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Spectral resolution of more than one chlorophyll electron donor in the isolated Photosystem II reaction centre complex

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Prolonged illumination of the isolated Photosystem II reaction centre under aerobic conditions causes a selective photodestruction of chlorophyll which absorbs maximally at 680 nm. Concomitant with this effect is a loss of photochemical activity. When oxygen is absent, the reaction centre is no longer damaged by illumination. Protection of the 680 nm absorbing chlorophyll against photodamage and maintenance of photochemical activity can also be achieved if silicomolybdate (SiMo) is present as an electron acceptor, although in this case there is an irreversible bleaching at 670 nm, whether oxygen is present or not. We therefore suggest that there are two chlorophyll species in the reaction centre absorbing at 670 nm and 680 nm, respectively. The latter we attribute to the primary donor P680 and the former to an accessory chlorophyll. It seems highly likely that in the absence of SiMo but under aerobic conditions, the photodestruction of P680 involves singlet oxygen generated from the P680 triplet. When SiMo is present the yield of the triplet is significantly reduced (Nugent, J.H.A., Telfer, A., Demetriou, C. and Barber, J. (1989) FEBS Lett. 255, 53–58) due to electron transfer to the acceptor and thus this mode of photodegradation is reduced. However, the accumulation of $P680^+$ when SiMo is present facilitates the oxidation of the accessory 670 nm chlorophyll which seems to result in its photodestruction by a mechanism not involving oxygen.

Introduction

The first reports that it was possible to isolate a Photosystem II (PS II) reaction centre complex containing the D1 and D2 polypeptides, but free of proteins which bind light-harvesting chlorophylls, such as CP47 and CP43 [1,2], were of considerable importance because they gave experimental backing to the concept that the structure of the reaction centres of PS II and purple photosynthetic bacteria are similar [3,4]. The isolated PS II reaction centre differs, however, from the isolated bacterial system in that it does not have an H-subunit, but instead contains the α- and β-subunits of cytochrome b-559 [5,6] and the 4.8 kDa product of the *psb*I gene [6,7]. Moreover, unlike its bacterial cousin, it did not, when isolated, bind the quinones that constitute the secondary acceptors [1,2]. Important functional differences between the bacterial and the PS II reaction centres are the oxidising potentials created by charge separation and the source of electrons that reduce the oxidised primary electron donors.

Unlike the bacterial system, the PS II reaction centre contains two tyrosine residues (Tyr_{161} and Tyr_{160}) on the D1 and D2 polypeptides, respectively, one of which (Tyr_z on D1) is thought to act as the intermediate between water oxidation and the reduction of $P680^+$ [8–10]. However, so far this intermediate shows little detectable activity in the isolated PS II reaction centre [11,12]. In the bacterial reaction centre the primary electron donor, which is known to be a special pair of bacteriochlorophyll molecules, when oxidised, creates a potential of about 0.4 V, whereas the redox potential of the primary oxidant in PS II, $P680/P680^+$, is at least 1.1 V [13]. For this reason PS II is able to oxidise water ($E_0' \simeq 0.81$ V). This potential is, however, also sufficiently positive to oxidise antenna chlorophyll. Chlorophyll photo-oxidation was originally observed by Visser

Abbreviations: Chl, chlorophyll; D1, CP47, CP43 and D2, products of the *psb*A, *psb*B, *psb*C and *psb*D genes; EPR, electron paramagnetic resonance; FWHM, full width at half maximum; P680, primary electron donor in PS II; PS II, Photosystem II; Q_A and Q_B, secondary quinone electron acceptors in PS II; SiMo, silicomolybdate ($SiMo_{12}O_{40}^{4-}$).

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and Rijgersberg [14], and recently, the identity of antenna chlorophyll(s) which can be oxidised in PS II has been discussed extensively in a paper by Thompson and Brudvig [15]. Their experiments employed PS II particles containing a large number of chlorophyll-binding polypeptides but they speculated that antenna chlorophyll bound to the D1 and D2 polypeptide (by analogy to the accessory bacteriochlorophylls on the L and M subunits [16] in the purple bacterial reaction centre) may be oxidised by $P680^+$.

In a preliminary paper [17] we reported the light-minus-dark difference spectrum of the isolated PS II reaction centre (approx. 5–6 Chl/2 Pheophytin, see Ref. 18) induced in the presence of the artificial electron acceptor, silicomolybdate (SiMo). This showed a maximum reversible bleaching at 680 nm with a shoulder at about 670 nm. We suggested that this spectrum might be due to oxidation of accessory chlorophyll(s) bound to D1 and/or D2 in addition to P680. In these preliminary experiments [17], measurement of the light-minus-dark absorption difference spectrum was hampered by the instability of the PS II reaction centre isolated in Triton X-100. In the absence of acceptor, bright light was found to bring about considerable irreversible bleaching of chlorophyll, initially peaking at about 680 nm but gradually shifting to the blue [17]. The bleaching of this chlorophyll was correlated with a loss of the ability to show primary charge separation [19] and the reversible light-induced absorption decrease in the presence of SiMo [17].

Recently, stability problems (both thermal and light-induced) which are encountered with the PS II reaction centres isolated in Triton X-100 [20,21], have been overcome by combining exchange of the reaction centres into other detergents [19–22] with the use of anaerobic conditions during measurement of light-induced phenomena [19,23].

In this paper we have used isolated PS II reaction centres which have been exchanged into dodecyl maltoside to investigate thoroughly the irreversible and reversible light-induced absorption changes which occur under aerobic and anaerobic conditions both plus and minus SiMo. We can thus identify and measure the absorption characteristics of the chlorophyll species which are photo-oxidised in PS II.

Materials and Methods

The PS II reaction centre was isolated from pea thylakoids using a procedure similar to that given in Chapman et al. [21], in which the second chromatographic step involved exchange from 0.2% Triton X-100 into 2 mM dodecyl maltoside. Reaction centres were either suspended in 50 mM Tris-HCl (pH 8.0 at 4°C) without detergent, resulting in a 50–100-fold dilution or, where indicated, in the same buffer with 2 mM dodecyl maltoside. Silicomolybdate, obtained from Pfaltz and Bauer, was usually added at 0.5 mM. Oxygen was removed from the samples using a glucose/glucose oxidase trap as described by Crystall et al. [19]. Samples were incubated for 5 min at 4°C to ensure complete anaerobiosis.

Absorption spectra were recorded on a Shimadzu MPS 2000 or an SLM Aminco DW 2000 spectrophotometer. Light–dark absorption difference spectra were recorded on a Perkin Elmer model 554 UV-Vis or the SLM Aminco DW 2000 spectrophotometer equipped for side illumination with a 150 W tungsten lamp. The actinic light was transmitted through a Corning 4-96 filter and a Calflex C heat filter. The intensity at the cuvette was 800 $\mu E \cdot m^{-2} \cdot s^{-1}$. The photomultiplier was protected from scattered actinic light by a Schott RG660 glass filter. The optical pathlength was 10 mm and the cuvette volume was 1 ml. Preillumination with bright white light was by an unfiltered tungsten lamp delivering 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$ (unless otherwise indicated in the text) to the sample which was maintained at 4°C.

Results

Irreversible light-induced absorption changes: photodamage

Fig. 1 shows the changes in absorption spectra and the Q-band absorption maxima of the isolated PS II reaction centre complex as a function of time of preillumination with strong white light. It also shows the effect of the presence and absence of oxygen and the electron acceptor, SiMo. As found previously [2,17,22], under aerobic conditions and in the absence of electron acceptor (Fig. 1A), there is a gradual loss of absolute absorbance accompanied by a blue shift in the wavelength of the red peak (see insert, Fig. 1A). This change of the red maximum is due to selective loss of a long-wavelength form of chlorophyll (presumably the chlorophyll(s) which constitute P680 – see Ref. 17) and is accompanied by a loss of photochemical activity [17] and the optical activity as measured by CD [24]. Fig. 1B shows that the removal of oxygen from the sample essentially prevents the photo-induced damage, as judged by the very small change in the absorption spectrum of the sample.

In Fig. 1C it can be seen that although the presence of SiMo during preillumination prevents the large blue shift of the red peak, it does not protect against an overall decrease in absorbance. The extent of this decrease is, however, less when oxygen is removed from the sample and under these conditions there is a small red shift in the absorption peak (Fig. 1D). This suggests that an additional chlorophyll species is damaged by light, when SiMo is present, and that this species has a red maximum which is at a shorter wavelength than that of chlorophyll(s) attributed to P680.

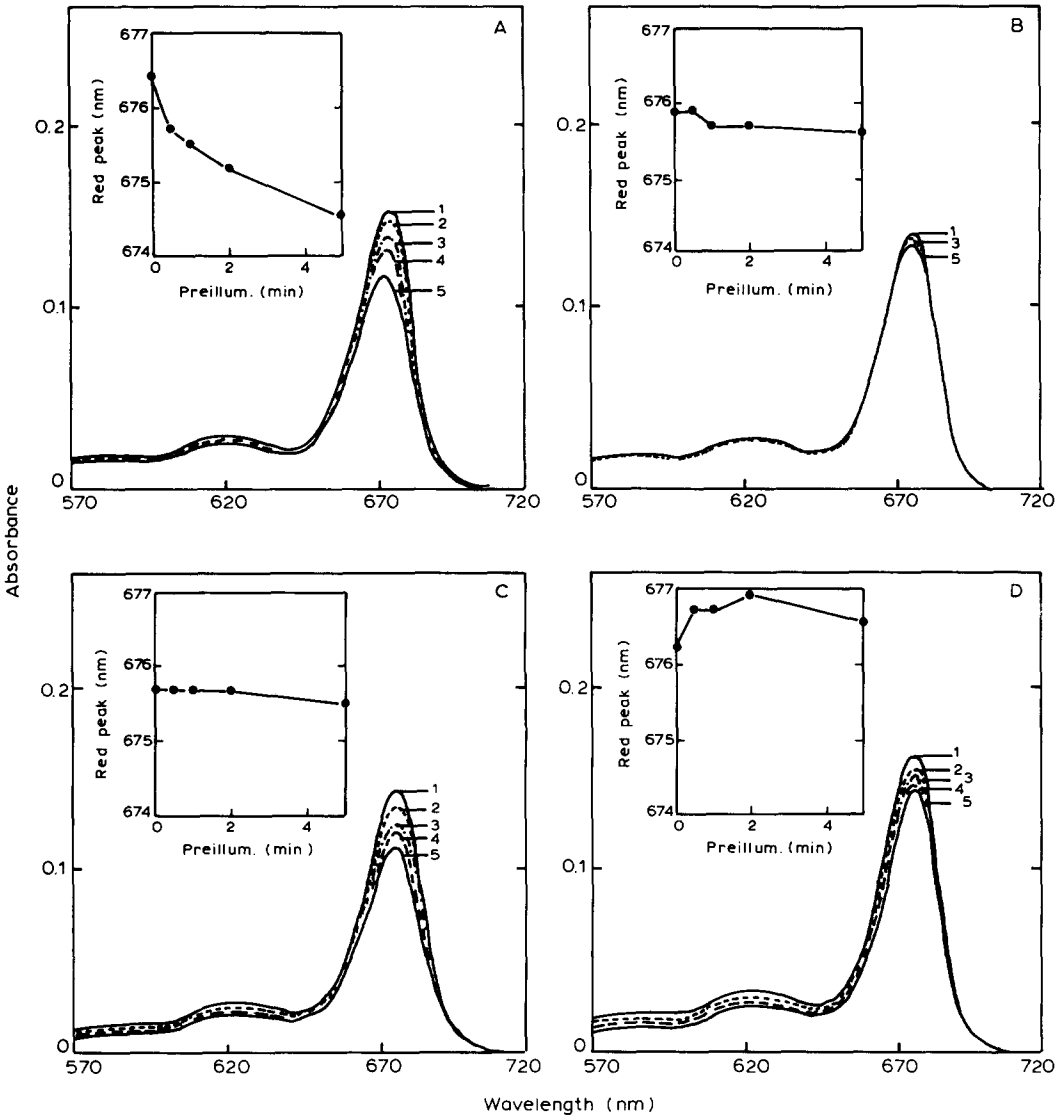

Fig. 1. The effect of white light pretreatment on the absorption spectrum of PS II reaction centres in the presence and absence of oxygen and SiMo, plus oxygen (A, C); plus SiMo (C, D). Spectra 1–5 are after 0, 0.5, 1, 2 and 5 min light treatment, respectively. The inserts show the effect of time of preillumination on the red absorption maximum wavelength. In spectra C and D a general absorbance increase due to reduction of SiMo was offset by subtraction of the absorption increase at 720 nm. Chlorophyll concentration 2 $\mu g \cdot ml^{-1}$. Slit-width 1 nm. Light pretreatment as described in Materials and Methods.

In Fig. 2 we show the results of a similar experiment to that of Fig. 1. The data are, however, presented as the difference between the spectra of the light-pretreated samples and the initial dark absorption spectra, in order to investigate in more detail the pigment species which undergo photodamage. Fig. 2B shows that when there is no acceptor present, careful treatment to remove all oxygen results in complete stability to bright white light. In the remaining three sets of difference spectra it appears that two pigment species absorbing maximally at 670 nm and 680 nm, are involved in the irreversible bleaching brought about by strong white light. The wavelength maxima of these two species were confirmed by second derivative spectra. The insets of Fig. 2 show that the relative extent of the decrease of A_{670} and A_{680} depends on the presence and absence of oxygen or SiMo. When SiMo is absent, the effect of illumination of aerobic samples causes a preferential bleaching at 680 nm compared with 670 nm (Fig. 2A). The addition of SiMo under aerobic conditions increases the rate of change of A_{670} (Fig. 2C). Absence of oxygen when SiMo is present, however, appears to protect the 680 nm species more than the 670 nm species in the early stage of photodamage treatment (Fig. 2D). Under these conditions the preferential bleaching of A_{670} seen in the presence of SiMo is gradually lost with time of illumination (Fig. 2D). The insert in Fig. 2C shows that this is also the case, but to a lesser extent, when oxygen is present with SiMo. We have previously reported [17] that the protective effect

of SiMo results in the irreversible destruction of this acceptor, so it is not surprising that its effectiveness at minimising P680 photodamage decreases with time. Overall, the analyses of the difference spectra in Fig. 2 suggest that two different species of chlorophyll absorbing maximally at 680 nm and 670 nm can be photodamaged. We assign the former to the primary electron donor, P680, and the latter to an accessory chlorophyll, which we designate Chl670. The data of Fig. 2 are consistent with the changes in position of the red peak and absorbance decreases which were seen in the experiments of Fig. 1. In Fig 2C and D a background increase in absorbance during preillumination is due to a low level of reduction of SiMo (see later).

The above experiments were carried out at 'low' detergent concentration as the reaction centres, originally isolated in 2 mM dodecyl maltoside, were diluted 50-fold in detergent-free buffer. However, if 2 mM dodecyl maltoside was present in the suspension medium, essentially the same spectral pattern of photodamage was found (see Ref. 24).

Fig. 3 shows an experiment carried out under aerobic conditions, in which photodamage of the reaction centre was brought about using blue light of a lower intensity than the white light used in the experiments of Figs. 1 and 2. This 'mild' photodamage treatment emphasises the decreased sensitivity of A_{680} and increased sensitivity of A_{670} to photodamage afforded by the presence of SiMo, at least in the initial stages of photodamage. The full width half maximum (FWHM) for the decrease at 680 nm which is seen in the absence of SiMo is about 12 nm (Fig. 3A). Fig. 3B indicates that in the presence of SiMo the decrease at 670 nm seen after 1 and 2 min photodamage is about 13 nm FWHM but also shows that the relative proportion of A_{680} bleaching increases with time of photodamage, leading to an increase in the FWHM.

Reversible light-induced absorption changes: stabilisation of charge separation

The PS II reaction centre isolated in Triton X-100 has been shown to exhibit reversible light-induced changes in the presence of the electron acceptor, SiMo [2,12,17], which were suggested to be due to stabilisation of charge separation and hence the photoaccumulation of oxidised electron donor(s). These absorption changes, which were maximal at 680 nm but also showed a distinct shoulder at about 670 nm, were suggested to

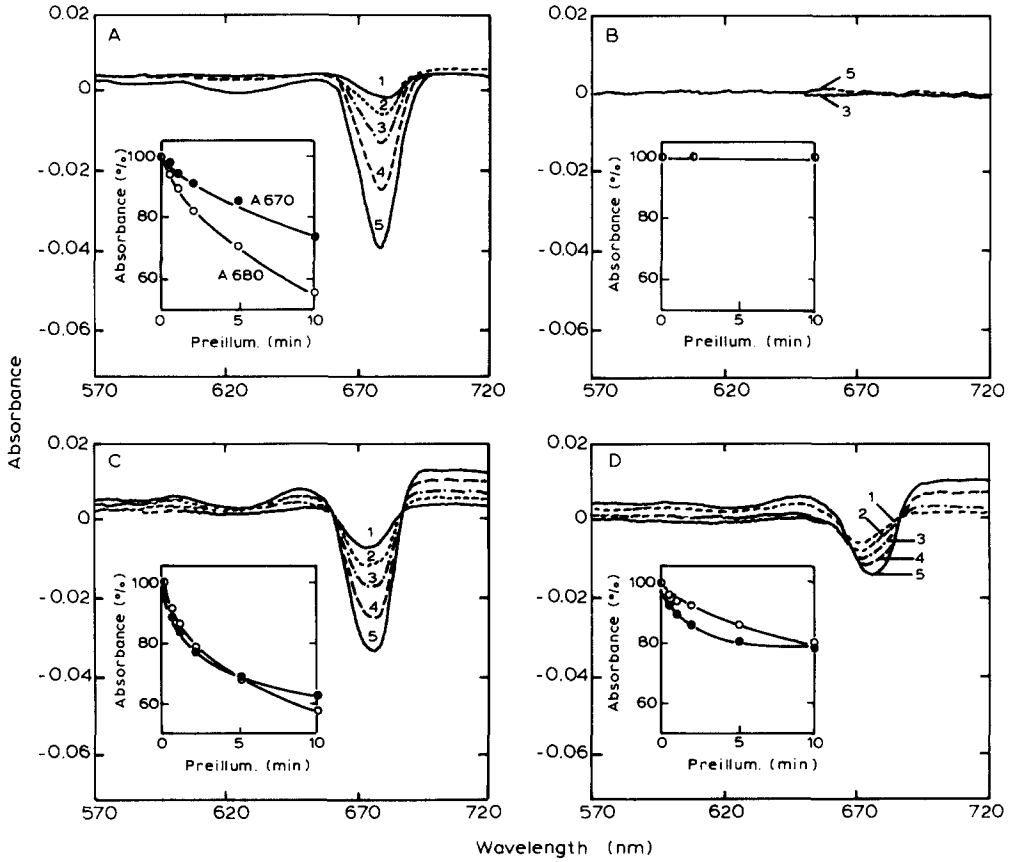

Fig. 2. The effect of photodamage treatment (as in Fig. 1) on the absorption spectrum of PS II reaction centres, plus oxygen (A, C); plus SiMo (C, D). Spectra 1–5 are the difference between light pretreatment for 0.5, 1, 2, 5 and 10 min respectively, minus the spectrum before light treatment. The general absorbance increase seen during preillumination is due to net SiMo reduction. The insets show the relative decrease in absorbance at 670 nm (●) and 680 nm (○) plotted as the percentage of the initial absorbance after subtraction of the increase at 720 nm. Chlorophyll concentration was 3.3 $\mu g \cdot ml^{-1}$. Slit-width 1 nm.

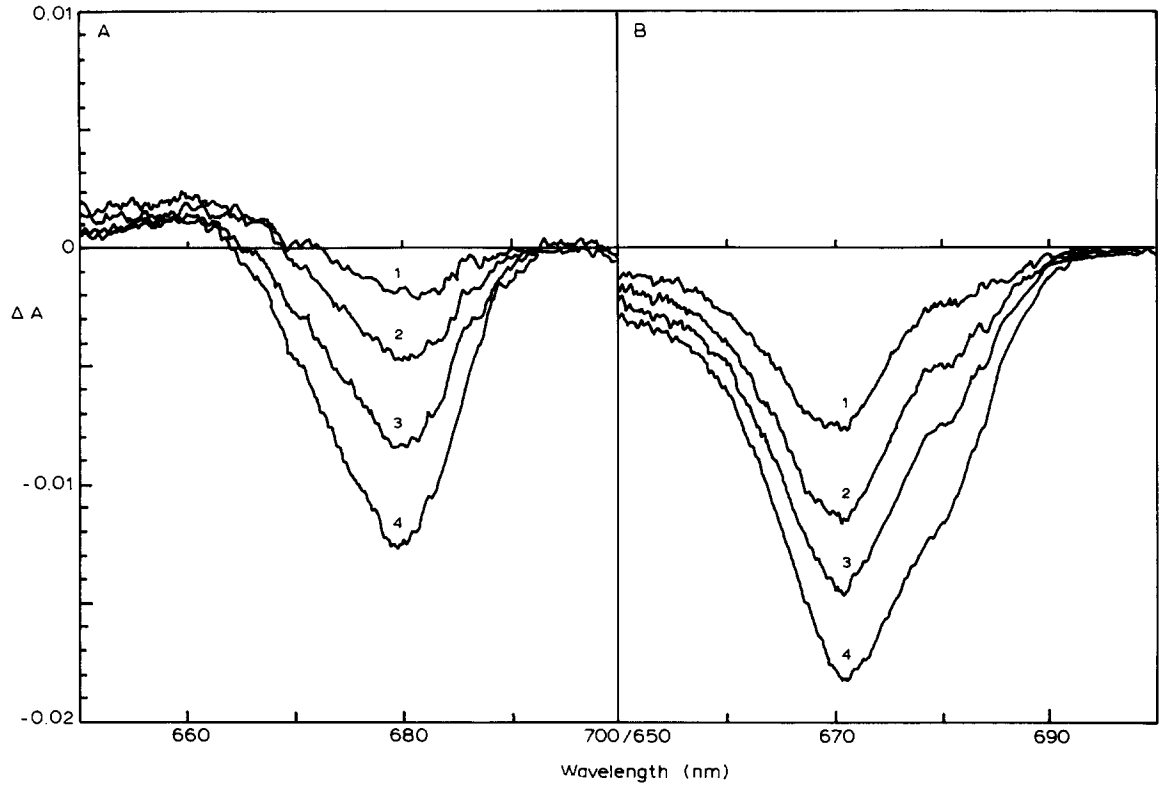

Fig. 3. The effect of mild photodamage treatment (360 $\mu E \cdot m^{-2} \cdot s^{-1}$ blue light, Corning 4-96 glass filter) on the absorption spectrum of PS II reaction centres under aerobic conditions minus (A) and plus (B) SiMo. Spectra 1–4 are the differences between light pretreatment for 1, 2, 3 and 4 min respectively and the initial dark spectra. A general absorbance increase due to reduction of SiMo was offset by subtraction of the A_{720} change.

be due to the photo-accumulation of an oxidised monomeric chlorophyll in addition to $P680^+$ [17]. The reversible absorption changes were measured previously under aerobic conditions [17]. Because anaerobic conditions completely protect against irreversible light-induced loss of absorbance (Figs. 1 and 2), we have now investigated the effect of the absence of oxygen on the ability of the isolated PS II reaction centre to show SiMo-dependent charge stabilisation. In Fig. 4A it can be seen that, in the presence of oxygen and SiMo, the reversible light-induced absorption decrease at 680 nm is accompanied by an irreversible decrease. The latter decrease is eliminated by anaerobic conditions, whereas the reversible change was essentially unaffected (Fig. 4C). As expected from the control experiments of Figs. 1 and 2, when SiMo was absent there was an irreversible absorption decrease (Fig. 4B) which was inhibited by the absence of oxygen (Fig. 4D).

We frequently found that when the samples were illuminated initially, whether anaerobically or aerobically, there was a small reversible decrease in A_{680}, but that after a period of illumination (approx. 30–60 s) the maximum extent of the reversible decrease was established (data not shown). The extent of this effect was found to be highly dependent on the batch of SiMo used for experiments and seems to be related to its variable degree of partial reduction. Hence in most experiments in the presence of SiMo, e.g., that of Fig. 4, we gave a brief preillumination with the exciting light in order to establish the maximum reversible absorption change before making measurements.

Such pretreatment was given in order to obtain the SiMo-dependent light-minus-dark absorption difference spectrum of PS II reaction centres under anaerobic conditions (Fig. 5). The spectrum obtained (Fig. 5) is essentially the same as that reported by Telfer and Barber [17] under aerobic conditions. The only difference is that we now find a nearly uniform increase in absorbance between 720 nm and 840 nm. However, this was found not to be due to the anaerobic conditions, but to a poor signal to noise ratio when using low levels of reaction centre. Using a higher chlorophyll concentration and a wider slit-width, we now always obtain spectra as in Fig. 5. Provided that care was taken to prevent photodamage, the spectrum was found to be independent of: (a) the presence or absence of oxygen; (b) whether the preparation had been detergent exchanged; and (c) the presence of 2 mM dodecyl maltoside in the reaction medium. Consequently, we conclude that the shoulder (at about 670 nm) is an inherent feature of this spectrum.

Using stabilising conditions which prevent the slow irreversible changes in absorbance seen in Fig. 4A (i.e., anaerobiosis and maltoside-exchanged reaction centres),

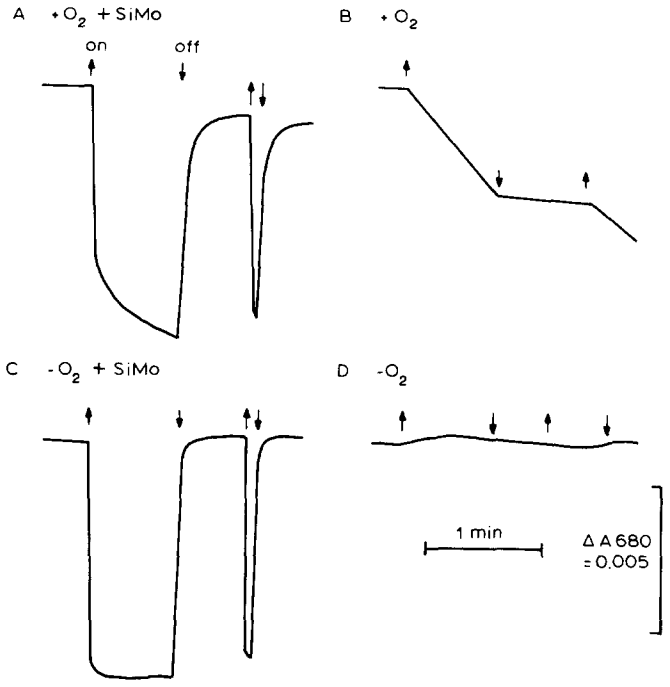

Fig. 4. Light-induced absorbance changes at 680 nm in PS II reaction centres, plus oxygen (A, B); plus SiMo (A, C). Samples were pretreated for 1 min with the blue excitation light to establish the maximum reversible absorbance changes at 680 nm. Chlorophyll concentration 2.7 $\mu g \cdot ml^{-1}$. Slit-width 4 nm.

we were able to obtain a light-minus-dark difference spectrum by continuous scanning rather than point by point (data not shown) and second derivative spectra confirm that the shoulder (Fig. 6A) is due to a component with a peak absorbance at 670 nm. For direct comparison, Fig. 6B shows a second derivative spectrum of the irreversible decrease in absorbance induced by illumination in the presence of SiMo, which indicates the bleaching of two components with peak absorbance at 670 and 680 nm.

In Fig. 3 we demonstrated that photodamage conditions can be chosen which lead to preferential irreversible bleaching at either 670 nm or 680 nm. We, therefore, investigated the correlation between this irreversible bleaching and the reversible light-induced changes at 670 nm and 680 nm. Fig. 7 shows the reversible absorption changes (ΔA_{670} and ΔA_{680}) of samples preilluminated in the absence of SiMo. When this was carried out under aerobic conditions, the ability of the reaction centres to show SiMo-dependent reversible absorption changes at 680 nm and 670 nm was severely and proportionally inhibited by preillumination (Fig. 7A). Anaerobic conditions completely prevented this light-induced loss of photochemical activity (Fig. 7B). Fig. 8A shows the effect of preillumination carried out in the presence of SiMo and oxygen which brings about a preferential bleaching at 670 nm. In this experiment, it can be seen that there are two phases to the changes in absorbance. The initial increase is the trivial effect due to the light requirement for establishing the maximum yield of absorbance change. However, over a longer time scale (> 30 s preillumination), there is a gradual decline in the extent of the reversible absorption decrease, with ΔA_{670} being affected to a greater extent than ΔA_{680}. Essentially the same results were obtained when the preillumination was carried out under anaerobic conditions. The differential inhibition of the two components, and their sensitivity to anaerobic conditions, is emphasised in Fig. 8B, which plots the ΔA_{670} to ΔA_{680} ratio and shows that, after 8 min preillumination, the change at 670 nm reduces from

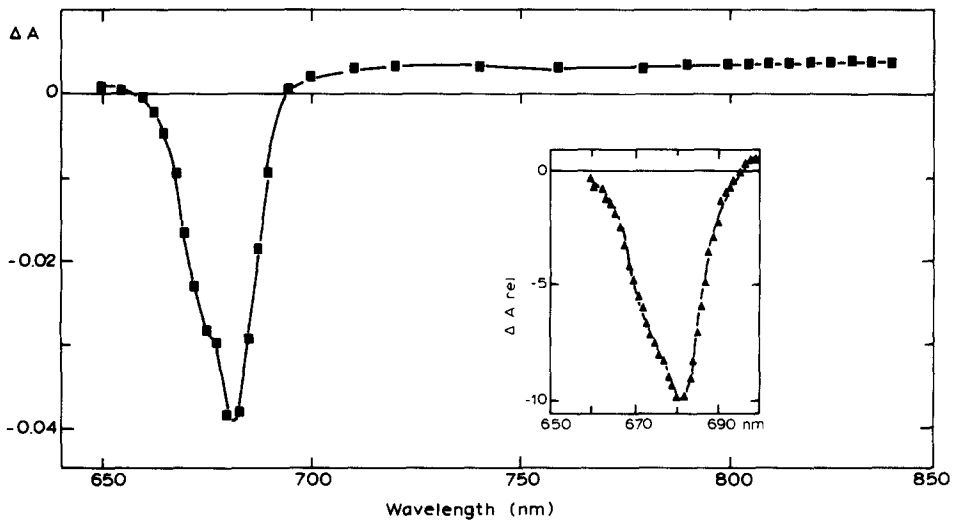

Fig. 5. Light-minus-dark (after light) absorption difference spectrum of PS II reaction centres (8 $\mu g \cdot ml^{-1}$). SiMo concentration 1 mM. Slit-width 4 nm (insert 1 nm). Samples were initially preilluminated with the blue excitation light for 30 s before measuring the point by point spectrum induced by 5 s flashes of blue light (800 $\mu E \cdot m^{-2} \cdot s^{-1}$). Loss of absorbance during measurement was corrected for by checking ΔA at 680 nm periodically, as described in Ref. 10.

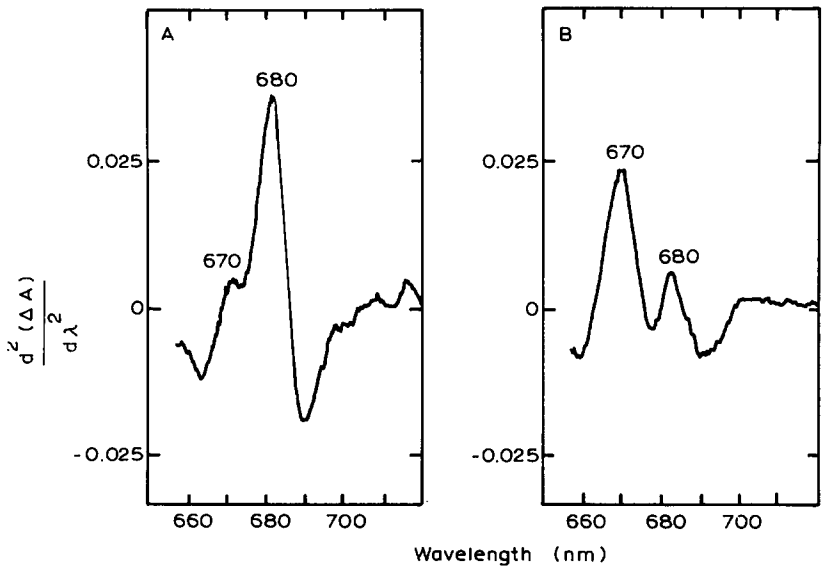

Fig. 6. Second derivative spectra of light-induced reversible (A) and irreversible (B) absorption changes in PS II reaction centres seen in the presence of SiMo and absence of oxygen. Chlorophyll 2 $\mu g \cdot ml^{-1}$.

approx. 50% to approx. 35% of the 680 nm change. These data strongly support the suggestion that the light-induced spectrum shown in Fig. 5 is due to the accumulation of two distinct oxidised chlorophyll components.

Discussion

In the presence of oxygen, but with no artificial electron acceptor present, preillumination of the isolated PS II reaction centre causes a preferential destruction of chlorophylls which absorb maximally at 680 nm. This effect is accompanied by a loss of photochemical activity as measured by $P680^+$ photoaccumulation (Fig. 7A), electron transport [21,22], P680 triplet formation [25] and recombination fluorescence [19]. Therefore it seems that the damage involves the chlorophyll(s) which constitute the primary electron donor, P680. When oxygen is totally removed from the medium, selective photodamage is prevented i.e., both the absorption

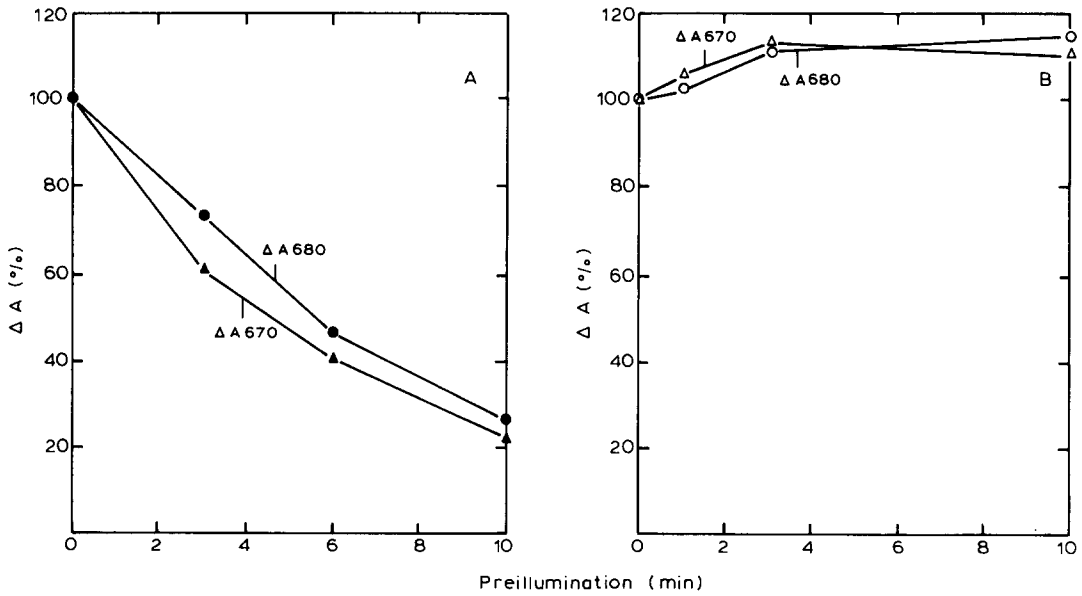

Fig. 7. Effect of light pretreatment in the absence of SiMo under aerobic (A) and anaerobic (B) conditions on the relative reversible light-induced absorbance changes at 670 nm (triangles) and 680 nm (circles) seen in the presence of SiMo. Results are expressed as the % of the maximum ΔA. 0.5 mM SiMo was added after the preillumination treatment and the samples were incubated in the dark for 5 min followed by 1 min excitation with blue light before measurement of the reversible light-induced absorption changes. Chlorophyll concentration 2 $\mu g \cdot ml^{-1}$. 2 mM dodecyl maltoside was present throughout the experiment.

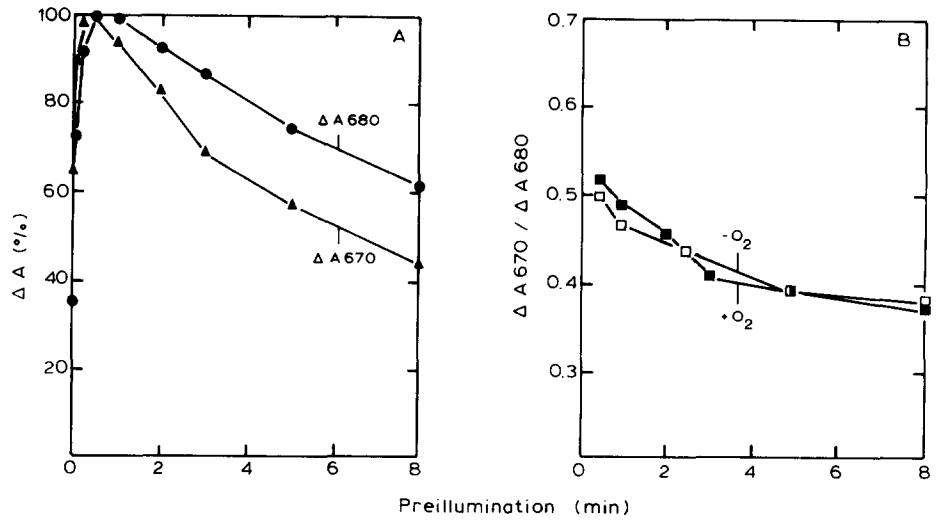

Fig. 8. (A) Effect of light pretreatment in the presence of SiMo under aerobic conditions on the relative reversible light-induced absorption changes at 670 nm (triangles) and 680 nm (circles). Results are expressed as the % of the maximum ΔA. Light pretreatment was carried out progressively on the same samples. (B) shows the ratio of ΔA_{670} to ΔA_{680} against light pretreatment time both plus (closed symbols) and minus (open symbols) oxygen. Chlorophyll concentration 2 $\mu g \cdot ml^{-1}$. SiMo concentration 1 mM. 2 mM dodecyl maltoside was present throughout the experiment.

characteristics and photochemical activity are unaffected. Flash absorption studies by Mathis et al. [26] and by Giorgi et al. [25], indicate that under aerobic conditions the P680 triplet state, generated by radical pair recombination, is quenched by molecular oxygen. This almost certainly results in the formation of highly toxic singlet oxygen. We suggest that singlet oxygen preferentially attacks the conjugated bond systems of the chlorophyll(s) of P680 and consequently causes the spectroscopic bleaching and photochemical inactivation of this pigment. This conclusion is supported by the fact that when oxygen is absent, the process of primary charge separation and recombination does not result in damage to any pigments of the PS II reaction centre. When the electron acceptor SiMo is present, the action of light is to allow the accumulation of $P680^+$ [2,17]. Because of this the recombination reaction is inhibited and therefore the yield of the P680 triplet is decreased [11,12,25]. The extent to which triplet formation is inhibited by SiMo is dependent both on the concentration present [12] and its degree of oxidation i.e., it is more effective if preoxidised with ferricyanide [11].

In this paper we have shown that the presence of SiMo during preillumination protects partially against photodestruction of the P680 chlorophyll whether oxygen is present or not. However, under these conditions, there is an additional selective bleaching of a chlorophyll species which absorbs maximally at 670 nm. The simplest interpretation of these results is that the photoaccumulation of $P680^+$ is accompanied by the oxidation of an accessory chlorophyll species characterised by its red absorption peak at 670 nm. It seems that this latter oxidation causes a gradual destruction of the accessory chlorophyll by a mechanism which occurs under anaerobic as well as aerobic conditions. The greater sensitivity of $Chl670^+$ to degradation may reflect a slower turnover rate (longer lifetime) of this species compared to $P680^+$. Such a suggestion is supported by the reversible light induced absorption changes measured with SiMo present. In this case, the light-minus-dark difference spectrum contains two chlorophyll components which can be assigned to the formation of $P680^+$ and $Chl670^+$. Photodegradation under aerobic conditions in the absence of SiMo, which leads to a specific loss of P680, has an equally inhibiting effect on the ability of the reaction centres to show light-induced reversible changes (with SiMo as an acceptor) at 680 nm and 670 nm (Fig. 7). This seems to indicate an interdependence of the Chl670 and P680 oxidations. If, however, photodegradation is carried out in the presence of SiMo, the Chl670 can be preferentially destroyed as shown by the change in ratio of ΔA_{670} to ΔA_{680} in Fig. 8B. These data suggest that Chl670 is a secondary electron donor to $P680^+$, which is a conclusion consistent with previous EPR [11,12] and low temperature absorption [12] studies. This secondary donor, which is irreversibly oxidised at 4 K has the EPR characteristics of a monomeric chlorophyll [11,12]. The low temperature (245 K) optical absorption studies of Takahashi et al. [12] indicated that the irreversible and reversible oxidations observed when SiMo was an acceptor involved chlorophylls which absorb maximally at 665 nm and 677 nm respectively. These values are at shorter wavelengths than reported here but may reflect a low temperature blue-shift in the absorption peaks.

The isolated PS II reaction centre used in our experiments does not contain the quinone acceptors, Q_A and Q_B. It does, presumably, contain the two tyrosine residues in the D1 and D2 polypeptides which can act as secondary electron donors to $P680^+$ [8–10]. However,

even with SiMo present, they are not extensively photo-accumulated in their oxidised forms [11,12]. Other possible donors to $P680^+$ are cytochrome *b*-559 [27] and β-carotene [28]. In the isolated PS II reaction centre, cytochrome *b*-559 is present in its low potential form and is therefore already oxidised in the dark. Whether β-carotene can act as an electron donor in this isolated complex is unknown, but the carotenoid triplet yield is low and does not involve quenching of the P680 triplet [29,30].

Our results seem to indicate that a secondary donor to $P680^+$ in the isolated PS II reaction centre is a monomeric chlorophyll which we have designated Chl670. This chlorophyll is presumably bound to the D1 or D2 protein but the broader absorption bleach in the red region seen with larger particles [31,32] may include additional photo-oxidised chlorophylls bound to other polypeptides. Chl670, first reported here, may be the chlorophyll donor to $P680^+$ suggested by Thompson and Brudvig [15] to be involved in a cyclic electron transfer pathway around PS II which protects against photoinhibition. In their scheme, the oxidised chlorophyll is reduced by cytochrome *b*-559. Oxidised cytochrome can thus be reduced by Q_A or directly by pheophytin and the cycle therefore would help to protect PS II by dissipating excess excitation.

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