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Regular paper

Photosystem II electron transfer cycle and chlororespiration in planktonic diatoms

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Abbreviations: DCCD – N,N'-dicyclohexyl-carbodiimide; NPQ – non-photochemical fluorescence quenching; PS I and PS II – Photosystem I and II; Q_A – primary quinone acceptor of PS II; S_0 to S_3 – oxidation states of the water evolving complex; Y_D – tyrosine D of the PS II reaction center responsible for EPR signal II slow; Y_{SS} – steady-state oxygen yield per flash; Y_Z – tyrosine Z of the PS II reaction center secondary electron donor

Abstract

The dominance of diatoms in turbulent waters suggests special adaptations to the wide fluctuations in light intensity that phytoplankton must cope with in such an environment. Our recent demonstration of the unusually effective photoprotection by the xanthophyll cycle in diatoms (Lavaud et al. 2002) also revealed that failure of this protection led to inactivation of oxygen evolution, but not to the expected photoinhibition. Photo-oxidative damage might be prevented by an electron transfer cycle around Photosystem II (PS II). The induction of such a cycle at high light intensity was verified by measurements of the flash number dependence of oxygen production in a series of single-turnover flashes. After a few minutes of saturating illumination the oxygen flash yields are temporarily decreased. The deficit in oxygen production amounts to at most 3 electrons per PS II, but continues to reappear with a half time of 2 min in the dark until the total pool of reducing equivalents accumulated during the illumination has been consumed by (chloro)respiration. This is attributed to an electron transfer pathway from the plastoquinone pool or the acceptor side of PS II to the donor side of PS II that is insignificant at limiting light intensity but is accelerated to milliseconds at excess light intensity. Partial filling of the 3-equivalents capacity of the cyclic electron transfer path in PS II may prevent both acceptor-side photoinhibition in oxygen-evolving PS II and donor-side photoinhibition when the oxygen-evolving complex is temporarily inactivated.

Introduction

Although marine diatoms contribute substantially to global primary production (van den Hoek et al. 1995), their bioenergetics has received relatively little attention. Diatoms often dominate the phytoplankton in turbulent waters (Harris 1986), where vertical mixing continuously exposes them to large fluctuations in light intensity on time scales of minutes to days (Lewis et al. 1984). They must be able to survive long periods of darkness when carried down by deep mixing as well as short periods of full sunlight when they hit the surface. They have indeed been shown to survive many days of darkness without carbon assimilation (Griffiths 1973) and to cope with a wide variation in light intensity (Richardson et al. 1983). Their main protective mechanism against over-excitation appears to be non-photochemical quenching of the chlorophyll excited state (NPQ) (Ting and Owens 1993) associated with xanthophyll de-epoxidation (Arsalane et al. 1994; Olaizola et al. 1994). By a quantitative study of the quenching by NPQ, both with open (Q_A oxidized) and with closed (Q_A reduced) photosystem II (PS II) reaction centers, we recently demonstrated that the xanthophyll cycle provides the planktonic diatom *Phaeodactylum tricornutum* with a unusually effective and adaptable mechanism to down-regulate PS II activity at high light intensity (Lavaud et al. 2002). Illumination for an hour at a light intensity corresponding to full sunlight (50 times the intensity at which the cells were grown) was required to inactivate a large fraction of PS II, as measured by the yield of O_2 production upon illumination by repetitive single-turnover flashes. Surprisingly, the chlorophyll fluorescence yield increase associated with photoreduction of Q_A was not affected. When the oxygen evolving complex is not functional, charge separations in PS II would be expected to lead to rapid photo-oxidative destruction, either directly by the strong oxidants resulting from charge

separation or indirectly by charge recombination to the triplet state, leading to singlet oxygen production (Aro et al. 1993). The only likely way to avoid that would seem to be an electron transfer cycle around PS II.

Cyclic electron flow around PSII, presumably via cytochrome b_{559} (for review see Whitmarsh and Pakrasi 1996), has been suggested earlier as a photoprotection mechanism that could retard both acceptor and donor side photoinhibition (Nebdal et al. 1992; Barber and De Las Rivas 1993). Such a cycle was shown to occur *in vivo* in the green alga *Chlorella pyrenoidosa* at high light intensities (Falkowski and Kolber 1986). Prasil et al. (1996) studied its expression in a temporary decrease of O_2 production by saturating flashes during the first tens of seconds after a light to dark transition, and concluded that the cycle occurs as long as the plastoquinone pool is reduced.

In order to verify the possible induction of an electron transfer cycle in *Phaeodactylum tricornutum* at high light intensity, we have also investigated the flash number dependence of the O_2 yields in a series of single turnover flashes. For dark-adapted cells, these yields initially show a period 4 oscillation with flash number due to the 4-state oxidation cycle of the O_2-evolving complex (Joliot et al. 1969; Kok et al. 1970). To produce an O_2 molecule, 4 consecutive charge separations in the same PS II reaction center are required, each raising the oxidation state ('S-state') by one equivalent. S_0 and S_1 are stable, but the higher S-states decay to S_1 in a few minutes darkness, resulting in an oscillating O_2 yield with maxima on flash numbers 3, 7, etc. The oscillation is damped in a few cycles to a constant steady state yield, Y_{SS}, due to loss of synchrony caused mainly by a non-zero probability of 'misses', usually near 10 %. The oscillation pattern is extremely sensitive to the miss probability and would

change significantly already if only a few percent of the electrons would come from a cyclic electron transfer pathway rather than from water oxidation.

After exposure to high light intensity a very high miss probability that disappeared in approximately 15 flashes, quite similar to that reported by Prasil et al. (1996) for *Chlorella*, was indeed observed. In *Phaeodactylum* and other planktonic diatoms, it could still be measured after very long dark times and its properties suggest a somewhat different interpretation, but nevertheless provide evidence for a photoprotective electron transfer cycle around PS II at high light intensity.

Materials and methods

Cultures

Phaeodactylum tricornutum Böhlin and other marine diatoms: *Cylindrotheca fusiformis*, *Cylindrotheca cryptica* and *Navicula* sp. were grown photoautotrophically at 18°C in sterile natural seawater F/2 medium (Guillard and Ryther 1962) in 300 mL batches, continuously flushed with sterile air (except for *Navicula* sp. which is a benthic species)and illuminated at 40 $\mu E.m^{-2}.s^{-1}$ by white fluorescent tubes in a 16 h light - 8 h dark cycle. The cells were harvested in exponential phase, resuspended in their culture medium at 10 µg Chl *a*.mL^{-1}, and kept continuously stirred under low light at 18°C until use. All measurements were done at 18°C. Before measurement 4 mM sodium bicarbonate was added.

Measurement of oxygen evolution

O_2 production on illumination by a series of short (5 µs) saturating flashes (Strobotac, General Radio) fired at 2 Hz was measured polarographically by a rate electrode (Joliot and Joliot 1968) as described (Lemasson and Etienne 1975), after 7 min settling of the

cells in the dark. For the experiments shown by the diamonds in Figure 3A, the setup was modified to allow saturating illumination via an optic guide that could be removed within a few seconds before the flash series. Illumination was provided by a 150 W quartz-iodine lamp through a heat filter. The O_2 deficit was quantified as 20 x Y_{SS}, the steady-state flash yield, minus the sum of signals in a 20-flash series, expressed in units Y_{SS}. The contribution to the deficit resulting from normal S-state deactivation during dark-adaptation was corrected for by subtraction of the deficit in a non-illuminated sample.

Fluorescence yield measurements

Chlorophyll fluorescence yield changes were recorded by a modified (Ritz et al. 1999) PAM 101 module (Walz). The cells were dark-adapted for 20 min before actinic illumination of adjustable intensity, as measured by a PAR sensor (Licor), and the development of NPQ was probed by 600 ms pulses of 4 $mE.m^{-2}.s^{-1}$ white light from a quartz-iodine lamp admitted by an electronic shutter (Vincent Uniblitz). NPQ was calculated as $(F_m-F_m')/F_m'$ (Krause and Weis 1991).

Results

Fig.1A First mention

Typical O_2 flash yield measurements are shown in Figure 1a. *Phaeodactylum tricornutum* cells were allowed to settle in darkness on a rate electrode and the polarographic current changes induced by a series of single-turnover flashes were recorded. The dashed line shows the classical period four oscillation of the O_2 yield with flash number due to the 4-step redox cycle of the O_2-evolving complex (Joliot et al. 1969; Kok et al. 1970). The damping to a constant steady-state yield, Y_{SS}, results from a normal probability of about 10 % that a photoreaction fails to contribute to H_2O

oxidation. Early in the sequence this 'miss probability' was much higher in cells that had been exposed to saturating light for 5 min, but it returned to 10 % during the flash series so that Y_{SS} was the same (solid line). This phenomenon is better visualized by plotting the sums of 4 consecutive flash yields in order to remove most of the oscillatory pattern (Figure 1b). The solid symbols show that it took about 15 flashes before the normal O_2 yield was fully recovered in the illuminated sample.

Fig.1B First mention

We quantify the oxygen deficit as 20 Y_{SS} minus the total oxygen production of the 20 flashes and subtract the O_2 deficit of the non-illuminated sample, which is 0.8-1.0 Y_{SS}. This is expected because the higher S-states are reduced to S_1 in the dark, so the average number of stored oxidizing equivalents is expected to decrease from approximately (0+1+2+3)/4 = 1.5 in the steady state to (0+1+1+1)/4 = 0.75 after dark-adaptation. The difference of 0.75 equivalents should cause a deficit of 0.83 Y_{SS}, taking the 10 % miss probability into account. It will be somewhat larger because the miss probability and hence the steady state S-state concentration is higher for high S-states than for low S-states (de Wijn and van Gorkom 2002). The deficit in the illuminated sample in Figure 1 was higher than that in the non-illuminated sample by 2.8 Y_{SS}, or 2.5 electrons per PS II. The shape of the oscillation pattern does not suggest a phase delay by 3 flashes but rather an increased miss probability that gradually disappears in 15 flashes. From our previous study (Lavaud et al. 2002) we know that the large extra miss probability during the first S-state cycle in these conditions cannot be attributed to a non-saturating flash energy or to the presence of closed PS II centers. It appears, therefore, that the O_2 deficit is due to the presence of reductants that can compete with water as an electron donor to PS II. Decreasing the flash frequency did not change the deficit, however. Similar data were obtained with other planktonic marine diatoms from

turbulent coastal waters of the Northern Atlantic (*Cylindrotheca fusiformis* and *Cyclotella cryptica*), but not or much less with a benthic diatom (*Navicula* sp.), a green alga (*Chlorella fusca*) and a cyanobacterium (*Synechococcus* sp.). The duration and intensity of illumination producing the maximum O_2 deficit were rather different for the different species but it was for the planktonic diatoms that the O_2 deficit was the highest.

The O_2 deficit was induced by conditions similar to those that elicit NPQ, suggesting a connection to photoprotective mechanisms, but there are clear differences in detail. Figure 2a shows that it appeared earlier and continued to rise longer. At much longer times, while NPQ continues to increase slowly (Lavaud et al. 2002), Y_{SS} and the O_2 deficit decreased in parallel (Table 1), i.e. the deficit per active PS II remained unchanged. This observation indicates that the electron donors responsible for the O_2 deficit are intrinsic components of PS II and not a common pool accessible to all remaining active PS II centers. Figure 2b shows the relation between O_2 deficit and NPQ when their amplitude was varied using either different light intensities (solid symbols) or different concentrations of an uncoupler (NH_4Cl) (open symbols). Presumably both conditions affect NPQ primarily via the extent of the steady-state pH gradient maintained during illumination. When the pH gradient is lowered by decreasing the electron transport rate (using a lower light intensity) or by uncoupling (which probably increases the electron transport), a similar decrease of O_2 deficit with decreasing NPQ is seen. Therefore the deficit appears to depend on the pH gradient, but not in the same way as NPQ: a deficit is detectable already at a pH gradient too small to induce NPQ. Low concentrations of N,N'-dicyclohexyl-carbodiimide (DCCD) enhanced NPQ but decreased the deficit (Table 1). DCCD can have multiple effects but

Fig.2A First mention

Tab.1 First mention

Fig.2B First mention

since its most obvious effect at low concentration is to inhibit the dissipation of the pH gradient by the ATP-synthase (McCarty and Racker 1967; Shoshan and Selman 1980), these results suggest that ATP synthesis is required for induction of the O_2 deficit.

Fig.3A First mention

After illumination, the size of the O_2 deficit changed in complicated ways (Fig. 3a). The open symbols in panel A show that recovery from NPQ was largely complete in 5 min, independent of illumination time. Decay of the O_2 deficit (solid symbols) was much slower and depended strongly on illumination time. Two components can be distinguished: a rapid decay starting after a lag time that also depended on illumination time, followed by a much slower component. Addition of DCCD after illumination led to a slower decay of the O_2 deficit and to an increase of NPQ after its initial post-illumination decrease (not shown). The first point of these O_2 data is at 7 min after illumination, the time required for settling of the cells on the electrode. The diamonds represent a light treatment equivalent to that for the circles but which was applied directly on the rate electrode. These data show that the deficit was fully developed immediately after illumination and did not decrease during a half-hour in the semi-anaerobic conditions on the electrode.

Fig.3B First mention

Figure 3b shows the O_2 deficit as a function of dark time after a fixed illumination time of 5 min at different light intensities (solid symbols). Here a comparison is made to the rate of O_2 consumption (open symbols, dashed lines), revealing a rather similar pattern. These data were obtained from O_2 concentration measurements by a Clark electrode; a typical trace is shown in the inset. Most or all of the O_2 and hence of the photosynthetic products made during the high intensity illumination are consumed again within tens of minutes after illumination. Apparently the size of the O_2 deficit, when below its maximum of 3 electrons/PS II, reflects the rate

of this consumption or the amount of photosynthetic products left. This suggests that the electrons reducing the PS II components responsible for the O_2 deficit in this case originated ultimately from the photosynthetic products generated during the light treatment. That would also account for the suppression of the O_2 deficit by DCCD when added before but not when added after illumination.

Figure 4 shows the kinetics of re-appearance of the O_2 deficit in a second flash series as a function of dark time, on the electrode, after it was removed by a first series of 20 flashes. Depletion of the electron source by (chloro)respiration is slow in the semi-anaerobic conditions on the polarized electrode and should not become limiting on this time scale (see Fig. 3a, diamonds). The deficit increased with a half time 2 min to the same value as in the first flash series. This is not shorter than the lifetime of the high S-states, which limits the minimum light intensity where PS II can function, and will not decrease the efficiency of photosynthesis in low light. The appearance of the deficit after strong illumination could not be resolved (diamonds in Figure 3a) and must be much faster than its measurement (a few seconds). This suggests that immediately after, and presumably during, high intensity illumination the time required for reduction of the PS II components responsible for the O_2 deficit must be at least several orders of magnitude shorter than 2 min and might well be short enough to play a photoprotective role.

Discussion

The large deficit that appears after saturating illumination in the O_2 yields of a series of single turnover flashes could not be attributed to a decreased flash yield of stable charge separation and shows that a substantial fraction of the charge separations did not

contribute to water oxidation. Therefore competition by an alternative electron donor had to be assumed. The O_2 deficit of up to 3 Y_{SS} is much larger than can be accounted for by possible changes in steady state S-state distribution or deactivation kinetics induced by high intensity illumination.

Cofactors involved

The proportional decrease of Y_{SS} and the deficit by long exposure to high light intensity shows that the alternative electron donors causing the deficit in O_2 production are probably endogenous PS II components that are not exchangeable between different PS II centers. Perhaps reduction of S_1 to S_0 might account for a fraction of the deficit, but it is not possible to attribute the deficit to an over-reduced 'S_{-2}' state of the oxygen evolving complex itself, because the oscillation pattern shows a decreased efficiency rather than a delay in O_2 production. The PS II reaction center contains several cofactors known to play a role as alternative electron donors in specific experimental circumstances, especially at cryogenic temperatures: β-carotene, Chl_Z, and cytochrome b_{559} (Butler et al. 1973; Visser et al. 1977; Schenk et al. 1982; Thompson and Brudvig 1988). Tyrosine D (Y_D) is the only PS II component that normally competes with, but does not completely out-compete, the electron transfer reactions leading to water oxidation. The high S-states can oxidize it in seconds (Babcock 1987), presumably via their redox equilibria with the secondary electron donor Y_Z and the primary electron donor P_{680}. Y_Z^{ox} can oxidize Y_D in a ms (Boussac and Etienne 1982) and this reaction competes with the 2 ms reduction of $Y_Z^{ox}S_3$ to Y_ZS_0 by water (van Leeuwen et al. 1993). Normally Y_D remains in its oxidized tyrosyl radical form during photosynthesis, but we found no literature data to confirm that for diatoms. If high light intensity would

enable its rapid reduction, e.g. via Cyt b_{559} and one other component, a cyclic electron transfer path would result that could explain both the O_2 deficit reported here and the lack of photo-oxidative damage when the oxygen evolving complex is inactivated (Lavaud et al., submitted).

Reduction of the alternative electron donors during illumination

In the conditions of Figure 4, the reduction rate of the alternative electron donors is approximately 1 equivalent per PS II per minute. With an oxidation rate near 1 equivalent per S-state cycle and a reasonable light-saturated rate of O_2 evolution of the order of 10 S-state cycles per PS II per second, the alternative electron donors should be in their oxidized state during saturating illumination. The O_2 deficit measured in seconds after illumination, however, shows that the alternative electron donors were largely in the reduced state. At high light intensity their oxidation is unlikely to be slower, so their reduction must be very much faster than in the dark.

Electrons reducing the endogenous PS II components that act as alternative electron donors would be expected to come from Q_A, Q_B, or the plastoquinone pool. The steady state reduction level of the plastoquinone pool remains unusually low on illumination of *P. tricornutum*, in spite of normal antenna sizes and relative amounts of PS I and PS II (Owens 1986; Caron et al. 1987) and the absence of a 'state 1 - state 2 transition' (Owens 1986). This would be consistent with an electron transfer cycle around PS II that, at saturating light intensity, is not slow compared to linear electron flow to PS I. A time constant of the order of 10 ms would be indicated, long enough not to interfere with commonly used methods to determine the extent of photochemical quenching, but short enough to prevent acceptor side photoinhibition (Nebdal et al.

1992) and keep some of the alternative electron donors in the reduced state. Those would decrease the quantum yield of O_2 evolution substantially and would also account the non-linearity between O_2 evolution and photochemical quenching at high light intensity that Geel et al. (1997) attribute to a redox state dependent Mehler-type O_2 reduction.

Reduction in the dark

Prasil et al. (1996) studied a similar post-illumination decrease of O_2 flash yields seen in the green alga *Chlorella pyrenoidosa* on a time scale of seconds, which they attributed to a Cyt b_{559}-switched cyclic electron transport around PS II. The main difference with our data seems to be the absence of period 4 oscillations due to the absence of a dark-adaptation time between illumination and flash series. The recovery to normal values after illumination was attributed to a decreasing number of centers in which the cycle was switched on and was related to the oxidation of the plastoquinone pool. Indeed a faster recovery of the oxygen yield per flash during the flash series was observed with background illumination by far-red light that selectively excited PS I, and a slower recovery was obtained in anaerobic conditions. Measurements at a different flash spacing than 2.5 s were not reported, but would not be expected to show a much different oxidation time of the pool in this case.

The O_2 deficit in *P. tricornutum* did not depend on flash frequency, so the time required to recover Y_{SS} yields was proportional to the spacing of the flashes. Apparently it is the flashes that cause the recovery, not the time spent between them. If the deficit in O_2 production is attributed to cyclic electron transport during the flash series, as proposed by Prasil et al. (1996), it implies a temporary shortage also in reduction of the

plastoquinone pool by PS II compared to its oxidation by PS I. So the flash series itself might indeed cause some oxidation of the pool if it was in the reduced state. Since the deficit accounts for at most 3 electrons/PS II, however, an unlikely small pool size or PS II/PS I ratio would have to be assumed to explain the recovery of normal Y_{SS} yields by PS I-induced oxidation of the total plastoquinone pool. If only an arbitrary fraction of the pool was involved an invariable maximum O_2 deficit of 3 Y_{SS} would not be expected. We conclude that cyclic electron transfer during the flash series is unlikely to explain our results.

After a few minutes darkness, the reduction of the alternative electron donors appeared to depend on the total pool of reducing equivalents accumulated by water oxidation during the preceding illumination. After saturating illumination an accelerated O_2 uptake was observed that consumed approximately the same amount of O_2 as had been produced in the light (Fig. 3b). The diatoms are evidently unable to use the temporary >10-fold excess of light, compared to the light intensity at which they were grown, for net carbon fixation on this time scale. Instead, they seem to use the accumulated reducing equivalents as fuel for subsequent (chloro)respiratory ATP synthesis in the dark. Such a process could extend the useful dynamic range of photosynthetic electron transport. This could be an important advantage at fluctuating light intensity, e.g. in allowing carotenoid biosynthesis to proceed (Bennoun 2001; Carol and Kuntz 2001). We have shown earlier that the capacity of photoprotection by NPQ in *P. tricornutum* is proportional to the diadinoxanthin content of the cells.

A temporary storage of excess reductant for later use was recently proposed to be the function of the bi-directional hydrogenase in a cyanobacterium (Appel et al. 2000). H_2 seems in fact the most, if not the only, likely reductant to have direct access to the

donor side of PS II and cause an O_2 deficit. The electron source involved here cannot be H_2, however, since it was not washed from the sample on the rate electrode. Instead, the reducing equivalents are presumably accumulated in the form of reduced carbon compounds produced by the Calvin cycle or C_4 metabolism (Reinfelder et al. 2000) and should have no access to the donor side of PS II. The electron transfer pathway reducing the alternative electron donors in PS II is probably very indirect in this case.

When added after illumination, DCCD caused not only to a slower decay of the O_2 deficit but also an increase of NPQ after its initial post-illumination decrease. This indicates that the oxidation of the accumulated photosynthetic products takes place at least in part in the chloroplast via the chlororespiration coupled to ATP synthesis reported earlier (Caron et al. 1987; Ting and Owens 1993; Jakob et al. 1999). Chlororespiration is thought to proceed via the plastoquinone pool (Bennoun 2001), so in principle the same electron transfer pathway from plastoquinol to the PS II donor side could be involved in the dark as well as in the light, only at a 10^4 times slower rate. This rate difference is much too large to be attributed merely to a lower steady state plastoquinol concentration if chlororespiration can build up a sufficiently large pH gradient to induce substantial NPQ in the dark. An effective switching mechanism must exist to ensure that the cycle operates only at high light intensity. Cyt b_{559} has often been postulated to play a role in such a switching mechanism (Thompson and Brudvig 1988; Nebdal et al. 1992; Allakhverdiev et al. 1997).

Concluding remarks

We have presented evidence for the induction of an electron transfer cycle around PS II in planktonic diatoms at high light intensity. With an estimated rate of 10^2

s^{-1} at saturating light intensity acceptor-side photoinhibition due to over-reduction of Q_A is prevented and the cofactors involved in the cycle are kept largely in the reduced state that competes with water as an electron donor. This is tentatively attributed to electron transfer from Y_D to Y_Z^{ox} that competes with the 2 ms reduction of $Y_Z^{ox}S_3$ to Y_ZS_0 by water and that will also prevent photo-oxidative damage when the O_2-evolving complex is inactivated. The fact that we can still see the competition long after the cycle has been shut down is attributed to a very slow, physiologically irrelevant leak of electrons from the chlororespiratory pathway in the dark to the cofactors responsible for the PS II electron transport cycle at high light intensity.

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Figure legends

Figure 1. (a) Oxygen production in a series of single-turnover flashes by dark-adapted cells (dashed line) or after 5 min saturating illumination (450 $\mu E.m^{-2}.s^{-1}$) in the PAM vial (solid line). In panel (b) the sums of 4 successive flash yields are plotted versus flash number to reveal the gradual decrease of the miss probability in the light-treated cells.

Figure 2. (a) Induction of the O_2 deficit (circles) during saturating illumination in the PAM vial, compared to that of non-photochemical quenching (triangles). Panel (b): relationship between the O_2 deficit and NPQ were lowered by increasing the light intensity used during a 5 min illumination (50, 150, 250, 350, 450 $\mu E.m^{-2}.s^{-1}$ from left to right, solid symbols) or by adding to the cells increasing concentrations of NH_4Cl before the dark incubation (3.5, 2.5, 1, 0 mM, from left to right, open symbols).

Figure 3. O_2 deficit (solid symbols) as a function of time after illumination. Panel (a): Illuminated for 1, 5, or 15 min (squares, circles, triangles) in the PAM vial at an intensity of 450 $\mu E.m^{-2}.s^{-1}$, NPQ measured (open symbols) and samples taken 7 min before O_2 measurement to settle on the O_2 electrode. Diamonds: as circles but illuminated on the electrode. Panel (b): Illuminated for 5 min at 70 (diamonds), 250 (triangles) and 450 $\mu E.m^{-2}.s^{-1}$ (circles). Open symbols: O_2 consumption (μmol O_2.mg Chl $a^{-1}.h^{-1}$) measured with Clark electrode. Inset: typical trace of O_2 concentration measurements by a Clark electrode before, during (between the arrows) and after a 5 min illumination at 450 $\mu E.m^{-2}.s^{-1}$. The zero point is arbitrary (offset substracted).

Figure 4. O_2 deficit as a function of dark time on the electrode after its removal by a first flash series (which had the deficit shown by the open circle at 7 min after saturating

illumination). It is initially negative due to the lower oxidation state of the O_2 evolving complex in the dark-adapted reference.

Table 1 Effects of saturating light on NPQ and O_2 flash yield

	NPQ	Y_{SS}	O_2 deficit
5 min Light	1.6 ± 0.1	0.98 ± 0.2	2.8 ± 0.2
60 min Light	2.5	0.69	1.7
DCCD + 5 min L	2.1	0.95*	1.5

Effects of illumination (in the PAM vial) at 450 $\mu E.m^{-2}.s^{-1}$ on 20 min dark-adapted *P. tricornutum*. NPQ: non-photochemical quenching expressed as $(F_m–F_m')/F_m'$. Y_{SS}: steady state O_2 flash yield measured after 7 min sedimentation on the electrode, normalized to non-illuminated control. DCCD was added at 10 µM at the start of the 20 min dark-adaptation. *DCCD decreased the dark-adapted Y_{SS} substantially. The O_2 deficit is given in units Y_{SS} of the non-illuminated control.

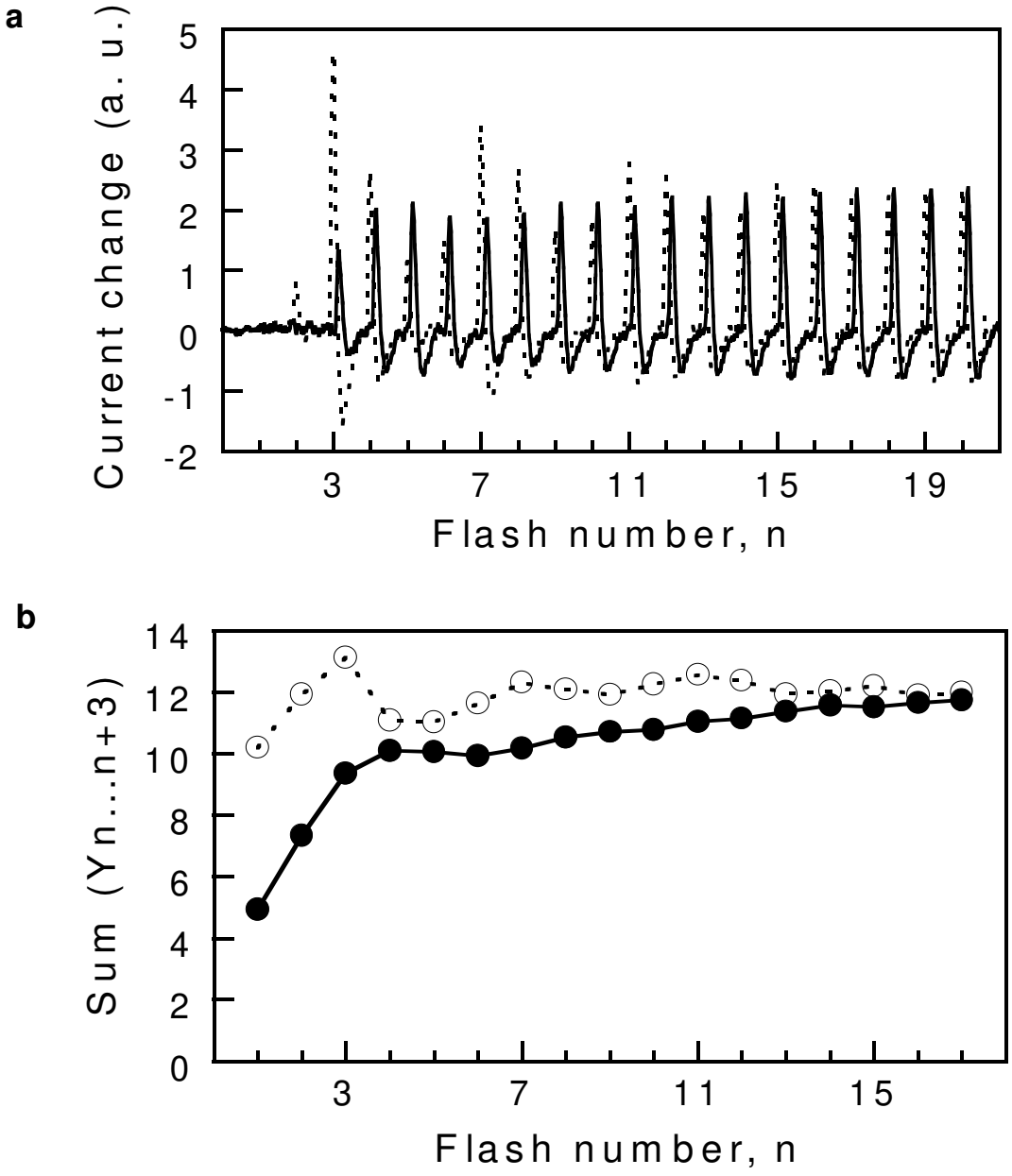

Figure 1
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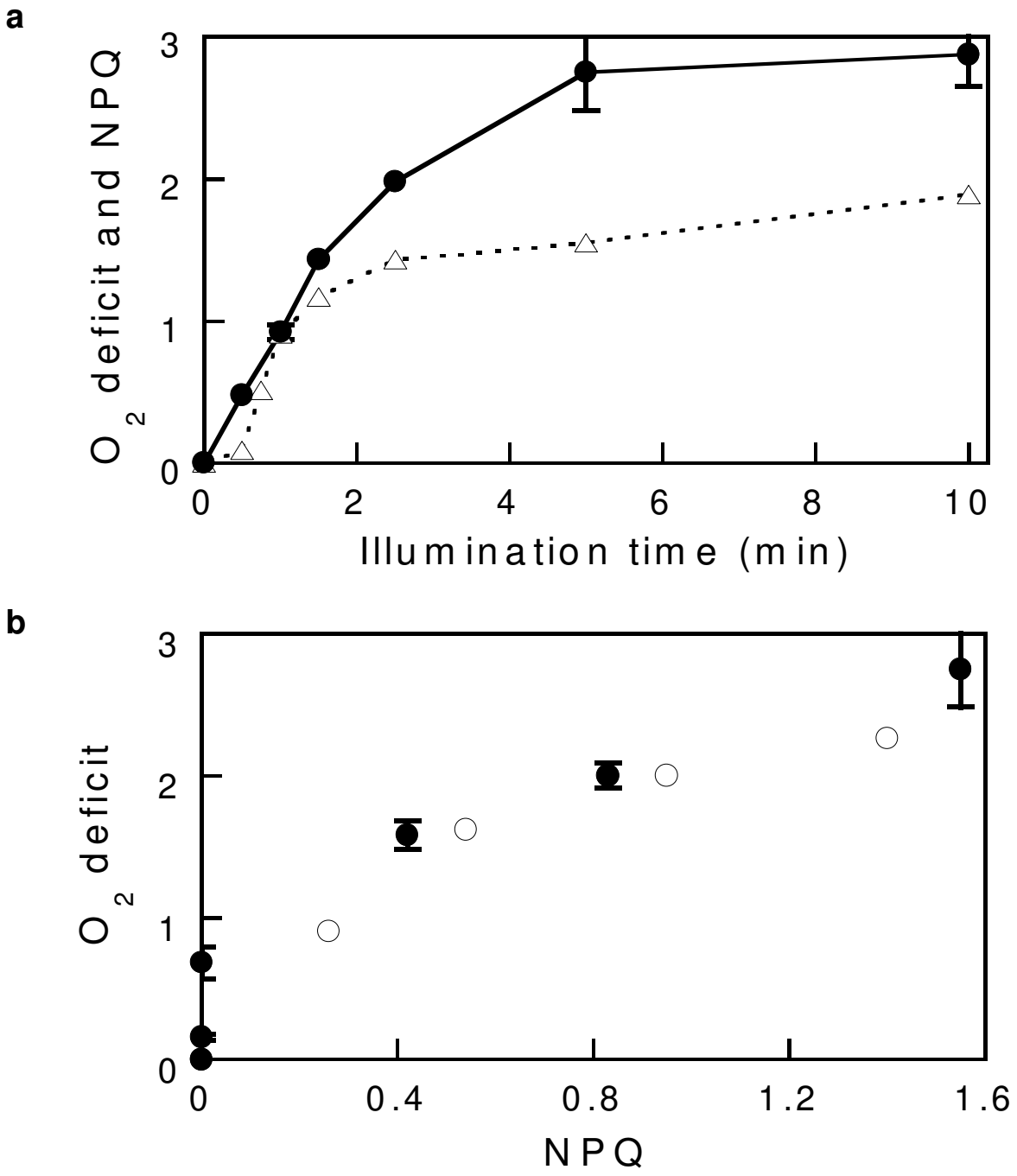

Figure 2
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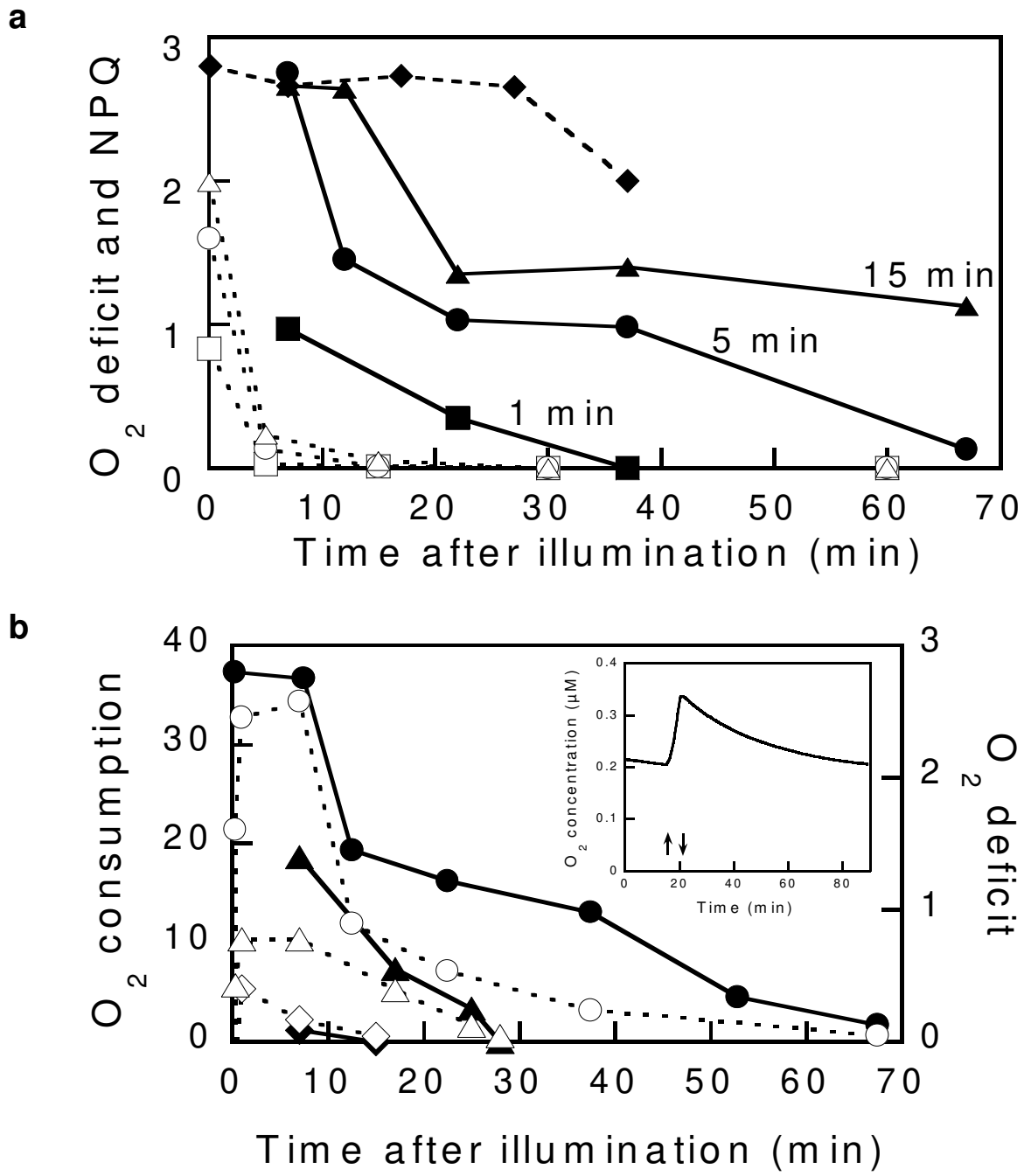

Figure 3
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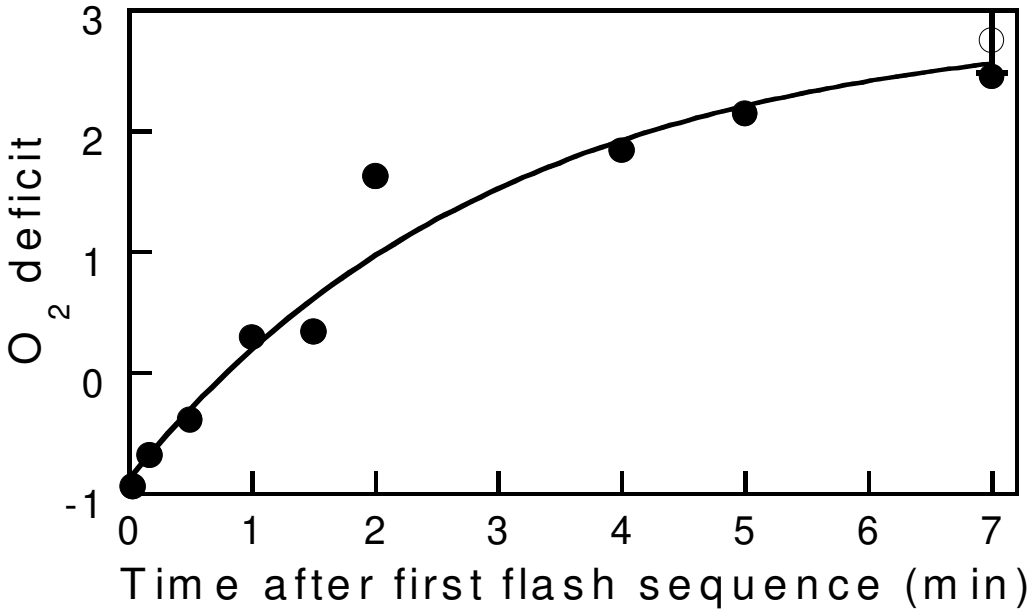

Figure 4
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