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ENHANCEMENT STUDIES ON ALGAE AND ISOLATED CHLOROPLASTS

PART I. VARIABILITY OF PHOTOSYNTHETIC ENHANCEMENT IN *CHLORELLA PYRENOIDOSA*

W. P. WILLIAMS and Z. SALAMON*

Biophysics Laboratory, Chelsea College, University of London, Manresa Road, London SW3 6LX (U.K.)

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SUMMARY

Studies of the variability of enhancement in *Chlorella pyrenoidosa* confirm the existence of two types of variability: a very slow diurnal variation linked to the growth cycle and a much more rapid adaptive response to the immediate incident light conditions (State I–State II transitions). Measurements of the wavelength dependencies and relative contributions of these two types of variability suggest that they may be linked.

A close examination of the enhancement signals associated with the State I–State II transition reveals that the transitions can take place in any one of three ways: by a change in Photosystem II efficiency alone, by a change in Photosystem I efficiency alone or by a simultaneous change in the efficiencies of both photosystems.

Measurements of the rates of transition between State I, State II and the dark adapted state, Dark, suggest that the behaviour of State II and Dark are normally, but not always, identical. The transitions between the three states were found to be first order. For those samples exhibiting the same behaviour in Dark and State II, the rate of the State I–State II transition was found to be independent of the wavelength of Light II, suggesting that the return from State I to State II is essentially a dark process and that the driving force for the adaptive transition is the over-stimulation of Photosystem I.

Finally, a model is proposed, involving an antagonistic control of the quantum yields of photochemistry of the two photosystems, that is capable of explaining the links between the two types of variability, their wavelength dependencies and the shapes of the individual enhancement signals.

Abbreviations: PSI, Photosystem I; PSII, Photosystem II.

* Permanent address: Physics Department, Technical University of Poznan, Poznan, Poland.

INTRODUCTION

The enhancement of the oxygen evolution efficiency of light predominantly absorbed by one photosystem by the superposition of light predominantly absorbed by the other was first reported by Emerson et al. [1]. Since then the phenomenon has been extensively studied in many laboratories [2].

The majority of enhancement studies have been performed on whole cell systems, most commonly on the unicellular alga *Chlorella pyrenoidosa*. In recent years these studies have been extended to isolated chloroplasts. Initially the results of such studies proved to be extremely variable, some workers observing enhancement [3–5] and others failing to do so [6, 7]. It is now recognised that this variability arose from the fact that the chloroplasts used in such studies were broken or badly damaged on isolation and that the observation, or lack of observation, of enhancement was dependent on the presence, or absence, of certain co-factors [8–10].

The inherent variability of the enhancement phenomenon is not a new concept. The variation of enhancement with cell development in synchronously grown algal cultures is well documented [12–14]. These changes, which are extremely slow, taking place over periods of hours, appear to be firmly linked to the growth cycle of the cells. Bonaventura and Myers [15], however, working with unsynchronised cultures of *Chlorella*, have reported a more rapid type of change that takes place as a direct response to the distribution of incident light between the two photosystems. They observed that illumination with light predominantly absorbed by Photosystem I (Light I), led to a transition, over a period of minutes, from a state characterised by a low enhancement (State II) to a state characterised by a higher enhancement (State I). This change was reversed on illumination with light predominantly absorbed by Photosystem II (Light II). Measurements of the chlorophyll *a* fluorescence of intact chloroplasts [16–20] suggest that similar changes might also occur in intact chloroplasts but direct evidence for such changes based on enhancement studies has not as yet appeared in the literature.

The experimental results described in the present paper, Part I, suggest that the slow changes in enhancement linked with the algal growth cycle and the much more rapid State I–State II transitions are related phenomena. Studies of the change of enhancement in State I and State II with wavelength and time, of the shape of the individual enhancement signals and of the rates of transition between the two states suggest that both phenomena can be interpreted in terms of changes in the relative photosynthetic efficiencies of Photosystem I and Photosystem II brought about by changes in the concentration of a central control factor. In Part II, we report a series of experiments, performed in co-operation with Barber and Mills [11] on enhancement in intact chloroplasts, that suggest that this factor is likely to be Mg^{2+}.

MATERIALS AND METHODS

Algae culture

Cl. pyrenoidosa strain 211/8c from the Culture Collection of Algae and Protozoa, University of Cambridge, was cultured in Knops medium at a temperature of 27 °C under a 10 h dark–14 h light cycle. The cultures were usually started fresh from

agar-slope cultures and samples were collected at different stages throughout the log-phase growth period.

Oxygen evolution measurements

Oxygen evolution measurements were made using an a.c. oxygen electrode system similar to that described by Bonaventura and Myers [15]. The light sources were two 500 W projector lamps. Light I and Light II were isolated by the use of 705 nm and 645 nm Balzer Filtraflex Interference Filters (half-band widths 10–15 nm). Light II was chopped at about 4 Hz by a Brookdeal Model 479 Light Chopper; Light I was unchopped. The a.c. signal from the oxygen electrode was measured with the aid of a Brookdeal Model 9401 Lock-in Amplifier.

The circulating medium in the electrode system was 0.1 M KCl, 0.05 M phosphate buffer (pH 7.0), 10 mM KNO_3, 6 mM $MgSO_4$, 0.3 mM $KHCO_3$. Measurements were made at 22 °C. Samples were equilibrated for at least 15–20 min on the electrode surface prior to measurement.

The intensities of Light I and Light II, average intensity in the case of the chopped beam, were measured using an Eppley Thermopile. The light intensities varied from experiment to experiment but were usually about 80 $\mu W \cdot cm^{-2}$ (Light II) and 100 $\mu W \cdot cm^{-2}$ (Light I). Values for individual measurements are quoted in the relevant figure captions. It was often necessary to pre-illuminate samples in unchopped background light to ensure that they were in a given state at the start of a measurement. The preillumination intensities used were always 170 $\mu W \cdot cm^{-2}$ (Light II) and 240 $\mu W \cdot cm^{-2}$ (Light I). The required pre-illumination times varied from sample to sample. Checks were made to ensure that the algae were completely equilibrated in the relevant state. If measurements were made on dark-adapted algae, checks were again made to ensure that full dark-equilibration had been achieved.

RESULTS

Enhancement ratio

The a.c. electrode is sensitive only to oxygen evolution stimulated by the modulated Light II beam. Enhancement using this type of apparatus is most conveniently expressed in terms of the enhancement ratio, E, introduced by Sinclair [9]:

$$E = \frac{\text{a.c.}O_2 \text{ evolution in Light II plus Light I}}{\text{a.c.}O_2 \text{ evolution in Light II alone}} \quad (1)$$

The enhancement ratio reflects the enhanced efficiency of the Photosystem II component of Light II resulting from the increased availability of Photosystem I products on the superposition of the Light I beam. It is closely related to, but not identical with, the various values of enhancement measured under the corresponding conditions using a d.c. electrode system [2].

Our enhancement signals, like those previously reported by Bonaventura and Myers [15] were usually made up of two components: a fast initial rise, limited only by the response time of the instrument, which reflected the instantaneous state of the algae; and a slower component corresponding to the adaptation of the algae to the change in incident light. A typical signal is shown in Fig. 1.

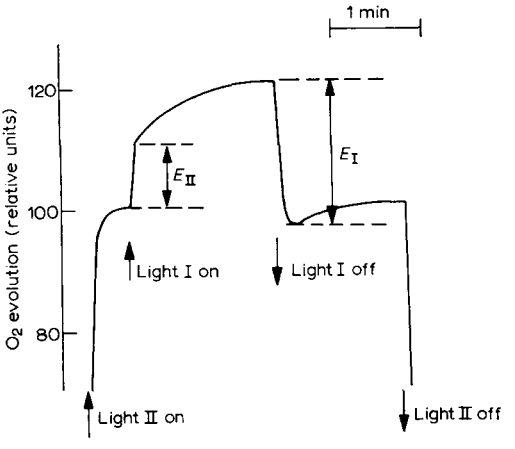

Fig. 1. Typical enhancement signal obtained by the superposition of Light I (intensity 100 μW · cm^{-2}) upon Light II (intensity 80 μW · cm^{-2}). The sample was pre-illuminated in unchopped background light directly prior to measurement to ensure that it was initially in State II. The values of E_{II} and E_I characterising the instantaneous enhancement in State I and State II respectively are given by the ratio of the indicated levels of oxygen evolution. Details of the preillumination regimen are given in the Methods section.

We shall refer to the value of E for the fast component as E_I, E_{II} or E_D, depending on whether the algae were pre-illuminated in Light I (State I), pre-illuminated in Light II (State II) or dark adapted (Dark) in the period directly preceding measurement. The change in value of the enhancement ratio accompanying adaptation, the slow component, will then be given by expressions of the type:

$$\Delta E_{II-I} = E_I - E_{II} \tag{2}$$

where the subscripts associated with ΔE refer to the initial and final states involved in the adaptation (cf. Fig. 1).

Absolute determinations of E are extremely difficult. At low light intensities the measurements are complicated by the Kok effect [21] and at higher intensities by the decreasing quantum efficiency of oxygen evolution. A typical plot of E_{II} as a function of the intensity of Light II is shown in Fig. 2. The intensity of Light I used to

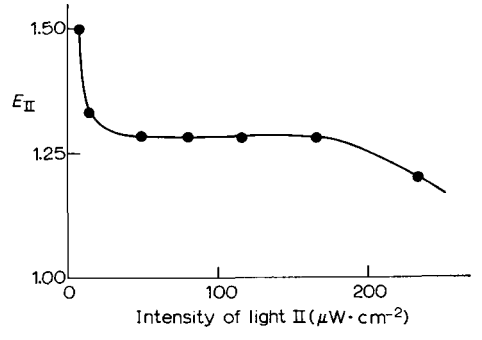

Fig. 2. Typical plot of the variation of enhancement ratio with the intensity of Light II. All measurements were made under State II conditions. The intensity of the enhancing Light I beam was adjusted to give the maximum enhancement for each individual measurement.

stimulate enhancement in these measurements was adjusted so as to yield maximum enhancement at each individual Light II intensity. The intensity curves varied somewhat from sample to sample, but always showed a sharp rise in E at low Light II intensities and a gradual fall in E at high intensities. A clear plateau region was normally detectable and the Light I and Light II intensities used for a given sample were chosen so as to ensure that enhancement measurements were made in the middle of this region.

Variability of enhancement ratio

Bonaventura and Myers [15] using unsynchronised cultures, reported enhancements of the order of 20–30 % in State I ($E_I = 1.2$–1.3), and of the order of 10 % in State II ($E_{II} \simeq 1.1$). If we used algae grown under similar conditions, we obtained similar values. However, as our interest lay in the variability of E, we chose to vary our growth conditions in order to obtain cells of varying age, pigment composition and degrees of synchronisation. Using cells grown from fresh agar-slope inoculations (a condition likely to encourage synchronisation), we obtained enhancement signals that differed from theirs both in size and shape.

Our samples yielded enhancement signals that differed from those reported by Bonaventura and Myers in two distinct ways. First, the rate and signal shape associated with the slow component of the enhancement signal varied very markedly from culture to culture. We shall return to this in a later section. Second, the relative contributions of the fast and slow components to the enhancement signal were extremely variable. This latter variation appeared to be associated primarily, but not exclusively, with changes in the fast component of the signal.

The value of E associated with the fast component of the enhancement signal varied not only from sample to sample but also for the same sample during the course of the day. A sample giving a value for E_I of 1.1 in the morning might, for example, give a value of 1.4 six h later. These changes could be followed either by leaving the sample on the electrode or by taking fresh samples from the culture tube during the course of the day. They were thus clearly associated with the growth cycle of the algal cells and not a consequence of changes in the cells on the electrode. This type of variability was maximal in young cultures inoculated direct from slope cultures and minimal in old cultures, approaching the end of their log-phase growth, and in cultures started by re-inoculation from earlier liquid grown cultures. These large variations in E over the daily growth cycle and through the life of the culture were thus clearly reflections of the diurnal rhythms in enhancement of the type reported by Senger and Bishop [12] arising from a partial entrainment of our algal cultures.

Our partially synchronised cultures thus appeared to show two distinct types of enhancement variability: a slow change in E dependent on cellular metabolism which is insensitive, and a much faster change ΔE which is sensitive, to the immediate incident light conditions. Under conditions of unsynchronised growth, the growth cycle-linked enhancement is averaged through the cell population and no diurnal variation is observed.

Wavelength dependence of E and ΔE

The wavelength dependence of E was measured by varying the wavelength of Light II whilst keeping the wavelength of Light I fixed at 705 nm. The intensity of

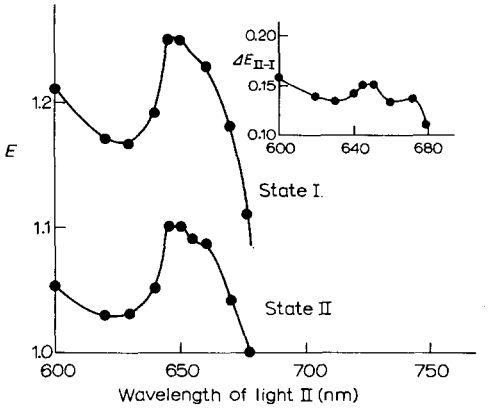

Fig. 3. Wavelength dependence of enhancement ratio for State I and State II for a sample showing little or no diurnal variation. The intensity of Light II beam for $\lambda = 645$ nm was 75 $\mu W \cdot cm^{-2}$. Its intensity at the other wavelengths was adjusted so as to yield the same rate of oxygen evolution as at 645 nm. The wavelength and intensity of Light I were kept at the standard value ($\lambda = 705$ nm, 100 $\mu W \cdot cm^{-2}$) throughout. The wavelength dependence of ΔE_{II-I}, the increase in enhancement ratio on going from State II to State I, is shown in the inset to the figure.

Light II at the different exciting wavelengths was adjusted to yield the same rate of oxygen evolution. This minimised distortions that might otherwise have arisen as a consequence of changes in position on the enhancement-intensity curve (cf. Fig. 2). The resolution of the resulting "enhancement spectrum" was limited by the half-band widths (10–15 nm) of the interference filters used to isolate Light II. It was, however, sufficient to allow comparison with other such spectra already in the literature.

The wavelength dependence of E in State I and in State II for a sample exhibiting little or no diurnal variation is shown in Fig. 3. Comparison of these results with earlier enhancement spectra is complicated by the fact that the enhancement ratio measured in this investigation differs from enhancement measured using d.c. techniques. The present plots do, however, mirror the main features of the earlier results [8, 22, 23], in that they show maxima at about 650 and 670 nm and a rapid decrease in enhancement above 670 nm. The corresponding wavelength dependence for ΔE_{II-I} is plotted in the inset to the figure. ΔE_{II-I} shows a small but reproducible wavelength dependence that closely resembles that of the enhancement curves.

The wavelength dependence of E_I for a sample exhibiting an appreciable diurnal variation is shown in Fig. 4. The measurements were restricted to State I so as to minimise effects due to changes in enhancement during the period of measurement. Again the two curves are similar in appearance to published enhancement spectra. The wavelength dependence of the diurnal change in E_I, denoted by $\Delta E_I'$, is shown in the inset to the figure. It, like the ΔE_{II-I} curve in Fig. 3, resembles the individual enhancement curves. The match is not quite so good as that for the State I–State II curves, but this can probably be attributed to time dependent changes in enhancement during the course of the measurements. The diurnal and the adaptive changes thus both appear to be constant fractions of the instantaneous enhancement level. The possible significance of this will be examined in the Discussion section.

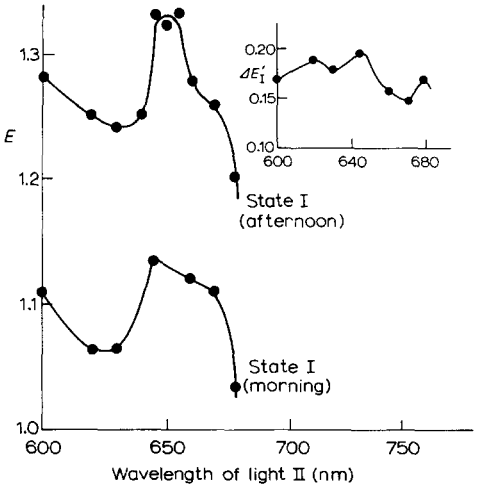

Fig. 4. Wavelength dependence of the enhancement ratio for a sample showing appreciable diurnal variation. The wavelength dependence was first measured shortly after placing the sample on the electrode (morning) and again 6 h later (afternoon). All measurements were made under State I conditions. The intensity of Light II at $\lambda = 645$ nm was 65 $\mu W \cdot cm^{-2}$, otherwise all conditions were as described in the caption to Fig. 3. The wavelength dependence of the increase in the State I enhancement ratio between the morning and afternoon measurements, $\Delta E_I'$, is shown in the inset.

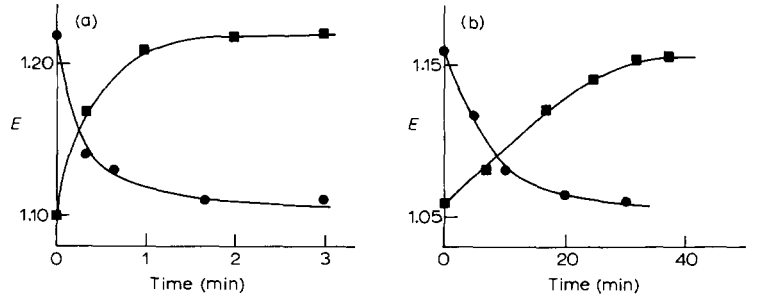

Fig. 5. Plots of the time courses of State I–State II transitions. Plot (a) shows a rapid transition, effectively completed in under a minute, and plot (b) shows a much slower transition. Samples were initially pre-illuminated to ensure that they were in State II. The development of State I in Light I was followed by switching off Light I for short periods to sample the Light II level. The reverse transition was followed by sampling the enhancement signal for short periods, as the sample returned from State I to State II, following the removal of Light I. The intensities of Light II and Light I were 80 $\mu W \cdot cm^{-2}$ and 100 $\mu W \cdot cm^{-2}$ for plot (a) and 60 $\mu W \cdot cm^{-2}$. and 110 $\mu W\, cm^{-2}$ for plot (b).

Signal shape and State I–State II transitions

We noted earlier that the shape of the enhancement signals varied from sample to sample. Part of this variation can be accounted for in terms of the varying contribution of the instantaneous enhancement, E, associated with the stage of the growth cycle. Another factor is the wide variation that can take place in the rate of the slow component. We observed transition times for the completion of the State I–State II transition varying from less than 1 min up to nearly 1 h (cf. Fig. 5). The effect of such changes can, however, be readily followed by measuring the enhancement

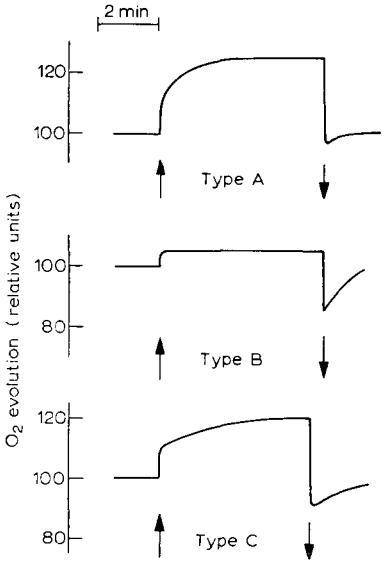

Fig. 6. Examples of the three different types of enhancement signal observed for *Chlorella*. The traces show the rate of oxygen evolution in Light II alone and in Light II+Light I. The arrows indicate the switching on and off of Light I. Samples were initially in State II and all measurements were made under the standard conditions outlined in the Methods section. See text for explanation of signals.

signal throughout the duration of the transition and replotting it on an appropriately compressed or expanded time scale.

After due allowance for these two factors, three distinct types of enhancement signal could be identified. Typical examples of each are shown in Fig. 6. The difference between the signals lies in the way that the oxygen evolution levels change during the State II–State I transition. In signals of Type A, the rate of oxygen evolution in Light II alone remains effectively constant in both State I and State II, whilst the rate in Light I+Light II is variable. In signals of Type B, the rate of oxygen evolution in Light I+Light II remains effectively constant in both State I and State II, but the rate in Light II alone is variable. In signals of Type C, the type reported by Bonaventura and Myers [15], the rates of oxygen evolution are variable in both Light II alone and Light II+Light I. These changes, as we shall detail in the Discussion section, are clearly reflections of variations in the relative efficiencies of the two photosystems.

Most of our samples showed signals that were exclusively, or at least predominantly, Type A or Type B. Type C signals were comparatively rare. All enhancement signals are average responses from the particular cell population present in the given sample. The relative rarity of Type C responses could be interpreted as meaning either that the partially synchronised cultures used in our measurements were normally at the extreme ends of a continuum of signal shapes varying from Type A through Type C to Type B, or that Type C signals are merely reflections of mixed populations of cells that individually show Type A or Type B responses. In either case, the Type C response can be considered as a compromise between two extremes and as such we will for the present limit ourselves to a consideration of Type A and Type B signals.

Relationship between State I, State II and Dark

The study of State I–State II transitions is complicated by the fact that the rates of oxygen evolution in both these states can potentially be influenced by the incident illumination. This difficulty can be overcome, if we relate all oxygen evolution levels to a standard state. The most convenient state for such purposes is the dark adapted state.

Bonaventura and Myers [15] reported that dark adapted cells showed some of the responses normally associated with State I. In particular, they noted that initial level of oxygen evolution in Light II was lowered. We investigated the relationship between the dark adapted state, Dark, and State I and State II for algae showing Type A and Type B responses. In order to do this we measured the initial rates of oxygen evolution in Light II and in Light II+Light I for dark adapted, Light I pre-illuminated and Light II pre-illuminated algae. The various rates, normalised to the Light II rate for dark adapted algae, together with the corresponding values of E and ΔE are set out in Table I.

TABLE I

Values of the relative rates of oxygen evolution of dark adapted, State II adapted and State I adapted algae for samples showing Type A and Type B responses and their corresponding enhancement ratios.

	Rate of O_2 evolution (relative units)		Enhancement ratio
	Light II	Light II+Light I	
Type A			
Dark	100	110	$E_D = 1.10$
State II	103	119	$E_{II} = 1.16$
State I	100	122	$E_I = 1.22$
Type B			
Dark	100	100	$E_D = 1.00$
State II	95	100	$E_{II} = 1.05$
State I	81	100	$E_I = 1.23$

In most cases the rates of oxygen evolution in State II and the Dark state were identical, but in a significant number of cases they differed. The slow variations in E inherent in our samples make it difficult to be certain of the significance of the small differences in Light II stimulated oxygen rates taken from successive measurements. We consistently found the rate of oxygen evolution in Light II for algae showing Type B responses to be slightly lower in State II than in the Dark state, but were unable to reach a firm conclusion regarding the relative rates for algae showing Type A responses.

The differences in enhancement ratio, E, between Light II and dark adapted algae could, as they simply involved the ratio of two levels of oxygen evolution before and after the superposition of Light I, be measured much more accurately. The values quoted in Table I clearly show that the Dark state lies closer to State II than to State I. In all our measurements with partially synchronised algae, independent of the type of response, we observed $E_I > E_{II} \geqslant E_D$. In our experience the Dark state most

closely resembles State II for such samples. If there is a difference, it is in that the Dark state shows more pronounced State II characteristics than are achieved by Light II illumination. The inherent variability of the responses do not, however, allow us to rule out the possibility of different relationships between the various states in algae grown under different conditions.

The relationship between the Dark state and State II for those samples in which they show non-identical behaviour is particularly clearly illustrated in the trace shown in Fig. 7. In this particular experiment, the enhancement ratio for dark adapted algae was $E_D = 1.10$. Pre-illumination with Light II, or exposure of the dark adapted algae to two min of Light II illumination yielded a value of $E_{II} = 1.15$. Illumination with Light II thus led to an increase in enhancement ($\Delta E_{D-II} = 0.05$), an action normally associated with Light I. Plots of the rates of the time course of transitions between the three states for the same sample are shown in Fig. 8. The intermediate position of State II is again clearly apparent.

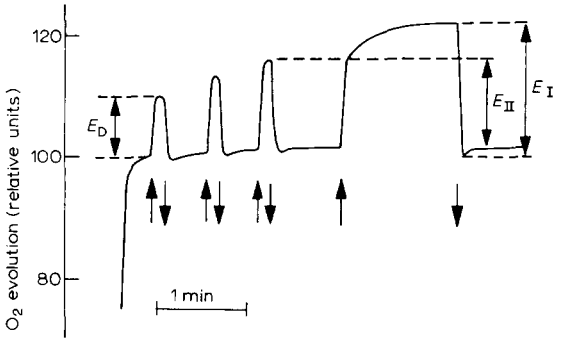

Fig. 7. Signal trace showing the relationship between the Dark state, State II and State I for a sample showing Type A responses for which the behaviour of Dark and State II are non-identical. The sample was initially dark adapted, see Methods. The initial enhancement was measured as soon as possible after switching on Light II. The transition from Dark to State II was then followed by sampling at intervals with Light I. After State II had been reached, Light I was left on so that the normal State II–State I transition could be followed. The arrows show the points at which Light I was switched on and off. E_D, E_{II} and E_I were calculated as the ratios of the indicated levels of oxygen evolution. The intensities of Light I and Light II were 100 $\mu W \cdot cm^{-2}$ and 80 $\mu W \cdot cm^{-2}$, respectively.

Sets of traces showing the relationship between the rate of oxygen evolution in both the initial and steady states, normalised to the dark adapted level in Light II, for samples showing Type A and Type B responses, are given in Figs. 9 and 10.

Influence of wavelength of Light II on State I–State II transitions

Algal samples showing Type A responses and samples showing Type B responses both yield "enhancement spectra" of the type shown in Figs. 3 and 4. The shapes of the individual enhancement signals and the rates for the State I–State II transitions can, however, show marked variations with wavelength. The nature of these changes is determined by the relationship between the Dark state and State II, but is independent of the type of response shown by the samples.

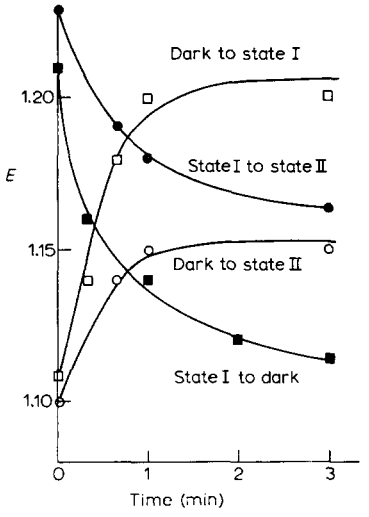

Fig. 8. Plots of the time courses of the transitions between State I, State II and Dark for the sample shown in Fig. 7. The sample was Dark, Light II or Light I adapted as described in the Methods section and the transitions between the different states were followed as described in the caption to Fig. 5.

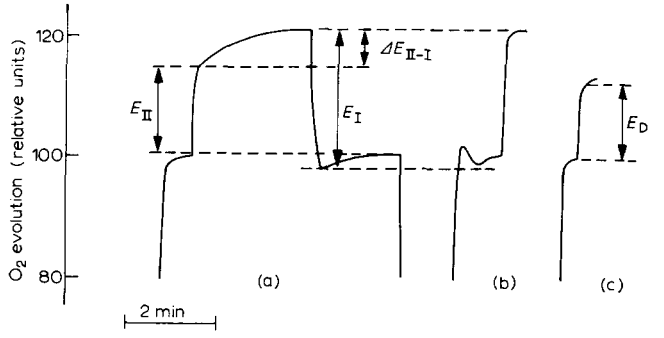

Fig. 9. A complete schematic of the relationship between the initial rate of oxygen evolution and the steady state levels in Light II alone and in Light II + Light I for a sample showing TYPE A responses. Normalised traces are shown for (a) Light II adapted (b) Light I adapted and (c) Dark adapted algal samples. The arrows indicating the switching on and off of Light I (100 $\mu W \cdot cm^{-2}$) and Light II (75 $\mu W \cdot cm^{-2}$) have been omitted to simplify the figure. E_D, E_{II}, E_I and ΔE_{II-I} can be calculated from the indicated levels in the normal way.

Light II dependence when Dark and State II show identical behaviour

If the behaviour of the Dark state and State II are identical, the wavelength dependence of the signal shape is very straightforward. The change in signal shape with varying wavelength of Light II is almost entirely due to the normal wavelength dependent variation of the fast component of the enhancement signal. A typical set of data, showing the magnitudes of the enhancement ratios in State I, State II and the transition rates between the two states for such a sample (measured for Light II of wavelength 630 and 645 nm), is given in Fig. 11a. Replotting this data on a logarithmic

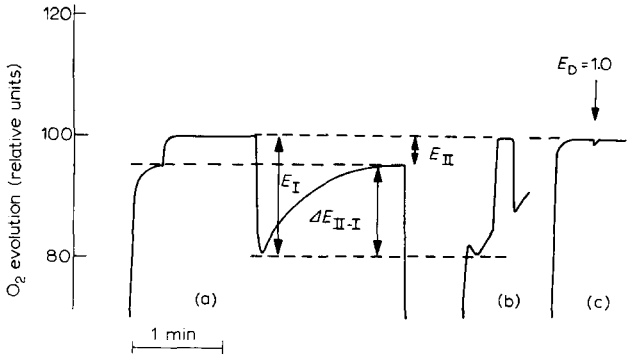

Fig. 10. A complete schematic of the relationships between the initial rates of oxygen evolution and the steady state levels in Light II alone and Light II + Light I for a sample showing Type B responses. Normalised traces are shown for (a) Light II adapted (b) Light I adapted and (c) Dark adapted algal samples. Again the arrows indicating the switching on and off of Light I (75 $\mu W \cdot cm^{-2}$) and Light II (65 $\mu W \cdot cm^{-2}$) are omitted.

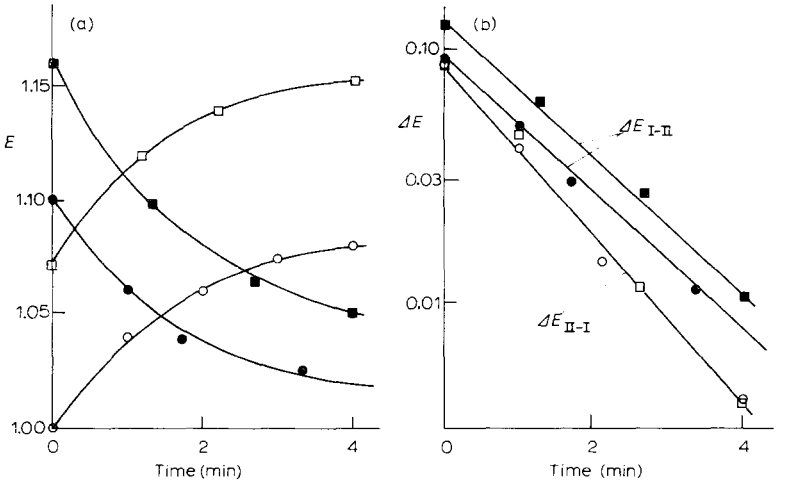

Fig. 11. Plots showing the time courses of State I–State II (Dark) transitions for a sample showing Type A responses for which the behaviour of Dark state and State II are identical. The plots were measured for Light II, $\lambda = 645$ nm (square symbols) and $\lambda = 630$ nm (circular symbols). The intensity of Light II at 645 nm was 65 $\mu W \cdot cm^{-2}$, the intensity at 630 nm was adjusted to give the same rate of oxygen evolution. The intensity of Light I was 75 $\mu W \cdot cm^{-2}$ throughout. The State I to State II transitions are given by the closed symbols and the State II to State I transitions by the open symbols. The transitions are plotted (a) linearly in terms of E and (b) logarithmically in terms of ΔE_{II-I} and ΔE_{I-II}.

scale (Fig. 11b), shows the transition rates to be first order. The magnitude of ΔE shows a slight wavelength dependence (cf. Fig. 3), but both the forward and the reverse transition rates between State I and State II appear to be effectively wavelength independent. Whilst this is not surprising in the case of the State II–State I transitions, which are both driven by Light I, some wavelength dependence might have been anticipated for the Light II driven State I–State II transitions.

Light II dependence when Dark and State II show different behaviour

If the behaviour of the Dark state and State II are not identical, the wavelength dependence of the shape of the enhancement signal and the transition rates between the various states are much more complex. Under these conditions, the changes in signal shape are made up of two contributions: the wavelength dependent variation of the fast component (common to all signals), and an additional change due to variations in the slow component. These latter differences were clearly illustrated in Fig. 8.

The wavelength dependence of the rates of State I to State II transitions for samples in which the behaviour of State II and Dark are non-identical is more complex than that for samples in which they coincide. A series of State I to State II transitions, measured for different wavelengths of Light II, are plotted on a logarithmic scale in Fig. 12.

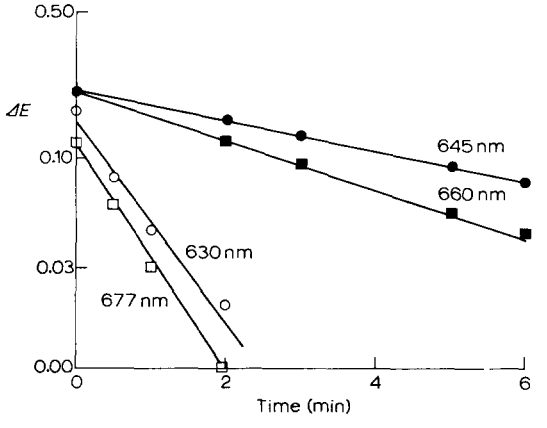

Fig. 12. Logarithmic plots of the time courses of a series of State I–State II transitions measured using Light II of different wavelengths for a sample showing Type B responses for which the behaviour of State II and Dark are non-identical. The intensity of Light II was 65 $\mu W \cdot cm^{-2}$ at 645 nm and the intensity of Light I was 75 $\mu W \cdot cm^{-2}$.

It is especially interesting to note that the slower transitions shown in Fig. 12 are associated with those wavelengths with the largest values of E (cf. Fig. 3). These wavelengths are those that would be expected to stimulate Photosystem II most strongly. The State I to State II transition thus does not seem to be a Light II driven process. We have never observed Light II illumination to speed up this transition. In our experience the rate of the State I to State II transition is always independent of, or slowed down by, Light II illumination. This suggests that control of the adaptative response is exerted by overstimulation of Photosystem I alone and that overstimulation of Photosystem II does not constitute an active response.

DISCUSSION

The intrinsic photochemical efficiencies of the independent half reactions comprising Photosystem II and Photosystem I, ε_{II} and ε_{I}, can be defined by:

$$\varepsilon_{II} = \alpha\Phi_{PSII} \quad (2)$$

$$\varepsilon_{I} = (1-\alpha)\Phi_{PSI} \quad (3)$$

where Φ_{PSII} and Φ_{PSI} are the quantum yields of photochemistry of the two photosystems and α is the fraction of absorbed quanta, absorbed in Photosystem II. These efficiencies determine the maximum possible turn-over rates of the two photosystems. Under normal conditions when the two photosystems are coupled in series, the rate of electron transport will be limited by the less efficient of the two photosystems and the quantum yield values will be appropriately altered. For light of wavelength less than 685 nm, Photosystem I is normally rate-limiting, hence the rate of oxygen evolution in Light II will be limited by the efficiency of Photosystem I. On superposition of Light I, Photosystem II will become rate-limiting and the rate of oxygen evolution will be limited by the efficiency of Photosystem II. Under the low-light conditions used in our experiments, the efficiency of the rate-limiting photosystem will be very close to the maximal efficiency defined by Eqn. 2 or 3.

The slow adaptive changes observed during the State I–State II transitions can thus be attributed to changes in the efficiencies of the rate-limiting photosystems. These changes could in principle be due to changes in α, to changes in the quantum yield terms or to simultaneous changes in both. Bonaventura and Myers [15] explained their results on the basis of changes in α. They justified this on the grounds that this was the simplest way of explaining the simultaneous antiparallel changes in the efficiencies of Photosystem I and Photosystem II (Type C responses) shown by their samples. Our observation that these changes in efficiency need not be coupled (Type A and Type B responses) suggests that they are more likely to be due to changes in the quantum yield terms.

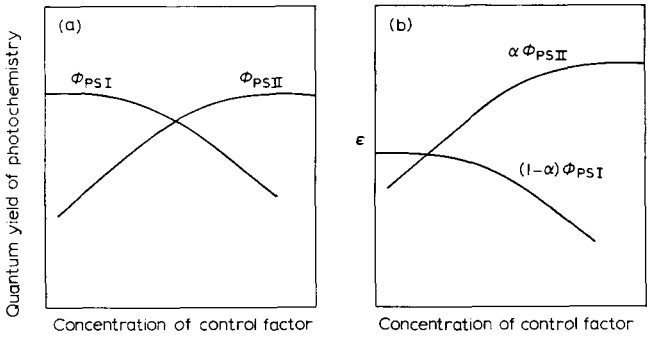

Fig. 13. (a) Sketches of the postulated dependence of the quantum yields of photochemistry, ϕ_{PSI} and ϕ_{PSII}, on the concentration of the factor controlling enhancement level. (b) Corresponding sketches of the photochemical efficiencies ε_I and ε_{II}, for the two photosystems.

A simple model capable of explaining all our observations is shown in diagrammatic form in Figs. 13 and 14. For the purposes of this model, we postulate the existence of a control factor that exerts an antagonistic effect on the values of Φ_{PSII} and Φ_{PSI}. The nature of this factor need not be stipulated but there is, as discussed

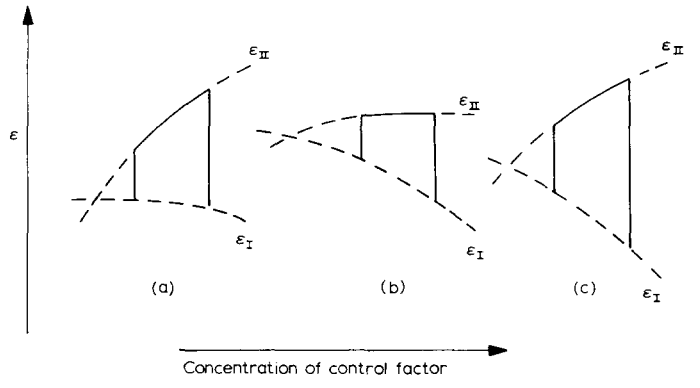

Fig. 14. Sketches showing how the Type A, Type B and Type C responses could be explained in terms of the position of the cross-over points of the ε_I and ε_{II} curves shown in Fig. 13b. See text for detailed explanation.

below, a good deal of evidence from other sources suggesting that Mg^{2+} might perform such a role. The dependence of Φ_{PSII} and Φ_{PSI} on the concentration of the control factor is assumed to be of the general form sketched in Fig. 13a. The Φ_{PSII} curve is assumed to be variable in the low, and saturated in the high, concentration region and the Φ_{PSI} curve variable in the high, and saturated in the low, concentration region. The corresponding curves for ε_{II} and ε_I are sketched in Fig. 13b.

The fast component of the enhancement corresponds to a change from a situation in which Photosystem I is limiting (Light II alone) to a situation in which Photosystem II is limiting (Light I+Light II). It is thus determined by the difference between the ε_{II} and ε_I values at a fixed concentration of control factor. The slow component corresponds to a change in efficiency of one, or both, photosystems under constant light conditions. It can thus be equated to a change in the relative values of ε_{II} and ε_I arising from a change in the concentration of the control factor. A State II to State I transition would correspond to a Light I driven increase in the concentration of the control factor and the corresponding State I to State II transition to the dark reversal of this increase.

The type of enhancement response will be determined by the relative positions of the efficiency curves. The existence of an initial fast component of enhancement in all our measurements means that initially $\varepsilon_{II} > \varepsilon_I$. Three different relationships between the ε_{II} and ε_I curves can be visualised under these conditions: ε_{II} variable with ε_I saturated, ε_{II} saturated and ε_I variable and both ε_{II} and ε_I variable. These correspond to Type A, Type B and Type C responses respectively. The condition $\varepsilon_{II} < \varepsilon_I$ was observed for intact chloroplasts (Part II) but not for *Chlorella*.

Let the values of ε_I and ε_{II} in Light II alone equal ε'_I and ε'_{II} and the values after adaptation to Light I equal ε''_I and ε''_{II}:

$$\varepsilon'_{II} = \alpha\Phi'_{PSII} \text{ and } \varepsilon'_I = (1-\alpha)\,\Phi'_{PSI} \tag{4}$$

$$\varepsilon''_{II} = \alpha\Phi''_{PSII} \text{ and } \varepsilon''_I = (1-\alpha)\,\Phi''_{PSI} \tag{5}$$

The Type A response corresponds to a situation in which Φ_{PSII} changes from Φ'_{PSII} to Φ''_{PSII} whilst Φ_{PSI} remains constant (cf. Fig. 14a). The Type B response

corresponds to a situation in which Φ_{PSI} changes from Φ'_{PSI} to Φ''_{PSI} whilst Φ_{PSII} remains constant (cf. Fig. 14b). In the Type C response both Φ_{PSI} and Φ_{PSII} change (cf. Fig. 14c). The conditions determining which of the possible responses will be exhibited by a given algal sample are not yet clear but such factors as variations in the relative pigment concentrations will certainly be important.

In addition to providing an explanation of the shapes of the enhancement signals, this model can also provide simple explanations for the wavelength dependencies of the instantaneous enhancement, E, and of the diurnal and adaptive variations in enhancement.

If the values of ε_I, ε_{II} etc. for Light II wavelength λ_x are designated by the subscript x, the enhancement ratios at a fixed concentration of control factor will be given by expressions of the form:

$$E_x = \frac{\alpha_x}{(1-\alpha_x)} \left[\frac{\Phi'_{PSII}}{\Phi'_{PSI}}\right] \tag{6}$$

The values of the quantum yield terms are wavelength independent. The wavelength dependence of E will, therefore, be determined by the wavelength dependence of α. Similarly, the values of ΔE will be given by expression of the form:

$$\Delta E_x = \frac{\alpha_x}{1-\alpha_x} \left[\frac{\Phi''_{PSII}}{\Phi''_{PSI}} - \frac{\Phi'_{PSII}}{\Phi'_{PSI}}\right] \tag{7}$$

and ΔE will thus show a similar wavelength dependence to E. The ratio of $\Delta E/E$ will of course be wavelength independent.

If we now attribute the diurnal variation in E, $\Delta E'$, to a diurnal variation in the concentration of control factor, the two types of enhancement variability will clearly have a common origin (i.e. a shift between two different fixed levels of the control factor). The only difference between them will be the driving force, in one case cellular metabolism, in the other Light I. The similarity in their wavelength dependencies thus follows directly.

The idea of a control factor acting in an antagonistic manner on the relative photochemical efficiencies of Photosystem II and Photosystem I, either by changing α or by changing the quantum yield of one or both of the photosystems has been proposed by many authors. A large rapidly growing body of evidence already exists suggesting that Mg^{2+} can act in this manner [20, 24–35]. The way in which control is exerted, by influencing spill-over of excitation energy [23–30], by shifting pigments from one system to another [15, 31–33], by changing the equilibrium constant between the two systems [34] or by altering the fluorescence quenching properties of Photosystem II [19, 35] is, however, still a matter of active dispute.

Several attempts have been made at directly measuring the Mg^{2+} dependence of NADP linked oxygen evolution and of the half reactions associated with the individual photosystems in isolated chloroplasts [24, 25, 32]. The interpretation of such measurements is complicated both by the difficulty in establishing the correct measuring conditions (pH, ionic strength, concentration of co-factors, etc.), and the difficulty of ensuring that all the relevant Mg^{2+} sensitive steps are included in the part reaction under study. Evidence is, however, emerging that the efficiency of Photosystem II linked electron transport is increased [24, 25], and that the efficiency

of certain Photosystem I linked electron pathways is decreased [24, 32], by high concentrations of Mg^{2+}.

Barber et al. [36] have suggested that the level of Mg^{2+} within the compartments of the chloroplast might be determined by chloroplast ATP levels. This would provide a direct linkage between enhancement and the energy transducing elements of photosynthesis. Senger and Frickel-Faulstich [14] have recently suggested a link between cyclic photo-phosphorylation and the growth cycle-linked enhancement changes. Our results suggest that the growth cycle-linked and the adaptive enhancement changes are very closely related. They also indicate that the adaptive change is primarily a Light I driven response. This would tend to support the idea that control of enhancement, if it is indeed photo-phosphorylation linked, is exerted via a cyclic pathway.

The picture that appears to be emerging from our investigations is that of a two-level control system designed to respond to the varying energetic requirements of the parent cell in which the basic energy balance of the cell is determined by a cellular metabolism linked control system on top of which is superimposed a fine control allowing adaptation to incident light conditions. A full analysis of this model must, however, rest on a detailed study of the variability of both the adaptive and growth-cycle linked enhancement changes in fully synchronised algae cultures. Studies along these lines are at present in hand.

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