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Effect of redox state on the dynamics Photosystem II during steady-state photosynthesis in eucaryotic algae

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Using a pump and probe-flash technique we simultaneously measured changes in fluorescence yields ($\Delta\phi$) and the yield of oxygen produced by the pump flash (Y) during steady-state photosynthesis in continuous background light in two species of eucaryotic algae, *Chlorella pyrenoidosa*, a chlorophyte, and *Chaetoceros gracilis*, a diatom. By varying the pump-flash intensity, we examined the flash-intensity saturation curves for $\Delta\phi$ and Y. When a small fraction of Photosystem (PS) II traps is closed by a blue-green background light the flash-intensity saturation curves for $\Delta\psi$ and Y closely followed a cumulative one-hit Poisson function. However, in cells exposed to a far-red background light (more than 720 nm) the flash-intensity saturation curve for $\Delta\phi$ (but not Y) rose faster than predicted by a one-hit Poisson model. These results suggest that energy transfer between PS II reaction centers is moderated by the spectral quality of a continuous photon flux. Using saturating pump flashes we calculated the relative fraction of open/closed PS II reaction centers as perceived from the donor and acceptor sides as the background irradiance was changed. The relative changes in $\Delta\phi$ and Y were highly correlated at low and moderate levels of continuous background light, regardless of spectral quality. At high continuous photon fluxes, however, Y declined faster with increasing background irradiance than $\Delta\phi$. Based on kinetic data it appears that the uncoupling between Y and $\Delta\phi$ at high photon flux densities is largely due to cyclic electron flow around PS II, which is negligible at subsaturating background irradiance levels. Cyclic electron flow around PS II accounted for 15–28% of the linear electron flow and may reduce damage to PS II reaction centers at supraoptimal irradiance levels.

Introduction

Since the discovery of state transitions in *Chlorella* by Bonaventura and Myers [1] it has become increasingly clear that light-absorption characteristics and photochemical energy-conversion processes in the photosynthetic apparatus are modified on relatively short time-scales in response to wavelength as well as the intensity of continuous photon fluxes. The functional plasticity of PS II has, on a structural level, been partially explained by redox-driven protein kinases

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Abbreviations: $\Delta\phi$, change in fluorescence yield ($=(F_s-F_p)/F_p$), where F_p is the fluorescence yield induced by a weak probe flash and F_s is the fluorescence yield induced by a probe flash following a 'pump' flash; Chl, chlorophyll; LL cells, low-light cells; HL cells, high-light cells; PS I and PS II, Photosystems I and II; P, steady-state photosynthesis measured with a continuous background light; P_{max}, maximum steady-state photosynthetic rate; Y, the relative yield of oxygen produced by a single-turnover flash; Ant2P, 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene.

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[2], which appear to modify the function of specific structural elements. In addition to influencing the distribution of excitation energy between Photosystem II and Photosystem I [3,4], phosphorylation has been suggested to influence the transfer of excitation energy between PS II centers [5,6], and to induce cyclic electron flow around PS II in vitro [7]. Using a pump and probe-flash technique [8], we examined the potential influence of intersystem redox state on the transfer of excitation energy between PS II reaction centers and cyclic electron flow in vivo in two species of eucaryotic algae, the chlorophyte *Chlorella pyrenoidosa* and the diatom *Chaetoceros gracilis.*

Evidence for energy transfer between PS II reaction centers has been primarily based on sigmoidal characteristics of the fluorescence induction curve in the presence of DCMU [5,9,10]. Results of such experiments with *Chlorella* have indicated that the probability of excitation energy transfer from closed reaction centers to open ones is high [9,10]. However, induction curves are complex, and have also been used to argue for the existence of heterogenous absorption cross-sections of PS II units [11]. One potential problem with induction curves is that, if even a small fraction of reaction centers turns over more than once, the curve will give the appearance of excitation-energy transfer. This problem can be avoided by using single-turnover saturating flashes and measuring the flash-intensity saturation curve [8]. Results from one such study with *Chlorella* [12] showed that the flash-intensity saturation curve for the change in fluorescence yield is exponential and closely follows a cumulative one-hit Poisson distribution, implying that the probability of excitation-energy transfer is low. These apparently contradictory results imply that fluorescence induction curves and flash-intensity saturation curves are not directly comparable.

Like energy transfer between the reaction centers of PS II, cyclic electron flow around PS II has been postulated and experimentally examined numerous times [7,13–19], but existence of such a cycle has proved difficult to establish under physiological conditions without the use of specific inhibitors of electron transport. The cycle has been suggested to occur at very high continuous irradiance levels, when a large fraction of PS II traps is closed [14,17,18] and very low irradiance levels which spectrally overlap the absorption cross-section of PS II [13,15,19]. These irradiance conditions presumably lead to the reduction of the plastoquinone pool on the acceptor side of PS II. Under these conditions electron carriers on the donor side of PS II may compete with intersystem electron carriers for electron equivalents in PQH_2. If this occurs, photon absorption by PS II would not result in the oxidation of water, but would still allow electrons to be transferred from P-680 to Q_a. To complete the cycle, Q_a must be reoxidized by an electron carrier which transfers electrons back to the donor side of PS II. Thus PS II would appear to be simultaneously closed on the donor side (in that O_2 could not be produced) and open to the acceptor side (in that fluorescence could be quenched). One way of assaying the fraction of open/closed PS II traps at an instant in time is to measure simultaneously the oxygen produced by a short saturating flash [12] and change in fluorescence yield of a weak 'probe' flash preceding and succeeding an actinic 'pump' flash [8]. By measuring both oxygen and fluorescence flash yields simultaneously it is possible to follow the fraction of open/closed PS II traps as perceived from the oxidizing and reducing sides of PS II.

We examined the effect of redox state, photon flux density and spectral quality on oxygen flash yields and the change in fluorescence yields. By varying the intensity of the pump flashes we calculated the extent of energy transfer between reaction centers II based on the deviation of the flash-saturation curves from a Poisson model. We further calculated the apparent relative absorption cross sections of PS II. Our results suggest that: (a) the extent of energy transfer between PS II reaction centers varies by a factor of about 2 and is small under reducing conditions, but increases under conditions which favor the oxidation of PQH_2; (b) at low and moderate photon flux densities oxygen flash yields and fluorescence flash yields measured with saturating pump flashes are highly coupled, regardless of the spectral quality of the background light; however, (c) at high continuous photon-flux densities oxygen flash yields decline more rapidly than fluorescence yields. In other words: electrons may cycle around PS II at high photon-flux densities. Cyclic electron flow may help to reduce photodamage to PS II, but is independent of spectral quality.

Materials and Methods

Chlorella pyrenoidosa, Emerson strain, was grown in minimal media supplemented with A5 trace metals at 23° C in continuous light supplied by a tungsten source [17]. Growth irradiance was adjusted to $2.4 \cdot 10^{15}$ quanta $\cdot$ $cm^{-2} \cdot s^{-1}$ (low light (LL) cells) or $3.9 \cdot 10^{15}$ quanta $\cdot$ $cm^{-2} \cdot s^{-1}$ (high-light (HL) cells). The 3 cm thick, oblong, glass culture vessel permitted even illumination of the culture during growth. The cultures were aerated with 1% CO_2 in air and only cells in exponential growth were used for experiments. *Chaetoceros gracilis* was grown at 23° C in artificial sea water medium, ASP_2, supplemented with A5 trace metals. Cells were grown at $9 \cdot 10^{15}$ quanta $\cdot$ $cm^{-2} \cdot s^{-1}$ and aerated with sterile air.

To measure oxygen flash yields, Y, and steady-state photosynthetic rates, P, cells were settled one layer thick on the platinum surface of an oxygen-rate electrode. The electrode design is identical to that described by Wang and Myers [20]. The electrode was covered with a cellophane membrane, over which medium enriched with 1.5% CO_2 in N_2 (anaerobic condition) or air (aerobic condition) continuously flowed at rate of 15–20 ml/min. Continuous background light was projected onto the sample from two possible sources. The light from a 500 W Ushio xenon source was passed through a water filter and then blue-green light (light 2) was isolated with a Corning 4–76 and a 4-96 and a heat absorbing filter. This light was reflected by a beam splitter, behind which a lambda 190SB quantum sensor was mounted. The reflected light was passed through a dichroic mirror, mounted at 45° to a Hammamatsu R-375 2-inch end-window photomultiplier tube. The dichroic mirror passed shorter wavelength blue light to the sample and reflected longer-wavelength red light from the sample to the photomultiplier tube. The blue light, which was focussed and reflected onto the sample, was attenuated to desired intensities with neutral density filters. A second continuous light was supplied from a 150 watt tungsten halogen source. Far-red wavelengths (light 1) from this source were isolated with a 720 nm sharp cut filter, a heat filter and a Lucite disc which served to block infrared light. The photomultiplier tube was protected with two 680 nm interference filters (2 nm half band pass), a 680 nm sharp cut filter and two infrared absorbing glass filters.

Flashes were provided from two Sugawara MS-230 xenon, bulb-type flash lamps, mounted above the sample. Blue-green light was isolated from both flash lamps with Corning 4-76 and 4-96 filters. The actinic saturating flash had a rise time of less than 1.5 μs and a duration of 4 μs at 50% peak intensity. The probe flash had a rise time of 0.2 μs and a duration of 1.5 μs at 50% peak intensity. The maximum intensity of the saturating flash was measured at $4 \cdot 10^{14}$ quanta $\cdot$ cm^{-2} and the probe flash was 1% of the saturating flash intensity. Flash pairs were triggered with a digital clock.

The current corresponding to the oxygen evolved from the saturating flash was amplified by a fast-response operational amplifier and the amplified signal was digitized with 10 bit resolution by a Kawasaki Electronics TM 1410 transient memory data logger. The oxygen flash yield reached a peak within 1 ms and the data from 16–32 flashes were summed. The flash profile, composed by 1024 data points, was displayed on an oscilloscope. The peak height of the flash yield was measured with a reproducibility of ±5% on replicate measurements on the same sample.

To measure changes in variable fluorescence yields, $\Delta\phi$, the fluorescence yield of 16–32 probe flashes was measured before (F_p) or following a saturating flash (F_s). The delay time between the pump flash and probe flash was 70 μs, unless otherwise stated. Extreme care was taken to ensure absolute linearity of photomultiplier tube response, which became critical at high continuous background irradiance levels. Stray light from the blue-green background sources was undetectable at the gains used and stray light from the far-red source at maximum intensity was less than 10% of the fluorescence signal from F_p. This system is essentially identical to one described previously [17]. The change in fluorescence yields was calculated from the expression

$$\Delta\phi = \frac{F_s - F_p}{F_p}$$

Light from the continuous background sources was measured at the surface of the oxygen electrode with a Yellow Springs Instruments (YSI) Model 65A radiometer and intercalibrated with a lambda LiCor 185 quantum meter interfaced with a model 190SB quantum sensor. Light intensity for the saturating flash was measured with the YSI radiometer and calculated from regression analysis of flash frequency vs. energy.

Results

Flash intensity saturation curves for Y and $\Delta\phi$

Flash intensity saturation curves were measured for Y and $\Delta\phi$ simultaneously. Representative flash saturation curves for a typical experiment are shown in Fig. 1 (a–c) for *Chlorella* under aerobic conditions. The saturation curves were fit by non-linear least squares regression analysis to a cumulative one-hit Poisson distribution,

$$Y/Y_{\max}(\text{or } \Delta\phi/\Delta\phi_{\max}) = 1 - e^{-\sigma E} \tag{1}$$

where E is the relative flash energy and σ is the relative absorption cross-section of PS II ($\sigma_{\text{PS II}}$). Except in dark-adapted cells, the flash intensity saturation curves for Y fit a cumulative one-hit Poisson function with extremely high correspondence. In dark-adapted cells, however, the flash intensity saturation curve for Y rose more steeply

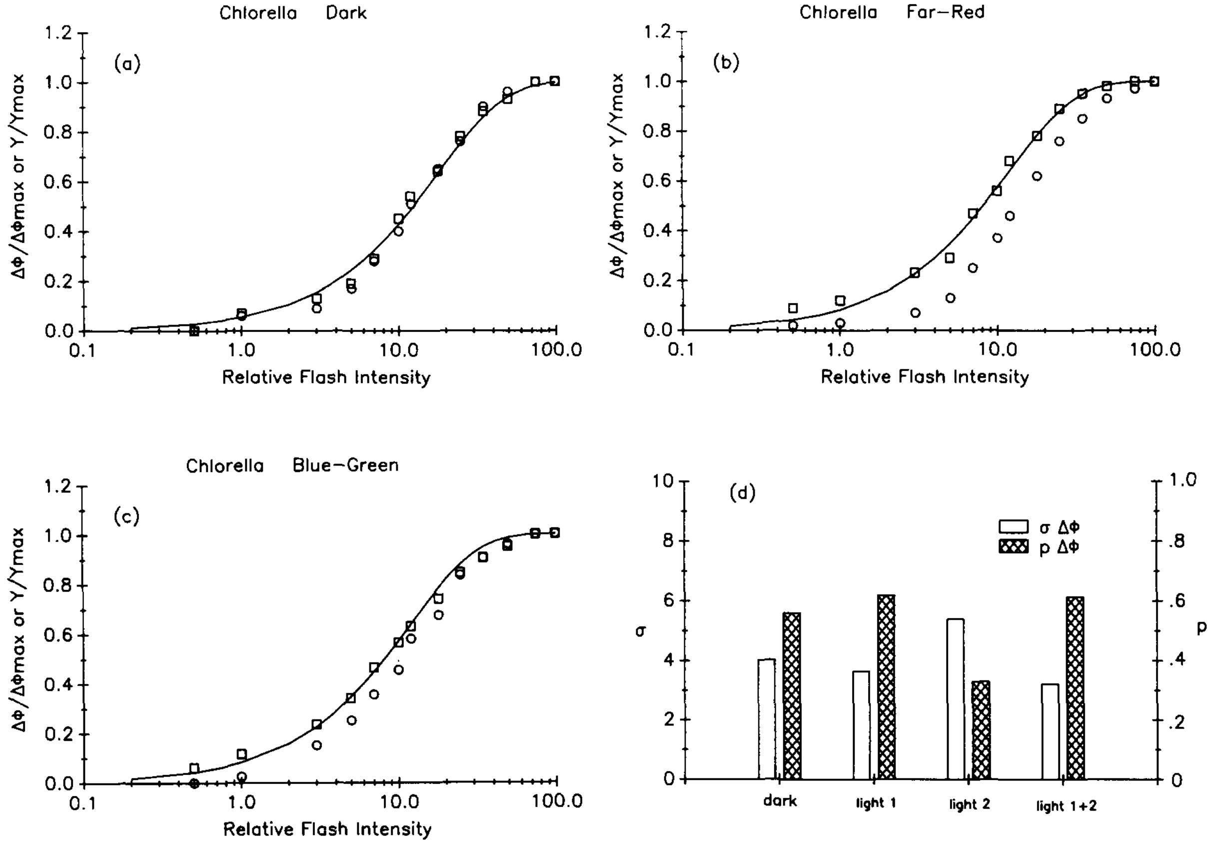

Fig. 1. Representative flash intensity saturation curves for Y (□) and $\Delta\phi$ (○) in *Chlorella pyrenoidosa* at 23° C under aerobic conditions in the (a) dark and (b) with a far-red background (more than 720 nm) source providing a continuous photon flux density of $1.0 \cdot 10^{16}$ quanta·m^{-2}·s^{-1}. (c) The deviation between flash intensity saturation curves for Y and $\Delta\phi$ in *Chlorella* measured in the dark (○), with a blue-green background light of $1.0 \cdot 10^{14}$ quanta·m^{-2}·s^{-1} (△) and with a far-red background light of $1.0 \cdot 10^{16}$ quanta·m^{-2}·s^{-1} (□) with an interval between pump flashes of 2 s and a 70 μs delay between pump and probe flashes. (d) Results of model calculations (see Appendix) for the probability, P, of transfer of excitation energy between reaction centers II and the calculated geometrical absorption cross-sections under a variety of conditions in *Chlorella*.

than predicted from the Poisson function (compare Fig. 1a with Fig. 1b). A similar situation can be observed for flash-intensity saturation curves for oxygen in the data of Ley and Mauzerall (e.g., Fig. 7 in Ref. 12). The deviation between the measured flash intensity saturation curve and that predicted by a cumulative one-hit Poisson function in dark-adapted cells may be caused the decay of some reaction centers from S_3 to lower S states in the 2 s interval between pump flashes. To examine that possibility, we measured the effect of the time delay between saturating pump flashes on Y and $\Delta\phi$ (Fig. 2). In *Chlorella*, Y remained constant up to 1.0 s and then decayed by a first-order process, with a half-time for the decay of 13 s. After 2 s between pump flashes 5–20% of the S_3 had decayed, depending on the experiment. In the presence of a continuous background light the reduction in Y does not occur because S_3 can be repopulated between flashes by photons absorbed from the continuous light source (see below).

The flash intensity saturation curves for $\Delta\phi$ invariably lagged behind Y at low flash intensities and rose more steeply at higher flash intensities than a cumulative one-hit model predicts (Fig. 1a–c). The deviation from a one-hit Poisson function was greatest for cells exposed to far-red light (Fig. 1b) and least for cells exposed to blue-green light (Fig. 1c). This deviation cannot be ascribed to the decay of the S states, as $\Delta\phi$ is relatively constant after 90 s between pairs of saturating pump flashes (Fig. 2). The deviation between a cumulative one-hit distribution and the observed distribution for the change in fluorescence yields may be interpreted within the context of transfer of excitation energy between PS II reaction centers [12,21].

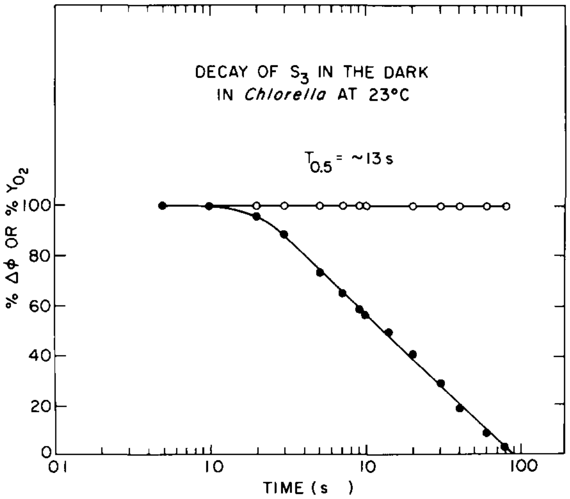

Fig. 2. The decay of S_3 in the dark in *Chlorella pyrenoidosa*. Y (●) and $\Delta\phi$ (○) were measured at time T_n, following a saturating pump flash at time T_0. The delay time between pump and probe flashes was 70 μs. In this representative experiment Y decayed with a half-time of 13 s while $\Delta\phi$ remained constant.

To analyze the transfer of excitation energy between PS II traps quantitatively we used a statistical model which assumes that every reaction center II, upon closing, may transfer the excess of excitation energy with a finite probability, p, to any other reaction center II, and the probability of the excitation energy being absorbed is proportional to the statistical level of 'openness' of the trap $(1 - \Delta\phi/\Delta\phi_{max})$ (see Appendix). In Fig. 1d we present calculated apparent relative absorption cross-sections for $\Delta\phi$, and the factor, p, which describes the probability of transfer of excess excitation energy between PS II reaction centers for $\Delta\phi$ under a variety of conditions. In this model σ is a geometrical parameter describing the probability of hitting a target σ by a flux of photons, as described by Poisson statistics [12,21]. The product of σ and p gives a parameter describing the intensity at which the process variable becomes light saturated. In the dark and in the presence of 10^{16} μmol quanta · $cm^{-2} \cdot s^{-1}$ of far-red light or a mixture of far-red and blue-green light (10^{16} and 10^{14} μmol quanta · $cm^{-2} \cdot s^{-1}$, respectively), p for $\Delta\phi$ was high, averaging 0.60. In contrast, in the presence of 10^{14} μmol quanta · $cm^{-2} \cdot s^{-1}$ of blue-green light alone, p for $\Delta\phi$ was 0.35. The changes in p were accompanied by corresponding inverse changes in σ (Fig. 1d).

The relationship between Y and steady-state photosynthesis

The effect of blue-green light on Y and steady-state photosynthetic oxygen evolution is shown in Fig. 3a and c for *Chlorella* and *Chaetoceros* under aerobic conditions. With a flash frequency of 0.5 s^{-1}, the addition of a weak background light (10^{13} quanta · $cm^{-2} \cdot s^{-1}$) caused Y to increase slightly from the dark-adapted value to a maximum, Y_{max}.

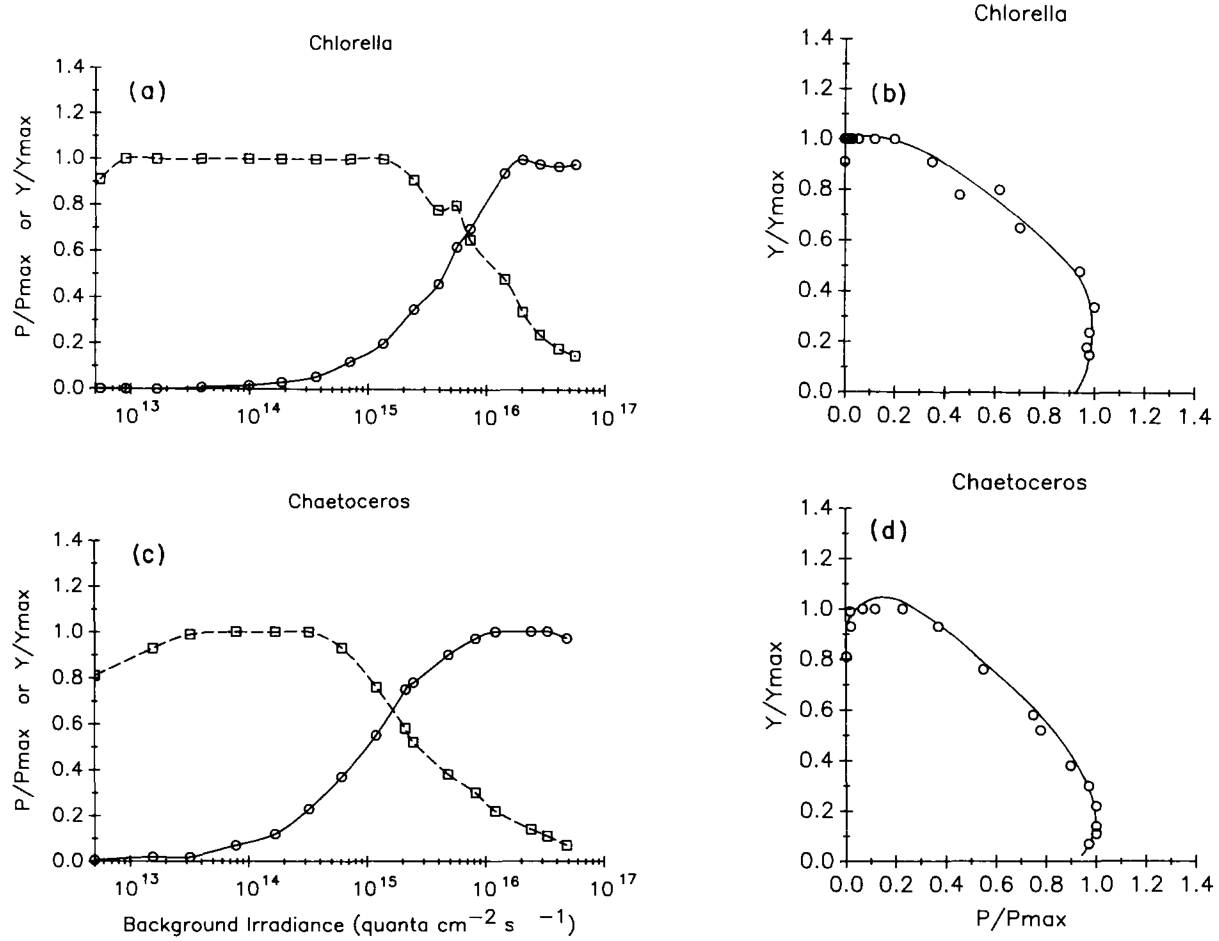

Fig. 3. The relationship between photon flux density, oxygen flash yields (□) and steady-state oxygen evolution (○) in *Chlorella pyrenoidosa* and *Chaetoceros gracillis* under aerobic conditions. Background irradiance was provided by a blue-green source (see text). The relationship between Y/Y_{max} and P/P_{max} is shown for both species.

Under aerobic conditions the extent of this enhancement varied between experiments, ranging from 5 to 20%. We attribute this increase to the repopulation of S_3 by the continuous photon flux (Fig. 2). As photon flux density increased beyond this activation level, Y remained relatively constant and then decreased at photon flux densities above 10^{15} quanta · cm^{-2} · s^{-1}. The inflection point at which Y decreased with increasing continuous irradiance levels corresponded to 20% of the light-saturated photosynthetic rate. As steady-state photosynthetic rates became light saturated Y continued to decline, and further decreases in Y were observed as cells were exposed to supra-optimal background irradiance levels.

The relationship between Y/Y_{max} and P/P_{max} is shown in Fig. 3b and d. This relationship is remarkably constant for both species and for HL and LL cells (data not shown) and is similar to that reported by Ley and Mauzerall for *Chlorella vulgaris* [12].

In eucaryotic algae light absorbed by Chls *b* and *c* is delivered to PS I as well as PS II [3,22]. We examined the absorption of our blue-green light ('light 2') by PS I, by comparing oxygen flash yield behavior under aerobic conditions with that obtained under anaerobic conditions. Under anaerobic conditions, with excess CO_2 available, the behaviour of Y at low levels of light 2 is quantitatively different from that observed under

TABLE I

Y/Y_{max} VALUES IN *CHLORELLA* CELLS UNDER AEROBIC AND ANAEROBIC CONDITIONS

Conditions	Y/Y_{max}			
	in the dark	in background light light 1 $1.1 \cdot 10^{16}$ μmol·quanta·$m^{-2} \cdot s^{-1}$	in background light light 2	
			$3 \cdot 10^{14}$ μmol·quanta·$m^{-2} \cdot s^{-1}$	$3 \cdot 10^{15}$ μmol·quanta·$m^{-2} \cdot s^{-1}$
Aerobic	0.9	1.0	1.1	0.5
Anaerobic	0.1	1.0	0.4	1.0

aerobic conditions (Table I, Fig. 4). It has previously been shown that under anaerobic conditions the plastoquinone pool is virtually completely reduced [23]. Anaerobic oxygen flash yields in the dark are low, averaging only 10% of Y_{max} obtained under aerobic conditions. Under anaerobic conditions oxygen flash yields increase markedly with increasing levels of light 2 up to a maximum at continuous photon fluxes of 10^{15} μmol quanta · $m^{-2} \cdot s^{-1}$. In cells exposed to far-red light, at levels which drive only 0.01% of P_{max}, Y is maximal under anaerobic conditions (data not shown). These data are easily explained by assuming that the light 1 oxidizes PQH_2, allowing more flash-driven electron equivalents to be donated to the acceptor side of PS II from water. The enhancement by moderate levels of light 2 (Fig. 4), is due to the excitation of PS I, which allows a fraction of PQH_2 to become oxidized. Thus, while the 'light 2' was absorbed by PS I, the photon flux density of the blue-green light required to oxidize PQH_2 was two orders of magnitude higher than that required to stabilize S_3 (compare Fig. 3a and 4).

The relationship between $\Delta\phi_{max}$ and steady-state photosynthesis

The effect of continuous light on $\Delta\phi_{max}$ and its components, F_p and F_s, was measured on the same sample at the same time with the same flash and background irradiance regime as the measurement of Y and P (Fig. 5). At low levels of light 2, both F_s and F_p increased from the dark-adapted values and $\Delta\phi_{max}$ decreased. When light 1 was added to light 2, the behavior of $\Delta\phi_{max}$ was qualitatively similar to that observed with the light 2 alone (Fig. 5). However, the rise in F_s observed with blue-green light at photon fluence rates of more than $3 \cdot 10^{15}$ quanta · $cm^2 \cdot s^{-1}$ was eliminated. Furthermore, with light 1, the high light induced quenching of F_s was only observed at light 2 intensities of more than 10^{16} quanta ·

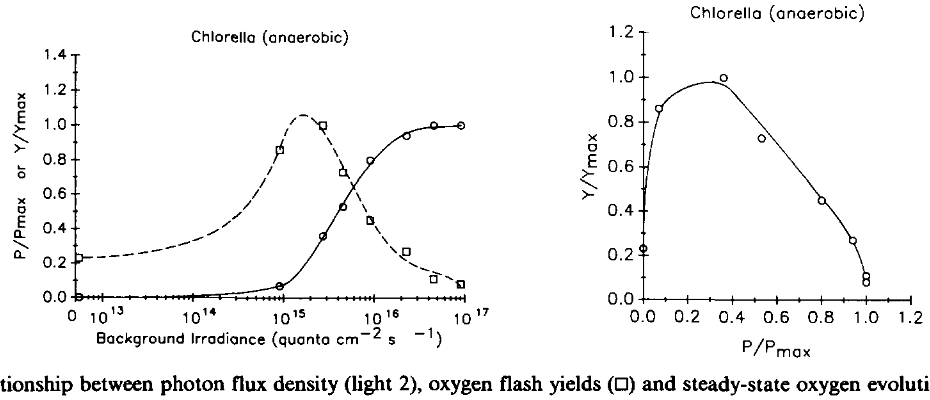

Fig. 4. The relationship between photon flux density (light 2), oxygen flash yields (□) and steady-state oxygen evolution for *Chlorella pyrenoidosa* (○) under anaerobic conditions. Note the large increase in Y/Y_{max} at low photon flux densities compared with aerobic conditions (Fig. 3).

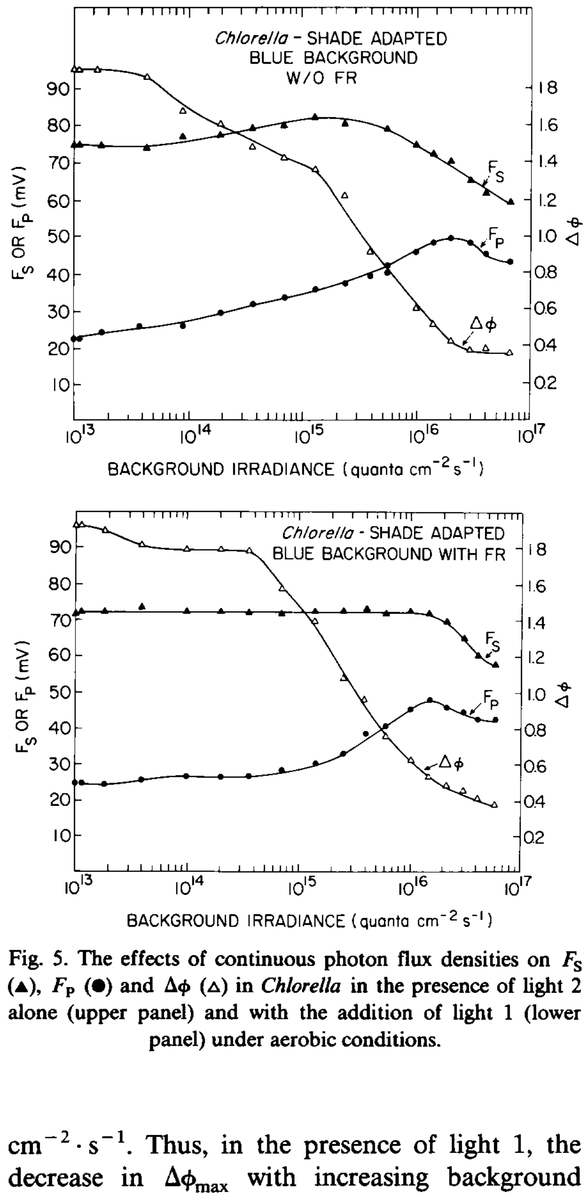

Fig. 5. The effects of continuous photon flux densities on F_S (▲), F_P (●) and $\Delta\phi$ (△) in *Chlorella* in the presence of light 2 alone (upper panel) and with the addition of light 1 (lower panel) under aerobic conditions.

cm$^{-2}\cdot$s^{-1}. Thus, in the presence of light 1, the decrease in $\Delta\phi_{max}$ with increasing background irrediance was due to completely to changes in F_p. At irradiance levels of more than 10^{16} quanta · cm$^{-2}\cdot$s^{-1} both F_s and F_p were quenched with and without far red light.

The decrease in $\Delta\phi$ induced by low continuous photon flux densities of light 2 did not correspond with steady-state oxygen production (Fig. 5). At irradiance levels of more than 10^{15} quanta · cm^2 · s^{-1}, $\Delta\phi$ decreased markedly, F_s reached a maximum at about $3\cdot10^{15}$ quanta · cm^2 · s^{-1} and decreased at higher irradiance levels, while F_p reached a maximum at approx. $2\cdot10^{16}$ quanta ·

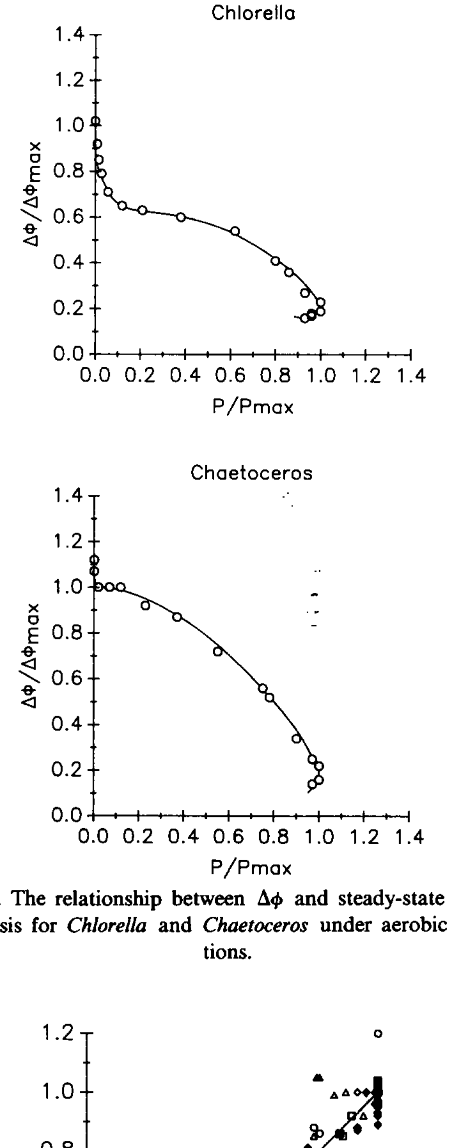

Fig. 6. The relationship between $\Delta\phi$ and steady-state photosynthesis for *Chlorella* and *Chaetoceros* under aerobic conditions.

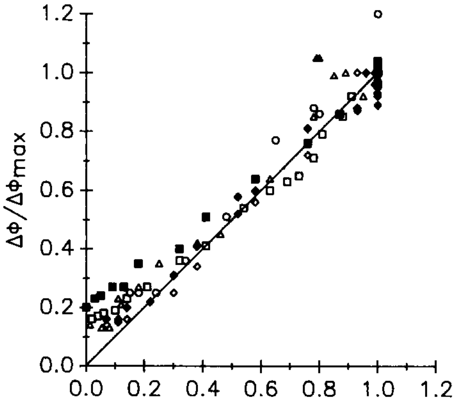

Fig. 7. The relationship between $\Delta\phi$ and Y under aerobic conditions in *Chaetoceros gracillis* in light 2 (△) and light 1 plus light 2 (○) in *Chlorella pyrenoidosa* in light 2 (■, □), light 1 plus light 2 (◆, ◇).

$cm^{-2} \cdot s^{-1}$ and then decreased. At very high photon flux densities $\Delta\phi$ remained relatively constant, averaging approx. 20% of the maximum value. The relationship between $\Delta\phi$ and P/P_{max} is shown in Fig. 6 and is similar to that between Y and P/P_{max} (Fig. 3b and d), with two important exceptions: (a) $\Delta\phi$ decreases from the dark-adapted value with the addition of weak background light 2, while Y increases; and (b) at supersaturating continuous photon flux densities, $\Delta\phi$ remained at 15–28% of $\Delta\phi_{max}$, while Y diminished to near zero.

Discussion

The concept of energy transfer between PS II reaction centers arose from an interpretation of the sigmoidal shape of fluorescence induction curves in the presence of DCMU [9]. Such a phenomenon, if it occurs, should be reflected in the flash-intensity saturation curve for the change in fluorescence yields as a deviation from a cumulative one-hit Poisson function [12]. Ley and Mauzerall [12] observed both sigmoidal DCMU-poisoned induction curves, and flash intensity saturation curves which closely (but not identically) fit a cumulative one-hit Poisson function. Under aerobic conditions the deviation of flash intensity saturation curve for $\Delta\phi$ from Poisson statistics is lower for cells under light 2, slightly higher in the dark, and highest under light 1 conditions. Assuming a single quencher of fluorescence, the deviations are not consistent with a hetrogeneous distribution of absorption cross-sections of PS II units [12]. The results of our model calculations suggest that the probability of transfer of excitation energy between PS II traps can vary by a factor of about 2, depending on the quality of background light (Fig. 1d).

While part of the discrepancy between Ley and Mauzerall [12] and Joliot and Joliot [9] may be due to differences in methodology, our results, measured with techniques essentially similar to those used by Ley and Mauzerall, suggest that physiological conditions may affect the 'connectivity' between PS II units. Kyle et al. [5] suggested that the extent of energy transfer between PS II traps could be modulated by the degree of phosphorylation of LHC II within thylakoids. Specifically, based on analyses of fluorescence induction curves in the presence of DCMU, they found that in vitro phosphorylation led to a reduction in energy transfer while dephosphorylation increased transfer efficiency. Our results, based on flash intensity saturation curves in unpoisoned cells, are consistent with those of Kyle et al., assuming that in experimental conditions of 10^{14} μmol quanta $\cdot m^{-2} \cdot s^{-1}$ light 2 the level of LHC II phosphorylation is increased, and at 10^{16} μmol quanta $\cdot m^{-2} \cdot s^{-1}$ light 1 LHC II is dephosphorylated. We have previously shown that these levels of background irradiance of light 2 and light 1 caused state transitions in *Chlorella pyrenoidosa,* as indicated by fluorescence transients [24]. We note that the background irradiance used by Ley and Mauzerall would be potentially absorbed by PS II, which appears to minimize energy transfer between PS II traps. It remains unclear how excitation energy is transferred between PS II reaction centers; however, it is presumably mediated by LHC II [24].

It is interesting that dark-adapted cells appear to have a slightly lower ability to transfer excitation energy between PS II units than cells exposed to far-red light. If energy transfer decreases as a consequence of a PQ-redox mediated kinase, these results suggest that dark-adapted cells, kept under aerobic conditions have a moderately reduced PQ pool. There is evidence that chloroplast respiration (chlororespiration) leads to reduction of some fraction PQ in *Chlorella pyrenoidosa* in the dark [26]. Furthermore, it has been shown that chlororespiration is sufficient to phosphorylate significantly thylakoid proteins in dark-adapted cells [22].

The relative flash yield of O_2 (Y/Y_{max}) and the change in fluorescence yield induced by a saturating pump flash ($\Delta\phi/\Delta\phi_{max}$) are highly correlated at moderate background irradiance levels, regardless of spectral quality (Fig. 7). The linear correlation between these processes in the presence of either light 1 or light 2 suggests that electron transport from H_2O to Q_a is highly coupled; i.e., there is no evidence of cyclic electron trnasport around PS II. However, at very low and very high irradiance levels the coupling between Y and $\Delta\phi$ is poor, and we examine this uncoupling within the context of hypothesized cyclic electron transport around PS II.

At low photon flux densities $\Delta\phi_{max}$ decreases more markedly than Y increases. If changes in $\Delta\phi_{max}$ were simply due to the reduction of Q_a, and it is assumed that Q_a is the sole fluorescence quencher in PS II at room temperature, the observed relationship between $\Delta\phi_{max}$ and Y cannot be attributed to cyclic electron flow around PS II at low photon flux densities. Excitation of PS II by a weak continuous source causes more fluorescence quenching than can be accounted by electrons originating from the donor side of PS II, regardless of the spectral quality of the light source. It is likely that low light induced changes result from the generation of a transthylakoid $\Delta\psi$ and/or pH gradient, which lead to changes in fluorescence yield [8].

At high photon flux densities Y decreases at a faster rate than $\Delta\phi$ (Fig. 7). This type of uncoupling beteen the formation of S_3 and the reduction Q_a can occur under physiological conditions for a number of reasons, including a back reaction between Q_a and P-680 [28,29], the Mehler reaction [30] and cyclic electron flow around PS II [17–19].

From delayed light emission studies it has previously been shown that the reoxidation of Q_a may be due to charge recombination but the back reaction does not populate S_3 [28,29]. However, under our experimental conditions, following a saturating flash, Y decays exponentially after a 1 s lag, with a half-time of 13 s, while $\Delta\phi$ remains constant (Fig. 2). Full activation of all PS II traps capable of forming S_3 should occur when approx. 1 photon is absorbed per RC II each second. At 10^{16} μmol quanta $\cdot$ cm$^2 \cdot$ s^{-1} of blue-green light, approx. 10^4 quanta per s are incident on reaction center II. These calculations suggest that at high continuous photon flux densities the contribution of a back reaction to the apparent uncoupling between Y and $\Delta\phi$ is negligible.

A second possible cause of the uncoupling between Y and $\Delta\phi$ at high photon flux densities is a stimulation of the Mehler reaction [30]. This is possible if oxygen produced in PS II by the saturating flash were consumed by PS I, which might occur if NADP were limiting. While we cannot absolutely exclude this possibility without isotope tracer data, we suggest that it is unlikely for the following reasons: (a) the Mehler reaction does not effectively compete with PS II photogenerated O_2 when CO_2 levels are high [30,31], which it was in our experiments; and (b) we measured Y after a 1 ms rise time, at the peak height, while the Mehler reaction has a relatively long time constant of several ms (Radmer, R., personal communication; see also Ref. 32).

A third possibility which may lead to an uncoupling between S_3 and Q_a is cyclic electron flow around PS II. This phenomenon could lead to the oxidation of Q_a and rereduction of P-680 through Z, without repopulating S_3. At very high photon flux densities the rate at which photons hit reaction center II is approx. tenfold greater than the turnover time of the water-splitting system (approx. 1.5 ms) [33,34]. However, at this hit rate 15–28% of Q_a appears to be oxidized as indicated by residual $\Delta\phi$. Weis and Renger [35] have shown that in trypsin-treated PS II membrane fragments, with an inactivated water-splitting system and blocked Q_A-Q_B electron transfer, Z becomes rereduced and in turn can reduce P-680 with a time constant of 15 μs. The oxidation of Q_a^- proceeds with a time constant of 200 μs. These results are consistent with reduction of Z by Q_a^-.

It has previously been suggested that cyclic electron flow around PS II might serve as a sink for excess excitation energy absorbed by PS II and thereby reduce photodamage to PS II reaction-centers [18]. We note that photoinhibition of steady-state oxygen evolution may still occur, in spite of the existence of such a cycle.

Our data do not address the issue of what specific electron carriers may mediate cyclic electron transport around PS II. A number of investigations have suggested that high potential cytochrome *b*-559 may be involved in such a cycle [14,19]; however, the measured turnover times of cytochrome *b*-559 appear to be too long to compete with linear electron transport significantly. To our knowledge, however, turnover of cytochrome *b*-559 has not been measured with a continuous saturating background light source. Packham and Barber [36] observed that when water oxidation is blocked by Ant5P, high-potential cytochrome *b*-559 is oxidized by low light and reduced by high light. This observation suggests tht if cyclic electron flow around PS II occurs at high light intensities it may in fact be mediated by cytochrome *b*-559. It would be interesting to see if the turnover of cytochrome *b*-559 increased markedly in high continuous background light.

Appendix

Assuming a homogenous absorption cross-section for PS II, and a single fluorescence quencher for each reaction center, target theory predicts that the flash intensity saturation curve for the change in fluorescence yield will be described by a cumulative one-hit Poisson distribution of a form

$$\Delta = \Delta\phi/\Delta\phi_{m_{\sigma}x}(I) = P[k > 0] = 1 - (1 - \sigma)^{I} \tag{A-1}$$

where k is the number of hits of target σ by photon density I [21]. The apparent deviation of the experimental data from above distribution toward higher Δ implies energy transfer between the PS II reaction centers. Such a phenomenon may occur when PS II units, with a currently closed reaction center, are hit by a second and subsequent photon with the result that the probe flash following a saturating flash has a higher fluorescence yield than would be expected if no excitation energy were transferred. The functional description of the energy-transfer phenomena in terms of a 'connectivity factor', which represents the probability of the transfer of an excitation encountering a closed reaction center II to another reaction center II has been given by Joliot and Joliot [9] and Paillotin [10].

We propose an alternative model to describe phenomenologically energy transfer between reaction centers II. Assume that every reaction center II, upon closing, may transfer the excess of excitation energy with a finite probability, p, to any other reaction center II, and that the probability of accepting the transferred energy by the reaction center (in terms of photoconversion) is proportional to the statistical level of 'openness' of the trap, $1 - \Delta$. The effect of energy sharing on Δ may be expressed as

$$\Delta = P[k > 0] + E[k > 1]E[\text{abs}] \tag{A-2}$$

where $P[k > 0]$ is the probability of at least one hit, $E[k > 1]$ is the expected value of more than one hit and $E[\text{abs}]$ is the expected value of absorbing the excess of excitation by currently open traps.

Assuming the Poisson statistics, one can express $P[k > 0]$ and $E[k > 1]$ as follows:

$$P[k > 0] = 1 - P[k = 0] = 1 - (1 - \sigma)^{I} \approx 1 - e^{\sigma I} \tag{A-3}$$

where I is the flash intensity and σ is the apparent absorption cross-section;

$$\begin{aligned} E[k > 1] &= \sum_{i=2}^{\infty} P[k = i]i = \sum_{i=1}^{\infty} P[k = i]i - P[k = 1] \\ &= \sigma I - \sigma I(1 - \sigma)^{I-1} \approx \sigma I - \sigma I\, e^{\sigma I} \\ &= \sigma I(1 - e^{-\sigma I}) \end{aligned} \tag{A-4}$$

The absorption of 'excess' excitation may consist of several steps of excitation transfer over actually closed traps, until an open one is found. Denoting the probability of transfer by p, and assuming that the probability of finding the closed and open traps is equal to Δ and $1 - \Delta$, respectively, the $E[\text{abs}]$ may be described as

$$\begin{aligned} E[\text{abs}] &= p(1 - \Delta) + p\Delta p(1 - \Delta) + \ldots + (p\Delta)^{n-1} p(1 - \Delta) \\ &= p(1 - \Delta)\sum_{n=0}^{\infty} (p\Delta)^{n} = \frac{p(1 - \Delta)}{1 - p\Delta} \end{aligned} \tag{A-5}$$

The nth term in Eqn A-5 describes the probability of absorbing the excitation after n transfer steps. Thus,

$$\Delta = 1 - e^{-\sigma I} + \sigma I(1 - e^{-\sigma I})\frac{p(1 - \Delta)}{1 - p\Delta} \tag{A-6}$$

where Δ is the average level of 'closeness' of traps before the transfer of the excitation. Every act of trapping will change this level (in both the microscopical scale and in above mathematical model), so the Δ will have to be calculated iteratively. Thus, when fitting the experimental data to Eqn. A-6 using the least-squares procedure, an internal iteration loop should be added to calculate the trial Δ. Rewriting Eqn A-6 to form

$$F = -\Delta + 1 - e^{-\sigma I} + \sigma I(1 - e^{-\sigma I})\frac{p(1 - \Delta)}{1 - p\Delta} \tag{A-7}$$

the iterative solution for Δ will take form:

$$\Delta_{k+1} = \Delta_k + \frac{F(\Delta_k)}{(\partial F/\partial\Delta)_{\Delta = \Delta_k}} \tag{A-8}$$

where

$$(\partial F/\partial \Delta)_{\Delta=\Delta_k} = -1+\sigma I(1-e^{-\sigma I})\frac{p^2-p}{(1-p\Delta_k)^2} \tag{A-9}$$

with Δ_0 calculated as

$$\Delta_0 = 1-e^{-\sigma I} \tag{A-10}$$

The iteration should be continued until a condition

$$\frac{|\Delta_{k+1}-\Delta_k|}{\Delta_k} < r \tag{A-11}$$

will be met with r being a small number. A satisfactory result can be obtained with r ranging between $1 \cdot 10^{-4}$ and $1 \cdot 10^{-8}$. As Eqn. A-7 is only mildly nonlinear, the number of necessary iterations of Eqn. A-8 satisfying the condition A-11 is usually less than 4. The resulting computational overhead is relatively small compared to the main part of the least-squares computations.

The derivatives of $\Delta\phi_{\max}$, σ and p, are calculated with Δ as a parameter calculated in the previous least-squares iteration step, with the initial values being the guesses. The initial fit function required to start the least-squares procedure is calculated as given by Eqn. A-10.

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