Characterization of the Light-Induced Oxygen Gas Exchange from the IC2 Deletion Mutant of *Synechocystis* PCC 6803 Lacking the Photosystem II 33 kDa Extrinsic Protein

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The absence of the extrinsic Mn-stabilizing 33 kDa protein in the IC2 mutant of *Synechocystis* PCC 6803 disturbs the redox cycling of the water splitting system and retards the formation of its higher S-states (I. Vass, K. Cook, S. Deak, S. R. Mayes, and J. Barber, Biochim. Biophys. Acta **1102,** 195–201 (1992)). We have performed analyses of the flash-induced oxygen exchange in the mutated cyanobacterium to clarify further the role of the 33 kDa protein. Under aerobic conditions, both the wild type and IC2 mutant show a relatively slow signal of oxygen rise on the first flash which is increased about twice by the addition of 10 μM DCMU and significantly diminished by lowering the oxygen concentration in the medium. According to action spectra measurements, this mode of apparent oxygen release is mediated by PS I and can be attributed to a light induced inhibition of respiratory activity. In contrast to the wild type, having the usual oxygen evolution flash pattern with a periodicity of four, the IC2 mutant shows a binary oscillation pattern of flash-induced respiratory oxygen exchange at a flash frequency 10 Hz, being dampened with DCMU or by a lower flash frequency (< 1 Hz). Oxygen evolution due to water splitting is clearly seen in the IC2 mutant when background far-red illumination is applied to saturate the signal due to respiratory inhibition, but a quadruple oscillatory component of flash-induced oxygen evolution appears only in the presence of artificial electron acceptors under partial aerobic conditions. The mutant possesses a higher PS I/PS II ratio compared to the wild type, as judged from both the flash-induced yields and quantum efficiencies of the steady-state rates of the oxygen exchange reactions. Estimates of antenna sizes indicate about a 20% decrease of optical cross-section at 675 nm of the PS II unit in IC2 mutants in comparison with the wild type. It is suggested that the absence of the 33 kDa protein leads to a modification of the PS II assembly and because of the slowing down of the S-state cycle, the rate of cyclic electron flow around PS II is enhanced. It seems that the absence of the 33 kDa protein in *Synechocystis* 6803 also disturbs energy transfer between adjacent PS II core complexes and may also alter their association with the phycobilisomes.

Introduction

Photosystem II (PS II) functions as a water-plastoquinone oxidoreductase [1–4]. The reduction of plastoquinone occurs within the reaction centre, consisting of the D1 and D2 proteins, while water oxidation involves a tetranuclear manganese cluster which sequentially stores positive charges as a result of electron donation to $P680^+$. $P680^+$ is the oxidized form of the primary donor of photosystem II and is also located in the D1/D2 heterodimer [5, 6]. The storage of oxidizing equivalents is necessary to catalyze the water splitting reaction giving rise to the oscillatory evolution of oxygen with a period of four [7]. The oscillatory pattern is observed by using flash excitation and can be explained by the S-state hypothesis [7].

In higher plants and eucaryotic algae, three extrinsic proteins are located on the donor side of PS II with apparent molecular masses of 33, 23, and 17 kDa. All three have been implicated in the water splitting reactions [4, 8]. However, the 23 kDa and 17 kDa proteins are not found in cyanobacteria and have been assigned roles involving the regulation of Ca^{2+} and Cl^- levels in or near

Abbreviations: D1, D2, product of the *psb*A and *psb*D genes, respectively, which are believed to form the heterodimer of the PS II reaction centre; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron); PS I, photosystem I; PS II, photosystem II; Q_A and Q_B, the primary and secondary quinone of PS II.

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the catalytic site [4, 8]. The 33 kDa protein, however, seems to be ubiquitous to all oxygenic organisms, including cyanobacteria, and is encoded by the *psb*O gene. From *in vitro* studies it has been concluded that this protein does not bind Mn but helps to stabilize the tetranuclear Mn cluster. Indeed, with isolated systems it is possible to remove the 33 kDa protein but maintain oxygen evolving ability as long as elevated levels of Ca^{2+} and Cl^- are present [9–12]. If the Ca^{2+} and Cl^- levels are, however, maintained at physiological levels then the Mn cluster is destabilized and oxygen evolution is readily inhibited.

More recently, mutants of the cyanobacterium *Synechocystis* PCC 6803, have been created which have had their *psb*O gene completely deleted [13–15]. These mutants are able to grow photoheterotrophically and evolve oxygen, indicating that the 33 kDa protein is not absolutely required for water splitting. Compared with wild type *Synechocystis* cells, the *psb*O-less mutant grew more slowly and evolved oxygen at a lower rate. Both fluorescence [13, 14] and thermoluminescence [16, 17] measurements indicated that the normal oxidation reactions of PS II in the mutant were perturbed in such a way as to slow down the advancement of the S-state cycle, particularly in the $S_3 \rightarrow S_0$ transitions [17]. In this paper we have investigated further the ability of the mutant to perform the S-state transitions by measuring oxygen yields in a series of saturating flashes.

Materials and Methods

Synechocystis PCC 6803 is a glucose-utilizing strain described in [18]. It was grown on agar plates consisting of either BG-11 [19] or Kratz-Mayers "C" [20] media supplemented with 5 mM glucose at 26–31 °C under continuous illumination of ~ 30 μE m^{-2} s^{-1} from day light fluorescent tubes. Liquid cultures (25 ml) were grown under the same conditions in 100 ml conical flasks. For photoautotrophic growth, the plates or flasks with the media free of glucose were placed in a hermetic glass box of about 10 litres volume with air +2% CO_2. The IC 2 mutant, lacking the *psb*O gene [15], was grown on agar plates with the same media supplemented with 5 mM glucose, in the presence of 25 μg ml^{-1} kanamycin to maintain the PS II lesion.

Light-induced oxygen gas exchange was measured with a bare-platinum electrode as described previously [21, 22]. A sample of 20 μl cell suspension, with a chlorophyll content of 0.2–0.3 μg, was placed on the 6 mm platinum disc in a groove, covered by cellophane membrane. The electrode chamber of ~ 30 ml was filled up with 50 mM Na-phosphate buffer and 50 mM KCl, pH 6.8, to the level of the membrane, retaining a free gas interspace over its surface with the given content of oxygen and carbon dioxide. In separate experiments with artificial electron acceptors added, we used a modified oxidant-proof electrode device, having an additional polyethylene membrane between the platinum electrode and a thin layer of the cell suspension. Samples were illuminated by either one or two beams from three light sources: a monochrometer, a "cool light" projection system through interference or cut-off filters with a light guide, and an ISS-100-3 M (U.S.S.R.) Xenon flash tube generating 1.8 μs flashes at a chosen frequency (up to 10 Hz) *via* a range of interference filters. The intensities of the light beams upon the electrode were varied by neutral density filters and measured by a calibrated thermoelement, RTN-20 S (U.S.S.R.), with correction for reflectance by the platinum. Polarographic signals were detected by a home-built amplifier at about 0.2 s time constant.

The number of chlorophyll molecules transferring excitation energy to the reaction centre of either PS II or PS I (antenna size) was determined from the light-saturation characteristics of specific signals from the photosystems following 1.8 μs flashes, according to previously described methods [23–25].

Chlorophyll concentration in N,N-dimethyl formamide extracts of the cyanobacterial cells was determined according to the equations of Inskeep and Bloom [26].

Results

Analyses of photoinduced oxygen gas exchange in cyanobacteria under low illumination is complicated by interaction of the photosynthetic and respiratory electron transport chains at the level of the plastoquinone (PQ) pool [27–32]. This leads to a light modulation of the rate of respiratory oxygen uptake superimposed on PS II mediated oxy-

gen evolution. This light-induced effect on respiration is due to PS I turnover and the consequential changes on the redox state of the PQ pool. Therefore, we selected conditions for the most complete separation of the different components of oxygen gas exchange in the wild type and IC2 mutant cells of *Synechocystis* 6803. Under aerobic conditions excitation of the dark adapted cells by a single microsecond flash would be expected to mediate only respiratory transients of oxygen gas exchange, since the water-oxidizing system produces a low level of oxygen on the first flash [7]. Fig. 1 shows the flash induced signals due to the inhibition of oxygen uptake in both the wild type and IC2 (note that the inhibition of oxygen uptake is observed as an increase in oxygen level induced by the flash). The magnitude and contour of the signals depended on growth conditions and physiological state of the cyanobacterium. After the addition of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which blocks the PS II-driven input of electrons to the plastoquinone pool, the integrated signal usually increased about twice (Fig. 1) (dotted lines). Similar flash triggered oscillations in the respiratory system have been observed in other cyanobacteria and green algae [33, 34]. On the other hand, when a continuous background illumination with far red light was given in order to saturate the decrease of the respiratory rate due to PQ oxidation, the same single flash excitation induced a rapidly relaxing signal of oxygen evolution due to water splitting by PS II (solid lines).

Fig. 2 shows the action of spectra for the "respiratory" and "photosynthetic" induced oxygen signals. As can clearly be seen, the former process is preferentially sensitized by long-wavelength pigment forms of chlorophyll belonging to PS I, while the action spectrum of the latter is characterized by a dominant contribution of phycobilin absorption which acts as the light harvesting system of PS II. Also worthy of note is that almost identical spectra were obtained for wild type und IC2 cells.

From the above results and discussions, the parameters of oxygen evolution and PS I induced inhibition of respiration may be used for quantitative analysis of functional units of PS II and PS I in the cyanobacterium. Integrated signals of the flash yield of these processes are therefore relative measures of the number of functionally active PS I and PS II reaction centres. The calculated parameters are given in Table I and reveal that, in comparison with wild type cells, there is a decrease of active PS II in the mutant cells by a factor of 2 to 5, which is accompanied by an increase (~20%) in active PS I for photoautotrophically grown cells. The data also indicate a PS I/PS II ratio of 2.5 to 4.0 in the wild type which increases dramatically to greater than 6 in the IC2 mutant.

Effective antenna sizes of photosynthetic units of PS I and PS II may be calculated from flash light saturation curves for the partial photoreac-

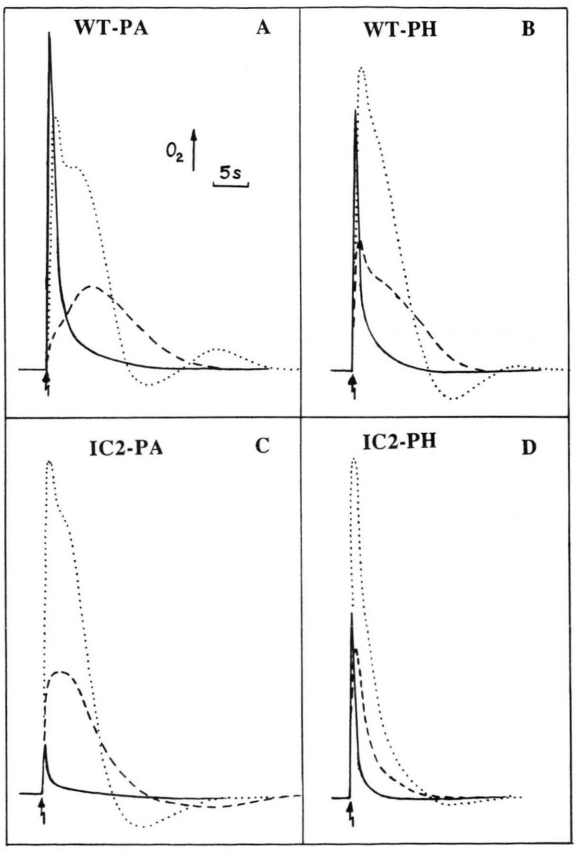

Fig. 1. Oxygen exchange transients induced by a saturating 1.8 μs flash in photoautotrophically (PA) or photoheterotrophically (PH) grown wild type and IC2 mutants of *Synechocystis* 6803. The data are normalized at flash onset. Flash-induced respiratory changes were recorded on dark-adapted cells without (dashed line) or with (dotted line) 10 μM DCMU in the assay medium of 50 mM Na-phosphate buffer and 50 mM KCl, pH 6.8, under air gas phase. Flash-induced oxygen evolution yield due to water splitting (solid line) in the same sample was recorded before the addition of DCMU on background of 2 W m^{-2} at 680 nm.

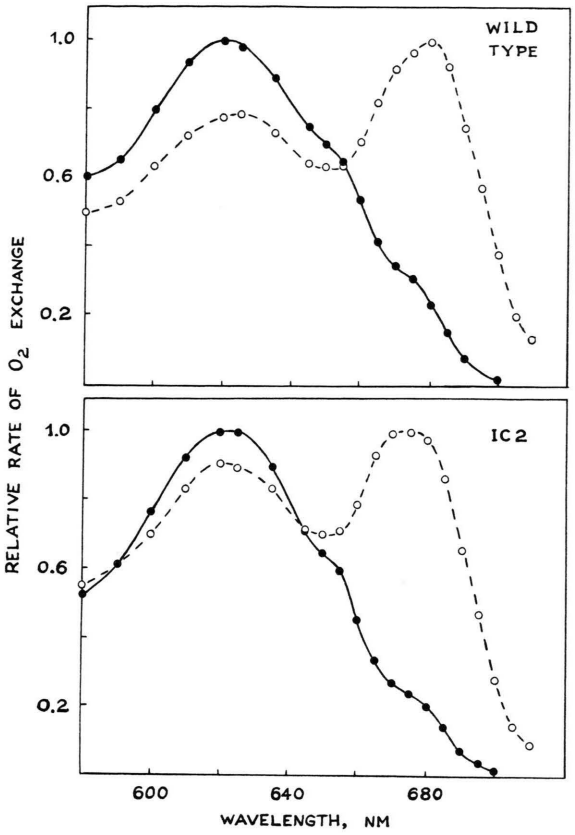

Fig. 2. Action spectra of light-induced oxygen exchange rates in photoheterotrophically grown wild type and IC2 mutant cells. The exciting monochromatic beam was <0.2 μE cm^{-2} s^{-1} with spectral halfband width of 2 nm. Oxygen evolution (closed circles) was measured on background of 2 W m^{-2} at 680 nm. Photoinhibition of respiratory rate (open circles) in the same sample was measured after the addition of 10 μM DCMU.

tions (Fig. 3) according to [25]. As shown in Table I, for photoautotrophically grown wild type and IC2 cells, the minimum photosynthetic units contained about 50 molecules of chlorophyll in both PS I and PS II complexes while the analysis of the data of Fig. 3 (see legend to this figure) indicates that the optimal cross-section for wild type PS II is about 20% greater than for IC2. It is possible that these apparent sizes correspond to distinct monomeric core complexes which do not transfer energy to neighbouring complexes. According to Rogner [35], PS I is extracted from *Synechocystis* 6803 as a monomer, in contrast to trimeric particles isolated from *Synechococcus*. On the other hand, the increase in the average size of PS II units in the wild type cells by about 20% may be due to energy migration within dimeric PS II core complexes, which are thought to be bound to

Table I. Photochemical parameters of the wild type and IC2 mutant of the cyanobacterium *Synechocystis* PCC 6803. Photosynthetic oxygen evolution (OE) and PS I-induced respiratory inhibition (RI) without and with 10 μM DCMU, respectively, were measured in the same sample with a platinum electrode in an assay medium of 50 mM Na-phosphate buffer and 50 mM KCl, pH 6.8. V is light-saturated rate of oxygen exchange, measured at 60 W m^{-2} of 580–700 nm or at 2 W m^{-2} of 680 nm for OE and RI, respectively. These measurements of OE were performed under a gas phase of Ar + 2% CO_2. Y is oxygen exchange yield induced by a saturating 1.8 μs flash and given in relative integrated units. Ø is maximum relative quantum yield of oxygen exchange under light-limiting conditions (see Fig. 4). The values of Y and Ø for OE were measured with a background illumination of 2 W m^{-2} at 680 nm. For comparison, all the values of V, Y and Ø are relative to a corresponding OE parameter of 100 in photoautotrophic wild type normalized to chlorophyll content. $1/\tau$ is turnover number per s of PS-specific oxygen exchange reaction calculated by division of V by Y. N is number of chlorophyll molecules in antenna of PS II or PS I calculated according to [25] by division of optical cross-section for PS-specific flash-induced oxygen exchange at 675 nm (Fig. 3) to optical cross-section of chlorophyll molecule *in vivo* at 675 nm in the cyanobacterium (2.54 $Å^2$).

Strain and growth conditions	V (arb. units)		Y (arb. units)		Ø (arb. units)		$1/\tau$ (s^{-1})		N	
	OE	RI	OE	RI	OE	RI	OE	RI	OE	RI
WT, photoautotrophic	100	0.6	100	260	100	190	160	0.5	52	39
WT, photoheterotrophic	18	1.3	65	240	62	240	44	1.1	48	37
IC2, photoautotrophic	12	2.4	14	310	18	310	136	1.6	57	47
IC2, photoheterotrophic	32	4.1	50	290	35	260	103	3.1	42	41

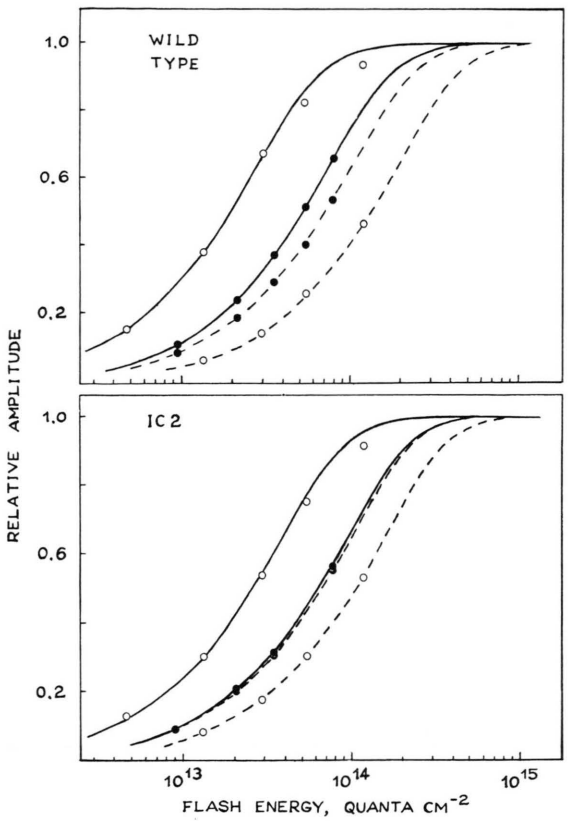

Fig. 3. Light saturation behaviour of the amplitude of flash-induced oxygen evolution (solid lines), measured with background illumination of 2 W m^{-2} at 680 nm, and PS I-induced inhibition of respiration (dashed line, with 10 μM DCMU) in photoheterotrophically grown wild type and IC2 mutants at 675 nm (solid circles) and 602 nm (open circles) normalized to corresponding maximum amplitude under saturating flash of about 2×10^{15} quanta cm^{-2} and 580–700 nm. The curves are fitted to cumulative one-hit saturation functions [22] using optical cross-sections of 350, 132, 100 and 52.5 Å^2 (from left to right) in the wild type, and 270, 107, 105 and 66 Å^2 in the IC2 mutant.

each phycobilisome in cyanobacteria [36–38]. This implies some possible structural distinctions in phycobilisome-PS II interactions in the mutant compared with the wild type. For example, in the absence of the Mn-stabilizing protein, the dimeric organisation of PS II may have a modified conformation with a lower probability of excitation energy exchange. The postulated structural changes are apparently not accompanied by a complete dissociation of the dimers, since photoinactivation of a considerable fraction of PS II units during photoautotrophic growth of the IC2 strain led to an increase in the average size of the remaining active PS II units by about 40%, possibly due to excitation energy transfer from inactive to active reaction centres within the dimeric complexes (data not shown). It is worthy of note that in the latter case the average size of the PS I unit also increased, possibly due to an increased spillover of excitation energy from the non-functional PS II units. The presence of a phycocyanin band at 630 nm in the action spectrum of the PS I induced decrease in respiration (Fig. 2) indicates an existance of spillover in the cyanobacterium, although direct energy transfer to PS I from phycocyanin may also occur.

Fig. 4 shows relative quantum yields of oxygen evolution and PS I induced inhibition of respiration in both the wild type and mutant cells as a function of photon flux at 625 and 675 nm. These parameters are a product of the number of functionally active photosynthetic units and their average optical cross-sections. Therefore, the corresponding plots contain information about the ratio of the spectral efficiency of the two photoprocesses under various light conditions. In agreement with flash measurements (Table I), this data also indicates a large increase in the ratio of PS I/PS II in the IC2 mutant compared with wild type cells. The drop of the relative quantum efficiency of PS I induced inhibition of respiration in both strains at high illumination indicates that the turnover rate of the respiratory chain is relatively slow (0.5 to 3 s^{-1}, Table I).

Light-saturated steady-state rates of oxygen evolution by the cells were measured after flushing the assay medium with a gas mixture consisting of argon plus 2% carbon dioxide. This condition was used in order to minimize the PS I induced effect on the oxygen signal due to changes in respiration rate. As shown in Table I, the maximum light-saturated rate is attained in photoautotrophically grown wild type cells, whereas in photoheterotrophic grown wild type and in both types of cultures of the IC2 mutant, the rate is reduced by a factor in the region of 3 to 10. This drop may be caused by the decrease of active PS II reaction centres as well as by changes in the balance between linear electron flow to carbon dioxide and the competitive processes of cyclic electron flow around PS I and/or photoconsumption of oxygen.

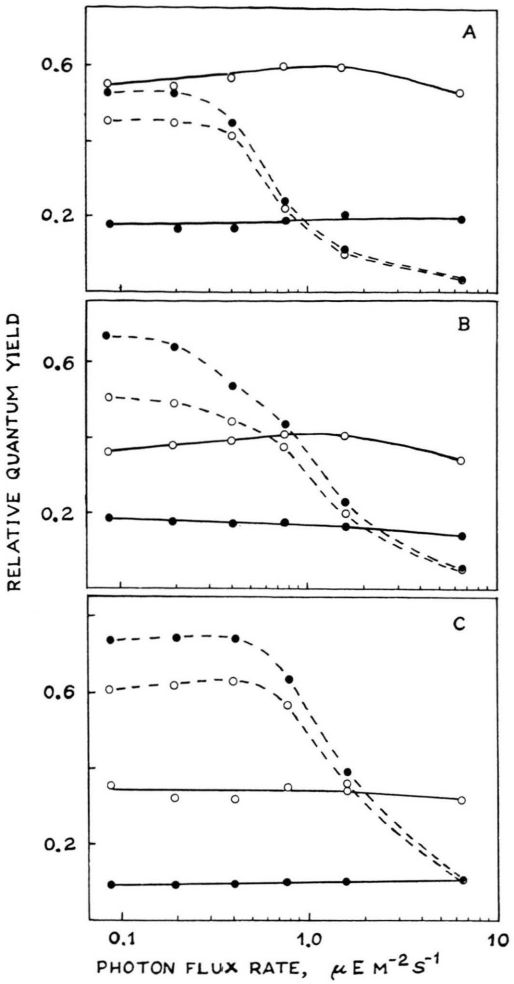

Fig. 4. Relative quantum yield of oxygen evolution measured with a background of 2 W m^{-2} at 680 nm (solid lines) and PS I-induced inhibition of respiration measured with 10 μM DCMU (dashed lines) *versus* photon fluxes at 675 nm (closed circles) and 625 nm (open circles) in: A, photoautotrophically grown wild type; B, photoheterotrophically grown wild type; C, photoheterotrophically grown IC2 mutants. The values of relative quantum yield were calculated by dividing the oxygen exchange rate by the corresponding photon flux value.

Indeed the abrupt decrease of the oxygen evolution rate of the wild type after transition from photoautotrophic to photoheterotrophic growth may be due to such a change in this balance.

We have found that light saturation of the PS I induced inhibition of respiration in DCMU-poisoned cells occurs at a low illumination level (< 5 μE m^{-2} s^{-1}) and that the maximum magnitude of the process does not exceed several per cent of the maximum rate of photosynthetic oxygen evolution. As shown in Table I, the capability of PS I to inhibit respiration in the IC2 mutant is 3 to 4 times greater than in the wild type cells, and increases after transition of both strains from photoautotrophic to photoheterotrophic nutrition. Any changes in the balance of electron influx and efflux influencing the redox state of the plastoquinone pool probably results in regulation of the levels of PS I and the activity (or amount) of a terminal oxidase in thylakoids of the cyanobacterium, in agreement with the findings of Fujita *et al.* [39–41].

Owing to the high PS I/PS II ratio, and the participation of both the photosystems in photoinduced oxygen gas exchange, the flash patterns of amperometric oxygen signals in *Synechocystis* 6803 differ from those in green algae and chloroplasts of higher plants. Taking into account different kinetics of oxygen evolution and PS I induced inhibition of respiration, we measured the patterns of both the amplitude of flash-induced oxygen signals and the integrated yield of oxygen per flash. Fig. 5 shows that the flash patterns are characterized by a substantial oxygen signal upon the first flash, despite giving prolonged dark adaptation. With the exception of this signal, which is more pronounced when integrated units are used (Fig. 5B), the oxygen amplitude flash pattern in the wild type cells show the usual Kok sequence of a damped oscillation with the periodicity of four and the first maximum occurring on the third flash (Fig. 5A). Differences in the ratio of the yields on the third and fourth flashes for photoautotrophic and photoheterotrophic cultures of the wild type cells may be due to an increase in the number of misses in the latter case, corresponding to a more reduced state of the plastoquinone pool.

A quite different flash-induced oxygen pattern is observed in the IC2 mutant under the same aerobic conditions (Fig. 5). It is characterized by a binary oscillation, being particularly pronounced in the integrated oxygen signals (Fig. 5B), which possibly arises from the operation of a two-electron gate on the acceptor side of PS II. This binary mode was often lost upon increasing the flash spacing from 0.1 to 1 s. Suppression of the binary oscillations after addition of DCMU (Fig. 6) indi-

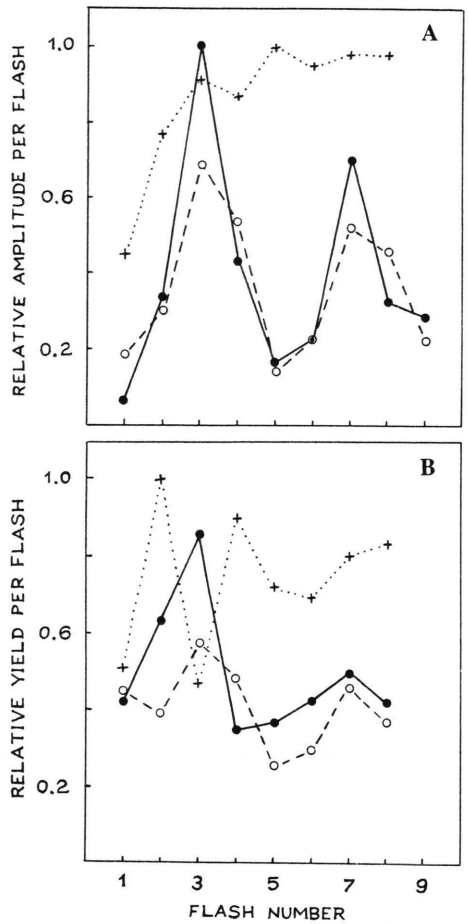

Fig. 5. Oxygen exchange patterns from cells exposed to a series of saturating flashes spaced 0.1 s apart under aerobic conditions. Amplitude (A) and total yield (B) per individual n-th flash were obtained by subtraction of the trace with n-1 flashes from the trace with n flashes recorded using a bare platinum electrode with a low-pass electronic filter of about 0.2 s time constant. Samples were kept in the dark for 15 min prior to the first flash and for 2 min prior to each following series of increasing number of flashes. The data were normalized to a maximum relative value on a chlorophyll basis. Closed circles, photoautotrophically grown wild type; open circles, photoheterotrophically grown wild type; crosses, photoheterotrophically grown IC2 mutants.

cates the necessity of PS II-driven electron influx to the plastoquinone pool for this effect.

Attempts to obtain the normal quadruple oscillation of flash induced oxygen evolution in the IC2 mutant by means of lowering oxygen partial pressure in the medium (in order to lower the dark respiration rate) were unsuccessful (data not

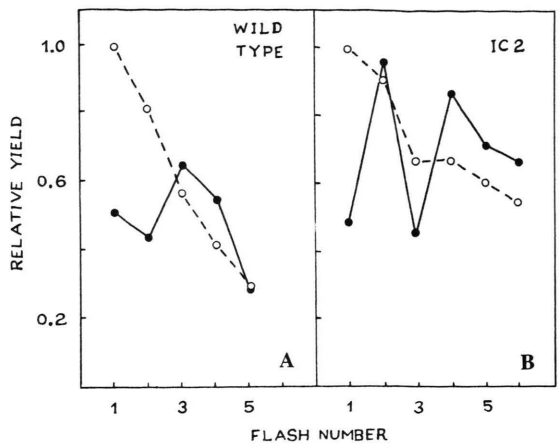

Fig. 6. Effect of the addition of 10 μM DCMU (dashed lines) on the pattern of flash-induced oxygen exchange in photoheterotrophic cells of the wild type (A) and IC2 mutant (B). Conditions are the same as in Fig. 5 and the solid lines represent no DCMU added.

shown). However, a similar oxygen flash pattern in the mutant and wild type cells was observed in the presence of external oxidants, such as 2,5-dichloro-*p*-benzoquinone plus sodium ferricyanide (Fig. 7). In this experiment we used a modified po-

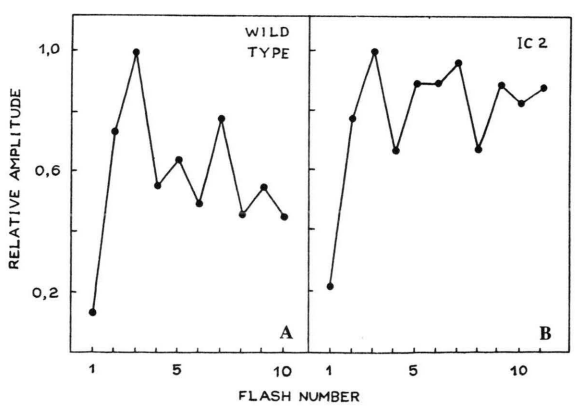

Fig. 7. Pattern of flash-induced oxygen exchange in photoheterotrophic cells of the wild type and IC2 mutant following 30 min incubation in an assay medium with 0.5 mM 2,5-dichloro-*p*-benzoquinone and 5 mM Na-ferricyanide under gas phase of Ar with a minor trace of oxygen. The data were obtained using an oxidant-proof platinum electrode with a polyethylene membrane and the flash frequency was 10 Hz. The dark pretreatment before giving the series of flashes was 1 min. Other information is the same as for Fig. 5. Under the conditions employed the maximum oxygen flash yield in IC2 mutants is 3–4 times lower than that in the wild type.

larographic device with a polyethylene membrane covering the platinum electrode. Although this arrangement decreased the sensitivity and time resolution of the signals, the oxygen flash pattern in the IC2 mutant showed a quadruple oscillation (maxima on the third and seventh flashes in Fig. 7B), modified by the binary oscillation. Relatively high oxygen flash yields for the first two flashes in both the wild type and mutant in this experiment were due to incomplete deactivation of S_3 and S_2 states of the water-oxidizing system (half times of 30 and 120 s, respectively) during 1 min dark intervals between the flashes.

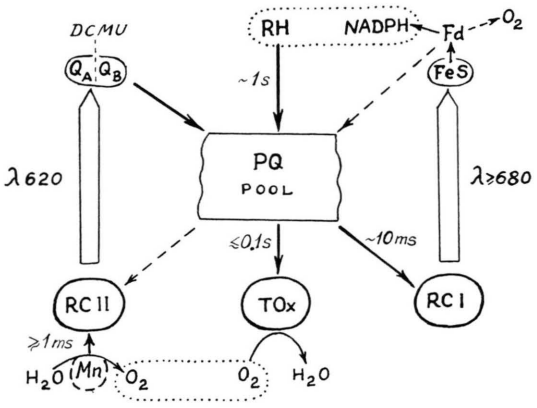

Fig. 8. Proposed scheme for the interrelation of photosynthetic and respiratory oxygen gas exchange in *Synechocystis* 6803. The plastoquinone (PQ) pool is reduced by PS II-driven water-splitting reactions *via* a one-electron Q_A and two-electron Q_B acceptors, or alternatively by a dehydrogenase complex of the respiratory electron transfer chain. The PQ pool is oxidized by PS I-mediated electron flow to low-potential acceptors (and pseudocyclic flow to oxygen) or by electron flow to a terminal oxidase (TOx). The scheme also implies the involvement of cyclic electron flow around both PS II (may be more available in the IC2 mutant) and PS I under certain conditions.

Discussion

Previous studies of *psb*O-less mutants of *Synechocystis* 6803 lacking the Mn-stabilizing 33 kDa extrinsic protein revealed their ability to grow photoautotrophically [13–15] unlike a similar mutant of the green alga *Chlamydomonas reinhardtii* [42]. However, the cyanobacterial mutants show a decrease (by 30–80%) of the oxygen evolving activity and an enhanced susceptibility to photoinhibition [14–16]. Furthermore, Vass *et al.* [16] and Burnap *et al.* [17], observed a heavily dampened abnormal non-oscillatory oxygen flash yield in *psb*O-less mutants of *Synechocystis* 6803.

Our results clearly demonstrate the complexity of the flash-induced oxygen gas exchange in *Synechocystis* 6803, where a significant PS I-mediated component occurs due to inhibition of respiration in both the wild type and IC2 mutant. Taking into account the diminished content of functionally competent PS II reaction centres in the latter, as well as a partially inhibited turnover of the perturbed water-splitting system during the transition from S_3 to S_0 state [16, 17] (that may enhance the cyclic electron flow around PS II), it is not surprising that the normal quadruple oscillatory pattern of the flash-induced oxygen evolution is not readily observed in the IC2 mutant under routine conditions. Indeed, we could only observe the pattern with maxima on the third and seventh flashes under partial aerobic conditions in the presence of artificial electron acceptors (Fig. 7).

In cyanobacteria, the PS II-driven electron input involves redox carriers common to the photosynthetic and respiratory electron transport chains (see Fig. 8) which explains the binary oscillatory pattern of the oxygen flash yields observed in Fig. 5B and 6B. This is because of the two electron/proton gating mechanism at the level of the secondary electron acceptor, Q_B [43] (similar to purple photosynthetic bacteria [44, 45]), which will stimulate respiratory activity. On the other hand, removal of the PS I-driven electron from the common redox pool induces inhibition of the respiratory activity. Thus, the recorded pattern of the flash-induced oxygen gas exchange in the cyanobacterium is a result of several different reactions (see Fig. 8), depending on their kinetics and the relative content of PS II, PS I and oxidative complexes. Besides, as mentioned above, the mutant and wild type cells may be distinguished by the rate of a cyclic electron flow around PS II.

An alternative explanation for the slow oxygen gas exchange component to that given above is possible. There are reports [46–48] of an additional mechanism of oxygen evolution, at least in the cyanobacterium *Oscillatoria chalybea,* which is linked to S_2-mediated decomposition of H_2O_2 produced in the immediate vicinity of the water-splitting system [49]. This type of oxygen signal

has some similar properties to those explained by respiratory modulations; a substantial yield upon a single flash and a binary oscillatory flash pattern [48]. However, like normal photosynthetic oxygen evolution, this H_2O_2 decomposition is sensitive to DCMU [47]. Experimental support for this mechanism comes from studies using subchloroplast PS II preparations which have been disturbed on the donor side [50, 51]. Although our data do not exclude the possibility of photoproduction of hydrogen peroxide in the wild type and mutant strains of *Synechocystis* 6803, with some relationship between the slow oxygen signal and H_2O_2 decomposition under certain conditions, the action spectrum (Fig. 2) evidently shows PS I-dependent behaviour of the phenomenon. Also its enhancement by the addition of DCMU is inconsistent with the peroxide mechanism.

In addition to the functional disturbance of the water-splitting system in the *psb*O-less mutant, our data indicate changes in the antenna size of the PS II units (Fig. 3, Table I). It is possible that structural modification of the PS II core/phycobilisome relationship occurs in response to the lack of the 33 kDa extrinsic protein, as previously suggested by Burnap and Sherman [13]. Furthermore, the 33 kDa protein may facilitate a proper assembly of the PS II core dimers in cyanobacteria [36–38], as well as in some mutants of the green alga *Chlamydomonas reinhardtii* lacking light-harvesting chlorophyll *a*/*b* complex [52].

Overall the data presented here is in general agreement with the conclusions of previous studies, that the absence of the 33 kDa protein in *Synechocystis* 6803 does not prevent the assembly of PS II complexes or prevent water splitting. Its absence, however, does reduce the number of PS II reaction centres capable of facilitating oxygen evolution compared to the wild type and also modifies the S-state cycle in such a way as to emphasize the complex nature and interplay of photosynthetic and respiratory electron flow in cyanobacteria. It is this complexity which can explain the abnormal flash yield patterns of oxygen evolution reported here and in previous studies with IC2 [16] and a similar *psb*O-less mutant of *Synechocystis* 6803 [17].

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