Biochimica et Biophysica Acta, 1020 (1990) 285–289
Elsevier

BBABIO 43302

Quantum efficiency for the photoaccumulation of reduced pheophytin in Photosystem II

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(Received 17 April 1990)

Key words: Photosynthesis; Photosystem II; Primary charge separation; Pheophytin reduction; Quantum efficiency

The quantum efficiency for the photoaccumulation of reduced pheophytin has been estimated using Photosystem II (PS II)-enriched membranes (with Q_A present) and isolated PS II reaction centres (with Q_A absent). Sodium dithionite was present in the reaction medium in order to reduce Q_A in the PS II membranes and also to act as an electron donor to photooxidised P680. The kinetic analyses of the rate of reduction of pheophytin, measured as absorption changes at 685 nm within the two systems, indicated that this reaction was three orders of magnitude more efficient in the isolated PS II reaction centre than in PS II-enriched membranes. When calibrated against the quantum efficiency of Q_A photoreduction within the PS II-enriched membranes, it was concluded that the absolute quantum yields for the photoaccumulation of pheophytin were 5.2% for the isolated PS II reaction centres and 0.005% for the PS II membranes. Although several factors could account for this very large difference it is suggested that, in part, a possible explanation is that there is a decrease in the quantum efficiency of primary charge separation within the PS II reaction centre when Q_A is present in its reduced state. Also discussed is the shape of the long wavelength reduced-minus-oxidised pheophytin difference spectrum obtained with the isolated PS II reaction centre in which any effects of the presence of reduced Q_A are avoided.

Introduction

Recently it has been possible to isolate a Photosystem II (PS II) reaction centre consisting of the D1 and D2 polypeptides, the apoproteins of cytochrome *b*-559 and the product of the *psbI* gene [1–3]. This complex is able to carry out the light-induced primary electron transfer reactions leading to the formation of the radical pair $P680^+Pheo^-$ where P680 is the primary electron donor and $Pheo^-$ is reduced pheophytin. Because the secondary quinone acceptors Q_A and Q_B are missing from this preparation, the radical pair back-reacts [4,5]. One route for this recombination gives rise to light emission in the form of delayed fluorescence [6]. If, however, sodium dithionite is present during the illumination period, electrons are donated to $P680^+$ and the state $P680Pheo^-$ accumulates [1,2]. Under these conditions the fluorescence emitted from the isolated reaction centre is significantly reduced [7]. A similar phenomenon is observed with intact membranes or with PS II-enriched particles [8,9]. In these cases the action of dithionite is two-fold; to act as donor to $P680^+$ and to prereduce Q_A so as to allow reduced pheophytin to accumulate upon illumination.

An interesting and important question which has not yet been fully resolved is whether the redox state of Q_A affects the efficiency of light to create the radical pair $P680^+Pheo^-$. That is, when Q_A is fully reduced, is the PS II reaction centre still an efficient trap? According to Schatz et al. [10] it is not, although the model of Klimov et al. [8,9] for the origin of variable fluorescence would say that it is.

In this communication we consider this question from data obtained from measuring the quantum yields for the formation of the state $P680Pheo^-$ in the isolated PS II reaction centre (without Q_A^-) and in PS II-enriched membranes (with Q_A^-). The experiments involve the measurement of the kinetics of the photoreduction of pheophytin using absorption difference spectroscopy.

Abbreviations: PS II, Photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea.

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Materials and Methods

The PS II reaction centre was isolated from peas using the procedure given by Chapman et al. [11] except the second chromatographic step was carried out using 3 mM dodecyl maltoside rather than Triton X-100, a procedure which greatly exhances the stability of the complex [6]. The isolated complex was characterized by its room temperature absorption spectrum (red peak at 676 nm) and its chlorophyll to cytochrome *b*-559 ratio. PS II-enriched membranes (BBY particles) were prepared from spinach using the procedure of Berthold et al. [12]. For all absorption difference measurements the reaction medium was contained in a glass cuvette with an optical pathlength of 1 cm. The reaction mixture was 10 μM chlorophyll, 2 mM $MgCl_2$, 35 mM NaCl, 20 mM Tris-HCl buffer (pH 8.0) and 6.3 mM freshly prepared and de-aerated sodium dithionite. Also contained in the reaction mixture were two redox mediators, 2 μM indigo disulphonate and 2 μM methyl viologen, and prior to adding the dithionite, the medium was degassed by a mild application of a vacuum. In the case of the PS II-enriched membranes the mixture also contained 0.01% Triton X-100, which helped to reduce the effect of particle flattening on the absorbance (under these conditions the differential flattening correction factor at 685 nm was 1.29). No such correction was necessary for the isolated PS II reaction centres and therefore no additional detergent was included in the medium. For the measurement of fluorescence induction kinetics using PS II-enriched membranes (BBYs), the reaction medium was 10 μM 3-(3,4-dichlorophenyl)-1,2-dimethyl urea (DCMU), 2 mM $MgCl_2$, 35 mM NaCl and 20 mM Tris-HCl (pH 8.0).

Absorption difference and fluorescence kinetic measurements were made using an apparatus which has been described previously [13]. The pheophytin photoreduction was induced by blue (Corning CS4-96) excitation light at intensities as indicated in the figures. For absorption difference measurements the measuring beam had a half-band width of 1.4 nm.

Results

Exposure of the isolated PS II reaction centre to illumination in the presence of 6.3 mM sodium dithionite induced an absorption transient in the red region of the spectrum (Fig. 1). The difference spectrum for this transient, shown in Fig. 2a, is similar to that previously reported for pheophytin photo-reduction (see Fig. 2b) although there are some interesting features which deserve discussion (see later).

Fig. 3 shows a semilog plot of a typical transient absorption signal at 685 nm measured with the PS II reaction centre. Using the same light intensity and chlorophyll concentration the PS II-enriched membranes (BBY particles) gave the 685 nm absorption transient shown in Fig. 4. Again, these data have been plotted semilogarithmically (Fig. 4, inset). The rate con-

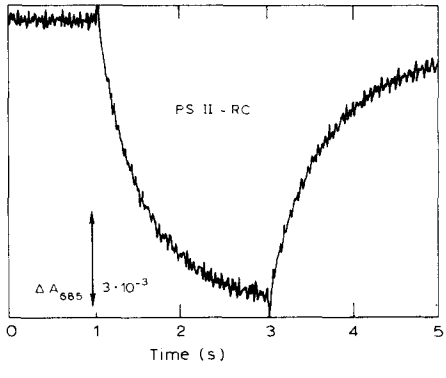

Fig. 1. Time-course of pheophytin photoreduction in isolated photosystem II reaction centres (PS II-RC) as indicated by an optical bleaching at 685 nm. The actinic light intensity was 950 μmol·m^{-2}·s^{-1} and had a duration of 2 s. The chlorophyll level was 10 μM.

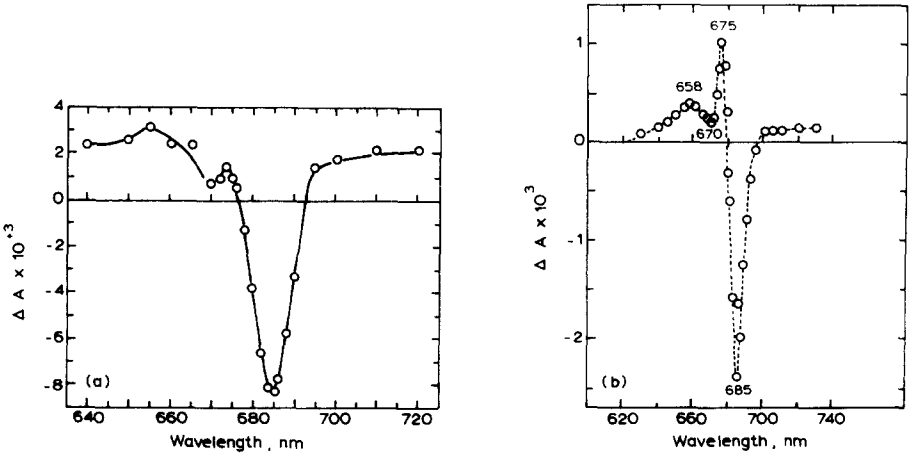

Fig. 2. Light-induced absorbance difference spectrum indicative of the photoreduction of pheophytin. (a) Photosystem II reaction centres (PS II-RC), (b) PS II-enriched membranes (BBY) taken from Ref. 20.

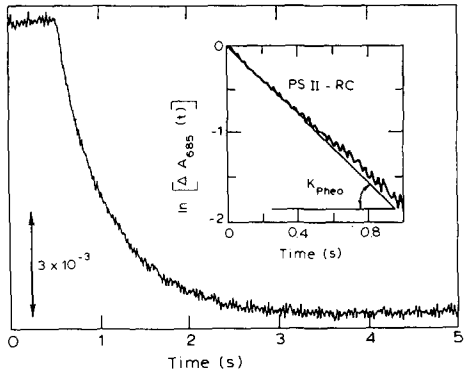

Fig. 3. Time-course of pheophytin photoreduction in PS II-RC as in Fig. 1 except for a longer period of exposure to the actinic light. Also shown is a semilog plot of the data which gives a rate constant for pheophytin reduction (k_{Pheo}) of 2 s^{-1}.

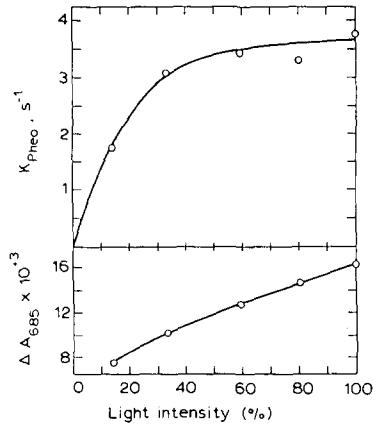

Fig. 5. Light intensity dependence of the rate (k_{Pheo}) and amplitude (ΔA_{685}) of photoreduction of pheophytin. The maximum light intensity corresponding to 100% was approx. 5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

stants obtained from these two semilog plots are 2 s^{-1} for the PS II reaction centre and 0.08 s^{-1} for BBYs.

The actinic light intensity used for the experiments shown in Figs. 1–3 was 950 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fig. 5 shows the light intensity dependence for the rate of pheophytin reduction in the isolated PS II reaction centre and the amplitude of the photoreduction. The fact that the former curve saturates at rates well within the time resolution of our instrument indicates that the rate of photoreduction of pheophytin has become limited by a dark reaction. The steady increase in the amplitude of the 685 nm bleaching with increasing light intensity presumably reflects the competition between the forward reaction and the relatively slow re-oxidation as indicated in the trace shown in Fig. 1.

In order to assess the quantum efficiencies of the light-induced reduction of pheophytin in the PS II systems being studied, the rate of Q_A reduction in BBYs was measured by monitoring the kinetics of the chlorophyll rise from F_0 to F_m (where F_0 is the initial, and F_m is the maximum fluorescence yield). In this experiment the reaction medium contained no sodium dithionite or mediators but the membranes were treated with 10 μM DCMU to avoid electron flow between Q_A and Q_B. To

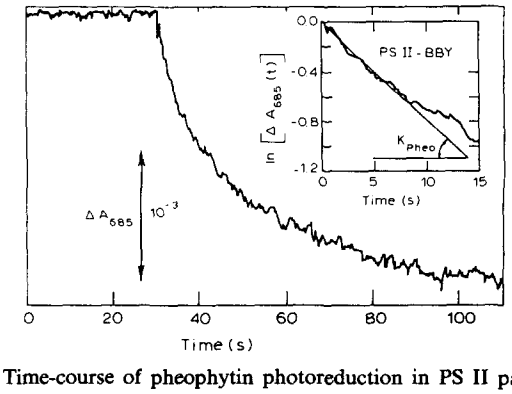

Fig. 4. Time-course of pheophytin photoreduction in PS II particles (BBYs, Ref. 12) under similar conditions to those of Fig. 1. The semilog plot gives k_{Pheo} of 0.08 s^{-1} (see Fig. 3 legend).

be able to resolve the kinetics of the fluorescence rise it was necessary to use a low actinic light intensity of 47.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The time-resolved fluorescence increase resulting from the photoreduction of Q_A is shown in Fig. 6 together with a semilog plot of the rate of growth in the area above the transient signal [14]. According to these data the photoreduction of Q_A in the PS II-enriched membranes occurred with a rate constant of 80 s^{-1}. If this rate constant is normalized to an actinic light intensity 950 $\mu mol \cdot m^{-2} \cdot s^{-1}$, then its value would be 1600 s^{-1}. Assuming the photochemical reduction of Q_A occurs with a quantum efficiency of one, then it follows that the quantum efficiency for the light induced reduction of pheophytin in BBYs, when dithionite and appropriate redox mediators are present, is 0.08/1600 or $5 \cdot 10^{-5}$. In the case of the PS II reaction centre it is clear that the quantum efficiency for the same reaction is higher. Assuming that there are 6 chlorophylls per PS II reaction-centre complex [15,16] and that BBYs contain 250 chlorophylls per PS II [14], then the observed rate constant of 2 s^{-1} for the photoreduction of pheophytin in the isolated PS II reaction centre corresponds to a normalized rate constant of $(250/6) \cdot 2$ s^{-1} or 83 s^{-1}). Again, assuming that the quantum efficiency for the photoreduction of Q_A is unity, then the quantum efficiency for pheophytin photoreduction in the PS II reaction centre is 83/1600 or 0.052 (5.2%). These results clearly indicate that the quantum efficiency for the reduction of pheophytin by light in the presence of dithionite is about 10^3-times greater in the isolated PS II reaction centre than in PS II-enriched membranes. In a previous study [7] which involved measuring the kinetics of the decrease in chlorophyll fluorescence as the reduced pheophytin accumulated, the difference in relative quantum yields was also found to be three orders of magnitude between isolated PS II reaction centres and PS II-enriched membranes or intact thylakoid membranes. Both studies are

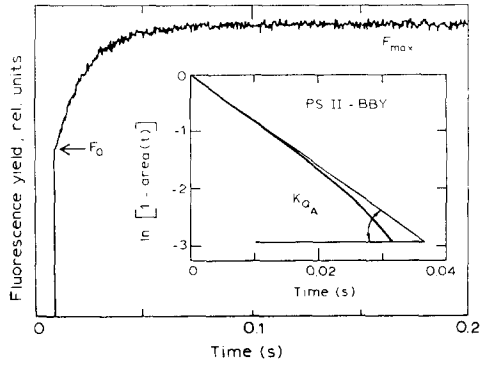

Fig. 6. Kinetics of the rise in chlorophyll fluorescence measured with a preparation of PS II-BBYs treated with 10 μM DCMU. Total chlorophyll concentration was 10 μM and the actinic light intensity was 47.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Also shown is the semilog plot of the rate of growth in the area above the transient signal which relates to the rate of photoreduction of Q_A (k_{Q_A}) (see Ref. 14).

consistent in that they clearly show that the photoaccumulation of reduced pheophytin occurs in the isolated reaction centre with a much higher efficiency than in PS II membranes where the secondary quinone acceptor Q_A is in its reduced state.

Discussion

In order to discuss the implications of the differences in quantum yield for the photoaccumulation of reduced pheophytin in the two experimental systems, it is useful to consider the following simple reaction scheme:

$$P680\ Pheo \xrightarrow{h\nu} P680^+\ Pheo^-$$

$$P680^+\ Pheo^- \xrightarrow{k'} P680\ Pheo$$

$$P680^+\ Pheo^- + D^- \xrightarrow{k} P680\ Pheo^- + D$$

where D^- is the reduced donor to $P680^+$. The quantum yield for $Pheo^-$ accumulation, γ, would therefore be given by

$$\gamma = \phi_p k[D^-]/(k' + k[D^-])$$

where ϕ_p is the quantum yield of primary photochemistry and k is the bimolecular rate constant expressing the interaction of $P680^+Pheo^-$ with the electron donor D^-. We have shown that there is a change in the quantum efficiency of the photoaccumulation of reduced pheophytin by three orders of magnitude between PS II reaction centres and a more complete PS II system. This very large difference could be the consequence of three possible factors; a decrease in k', an increase in k or an increase in the quantum yield of photochemistry ϕ_p. Indeed, it has been found from flash-absorption studies that recombination rate constant k' does seem to be greater for the intact PS II system as compared with the isolated reaction centre. For intact PS II the k' value has been estimated to be $5 \cdot 10^8\ s^{-1}$ [10] while for the isolated PS II reaction centres the value is $3 \cdot 10^7\ s^{-1}$ [4,6]. These values would, therefore, account for only a factor of about 20 difference in the quantum yields for $Pheo^-$ accumulation assuming $k' \gg k$, but much less if $k \approx k'$ or $k' < k$.

The value of k could be different for the two systems with dithionite having easier access to $P680^+$ in the isolated reaction-centre complex compared with the membrane system. The redox mediators, methyl viologen and indigo disulphonate, were included in the reaction medium to try to minimise this difference. Nevertheless, the light intensity curve does indicate that, even in the case of the isolated PS II reaction-centre complex, a dark-limiting step does occur at high light intensities (see Fig. 5). Further complications exist in that in the case of the isolated PS II reaction centre the electron donation is probably directly to $P680^+$ since to date we [17] and others [18] have been unable to observe significant levels of the secondary donor Z in its photooxidised state. In the case of BBYs, however, electron donation to $P680^+$ occurs from Z and how this is affected by the presence of dithionite is not known. Bearing in mind these complications, it is difficult to imagine that the interaction of D^- with $P680^+$ could be a difference of three orders of magnitude between the two experimental systems.

From the above discussion, it seems possible that the large difference in quantum yield for the photoaccumulation of reduced pheophytin between the isolated PS II reaction centre and the intact PS II unit within BBYs is partly due to a change in ϕ_p. One possible explanation is that when Q_A is present in its reduced state it exerts an electrostatic constraint on primary charge accumulation and reduces the quantum yield for the photoaccumulation of pheophytin. This conclusion is consistent with the work of Schatz et al. [10] and Schlodder and Brettel [19] who reported a lower quantum yield for radical pair formation ($P680^+Pheo^-$) when Q_A is reduced, compared with conditions when Q_A is in an oxidised state.

It is possible that the absence and presence of reduced Q_A may affect the shape of the $Pheo^-$-minus-Pheo difference spectrum. The spectra shown in Fig. 2 obtained with the isolated PS II reaction centre differs from that reported for BBYs by Demeter et al. [20] (see Fig. 2b). In contrast to the spectrum obtained with the isolated reaction centre, the BBY spectrum has a positive band peaking at about 674 nm, which is significantly greater than the positive band at 658 nm. Moreover, although the negative bands in the two spectra had similar peaks at about 685 nm, the intensity of the bands, relative to the positive absorption at 710 nm, is significantly different, with the BBYs having a 685/710

nm ratio of 3–4-times greater than the PS II reaction centre. Clearly, this difference has important implications regarding the use of an extinction coefficient derived from the $Pheo^-$-minus-Pheo spectrum of rBBYs [20] for estimating pheophytin reduction in the isolated PS II reaction centre. That the two difference spectra are dissimilar may not be due to the effect of Q_A since the spectrum shown in Fig. 2 is similar to that reported by Klimov et al. [8] for Q_A containing PS II-enriched particles poised at a redox potential of about −490 mV. Only when the redox potential was in the region of −200 mV did they observe a $Pheo^-$-minus-Pheo spectrum similar to that reported in Ref. 20.

Acknowledgements

We wish to thank Jeff A. Nemson, Francis Shanahan and Jill Farmer for technical support. We also thank the NSF and the Agricultural and Food Research Council for financial support. The work was performed while one of us (J.B.) was a Visiting Professor at U.C. Berkeley supported by a fellowship from the Miller Foundation.

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