Independently Acting Oxygen-Forming Complexes of Photosystem II in Cyanobacteria
A Study in the Filamentous Cyanobacterium *Oscillatoria chalybea*

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Cooperation, Oxygen-Forming Complex, Filamentous Cyanobacterium

It has been postulated that the oxygen-evolving centers of photosystem II do not operate independently in the cyanobacterium *Synechococcus leopoliensis* in contrast to those of the chlorophyte *Chlorella vulgaris* and the diatom *Phaeodactylum tricornutum* (Mauzerall and Dubinsky (1993), Biochim. Biophys. Acta **1183**, 123–129). Dependence would mean the existence of charge transfer among adjacent units and would be manifested by different saturation curves for the individual flashes of a sequence (different cross-sections), stronger damped oscillations and oxygen formation under the first flash, independently of the length of dark adaptation. We show in the present publication that in the filamentous cyanobacterium *Oscillatoria chalybea* the O_2-evolution pattern which shows an O_2-signal under the first flash (despite dark adaptation) can be explained within the heterogeneous Kok-model, assuming a non-standard initial S-state distribution (Bader, Thibault and Schmid (1983), Z. Naturforsch. **38c**, 778–792).

Introduction

The oxidizing equivalents produced by the photoreaction of photosystem II (PS II) and stored in the oxygen evolving complex (OEC) lead to the oxidation of water and O_2 release. The fact that the accumulation of four positive charges is necessary for the evolution of one molecule of oxygen has been observed for the first time by Joliot (1969) and theoretically described by Kok *et al.* (1970). Oxygen evolution under short saturating flashes as it is usually observed in chloroplasts or PS II reaction centers of higher plants gives a characteristic pattern with maximal O_2 yield under the third flash, practically no oxygen evolution under the first flash and only a minor one under the second flash. The damping of oxygen oscillations can be explained in the heterogeneous model by failure transitions between the sequentially oxidized steps (S-states) proposed by Delrieu (1974) or additionally by double advanced transitions in the homogenous model given by Kok *et al.* (1970). The shape of the O_2 pattern depends also strongly on the dark reactions and the time of dark adaptation (Vermaas *et al.*, 1984; Zimmerman and Rutherford, 1985). These models widely used in the literature assume no interactions between the oxygen evolving complexes.

Mauzerall and Dubinsky (1993) introduced two criteria to distinguish between independently and dependently operating oxygen evolving centers. By dependently operating systems they apparently mean centers for which a charge transfer among adjacent units exists. The two criteria introduced by Mauzerall and Dubinsky are (i) the saturating curves of oxygen evolution depending on the flash energy should be the same for individual flashes if the PS II OECs are independent and can vary only in the case of charge transfer between the PS II units; (ii) the more independent the OECs are, the more the oscillations will persist.

Mauzerall and Dubinsky have studied *Chlorella vulgaris*, *Phaeodactylum tricornutum* and *Synechococcus leopoliensis*. Using the above mentioned criteria they conclude that the oxygen evolving complexes in the cyanobacterium *Synechococcus leopoliensis* are not independent in contrast to

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Abbreviations: PS II, photosystem II; OEC, oxygen evolving complex.

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those of the chlorophyte *Chlorella vulgaris* and the diatom *Phaeodactylum tricornutum*.

We have performed similar experiments as those by Mauzerall and Dubinsky. We have measured oxygen evolution in *Chlorella kessleri* and *Oscillatoria chalybea* under the third flash varying the intensity of the 1st, 2nd and 3rd flash or of all of them using neutral filters with known transmission rates. Our computer simulations, based directly on the heterogeneous Kok model, are in a very good agreement with the experimental data. They confirm the hypothesis of Bader *et al.* (1983) on the non-standard initial S-state distribution in the case of the filamentous cyanobacterium. As long as the steady S-state in measurements of the effective cross section is not reached, the initial S-state distribution influences strongly oxygen evolution except that measured under variation of the intensity of the third flash (see Appendix).

Our earlier measurements of the temperature dependence of the O_2 yield under short saturating flashes in *Chlorella kessleri* and *Oscillatoria chalybea* seem to show that the interactions between PS II units on the level of OECs do not take place in the cyanobacterium *O. chalybea* (Burda *et al.*, 1996).

Material and Methods

Oscillatoria chalybea was cultured in the medium D of Kratz and Myers (1955). The cultures were grown at 26 ±1 °C in a 12 h light/12 h dark cycle under a light intensity of 1000 lux. The preparation of protoplasts and thylakoids from cells of the cyanobacterium was carried out as described by Bader *et al.* (1983).

Chlorella kessleri was cultured in a nutrient solution given by Kowallik (1963). The cultures were grown at 30 °C under a light intensity of 10 000 lux. The atmosphere contained 1.5% CO_2.

Flash measurements have been carried out by polarography with the "Three Electrode System" described by Schmid and Thibault (1979). Flashes were provided by a Stroboscope 1539 A from General Radio (Concord, Mass. USA). The flash duration at half intensity was approx. 8 µs. A sequence of three flashes spaced 300 ms apart has been given. The flash intensity was modulated by neutral filters (D from ITOS GmbH, Mainz, NG 11 and NG 12 from Schott). Flash experiments were carried out in the following series: a) 1st flash variable, 2nd and 3rd maximal intensity available (without filters), b) 2nd flash variable, 1st and 3rd maximal intensity available (without filters), c) 3rd flash variable, 1st and 2nd maximal intensity available (without filters) or d) all flashes variable (identical). It should be noted that in our figures 1 and 4 we give arbitrary units for oxygen evolved. Since the curves show no saturations, the unit 1.0 should not be understood as a maximal oxygen yield. Hence, all data for *Chlorella kessleri* and *Oscillatoria chalybea* are normalized to the highest amplitude of oxygen evolution observed under our experimental conditions.

Results and Discussion

A typical pattern of oxygen evolution for *Chlorella kessleri* differs from the one usually observed for *Oscillatoria chalybea*. In the case of *Chlorella* there is no oxygen release under the first flash and only a lesser O_2 yield under the second flash. The forth amplitude is comparable to the third one or sometimes higher. The pattern of oxygen evolution in the cyanobacterium preserves the high ratio of the forth to the third amplitude as in *Chlorella*, but exhibits signals already under the first two flashes. Additionally, the oscillations in *Oscillatoria chalybea* are more damped than in *Chlorella kessleri* (Bader *et al.*, 1983).

The theoretical description of the *Chlorella* pattern as well as that of the cyanobacterium using the standard Kok model (Thibault, 1978; Bader *et al.*, 1983) shows significant statistical deviations between the theoretical and experimental sequences. It looks as if the heterogeneous model, introduced by Delrieu (1974, 1983), provides sometimes a better agreement between the theoretical and experimental data. In our recent work we have shown that the state S_4 should be taken into account in the theoretical analysis, especially in the case of *Chlorella* (Burda and Schmid, 1996). However, for the computer simulations of the oxygen yield under the third flash in a sequence with alteration of the 1st, 2nd, 3rd flash or of all flashes, taking into consideration also the S_4 state, practically no quantitative and qualitative modification of the curves is observed. Therefore, we make our estimation of the initial S-state distribution (S_i) and the miss parameters (α_i) according to the 4 S-state heterogeneous model, omitting double hits (γ_i=0). The S_i and α_i collected in Table I are used in our further calculations.*

Table I. Miss parameters α_i and the initial S_i state distribution calculated from the 4 state heterogeneous Kok model for the oxygen pattern of *Chlorella kessleri* and *Oscillatoria chalybea*.

	Chlorella kessleri	*Oscillatoria chalybea*
α_0	0.006	0.002
α_1	0.028	0.140
α_2	0.561	0.51
α_3	0.047	0.096
S_0	0.259	0.164
S_1	0.720	0.68
S_2	0.026	0.080
S_3	0.0	0.076

The simulated curves of oxygen evolution under the first flash with the alteration of the 1st, 2nd, 3rd or of all three flashes for *Chlorella kessleri* and *Oscillatoria chalybea* are shown in Figs 1 and 2, respectively. One sees that in the cases when the 1st, 2nd or all flashes are variable the curves for *Chlorella* differ from those of the cyanobacterium, but they are exactly the same when only the third flash is varied (Figs 1a and 2a). The curves strongly depend on the initial S state distribution – especially on the population of the S_2 and S_3 states (see Appendix). The lower the population of the S_3 and S_2 states is, the closer are the curves for variable 1st or 2nd flashes to the curve of a variable 3rd flash (Figs 1 and 2). Consequently the curve for all variable flashes coincides with independently multiplying curves for variable 1st, 2nd and 3rd flashes.

The experimental curves for *Chlorella kessleri* and *Oscillatoria chalybea* are shown in Figs 3 and 4. There is no doubt that the theoretical curves based directly on the Kok hypothesis are in a good agreement with the experimental data.

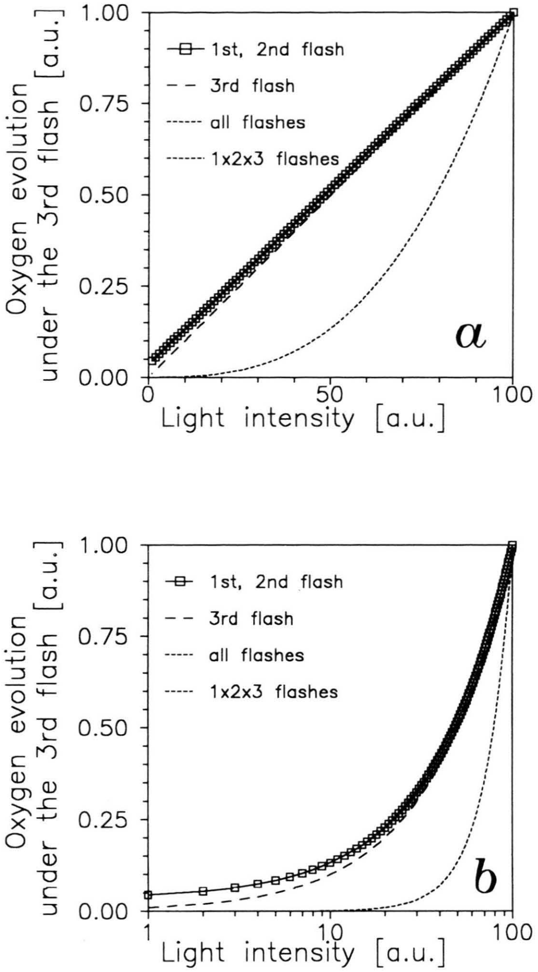

Fig. 1. Theoretical simulations of oxygen evolution under the third flash with the intensity attenuation of the 1st, 2nd, 3rd flash or of all flashes and a curve for the multiplied variable three flashes for *Chlorella kessleri.* The calculation is based on the transition parameters and the initial S state distribution given in Table I for *Chlorella kessleri.* The oxygen yield under the third flash, when the flash intensity of all three flashes is at the maximal intensity used under our experimental conditions, is taken as a reference signal. This means that the data are normalized to the highest amplitude of oxygen evolution. Fig. 1b shows the same data as in Fig. 1a but in a semilogarithmic plot.

Conclusions

The non-standard initial S-state distribution with a non-zero population of S_2 and S_3, proposed already by Bader *et al.* (1983), is a good explanation for the oxygen pattern observed for *Oscillatoria chalybea* differing considerably from that of *Chlorella*. The stronger damping of oscillations in the cyanobacterium pattern would be caused by a higher number of missed transitions. The assump-

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* In the paper by Forbush B., Kok B. and McGloin M. (1971), Photochemistry and Potobiology **14,** 307–321, the denomination of the transition probabilities is: α = miss, β = double hit and γ = successful transition.

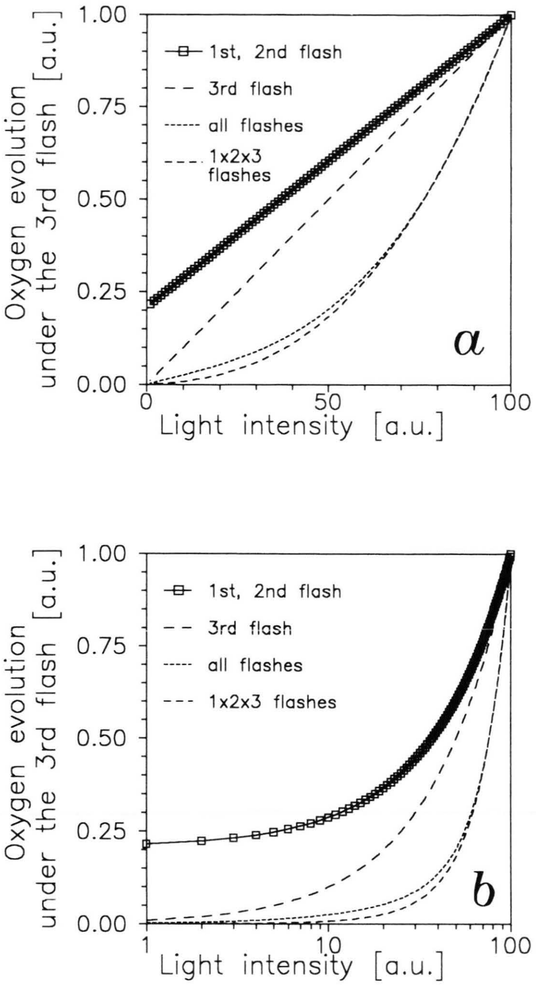

Fig. 2. Theoretical simulations of the oxygen evolution under the third flash with the attenuation of the 1st, 2nd, 3rd flash or of all flashes and a curve for the multiplied variable three flashes for the filamentous cyanobacterium *Oscillatoria chalybea*. The calculation is based on the transition parameters and the initial S state distribution given in Table I for *Oscillatoria chalybea*. The oxygen yield under the third flash, when all three flashes were at maximal intensity, is used as a reference signal. Fig. 2b shows the same data as in Fig. 2a but in a semilogarithmic plot.

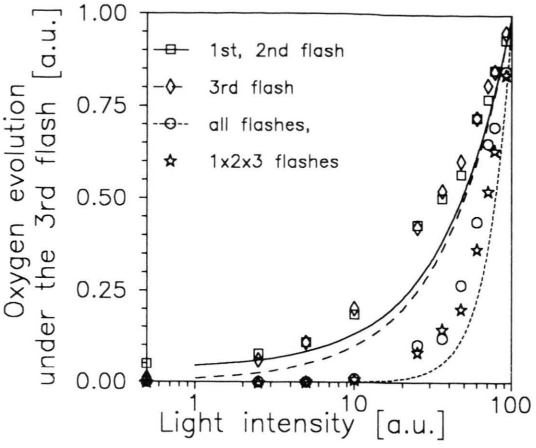

Fig. 3. Experimental data (given as point symbols) in comparison to the theoretical curves for oxygen evolution under the third flash with the attenuation of the 1st, 2nd, 3rd flash or of all flashes and for the curve for the multiplied variable three flashes for *Chlorella kessleri*. The light intensity scale is semilogarithmic.

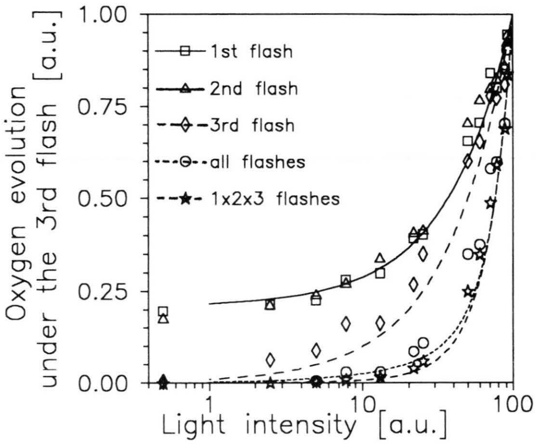

Fig. 4. Experimental data (given as point symbols) together with theoretical curves for oxygen evolution under the third flash with attenuation of the 1st, 2nd, 3rd flash or of all flashes and the curve for the multiplied variable three flashes for *Oscillatoria chalybea*. The light intensity scale is semilogarithmic.

tion of interactions and charge transfer between oxygen evolving complexes seems not necessary for the explanation of the data of cyanobacteria. The model of partial pair-wise interactions of the OECs would require that the highest population be in the S_0 state (62%) and the S_3 state (13%) which would be as stable as the S_1 state (18%). A high stability of the S_3 state is in contradiction with the idea of introducing a charge exchange between pairs of reaction centers which then should fournish the explanation of a significant signal already under the first flash (Mauzerall and Dubinsky, 1993). As has been shown already much lower S_3 occupations in darkness for *Oscillatoria chalybea* are sufficient to explain this phenomenon (Bader *et al.*, 1983; 1987; Burda and Schmid, 1996). Of course there is still the problem for the reason of the slower kinetic of the first two signals. According to Bader *et al.* (1983) the kinetic analysis of the first two signals shows that the composition of the signals is identical to the other signals of the sequence.

The temperature measurements on oxygen evolution under short saturating flashes in *Chlorella kessleri* and *Oscillatoria chalybea* seem to exclude the possibility of charge exchange on the level of OECs for the cyanobacterium (Burda *et al.*, 1996). However, the connectivity between the PS II units by sharing a common chlorophyll antenna is probable (Ley and Mauzerall, 1986; Mauzerall and Greenbaum, 1989). At any rate the idea of Kok that the oxidation of water is a four electron act requiring four photons trapped sequentially in reaction centers of PS II, assuming their independence, seems to be still valid also in the case of our filamentous cyanobacteria.

Appendix

To describe the transitions between S_i states under short saturating flashes we have used the heterogeneous model:

$$S^{(n+1)} = \mathbf{A}\,S^{(n)} \tag{1}$$

where

$$\mathbf{A} = \begin{pmatrix} \alpha_0 & 0 & 0 & \beta_3 \\ \beta_0 & \alpha_1 & 0 & 0 \\ 0 & \beta_1 & \alpha_2 & 0 \\ 0 & 0 & \beta_2 & \alpha_3 \end{pmatrix}$$

$S^{(n)}$ – is a vertical vector of the initial distribution of $S_i^{(n)}$ states (before the flash); $S^{(n)}=\{S_0^{(n)}, S_1^{(n)}, S_2^{(n)}, S_3^{(n)}\}$, $S^{(n+1)}$ - is a vertical vector of the new distribution of $S_i^{(n+1)}$ states (after flash); $\alpha_i+\beta_i=1$.

If $S^{(0)}$ is the S-state distribution after the dark adaptation period then the S-state distribution after two flashes can be calculated from:

$$S^{(2)} = \mathbf{AA}S^{(0)} \tag{2}$$

The amplitude of oxygen yield under the third flash is given by:

$$Y_3 = (1-\alpha_3)S_3^{(2)} \tag{3}$$

where $S_3^{(2)}$ is the population of the state S_3 after the second flash.

In the case when the flash intensity is modulated we have assumed that the transition between $S_i \rightarrow S_{i+1}$ states is proportional to the light intensity (to the transmission parameter of the filters – k):

$$S_{i+1} = k\,\beta_i S_i, \text{ where } \beta_i = 1-\alpha_i \text{ and } 0 \leq k \leq 1. \tag{4}$$

The transition matrix for the case of a variable flash intensity is:

$$\mathbf{B} = \begin{pmatrix} 1-k\beta_0 & 0 & 0 & k\beta_3 \\ k\beta_0 & 1-k\beta_1 & 0 & 0 \\ 0 & k\beta_1 & 1-k\beta_2 & 0 \\ 0 & 0 & k\beta_2 & 1-k\beta_3 \end{pmatrix} \tag{5}$$

We will show the exchangeability of **A** and **B** in the multiplication **AB**=**BA**. Therefore we use the decomposition of the matrix **B** and express **B** by **A** and a unit matrix **I**:

$$\mathbf{B} = k\,\mathbf{A} + (1-k)\mathbf{I}. \tag{6}$$

If the flash intensity is variable under the 1st or 2nd flash then oxygen evolution under the 3rd flash is expressed by:

$$Y_3 = (1-\alpha_3)S^{(2)}_{3(AB)}, \text{ where } S^{(2)}_{(AB)} = \mathbf{AB}\,S^{(0)} \text{ or } S^{(2)}_{(BA)} = \mathbf{BA}\,S^{(0)}. \tag{7}$$

From Eqns. (2), (3) and (7) it is clear that for the variable 1st and 2nd flash the amplitudes of the oxygen yield depend on the initial $S^{(0)}$-state distribution and should be the same because **AB**=**BA,** which can be shown as follows:

$$\begin{aligned}\mathbf{AB}&=\mathbf{A}(k\mathbf{A}+(1-k)\mathbf{I})=k\mathbf{AA}+(1-k)\mathbf{AI}=\\&=k\mathbf{AA}+(1-k)\mathbf{IA}=(k\mathbf{A}+(1-k)\mathbf{I})\mathbf{A}=\mathbf{BA}.\end{aligned} \tag{8}$$

We have used the property of the unit matrix **I** that for any matrix **A**, **AI**=**IA**.
For the variable 3rd flash:

$$Y_3 = k(1-\alpha_3)S^{(2)}_{3(AA)}, \text{ where } S^{(2)}_{(AA)} = \mathbf{AA}\,S^{(0)}. \tag{9}$$

Thus, the oxygen yield under the third flash appears independent on the initial S-state distribution. It depends only on the parameter k:

$$\frac{Y_3}{Y_3^{(S)}} = k \tag{10}$$

where $Y_3^{(S)}$ is the oxygen evolution under the third flash, when all flashes were saturating.

Acknowledgement

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