.49$ μamp is the change in O₂ current due to O₂ exchange on the fourth flash. However, it is preferable to extrapolate the actual maximum rate of O₂ reduction at the cathode for a given flash by correcting for the decay of the O₂ signal due to the preceding flash. For example, in Figure 1, the decay of the O₂ signal after the third flash is approximated by the line CE, where point E is the extrapolated value of the residual O₂ from the third flash at the time of the maximum O₂ signal from the fourth flash (point D). The difference $D - E$, which is 0.69 μamp in Figure 1, is the O₂ signal from the fourth flash after accounting for the residual O₂ from the third flash. This difference was calculated for each flash in the sequence and plotted in Figure 2. Actual decay curves were taken for each pulse in the sequence, and in all cases the linearly extrapolated decay for a given pulse (e.g. line CE) is the same within 0.5% as the measured decay to the point indicating residual O₂ for the following flash (e.g. point E).

To facilitate comparison between our results and those of other researchers, all values of O₂ per flash were normalized to the difference $B - A = 0.90$ μamp for the third flash in Figure 1. It has been well established that flash yield sequences obtained with intact algae are notably more damaged than those with chloroplasts, however, our O₂ exchange data for Ulva show good agreement with that of Forbush et al. (10) and Joliot et al. (13) for chloroplasts as shown in Figure 2, but is lowered in comparison to that of Diner (9) with Chlorella. A similar, damped four-step oscillation has also been obtained by measuring UV flash-induced absorbance changes in PSII membranes (7). This oscillation has been attributed to the reduction of Mn^{IV} ions in the O₂-evolving complex to Mn^{III} during the 1 ms $S₁ → (S₀ → S₁)$ transition. This flash yield sequence represents $S₁$ state turnover but is independent of electrode/O₂ exchange measurements. This sequence gives a fit to the Kok model with initial distribution of $S₀ = 25\%$, $S₁ = 75\%$, and with 9% misses and 9% double hits on all transitions.

To determine the magnitude of the uptake component of O₂...
exchange, photosynthetic water-splitting was inhibited by DCMU, which prevents the oxidation of $Q_t$ (the first stable electron acceptor in PSII) by electron transport. This produced a large $O_2$ uptake as shown in Figure 3 for varying flash frequencies, but inhibited $O_2$ evolution. This uptake occurs either during the 4 $\mu$s flash or in the subsequent dark interval. This uptake appeared earlier and was larger for higher flash frequencies. The uptake at 3.3 and 6.6 Hz reached its maximum rate in less than 2 s after the first flash, which coincides with the positions of the fifth and sixth flashes at 3.3 Hz, and the ninth and tenth flashes at 6.6 Hz. Since the amplitude of the uptake is approximately inversely proportional to flash frequency, and thus proportional to total light intensity, it appears that this uptake reflects the oxidation of a limited pool of reductants, most likely plastoquinones and other intersystem carriers, by PSI.

The results of the DCMU experiments suggest that the first minimum in the $O_2$ flash yield in Figure 2 is not only due to a four-step $O_2$ evolution, but contains a superimposed $O_2$ uptake component. This light-driven $O_2$ uptake which is not inhibited by DCMU has been associated with PSI activity (24, 27). The $O_2$ uptake in Figure 3 is clearly a PSI photoreaction since $O_2$ uptake associated with PSII would be suppressed by DCMU. This $O_2$ uptake during flash illumination has been shown to exist in tobacco chloroplasts (25) and was greatly enhanced by the presence of an exogenous electron acceptor such as 2,6-benzoquinone or ferricyanide.

Because of the magnitude of this DCMU-mediated $O_2$ uptake, it seems reasonable that an analysis of the $O_2$ exchange curve in terms of the $S$ state hypothesis can not be concerned solely with $O_2$ evolution, but must consider $O_2$ uptake as well. $O_2$ consumption has been shown to occur in both PSI and PSII and thus it is unlikely that the $O_2$ uptake will be exactly the same with PSII active or inhibited. As well, flash yield sequences obtained with PSII membranes containing exogenous electron acceptors may not be equivalent to $O_2$ evolution which occurs when both photosystems are functional. However, since the PSI $O_2$ uptake is most likely larger than that of PSII, a first approximation to actual $O_2$ evolution would be to determine the magnitude of the $O_2$ uptake and "correct" for this effect by adding it to the $O_2$ exchange curve. This results in an $O_2$ evolution curve which is shown in Figure 4a along with the extended $O_2$ exchange curve of Figure 1 for comparison. The $O_2$ evolution curve can then be plotted in terms of the $O_2$ yield as a function of flash number as described for Figure 2. This is shown in Figure 4b and compared with the uncorrected $O_2$ exchange flash yield sequence of Figure 2. The theoretical $O_2$ evolution flash yield is larger than that of
the evolution may be sufficient damping was evidenced for a four-step sequence familiar in the and Sciences programs (1).  

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