Dependence of the Flash-Induced Oxygen Evolution Pattern on the Chemically and Far Red Light-Modulated Redox Condition in Cyanobacterial Photosynthetic Electron Transport

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Flash-induced photosynthetic oxygen evolution was measured in cells and thylakoid preparations from the coccoid cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 and from the filamentous cyanobacterium *Oscillatoria chalybea.* The resulting characteristic flash patterns from these cyanobacteria can be chemically altered by addition of exogenously added substances like CCCP, DCPiP and inorganic salts. Potassium chloride, manganese sulfate and calcium chloride affected the sequences by specific increases in the flash yield and/or effects on the transition parameters. Chloride appeared to exert the strongest stimulatory effect on the oxygen yield. In comparison to chloride, both manganese and calcium did not significantly stimulate the flash amplitudes as such, but improved the functioning of the oxygen evolving complex by decreasing the miss parameter α. Particular effects were observed with respect to the time constants of the relaxation kinetics of the first two flash signals Y_1/Y_2 of the cyanobacterial patterns. In the presence of the investigated chemicals the amplitudes of the first two flash signals (Y_2 in particular) were increased and the relaxation kinetics were enhanced so that the time constant became about identical to the conditions of steady state oxygen flash amplitudes. The results provide further evidence against a possible participation of either PS I or respiratory processes to Y_1/Y_2 of cyanobacterial flash patterns. Dramatic effects were observed when protoplasts from *Oscillatoria chalybea* or cells from *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 were exposed to weak far red background illumination. Under these conditions, Y_2 (and to a smaller extent Y_1) of otherwise unchanged flash sequences were specifically modified. Y_2 was substantially increased and again the relaxation kinetics were accelerated making the signal indistinguishable from a Y_{ss} signal. From the mathematical fit of the sequences we conclude that S_2 contributes to 10–20% of the S-state distribution (in comparison to 0% in the control). Thus, far red background illumination might represent a valuable means for photosynthetic investigations where high amounts of S_2 are required like *e. g.* EPR measurements. In such experiments the corresponding EPR signals appeared substantially enhanced following far red preillumination (Ahrling and Bader, unpublished observations). Our results clearly show that the ‚controversial results' from parts of the literature suggesting the participation of different mechanisms (net oxygen evolution, inhibited uptake processes etc.) are *not* required to explain the flash-induced oxygen evolution in cyanobacteria: the seemingly ‚incompatible' conditions and conformations can be perfectly interconverted by different modulation techniques (chemicals, far red) of the respective redox condition within the water oxidation complex of photosynthesis.

Key words: Photosynthesis, Far Red Light, Cyanobacteria

Abbreviations: ABDAC, alkylbenzyldimethylammonium chloride; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; EPR, electron paramagnetic resonance; MSP, manganese stabilizing protein; OEC, oxygen evolving complex; Y_i, oxygen evolution amplitude following flash number i; Y_{ss}, steady state flash induced oxygen evolution.

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Introduction

Photosystem II and the oxygen evolving complex (OEC) operate as a water-plastoquinone-oxidoreductase and contain the intrinsic polypeptides D1 (*psb*A), D2 (*psb*D), CP43 (*psb*C), CP47 (*psb*B), and the α (*psb*E) and β (*psb*F) subunits of cyt b_{559}. Depending on the respective organism several extrinsic polypeptides are also important; in higher plants 3 such polypeptides of 16 kD (*psb*Q), 23 kD (*psb*P) and 33 kD (*psb*O) are associated with the lumenal regions of the OEC. Although it is generally accepted that the 33 kD polypeptide operates as a MSP (manganese stabilizing protein), the functions of the two smaller peptides are not quite clear. Beside their possible involvement in calcium- and chloride binding – under limiting conditions, in particular – a more general significance of the polypeptides for the 'covering' and protection of the inner parts of the OEC has been discussed by Homann (1987). In cyanobacteria where the two smaller higher plant polypeptides are missing the OEC might be more accessible to exogenous substances, gases etc. than is the case in higher plants. [It should be noted that for cyanobacteria other small polypeptides (12 kD, Cyt_{550}) have been described but their function may be completely different. *E. g.* Cyt_{550} binds only in the presence of both the 33- and the 12 kD polypeptides and the 12 kD polypeptide does not bind at all to the OEC in the absence of both the 33 kD- and the Cyt_{550}-component (Shen *et al.*, 1992; Shen and Inoue, 1993; Enami *et al.*, 1998).] However, cyanobacteria appear to interact in a very specific way with the surrounding gas atmosphere and the polypeptide composition of these organisms might in fact be seen in context with *e. g.* the cyanobacterial O_2/H_2O_2 cycle earlier described (Bader, 1994).

The above mentioned type of interaction can be regarded from two different points of view. First, there is the evolutionary aspect that oxygenic photosynthesis has been developed by filamentous cyanobacteria in a largely reducing atmosphere – although traces of O_2 must have been present even at that time as the oxidation of water requires catalytic amounts of oxygen with a cooperative mode of binding for its functioning (Bader and Schmid, 2000). Also, essential pigments like chlorophyll cannot be synthesized in the complete absence of O_2. The reaction from coproporphyrinogen III to protoporphyrinogen IX *i. e.* the formation of the vinyl groups out of the propionic acid side chains is catalyzed by the coprogen oxidative decarboxylase only in the presence of oxygen (Bogorad, 1966). In this context, the evolutionary significance of hydrogen peroxide as intermediate electron donor in some ecological niches has been proposed; the transition from inorganic salts to molecular water as electron source can in fact hardly be imagined in a single step (see discussions in Kasting *et al.*, 1985; Bader, 1994; Samuilov, 1997; Blankenship and Hartmann, 1998). Second, it must be kept in mind that oxygenic photosynthesis substantially increases the partial pressure of molecular oxygen at a site where (unregulated) oxidation processes appear highly problematic and might result in the undesired oxidation of vicinal components. (P_{680}^+ has a high positive redox potential of about 1.2 V.) It is well-known that reactive oxygen species are formed inside the OEC; P_{680} in the triplet state will form singlet oxygen and this singlet oxygen is not least formed by direct recombination of the radical pair $P_{680}^+Pheo^-$ (Booth *et al.*, 1990; Durrant *et al.*, 1990). It should be added that in the absence of molecular oxygen the decay of the P_{680} triplet state turned out much slower *i. e.* the excitation state was more stable than under oxygenic conditions (Rutherford, 1986). (The exceptionally high turn-over rate of the D1 protein can at least in part be explained by the protection against such detrimental oxidative processes.) Thus, the interaction of the OEC with molecular oxygen might help to keep the oxygen partial pressure in the immediate vicinity of the enzymatic process at a low level, in particular because of the high affinity of the complex towards oxygen. Taken the arguments together, it is clear that oxygenic photosynthesis requires small amounts of oxygen but has at the same time to limit the resulting and increasing partial pressure *via* some regulatory mechanism. The binding of atmospheric oxygen to the OEC with the subsequent formation of a peroxidic component might well represent such a regulatory mechanism and this peroxide played an essential role in evolution as 'transitory' electron donor (McKay and Hartmann, 1991; Bader, 1994).

Strong support for the idea of a facilitated interaction of the OEC with exogenous compounds

and the evolutionary significance of hydrogen peroxide in the development of oxygenic photosynthesis comes from investigations of Wydrzynski *et al.* (1996) who discussed that the structural integrity of the protein structure surrounding the catalytic site of the OEC finally determines whether molecular oxygen or hydrogen peroxide is formed as the result of photolytic activity. There is still debate whether in fact an intact OEC is needed for the decomposition of hydrogen peroxide yielding also substantial amounts of oxygen or whether this process might rather occur in impaired reaction centers (so that a concerted discussion of the involved processes might appear needless) (Samuilov, 1997). In the present paper we deal with the question whether modifications of both the external and the internal redox conditions affect the oxygen gas exchange of cyanobacteria trying to elucidate further the mechanistic background of flash-induced O_2 evolution amplitudes. In our earlier work we have shown that cyanobacteria appear to require very high salt concentrations for an optimal functioning of the photolytic water oxidation by principle (Bader *et al.*, 1992) possibly correlated to the relatively open structure of the ‚inner sanctum' (Homann, 1987) of the OEC.

Materials and Methods

Cyanobacteria

The experiments were carried out with cyanobacterial cells and thylakoid preparations from *Oscillatoria chalybea*, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942. The cultures were originally obtained from the 'Collection Nationale de Cultures de Microorganismes', Institut Pasteur, Paris (France) and from the 'Sammlung von Algenkulturen', Institut für Pflanzenphysiologie, Universität Göttingen (Germany) and are cultivated since years in the Department of Cell Physiology at the University of Bielefeld. Details of the cultivation and the preparations have been repeatedly described (Engels *et al.*, 1994; Abdel-Basset *et al.*, 1998; Exss-Sonne *et al.*, 2000). In the case of *Oscillatoria chalybea* protoplast preparations were obtained by mechanic homogenization of the filaments followed by enzymatic treatments using glucuronidase, cellulase and lysozyme according to Bader (1989). Cells of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 were directly used from 1–4 day-old cultures.

Flash electrochemistry

Photoevolution of molecular oxygen was measured on the 3-electrode-system developed by Schmid and Thibault (1979) and built by the Société d'Etude et de Construction d'Instruments Astronomiques in Manosque (France). The amplifier used was LH 0044 from National Semiconductor (Danbury, USA). Flashes with a duration of 5 μs, a frequency of 3.33 Hz and an intensity of 600 $\mu E \times m^{-2} \times s^{-1}$ were provided by stroboslave 1539A from General Radio (Concord, USA). The signals were processed by means of a laboratory-written program on an Atari Mega ST4 computer (Schulder *et al.*, 1992). Depending on the respective experiment amplitudes and/or 250 ms-integrals of the flash-induced oxygen evolution signals were taken as basis for *e.g.* calculation of the respective S-state distributions. Where indicated, far-red background illumination was applied by filtering the light from a standard Leitz projector through a 725 nm interference filter plus a 10% transmission or a 5% transmission neutral glass filter. This background light did not induce any detectable oxygen evolution by itself as evidenced in the respective control experiments.

Results and Discussion

Earlier studies in different laboratories have shown that cyanobacteria exhibit quite a number of peculiarities with respect to the flash-induced O_2 evolution pattern in the frame of the coherent Kok model (Kok *et al.*, 1970; Bader, 1994; Engels *et al.*, 1994). Amongst the principally weak oscillations and the first flash signals Y_1 and Y_2 have been intensively investigated in *Oscillatoria chalybea* by Bader (1994). Addition of various inhibitors (uncouplers, ADRY reagents, protonophors – *e.g.* CCCP) had no specific effects on Y_1 and Y_2 in relation to $Y_3 - Y_{ss}$ (Abdel-Basset and Bader, 1998) which has been used as proof for the homogeneity (or heterogeneity) of the cyanobacterial O_2 evolution amplitudes. However, with the aim of further investigating the effects of redox-active chemicals on the O_2 amplitudes in cyanobacteria we first calculated the dark distribution of S-states in the absence/presence of CCCP using the pro-

Table I. Effect of carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP) on the S-state distribution and on the transition probabilities of flash-induced O_2-evolution.

Assay	Model	S_{-1}	S_0	S_1	S_2	S_3	α	β	γ	Δ_q(%)
Control	4-states		46.8	49.1	≤ 0	4.1	31.4	68.6	6.3	0.54
	5-states	5.2	43.2	48.0	≤ 0	3.6	30.0	65.2	4.9	0.35
2.5 μM CCCP	4-states		74.7	19.0	≤ 0	6.3	38.7	49.2	12.75	2.28
	5-states	35.8	33.7	27.2	≤ 0	3.3	26.3	66.5	7.4	2.13

Mathematical fit of the photosynthetic oxygen evolution amplitudes in *Oscillatoria chalybea* using the computer simulation program developed by Thibault and Thiéry (1981) and Thiéry (1991). The program was run with 120 iterations under the assumption of 4- or 5 S-states, respectively. α – misses, β – successes, γ – double hits. The quality of the fit can be derived from the mean quadratic deviation values (%).

gram 'VOYONS' (Thibault and Thiéry, 1981; Thiéry, 1991). Table I shows the effect of CCCP on the dark distribution of the redox states in a thylakoid preparation from *Oscillatoria chalybea.* The activity of CCCP as an ADRY-reagent (Renger, 1972; Renger *et al.*, 1973; Hanssum *et al.*, 1985) cannot be detected in this case but a general shift of the S-state distribution to a more reduced condition is observed. This result was obtained with a cyanobacterial thylakoid preparation exposed to a CCCP-concentration of 2.5 μM which under theses conditions resulted in an about 50% inhibition of *all* oxygen amplitudes. However, a mathematical fit of the amplitudes in a 4-state Kok-model showed an increase in S_0 from 46.8 to 74.7% whereas a 5-state calculation yielded a strong participation of S_{-1} (35.8%). In either cases the reduced S-states were enriched at the expense of S_1. Both S_2 and S_3 and also the transition parameters were not affected.

Very different results are obtained with inorganic salts. In Fig. 1 a control sample having a sequence with specifically low Y_1 and Y_2 was chosen and the scale of the recording oscilloscope was adjusted to the optimal sensitivity in the presence of KCl. Thus, Figs. 1a–d are directly comparable. The maximal stimulation of the flash amplitudes was observed with KCl (0.4 M). This effect has been described already (Bader *et al.*, 1992) and can be interpreted in the sense that cyanobacteria like *Oscillatoria* normally live under salt limitation. Here, the altered accessibility of the OEC to exogenous substances due to the lack of the 16- and 23 kD extrinsic polypeptides might play a role leading also to a facilitated leakage of compounds, cofactors etc. from the inner parts of the OEC to the medium. The pattern itself is not changed as evidenced by the normalized (Y_{ss}) graphs based on the signal integrals in Fig. 2a. Although the signal Y_4 is increased to 320% of the control value in the presence of KCl, the shape of the normalized pattern is identical. Manganese chloride also stimulated the flash-induced O_2 evolution, although to a smaller extent – hence the effect is based on the presence of chloride and not of manganese. The other principal observation consisted of the strongly improved oscillation of the flash amplitudes. The maximum was increased and shifted from the fourth to the third flash and the first oscillatory minimum was decreased and shifted from the seventh to the sixth flash. A similar observation has been described for quaternary ammonium

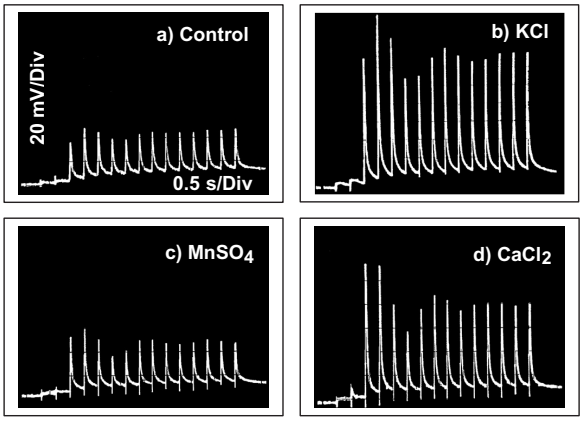

Fig. 1. Polarographic recordings of the flash-induced oxygen evolution in *Oscillatoria chalybea* in the absence and in the presence of inorganic salts. All salts were added giving a final concentration of 10^{-4}M. Following a sedimentation time of 30 min and a dark adaptation time of 10 min a train of 15 flashes was fired with a flash duration of 5 μs at a frequency of 3.33 Hz. The chlorophyll concentration was 30 μg/1 ml assay.

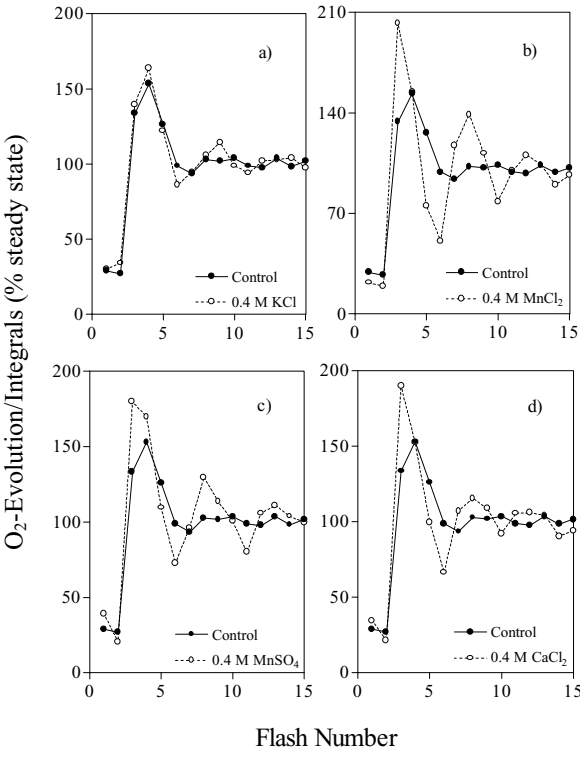

Fig. 2. Effect of inorganic salts on the oxygen evolution pattern in *Oscillatoria chalybea*. The integrated values of the flash signals were normalized to the steady state values of the respective sequences to specifically elucidate the effects on the oscillatory characteristics of the patterns. Other conditions as in Fig. 1.

salts (Bader, 1989) and explained by a substantial decrease of the miss parameter α which always appears to be very high (30–40%) for cyanobacterial flash sequences. In the case of KCl-addition, chloride seems to be the effective component rather than potassium, as $CaCl_2$ was nearly as effective as KCl and the addition of $MnSO_4$ clearly had the smallest effect. (Fig. 2). Table II shows the summary of the mathematical fit of the amplitudes in the absence/presence of the investigated salts from Fig. 2. First, it is observed that the assumption of an overreduced redox state S_{-1} can be neglected in the case of cyanobacterial S-state conditions. Neither was a significant amount of S_{-1} calculated for any of the experiments, nor was the quality of the fit – as evidenced by the mean quadratic deviation values – improved for the 5-state calculations. In fact the miss parameter was high and ranged around 40% and the population of the S_0-state was largely dependent on the presence of elevated amounts of KCl. At low KCl-concentrations the reaction centers were mostly in the S_1 state. In other terms, at low chloride concentrations the values for S_3 remain unaffected whereas a dark reduction from S_1 down to S_0 has to be regarded for increasing concentrations of chloride. Addition of $MnCl_2$ had a less pronounced affect with respect to the S-state distribution but decreased the number of misses (transition parameter α) significantly. This effect, however, was also observed to some extent following addition of KCl, $MnSO_4$ or $CaCl_2$, respectively. It might be

Table II. Effect of inorganic salts on the S-state distribution and on the transition probabilities of flash-induced oxygen evolution.

Assay	Model	S_{-1}	S_0	S_1	S_2	S_3	α	β	γ	Δ_q(%)
Control	4-states		8.8	83.5	≤ 0	7.7	38.7	54.5	6.0	1.60
	5-states	≤ 0	5.2	84.5	≤ 0	10.3	40.2	49.9	9.4	1.42
0.4 M KCl	4-states		40.9	49.4	3.3	6.4	24.4	70.5	3.6	1.90
	5-states	≤ 0	40.9	51.6	1.0	6.5	27.0	67.2	4.5	1.84
0.4 M $MnCl_2$	4-states	≤ 0	26.4	68.5	≤ 0	5.1	15.7	80.5	3.0	1.48
	5-states		26.2	68.7	≤ 0	5.1	16.0	80.1	3.3	1.46
0.4 M $MnSO_4$	4-states		28.2	62.9	0.3	8.6	21.1	75.7	1.8	2.48
	5-states	≤ 0	28.0	63.4	0.2	8.4	21.3	75.5	1.9	2.48
0.4 M $CaCl_2$	4-states		17.6	74.1	≤ 0	8.3	21.2	71.5	6.2	2.42
	5-states	7.7	18.0	66.8	≤ 0	7.5	19.8	74.6	3.9	2.48

Conditions and details see Table I.

concluded from the results summarized in Table II that chloride or manganese has to be present in relatively high concentrations to reduce the number of misses. One of the most striking observations and interpretations is that the lowered value for α is not only similar for any of the investigated salts but also about the same as we described for the quaternary ammonium biocide ABDAC (Bader, 1989). Moreover, all of these values are similar to the transition parameters in the case of higher plants or green algae without any additions. Thus, it looks as if just *two* possible conditions for the OEC are observed one belonging to the (functionally) suboptimal condition of the cyanobacterial OEC. Upon addition of (in)organic salts, however, the OEC can be structurally modified and functionally improved (with lower α-values) reaching another condition which is about the same as the evolutionary optimized OEC in green algae or higher plants under *in vivo* conditions. Photoactivation of the OEC in the sense of Mn-dependent modifications (Gleiter *et al.*, 1994; Qian *et al.*, 1997) does not seem likely in this context as we never observed any preflash-dependent enlargement of oxygen amplitudes with this cyanobacterium. However, under our conditions photoactivation as the result of 800 preflashes was easily detectable in both *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 and – even dramatically – in the respective MSP-free mutants (Engels *et al.*, 1994).

Furthermore, the addition of $MnCl_2$ and $CaCl_2$ (and to a smaller extent KCl) had a completely unexpected effect on both Y_1 and Y_2. Fig. 3 shows the first flash amplitudes with an amplified scale. The important obervations are that the signals under the second flash of the sequences are increased and -even more important- that the relaxation kinetics of the recorded signals are substantially enhanced. This is an essential point as the different time constants for Y_1/Y_2 on the one hand and for Y_3-Y_{ss} on the other had always been taken as an argument for the interpretation that Y_1 & Y_2 of a cyanobacterial sequence have to be attributed to another mechanism – distinct from the water splitting reaction in photosystem II – according to deviating interpretations (Méunier *et al.*, 1995). Under our conditions the relaxation times for Y_1/Y_2 have become indistinguishable from those for Y_3-Y_{ss} by the addition of the salts. This observation is not restricted to the investigated inorganic salts but can easily be obtained by the application of other chemicals. For the experiments depicted in Fig. 4 we scrutinized the first flash amplitudes in the presence of the standard electron acceptor

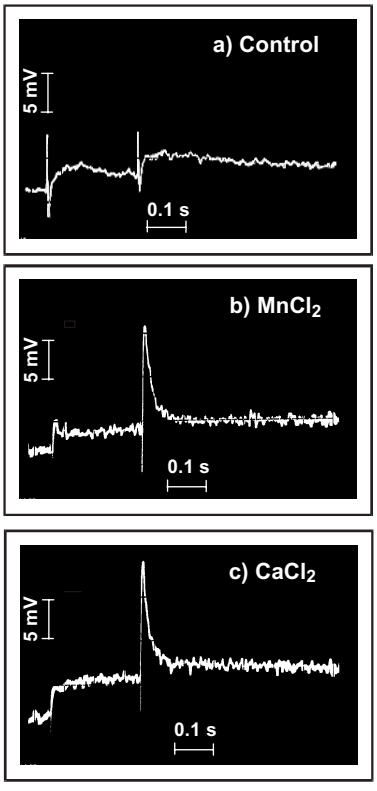

Fig. 3. Effect of manganese chloride (b) and calcium chloride (c) on the first flash amplitudes Y_1 and Y_2. The sequences were recorded with an unmodified flash frequency of 3.33 Hz with an extended time scale. Conditions as in Fig. 1.

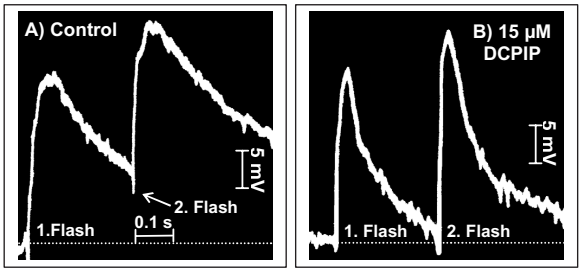

Fig. 4. Effect of 2,6-dichlorophenol indophenol (DCPiP) on the first flash amplitudes Y_1 and Y_2. The sequences were recorded with a flash frequency of 3.33 Hz with an extended time scale. Conditions as in Fig. 1.

DCPiP. Generally, a slight increase in the oxygen evolution amplitudes was observed (results not shown) what suggests that the electron flow through the photosystem has been optimized due to a simple improvement of the electron acceptor supply. When we analyzed the signal integrals instead of the amplitudes, however, we observed a faster signal relaxation. This holds true even in those cases when the amplitudes of Y_1/Y_2 were increased (Fig. 4). Again, the time constants for the recorded signals Y_1/Y_2 approach those normally observed for Y_3-Y_{ss}. From our results we conclude that all electrochemically detectable signals of a cyanobacterial O_2 flash pattern do in fact originate from a reaction mechanism within photosystem II. This interpretation includes the modulated participation of water oxidation and peroxide decomposition affected by conformational changes leading to an altered accessibility of the OEC (Bader, 1994; Wydrzynski *et al.*, 1996) but it supplies further arguments against the involvement of photosystem I or respiratory mechanisms as an origin of the initial oxygen amplitudes in cyanobacteria.

One possible argument against the above interpretation could be that addition of chemicals of any type always causes stress for the particles and this might in fact lead to more and complex consequences than just a modification of the redox conditions within the electron transport chain. Thus, we were looking for experimental conditions where a shift in the signal amplitudes could be achieved without any chemical stressor and such a condition might be the mere modification of the light regime. In this case, however, it is necessary to choose the best-suited wavelength together with an appropriate light intensity low enough not to induce water oxidation itself. This condition turned out to be a background illumination of 725 nm at an intensity of 0.7 and 0.4 $\mu E \times m^{-2} \times s^{-1}$, respectively. Under these conditions we observed the cyanobacterial flash sequences depicted in Fig. 5a/b. (For reasons of clarity we chose a control assay with specifically small Y_1/Y_2 amplitudes.) The steady state O_2 evolution is not affected by the background light, but there were dramatic effects in the region of Y_1 to Y_4. The maximum of the pattern is shifted from the fourth to the third flash and this hints at a modification of the dark distribution of S-states and/or the transition parameters. In the case of *Synechocystis* sp. PCC 6803 an identical picture with an even more pronounced enhancement of Y_2 was observed (Fig. 5c/d). In contrast to *Oscillatoria*, the *Synechocystis* control exhibited the maximum yield following the third flash anyway what might be interpreted in terms of a smaller contribution of α. The ratio Y_3/Y_4 was slightly increased by the light regime but not principally modified. Fig. 6 shows the corresponding experiment with another

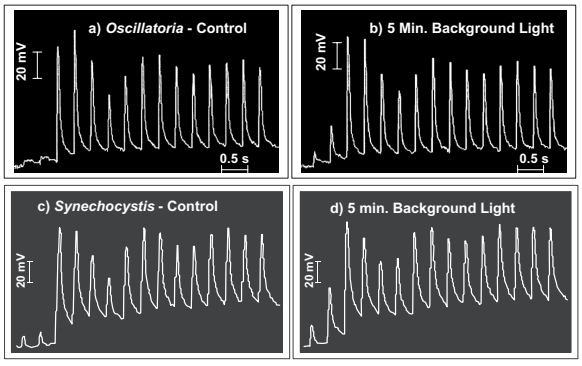

Fig. 5. Oxygen evolution pattern in *Oscillatoria chalybea* (a/b) and in *Synechocystis* sp. PCC 6803 (c/d) without (a/c) and with weak far red background illumination (b/d). The background light had an intensity of 0.7 $\mu E \times m^{-2} \times s^{-1}$ at 725 nm; it induced absolutely no oxygen evolution and was switched on 5 min before and during the firing of the flashes.

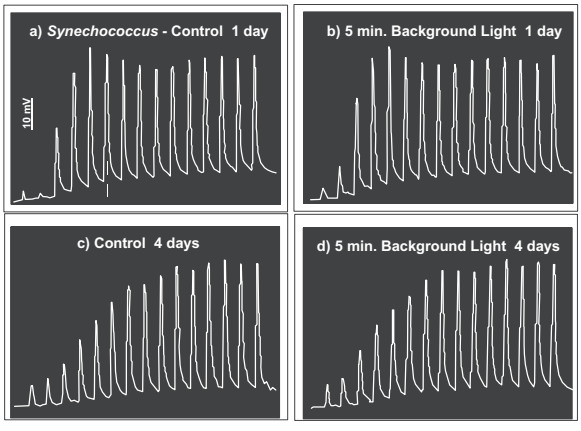

Fig. 6. Oxygen evolution pattern in a 1 day- (a and b) and a 4-day culture (c and d) of *Synechococcus* sp. PCC 7942 without (a and c) and with weak far red background illumination (b and d). The background light had an intensity of 0.7 $\mu E \times m^{-2} \times s^{-1}$ at 725 nm. Other conditions as in Fig. 5.

coccoid cyanobacterium, *Synechococcus* sp. PCC 7942. The typical flash pattern of this organism shows an extremely little oscillating sequence with an even more delayed Y_{max} in relation to other organisms. Beside the observation that again both Y_1 and Y_2 were significantly increased by the far red light it should be noted that both the maximum Y_5 and the steady state flash yield Y_{ss} were unaffected – according to the low light intensity of the background illumination (Fig. 6a/b). With *Synechococcus* sp. PCC 7942, however, the significance of the growth condition for the described effects was revealed. The far red effect on the flash amplitudes and the S-state distribution (*vide infra*) was only observed with 1–2 d old cultures after inoculation. Fig. 7c/d shows the identical experiment using cultures 4 days or more after inoculation. In this case, the transition parameter α (misses) was extremely enhanced so that no oscillation could be observed any more and under these conditions no effect of far red on Y_1/Y_2 appeared to be possible.

From Figs. 5 and 6 it is clear that the most convincing modification following far red background illumination lies in the region of Y_1/Y_2. Both flash amplitudes are increased, but the effect is largest and best seen for Y_2. Under optimal conditions Y_2 is stimulated 3–4-fold and, again, the relaxation is drastically accelerated (Fig. 7). The effect of a background light-induced stimulation of Y_1/Y_2 is of course dependent on the duration of the illumination prior to the analyzing sequence (Fig. 8). Fig. 8 shows in addition that for *Oscillatoria* the (in comparison slight) stimulation of the Y_1-amplitude appears to have different time constants than Y_2. For Y_1 we observed 100–150 s and for Y_2 about 50 s for optimal stimulation of the amplitudes. In this context the relevant argument is whether modification of the light regime might suffice to achieve a shift in the flash amplitude from Y_3/Y_{ss} to the Y_1/Y_2-region. Figs. 5–8 show that this is clearly the case. The data do not answer the question, however, which redox state is most affected by the background illumination. Therefore, we have fitted all sequence values in the mathematical program developed by Thibault and Thiéry (1981) and Thiéry (1991). Table III summarizes the results for both the amplitude and the integral values. The most important effect consists in the strong increase in the S_2 portion. This value which is normally calculated to lie around zero increased to 10–12% in the case of the amplitudes and even to 18% in the case of the integrals. A direct oxidative effect of the background illumination can be ruled out as we never observed any oxygen evolution by the far red light alone and – what might be more convincing – Table III shows that the portion of S_3 was not all increased. (Both

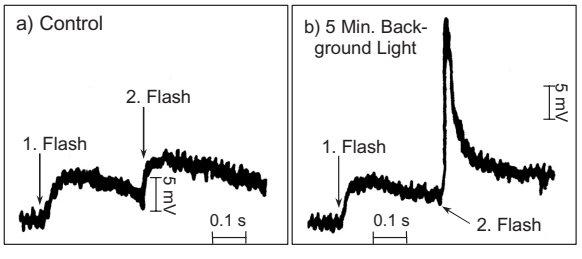

Fig. 7. Effect of weak far red background illumination on Y_1 and Y_2 of flash-induced oxygen evolution. The sequences were recorded at a flash frequency of 3.33 Hz with an extended time scale and increased sensitivity. Other conditions as in Figs. 5–6.

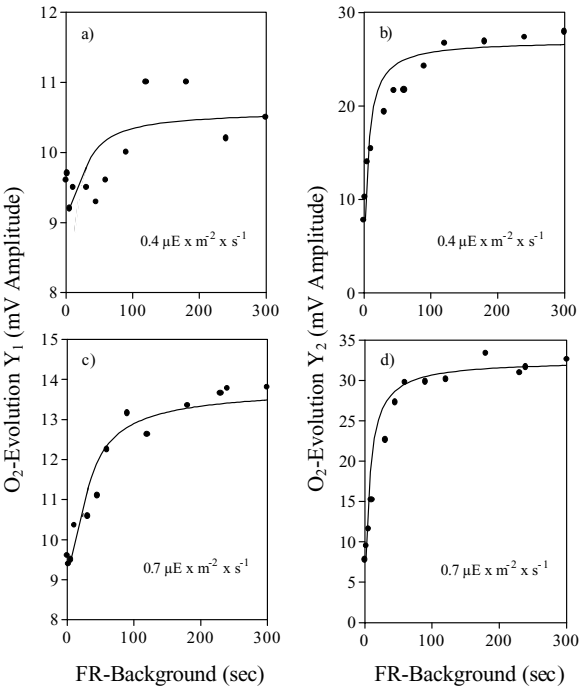

Fig. 8. Dependence of the far red irradiation time on the background light-dependent increase of flash amplitudes Y_1 (a and c) and Y_2 (b and d). The far red light had intensities of 0.4 $\mu E \times m^{-2} \times s^{-1}$ at 725 nm (a and b) and 0.7 $\mu E \times m^{-2} \times s^{-1}$ at 725 nm (c and d).

Table III. Effect of far red background light (725 nm) on the S-state distribution and on the transition probabilities of flash-induced oxygen evolution.

Assay	Model	S_{-1}	S_0	S_1	S_2	S_3	α	β	γ	Δ_q(%)
Control	4-states		34.8	61.2	≤ 0	4.0	24.9	70.9	4.4	1.72
Amplitudes	5-states	8.0	33.1	55.4	≤ 0	3.5	22.6	74.9	2.3	0.96
Far red	4-states		29.7	55.3	10.2	4.8	22.1	74.2	3.7	1.77
Amplitudes	5-states	7.2	26.7	49.0	12.4	4.7	20.4	77.8	1.14	1.65
Control	4-states		33.4	52.1	6.7	7.8	22.0	76.0	0.03	3.07
Integrals	5-states	≤ 0	36.7	55.1	0.4	7.8	25.2	70.1	4.1	2.03
Far red	4-states		22.5	50.7	18.1	8.7	19.0	80.5	0.5	3.63
Integrals	5-states	4.2	22.0	47.1	18.3	8.4	18.4	82.0	1.8	3.78

Conditions and details see Table I.

the small but possibly significant content of S_{-1} in this case and the transition parameters α, β and γ were not affected by the light treatment.) Thus, the far red light seems to specifically affect (increase) S_2; the detectable effect on Y_1 might be explained by the contribution of double hits and this in turn would explain why this effect is relatively small compared to the main effect on S_2. In the case of the 4d *Synechococcus* culture (Fig. 6c/d) the distribution of the redox states and/or the transition parameters were suboptimal (with respect to the function of the OEC) so that the far red dependent increase of the S_2 state which requires intact transition within the S-state system was completely lacking or at least not detectable.

Under optimal conditions, all three investigated cyanobacteria strongly reacted to a weak far red background illumination by a substantial modification of the S-state system *i. e.* an increase in the S_2 population to nearly 20%. Thus, far red illumination might offer an appropriate tool for a facilitated analysis of S_2-dependent techniques like EPR measurements which have been problematic in many cyanobacterial investigations thus far.

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