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Reconstitution of plastoquinone in the D1/D2/cytochrome *b*-559 photosystem II reaction centre complex

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Reconstitution of plastoquinone in the photosystem II D1/D2/cytochrome *b*-559 reaction centre complex, in the presence of the detergent Triton X-100, is reported. Illumination of the reconstituted system results in the reduction of cytochrome *b*-559, the process being partly herbicide-sensitive. In addition, the reconstitution of plastoquinone results in the ability of the isolated reaction centre to catalyse the photoreduction of 2,6-dichlorophenolindophenol in the presence of the exogenous electron donor diphenylcarbazide.

Photosynthesis; Photosystem II; Reaction center; Plastoquinone; Reconstitution; Cytochrome *b*-559

1. INTRODUCTION

There is now little doubt that the D1/D2/cytochrome (cyt) *b*-559 complex, first isolated from spinach chloroplasts [1] but now also from other plant material [2], is the reaction centre of photosystem (PS) II. The isolation of this complex gave proof for the proposals of Michel and Deisenhofer [3] and of Trebst [4] that the D1 and D2 polypeptides, in many ways, were homologous in structure and function to the L and M subunits of the purple bacteria [5]. This analogy of the two systems, however, is limited to the reducing side of the reaction centre and not to the oxidising side. The oxidation potentials of the primary donors of purple bacterial systems so far studied are in the region of 400 mV while the primary donor, P680, of the oxygenic PS II reaction centre must create an oxidising potential of at least 1000 mV. In contrast, the redox potentials created on the reducing side of the oxygenic and bacterial reaction centre seem to be very similar. Indeed, both use pheophytin-type molecules as primary stable electron acceptors at mid-point potentials of about −600 mV and both use quinones as secondary electron acceptors; Q_A at about −300 mV and Q_B at about 0 mV. Also of note is that both the bacterial and PS II reaction centres have a non-haem iron closely interacting with Q_A and Q_B.

When reaction centres are isolated and purified from purple bacteria, such as *Rhodobacter sphaeroides* or *Rhodopseudomonas viridis*, they always maintain firmly bound quinone corresponding to Q_A and Q_B. In contrast, the recently isolated PS II reaction centre complex does not retain Q_A or Q_B [1,6]. Like the bacterial counterpart, however, it does bind four chlorophylls and two pheophytin molecules and is able to catalyse primary charge separation [7–9]. Under certain circumstances the primary charge separation can be stabilized by the addition of various artificial electron acceptors and donors, leading to the photoaccumulation of reduced pheophytin [1,2] or of a chlorophyll cation, which is thought to be $P680^+$ [2]. Moreover, these exogenous additives can give rise to net electron transport [2,10]. Only very recently has it been possible to observe quinone-mediated electron transport and even in

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this case, the reaction was limited to the use of decylplastoquinone and to D1/D2/cyt *b*-559 complexes which had been subjected to detergent exchange with β-lauryl maltoside [10].

Here, we report new results concerning quinone-mediated electron transport catalysed by the isolated PS II reaction centre and show that it is possible to use the natural quinone, plastoquinone-9 (PQ-9), as an active component.

2. MATERIALS AND METHODS

PS II reaction centre complexes composed of D1, D2 and cytochrome *b*-559 were prepared from pea chloroplasts as described [10] and stored at −80°C. The complexes were thawed and resuspended in assay media as indicated in the figure legends. PQ-9 was a generous gift from Hoffman-La Roche (Basel) and was used without further purification. Plastoquinol-9 (PQH_2-9) was prepared from PQ-9 by the method of Rich [11]. Light-induced absorbance changes were measured with a Perkin-Elmer 557 dual-beam spectrophotometer as in [10] with side illumination of a 1 ml sample cuvette maintained at 4°C. Assays were carried out with PS II reaction centre samples containing 2 μg chlorophyll *a* in a medium of 50 mM Tris buffer (pH 8.0), 0.2% Triton X-100 unless described otherwise.

Light-driven reduction of cytochrome *b*-559 was monitored at 430 nm with 1 mM diphenylcarbazide (DPC) or 1 mM $MnCl_2$ as electron donors. Reduction of 2,6-dichlorophenolindophenol (DCPIP) (5 μM) was monitored at 590 nm and relative rates calculated using extinction coefficients determined by chemical reduction of DCPIP standards (by using dithionite) at the various pH values. Decylplastoquinone and PQH_2-9 were added to incubation media at 4°C, but PQ-9 was added to media at 30°C, mixed well and then cooled to 4°C before addition of sample and the artificial donors and acceptors.

3. RESULTS

We have previously shown [10] that on addition of decylplastoquinone to the D1/D2/cyt *b*-559 complex, in the presence of exogenous electron donors, a light-induced absorbance change at 430 nm is observed which reflects the reduction of cytochrome *b*-559. We demonstrated that this change represented a reduction of approx. 30% of the total cytochrome content. Under these conditions plastoquinone (PQ-9), which occurs naturally in the chloroplast thylakoid membrane, was ineffective. When the assay system described in [10] is used, very little (~10%) ascorbate-reducible cytochrome *b*-559 can be detected. We have now found that the ascorbate-reducible cytochrome *b*-559 can be significantly increased if the isolated PS II reaction centres are suspended in media containing various amounts of detergents. Fig.1A,B shows the amount of ascorbate-reducible cytochrome *b*-559 (expressed as a percentage of the total dithionite-reducible cytochrome) in media containing various amounts of the non-ionic detergent Triton X-100. It can be seen that the amount of ascorbate-reducible cytochrome increases with increasing detergent concentration. In fact, with concentrations of Triton X-100 of 0.2% and above, 90% of the cytochrome *b*-559 present in the PS II reaction centre is in an ascorbate-reducible form. Interestingly, under these same conditions we observed that reduced PQH_2-9 can also lead to the dark reduction of cytochrome *b*-559. The pH sensitivities of the dark reduction of the cytochrome by ascorbate and PQH_2-9 are shown in fig.2 where it can be seen that with optimal detergent levels, a full reduction of cytochrome *b*-559 was possible at about pH 8.0.

It is worthy of note that in the absence of added detergent, no cytochrome *b*-559 could be reduced in the dark by PQH_2. The requirement for detergent could be to allow accessibility by the quinol to the cytochrome within the protein complex but also it is very likely, as indicated by the results with ascorbate, that the detergent shifts the mid-point redox potential of cytochrome *b*-559 to a more favourable value (i.e. more oxidising). Such a shift in the redox potential of cytochrome *b*-559 in response to detergent and lipid additions has been noted previously for PS II-enriched preparations [6,12]. As expected even under the most optimal conditions PQ-9 does not reduce cytochrome *b*-559 in the dark. However, when added to the isolated D1/D2/cyt *b*-559 complex, PQ-9 facilitates a light-induced reduction of the cytochrome in a way similar to that observed previously with decylplastoquinone when the reaction centre was solubilized in β-lauryl maltoside [10]. A typical signal recorded at 430 nm is shown in fig.3 which also gives the concentration requirement for PQ-9. Compared with our previous work with decylplastoquinone, higher concentrations of PQ-9 were required in order to observe the effect. As before, to obtain the maximum signals the presence of a suitable PS II electron donor was necessary and we routinely used $MnCl_2$. With optimal conditions it was possible to photo-reduce

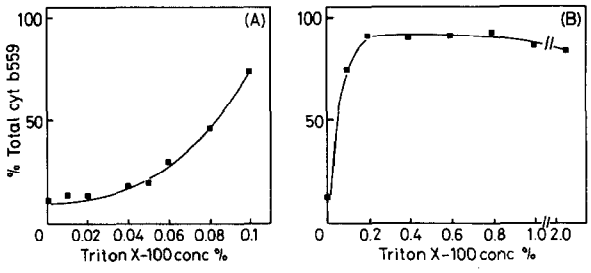

Fig.1. Ascorbate-reducible cytochrome *b*-559, expressed as a percentage of the total dithionite-reducible cytochrome, at various Triton X-100 concentrations. Samples contained photosystem II reaction centre complex (2 μg Chl·ml^{-1}) suspended in 50 mM Tris-HCl (pH 8.0), 3 mM ascorbate and Triton X-100. Detergent concentration: (A) 0–0.1%; (B) 0–0.2%.

about 62% of the total cytochrome *b*-559. For the dark reduction of the cytochrome by PQH_2 the concentration requirement for the quinol was much lower than for the light-induced reaction (cf. fig.4 with fig.3).

With no added quinone the isolated D1/D2/cyt *b*-559 complex can catalyse the photo-reduction of silicomolybdate [2] but cannot support a light-mediated reduction of the commonly used PS II artificial electron acceptor, DCPIP (see [10]) by DPC. However, under conditions when added

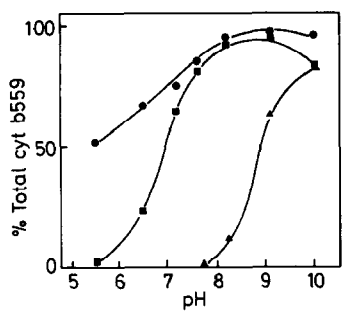

Fig.2. pH dependence of ascorbate (●—●) and plastoquinol-9 (■—■) reducible cytochrome *b*-559 expressed as a percentage of the total dithionite-reducible cytochrome. Samples contained 50 mM of an appropriate buffer, 2 μg Chl·ml^{-1} reaction centre complex, 0.2% Triton X-100 and either 3 mM ascorbate or 18 μM PQH_2. For comparison the pH dependence of ascorbate-reducible cytochrome *b*-559 under similar conditions but in the absence of detergent is also shown (▲—▲). The following buffers were used: Mes, Hepes, Tris, Tricine for increasing pH values. There were no significant differences due to the nature of the buffer.

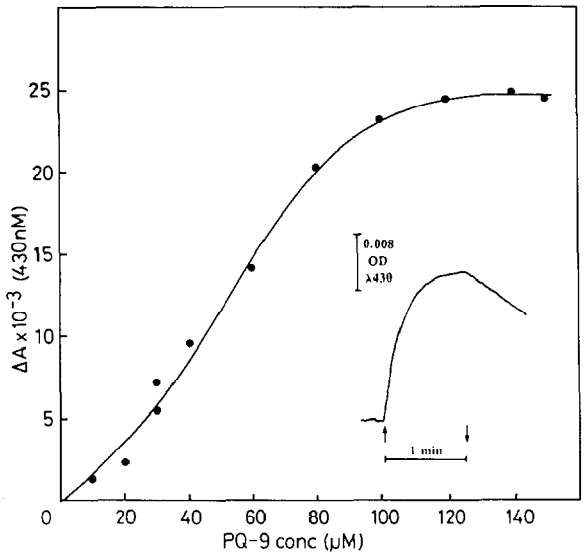

Fig.3. Shown as in inset is the light-induced absorbance change at 430 nm measured in samples containing reaction centre (2 μg Chl·ml^{-1}), 120 μM PQ-9 and 1 mM $MnCl_2$ in 50 mM Tris-HCl, pH 8.0, also containing 0.2% Triton X-100. Also shown is the plastoquinone concentration curve for the light-induced signal using an identical assay medium to that given above.

decylplastoquinol or PQ-9 can induce a photo-reduction of cytochrome *b*-559, this reaction can also occur. The dependence on decylplastoquinone concentration for the DPC to DCPIP reaction is shown in fig.5, while fig.6 shows that the pH optimum for this electron-transfer process is between 6 and 7. This value contrasts with the reduction of cytochrome *b*-559 which has an optimum at more alkaline pH. The maximum rate of DCPIP photo-

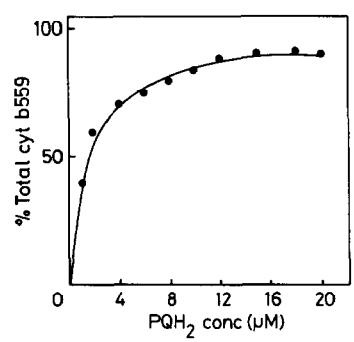

Fig.4. The effect of plastoquinol-9 concentration on the reduction of cytochrome *b*-559 in the dark. Samples contained reaction centres (2 μg Chl·ml^{-1}) in 50 mM Tris-HCl (pH 8.0) containing 0.2% Triton X-100.

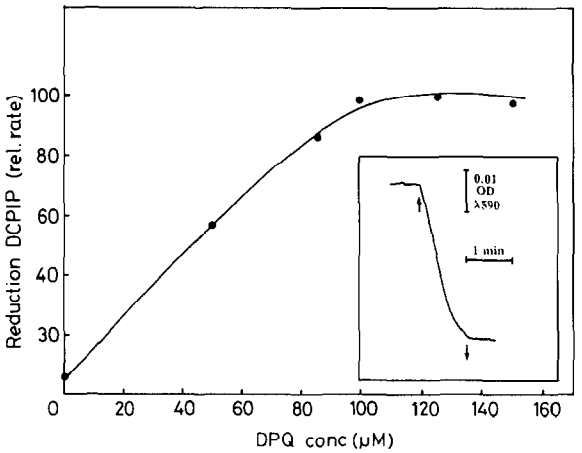

Fig.5. The inset shows the light-dependent reduction of 5 μM DCPIP assayed in reaction centre samples containing 2 μg Chl·ml^{-1}, suspended in 50 mM Tris-HCl (pH 8.0) containing 0.2% Triton X-100 and 25 μM decylplastoquinone (DPQ). Dephenylcarbazide (DPC) was used as an electron donor at 50 μM. Also shown is the DPQ concentration requirement of the DPC to DCPIP reaction.

reduction was very low, being approx. 500 equiv. electrons·mg Chl^{-1}·h^{-1} and was about half of that recorded when silicomolybdate was used as an electron acceptor [2].

In our previous work [10] we showed that the photo-reduction of cytochrome *b*-559 due to the presence of decylplastoquinone could be partially

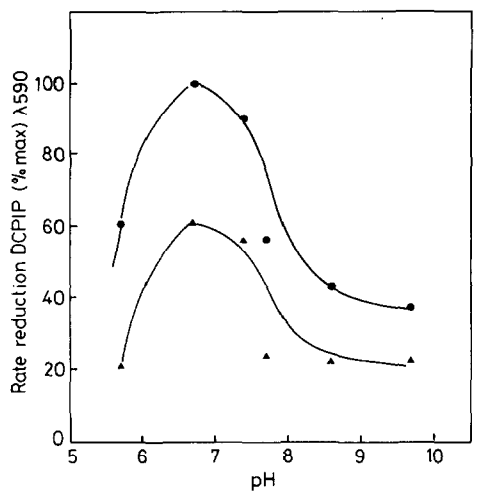

Fig.6. Light-induced reduction of DCPIP by PS II reaction centre in the absence (●) and presence of DCMU (▲). The reaction medium was as for fig.5 except that the pH was adjusted by using 50 mM Mes (for pH 5.0 to 7.5) and 50 mM Tris (for pH 7.5 to 10). DCMU concentration was 100 μM.

inhibited by the PS II herbicide, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). This inhibition required high concentrations of DCMU which probably reflects the low affinity of the isolated complex to bind herbicide [13]. We have found that the PQ-9-dependent reduction of cytochrome *b*-559 is also partially inhibited by high concentrations of DCMU. Such a partial inhibition was also noted for the quinone-catalysed light-driven electron transport from DPC to DCPIP (see fig.6).

4. CONCLUSION

We have shown that as with decylplastoquinone, the naturally occurring quinone of PS II, PQ-9, will support the light-induced reduction of cytochrome *b*-559. The ability of PQ-9 to act in this way is highly dependent on the level of Triton X-100 and the pH of the assay medium. Even then, high levels of this quinone must be added. The precise mechanism of the light-induced PQ-9-mediated reduction of the cytochrome is unclear but experiments involving chemical reduction in the dark indicate that the process occurs via the photo-reduction of the added quinone. Presumably, the added quinone is reduced by the photo-reduced pheophytin but the requirement for an exogenous electron donor indicates that the quantum yield for the reaction is low. The quinone could be reduced at either Q_A or Q_B or both, and the partial inhibition by DCMU indicates such an interaction. Nevertheless, the DCMU inhibition is not absolute even at higher concentrations and could reflect competition for either of the sites between quinone and the herbicide.

Further indication that the added quinones undergo photo-induced reduction by the PS II reaction centre complex is the finding that in the presence of added quinone, DCPIP will act as an electron acceptor. Under these conditions the light-mediated reduction of cytochrome *b*-559 is attenuated, suggesting that there is competition between the two terminal acceptors for electrons from the reduced quinone.

Although the above studies are an important step forward in an attempt to reconstitute the acceptor side of the isolated D1/D2/cyt *b*-559 complex, a thorough study is required to assess the interactions of the added quinones with the Q_A and Q_B sites. Such a study cannot be adequately

achieved with steady-state optical techniques but is possible with transient optical or EPR spectroscopy. The present results will hopefully provide a starting point for a more rigorous investigation of quinone reconstitution of the isolated PS II reaction centre.

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