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Quinone exchange in the Q_A binding site of Photosystem II reaction center core preparations isolated from *Chlamydomonas reinhardtii*

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We demonstrate here that it is possible, in Photosystem II reaction centers, to extract and replace plastoquinone-9 (PQ-9), the natural primary quinone electron acceptor, Q_A, by either another molecule of PQ-9 or by ubiquinone-9 (UQ-9), an isoprenolog of the quinone which acts in this role in purple photosynthetic bacterial reaction centers. The extraction of PQ-9 and the reconstitution with PQ-9 or UQ-9 were carried out simultaneously, in the presence of the detergent Triton X-100, on Photosystem II reaction center core preparations from *Chlamydomonas reinhardtii*. Quinone exchange is demonstrated unambiguously by the replacement of the light-induced $PQ^- - PQ$ ultraviolet difference spectra with that for $UQ^- - UQ$. That the semiquinones of UQ-9 and PQ-9 are photogenerated in the Q_A binding site is demonstrated by the following arguments: a local electrochromic band shift of pheophytin (C550), arising from the charge on Q_A^-, is observed in both cases. The ratio of $\Delta I/I_{325nm}$, the maximum ultra-violet absorbance change arising from the semiquinone anion (UQ^- and PQ^-), to that of C550, is approx. 6 in the case of PQ-9 centers and 3–4 in the case of UQ-9 centers. These ratios are consistent with the relative in vitro extinction coefficients of the PQ and UQ semiquinone anions at their absorption maxima in the ultraviolet, 13 and 8.2 $mM^{-1} \cdot cm^{-1}$, respectively (Bensasson, R. and Land, E.J. (1973) Biochim. Biophys. Acta 325, 175–181). $\Delta I/I_{325nm}$, a measure of semiquinone anion concentration, and $\Delta I/I$(C550) relax with similar rates whether the centers contain PQ-9 or UQ-9. Reaction centers which have lost the Q_A quinone are in a metastable state. Rapid reassociation of quinone restores primary charge separation. Reaction centers which do not rapidly reassociate with quinone lose irreversibly the ability to demonstrate primary photoactivity even after readdition of the extracted quinone.

Introduction

Cogdell et al. [1] and Okamura et al. [2], and more recently Gunner and coworkers [3], have shown that the primary quinone electron acceptor, Q_A, a ubiquinone-10, of reaction centers isolated from *Rhodobacter sphaeroides* may be replaced by a wide variety of benzo-, naphtho- and anthraquinones, many of which act as electron acceptors in the primary charge separation. These results have been a particularly rich source of information on the stereospecificity of quinone binding to the reaction center, the effects on binding of the

Abbreviations: Chl, chlorophyll; CP47, one of two major chlorophyll-protein complexes of PS II reaction centers; CP43, one of two major chlorophyll-protein complexes of PS II reaction centers; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron); PMSF, phenylmethane sulfonylfluoride; Mes, 4-morpholineethanesulphonic acid; PQ-9, plastoquinone-9; PS II, Photosystem II; RC, reaction center; Q_A, primary quinone electron acceptor; Q_B, secondary quinone electron acceptor; TX-100, Triton X-100; UQ-9, ubiquinone-9.

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nature of the quinoid ring substituent and the energetics of the primary charge separation.

A number of reports exist in the photosynthesis literature of extraction and reconstitution of plastoquinone-9 from the Q_A binding site of Photosystem II [4–8]. In a recent publication, Csatorday and coworkers [8] have argued that linolenic acid addition and removal can result in a reversible dissociation and reassociation, respectively, of PQ-9 from the Q_A site in PS II-enriched membrane fragments. Most reports, however, have involved organic solvent extraction of lyophilized membranes [4–6] and even in one case of Photosystem II particles [7]. We were unsuccessful, after many attempts, in reproducing the latter results with Photosystem II core preparations from *Chlamydomonas reinhardtii* and decided to try detergent extraction, successfully applied to bacterial reaction centers in the above-cited work [2,3]. Extraction and reconstitution with other than the natural plastoquinone-9 has not yet been demonstrated in Photosystem II (PS II) by any method, despite the considerable homology that exists with the bacterial reaction centers [9–13], particularly with respect to the electron acceptors. In this paper, we show that such substitution, while more difficult, is also possible in isolated PS II reaction center preparations with the aid of detergents.

Materials and Methods

PS II core particles were isolated from mutant F54-14 (lacking chloroplast ATPase and PS I reaction centers) of *Chlamydomonas reinhardtii* (a gift of Pierre Bennoun) and purified by sucrose-density gradient centrifugation as described previously [14]. The gradient band containing the PS II reaction centers in 0.03% Triton X-100 was diluted approx. 3-fold with 20 mM Mes (pH 5.9) containing 200 μM PMSF and centrifuged at 235 000 × *g* for 5 h at 5°C. The centers aggregated upon dilution below the critical micelle concentration of Triton X-100 (0.26 mM (0.017% w/v) [15]) and were collected in a pellet which was resuspended using a Potter homogenizer in a small volume of supernatant (final concentration, approx. 3 mg Chl per ml), frozen in liquid nitrogen and stored at −80°C. The particle suspension was thawed and generally redissolved in 0.5–1% Triton X-100, with other additions indicated in the figure legends.

The polypeptides present in this material are CP47 and 43 (51 and 47 kDa, the principal chlorophyll protein complexes), D2 (34 kDa), D1 (32 kDa), 33 kDa extrinsic donor-side polypeptide, and cytochrome *b*-559 (10 and 4 kDa).

The spectrophotometric measurements were performed using a flash-detection spectrophotometer based on an instrument designed by Joliot and coworkers [16]. Actinic flashes were provided either by a xenon flash (model FX199 EG & G, Salem, MA, 1 μs duration at half height) or a laser flash (Candela, Natick, MA, model SLL-150 dye laser with Oxazine 720, $\lambda_{max} = 693$ nm or a Candela model SLL-250 with Sulforhodamine 640, $\lambda_{max} = 645$ nm; 600 ns total duration in both cases).

Plastoquinone-9 (PQ-9) and ubiquinone-9 (UQ-9) were gifts of Ms. C. Guittard of Hoffmann-LaRoche S.A., Paris, and were used without further purification. Triton X-100 was Boehringer Mannheim membrane research grade. All other reagents were analytical grade.

Results

PS II reaction center particles (Fig. 1) were incubated at 300 μg Chl/ml (6 μM in reaction centers) for various times at 20°C in the presence of 0, 0.045 and 1% Triton X-100 (TX-100) and, at the latter concentration, in the presence and absence of added plastoquinone-9 (PQ-9) and *o*-phenanthroline. Primary charge separation was monitored at regular intervals by measuring saturating flash-induced photoreduction of Q_A at 320 nm in the presence of hydroxylamine. This reductant reduces the photooxidized donors, Z^+ and P-680$^+$, without reducing Q_A in the preceding dark period. This experiment (Fig. 1) shows a progressive decrease of the extent of Q_A photoreduction over a period of 21 h. Of the conditions shown in Fig. 1, the rates of activity loss were slowest and equivalent in the absence of added detergent and in the presence of 1% TX-100 containing 250 μM PQ-9 (40 times the concentration of endogenous quinone). Increasing the detergent concentration from 0 to 1% in the absence of added quinone or increasing the *o*-phenanthroline

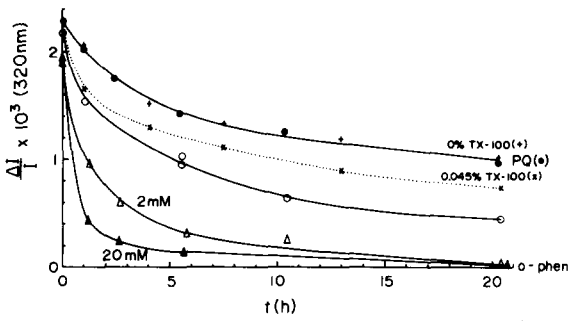

Fig. 1. Inactivation of photoreduction of Q_A upon incubation at 20 °C with and without PQ-9 and *o*-phenanthroline and at various detergent concentrations. PS II particles were maintained at 300 μg Chl/ml in 20 mM Tris-HCl (pH 8.0) at 20 °C for the indicated times in the presence of 0% (+), 0.045% (×) or 1% Triton X-100 (○, ●, △, ▲). The samples contained either no further additions (○, +, ×), or 250 μM PQ-9 (●), or 2 mM (△) or 20 mM (▲) *o*-phenanthroline (o-phen). At the end of the incubation period, the samples were diluted to 3 μg Chl/ml with 20 mM Tris-HCl (pH 8.0) for measurement. $K_3Fe(CN)_6$ (10 μM) was added and after 1 min in the dark 2 mM NH_2OH. After 2 additional min in the dark, a series of 14 saturating actinic xenon flashes were given 100 ms apart. $Q_A^- - Q_A$ was measured at 320 nm at at least 33 ms after the last actinic flash, by taking the light-minus-dark difference signal. Optical path length, 16 mm.

concentration at 1% TX-100 enhanced the inactivation rate.

The number of quinones per micelle for 6 μM reaction centers in 1% TX-100 (100 μM micelles [15]) and 0.045% TX-100, both in the absence of exogenous PQ-9, would be 0.06 and 2, respectively. In the presence of 250 μM PQ-9 in 1% Triton X-100 this value would be 2.5. In all three cases complete equilibration of PQ-9 between micelles and no preferential binding to reaction centers is assumed. We see therefore that there is a protective effect of a high ratio of PQ-9 per micelle. There is also a stimulated inactivation by *o*-phenanthroline, as in bacterial reaction centers, where this inhibitor facilitates the extraction of Q_A [2]. Both observations suggest that, at 20 °C, at TX-100 concentrations above its critical micelle concentration, PQ-9 is lost from the Q_A site. Addition of 250 μM PQ-9 to 1% TX-100 (2.5 PQ-9/micelle at 100 μM Triton micelles) during the incubation gives the same inactivation rate as no Triton at all and would maintain the Q_A site completely occupied by displacing the $PQ_{bound} \leftrightarrow PQ_{free}$ equilibrium toward the reaction center-PQ-9 complex.

PS II reaction centers were treated at 300 μg Chl/ml with 1% Triton X-100 at 15–20 °C for 24 h in the absence of added quinone as in Fig. 1 (○) or with added inhibitors like DCMU and *o*-phenanthroline to extract Q_A. Where used, the inhibitors were removed by washing through sucrose density gradient centrifugation and dialysis. The reaction centers were then supplemented with 250 μM PQ-9 (1% Triton X-100) and incubated at 22 °C. Little significant restoration of quinone photoreduction was observed even after several hours of incubation. While the experiments which follow will show that PQ-9 truly dissociates and reassociates with the Q_A binding site, it would appear that in order to restore photoactivity reassociation must occur within a short time of dissociation.

The assay conditions of Fig. 1 (ferricyanide and an excess of NH_2OH) do not allow the reaction center to recover following charge separation, as there is no oxidant available to reoxidize Q_A^-. Use of benzidine and ferricyanide together, however, allows the reaction center to recover following flash excitation. Benzidine reduces the photooxidized secondary donor, Z^+, within several tens of milliseconds, following charge separation, but has an E_m too high ($E_{m,7} = 0.55$ V) to reduce either Q_A or ferricyanide. The ferricyanide is thus free to reoxidize Q_A^- within 30 s of actinic flash excitation, allowing the reaction center to be restored to the initial dark-adapted state which preceded the actinic flash, permitting signal averaging.

Fig. 2 shows the $Q_A^- - Q_A$ difference spectrum obtained upon saturating flash illumination in the presence of ferricyanide and benzidine following incubation with 250 μM PQ-9 for 24 h at 15 °C in 1% Triton X-100, followed by 24 h at 4 °C. The experimental spectrum of Fig. 2 is compared to the pulse radiolysis difference spectra of $PQ^- - PQ$ of Bensasson and Land [17] shifted 4 nm toward the red and normalized to the biological spectrum at 320 nm. As reported by Van Gorkom [18], the biological spectrum closely resembles a red-shifted pulse radiolysis difference spectrum for the semiquinone anion minus quinone of PQ-9 [17]. An

identical spectrum was obtained using hydroxylamine as the donor to Z^+ and P-680$^+$.

Most of the quinone exchange experiments described here were carried out by first incubating at high detergent and quinone concentrations at elevated temperatures followed by 24 h of incubation at 4° C. While it was initially thought that the 4° C incubation would further such exchange,

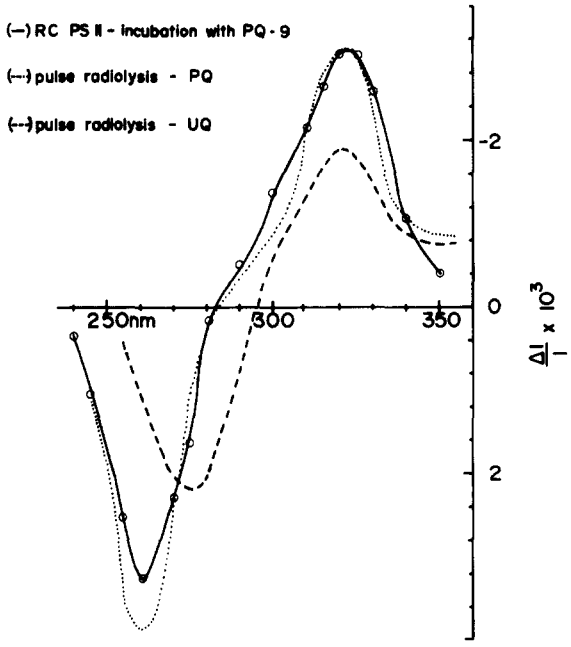

Fig. 2. $Q_A^- - Q_A$ difference spectrum obtained following incubation with PQ-9 for 24 h at 15° C plus 24 h at 4° C. PS II particles were maintained at 300 μg Chl per ml during the incubation in the presence of 250 μM PQ-9, 1% Triton X-100, 20 mM Tris-HCl (pH 8.0). The sample was diluted to 6 μg Chl per ml with 20 mM Tris-HCl (pH 8.0) in the presence of 10 μM $K_3Fe(CN)_6$ and 10 μM benzidine. $Q_A^- - Q_A$ was obtained as the average signal obtained from ten detecting flashes given at 200 ms intervals starting at 100 ms after a single saturating laser (693 nm) flash. Two experiments 40 s apart were averaged for each point. The initial amplitude at 500 μs after the actinic flash was 1.35 times the $\Delta I/I$ shown for these experimental points. The absorbance changes shown in the figure are 61% greater than those obtained for incubation under the same conditions without added PQ-9. The optical path length for these measurements was 16 mm. The pulse radiolysis difference spectrum for $PQ^- - PQ$ (·····) was shifted 4 nm to the red and normalized to the $Q_A^- - Q_A$ difference spectrum at 320 nm. The amplitude of the $UQ^- - UQ$ difference spectrum (- - - - -), also shifted 4 nm to the red, was determined by the ratio of the extinction coefficients ($PQ^- - PQ/UQ^- - UQ$) given by Bensasson and Land [17].

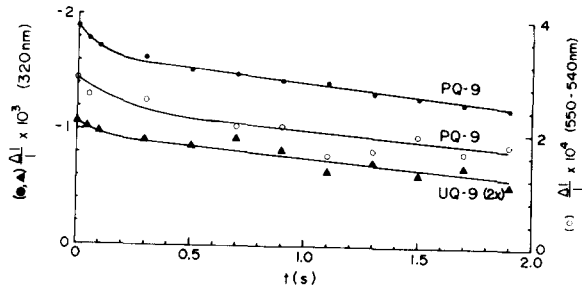

Fig. 3. Relaxation kinetics of $\Delta I/I_{320\,nm}$ and $\Delta I_{550-540\,nm}$ (C550) following incubation with PQ-9 or UQ-9 for 24 h at 20° C plus 24 h at 4° C. PS II particles were maintained at 300 μg Chl/ml during the incubation in the presence of 250 μM PQ-9 or 250 μM UQ-9, 1% Triton X-100, 20 mM Tris-HCl (pH 8.0). The sample was diluted to 3.4 μg Chl/ml with Tris-HCl (pH 8.0) in the presence of 10 μM $K_3Fe(CN)_6$ and 10 μM benzidine. A saturating laser (693 nm) flash was given and the reoxidation of Q_A^- was followed from 50 μs onward at 320 nm and by the difference 550 − 540 nm (C550). Both 320 nm and 550 − 540 nm measurements are shown for PQ-9, while only the former is shown for UQ-9. Flash-induced difference spectra (not shown) indicated that in the case of the UQ-9 incubated centers three out of four photoactive centers contained UQ, the rest contained PQ. For the sake of comparison, the amplitude of the 320 nm absorbance change is multiplied by a factor of two for UQ-9. Optical path length, 16 mm.

more recent experiments suggest that this step is probably not necessary.

A sample similar to that of Fig. 2 was incubated with 250 μM PQ-9 in 1% TX-100 for 24 h at 20° C followed by 24 h at 4° C. A saturating flash was given in the presence of ferricyanide and benzidine. Relaxation kinetics (Fig. 3) were measured at 320, 540 and 550 nm. The first wavelength detects PQ-9 semiquinone anion minus quinone. The difference 550–540 nm detects C550 [19], an electrochromic shift of pheophytin *a* induced by the negative charge on Q_A^- [20] and a linear indicator of the concentration of the latter species [21]. The ratio of the two signals, $(\Delta I/I_{320\,nm})/(\Delta I/I_{550-540\,nm}) = 6$ is similar to that reported by van Gorkom [18,20] and to that observed in untreated *Chlamydomonas* reaction centers (Diner, B.A., unpublished results). That this ratio does not change as a result of incubation and that it is constant throughout the relaxation argues that the PQ-9 reassociates with the Q_A binding site. The difference spectrum of $Q_B^- - Q_B$ shows a similar extinction coefficient to $Q_A^- - Q_A$ at 320 nm, but a 4-fold smaller C550-like electro-

chromic shift [22]. As the relaxation rates of Fig. 3 are equivalent, no functional quinone is present in the Q_B site (see also Ref. 23), despite the presence in this material of the acceptor-side non-heme iron [24,25]. Reoxidation of Q_A^- occurs via electron transfer to ferricyanide.

To prove conclusively that the quinone was actually extracted and reassociated with the Q_A binding site, PQ-9 was replaced by UQ-9 in an incubation experiment similar to that of Fig. 2. PS II reaction center core preparations at 600 μg chlorophyll/ml were incubated with 250 μM UQ-9 in 0.5% TX-100 at 20 °C for 18 h, followed by 24 h at 4 °C. Comparison of the pulse radiolysis spectra of Fig. 2 [17] shows that substitution of

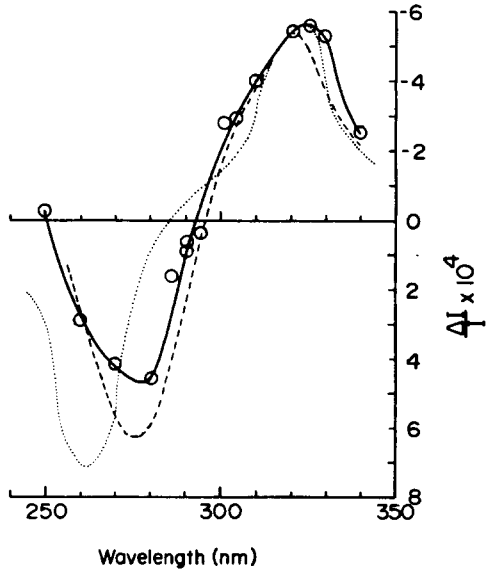

Fig. 4. Ultra-violet flash-induced difference spectrum (○) following exchange of PQ-9 with UQ-9. Reaction center core preparations (different batch from Figs. 1–3) at 600 μg Chl/ml were incubated in the presence of 250 μM UQ-9, 0.5% Triton X-100 in 20 mM Tris-HCl (pH 8.0), 200 μM PMSF for 18 h at 20 °C followed by 24 h at 4 °C. The sample was diluted to 12 μg Chl/ml with 20 mM Tris-HCl (pH 8.0) and 10 μM $K_3Fe(CN)_6$ and 10 μM benzidine were added. A single saturating laser flash (645 nm) was given and the average absorbance change measured between 49 ms and 1.1 s. Ten experiments were averaged with 40 s between each experiment. The initial amplitude at 49 ms after the actinic flash was 1.15 times the values shown in the figure. Optical path length, 10 mm. The dashed and dotted line spectra are the pulse radiolysis difference spectra of Bensasson and Land [17] for UQ^- – UQ and PQ^- – PQ shifted 5 nm to the red and normalized to the experimental spectrum at 320 nm.

UQ-9 for PQ-9 should shift the $Q_A^- - Q_A$ difference spectrum to longer wavelengths. That this is indeed the case is shown in Fig. 4, where light-induced absorbance changes are shown and compared to pulse radiolysis spectra normalized to the experimental spectrum (○) at 320 nm. The experimental points closely resemble the pulse radiolysis difference spectrum $UQ^- - UQ$ of Bensasson and Land [17] shifted 5 nm to the red and not at all that of $PQ^- - PQ$ shifted to the red by the same increment. UQ-9 has thus replaced PQ-9 as the primary quinone acceptor.

That UQ-9 is actually functioning in the Q_A-binding site is shown by observation of the Q_A^--induced electrochromic band shift of pheophytin, C550, both in untreated and UQ-9-exchanged centers. The C550 spectra of both of these are quite similar (Fig. 5). The spectrum observed in UQ-9-exchanged centers shows a reproducible dip at 540 nm, not observed in untreated centers. The source of this minor difference is unknown. Comparison (Fig. 6) of the relaxation of the light-induced C550 with that of $\Delta I/I_{325\,nm}$, a direct measure of $[UQ^-]$, shows these to be similar with a $t_{1/2}$ of 1.5–2 s further supporting the location of UQ^- in the Q_A binding site. There is a small (approx. 10%) slowly relaxing component in the $\Delta I/I_{325\,nm}$ which could be enhanced by increasing the detergent concentration above the critical micelle concentration and appeared to be associated with photoreduction of UQ not associated with the Q_A site.

The amplitude of the C550 signal in UQ-9 centers, an indicator of the fraction of centers remaining photoactive, is 3-times smaller than that of the untreated centers. $\Delta I/I_{325\,nm}$ is, however, about 5.3-times smaller for the same centers. This difference is consistent with the lower extinction coefficient for $UQ^- - UQ$ at the maximum ΔA (8.2 $mM^{-1} \cdot cm^{-1}$) in the UV as compared to $PQ^- - PQ$ (13 $mM^{-1} \cdot cm^{-1}$). The ratio of $\Delta I/I_{325\,nm}$ to that of $\Delta I/I$ (552–542.5) was 3–4 in the case of UQ-9 centers and approx. 6 for untreated or PQ-9-exchanged centers.

Additional arguments supporting the photoreduction of UQ-9 in the Q_A site is the similar rate of oxidation, by ferricyanide, of UQ^- and PQ^- in centers that were incubated and flashed in the presence of these quinones (Fig. 3) and the ability

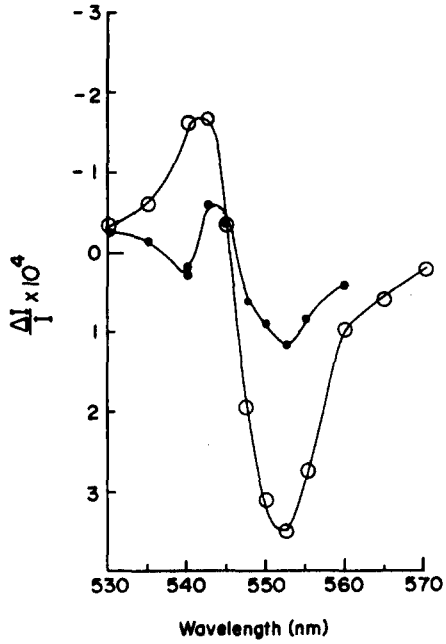

Fig. 5. Flash-induced difference spectra in the region of C550 for untreated (○) and UQ-9 (●) reaction center core preparations. The core preparations of Fig. 4 were prepared and assayed as in the preceding figure. Untreated centers were diluted to the same chlorophyll concentration (12 μg Chl/ml) and assayed under the same conditions (solutions and flash regime). Optical path length 10 mm.

to see light saturation of UQ^- and PQ^- formation as the actinic flash intensity is increased (not shown). In cases where quinones, associated with reaction centers, occupied sites other than that of Q_A, light saturation was not observed.

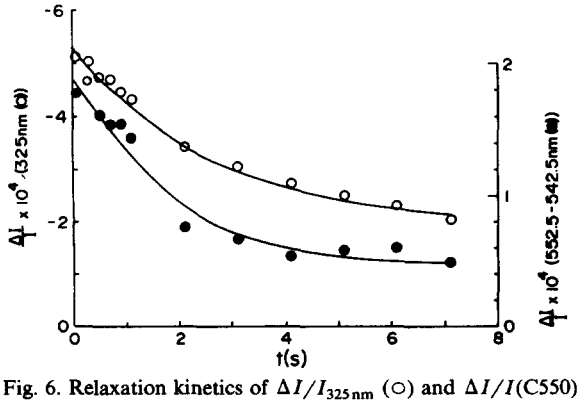

Fig. 6. Relaxation kinetics of $\Delta I/I_{325\,nm}$ (○) and $\Delta I/I$(C550) (●) in UQ-9 centers. Conditions (centers, solutions and flash regime) as in Fig. 4. The earliest time point is 49 ms after the actinic flash. Optical path length, 10 mm.

Discussion

The detection of a light-induced $UQ^- - UQ$ difference spectrum in PS II reaction centers, incubated in the presence of detergent and UQ (Fig. 4), indicates that UQ – 9 has replaced PQ-9 as the primary quinone electron acceptor and that quinone binds reversibly to the center in the presence of 1% Triton X-100. That UQ-9 is rebound to the same site as that left by PQ-9 is indicated: (a) by the observation of a C550 pheophytin band shift induced by the charge on Q_A^- (Fig. 5); (b) by the ratio of $\Delta I/I_{325\,nm}$: $\Delta I/I$(C550) of 3–4 rather than approx. 6 as in PQ-9-containing centers, consistent with the lower extinction coefficient for $UQ^- - UQ$ as opposed to $PQ^- - PQ$ (Figs. 4–6); and (c) by the similar relaxation kinetics of $\Delta I/I$(C550) and $\Delta I/I_{325\,nm}$ in the presence of ferricyanide, following light-induced formation of the semiquinone (Fig. 6).

The acceleration of the loss of Q_A photoreduction by *o*-phenanthroline is also consistent with reversible binding of the quinone to the Q_A site. This competitive inhibitor of quinone binding to the Q_B site [9,26–28] has been shown to accelerate UQ-10 extraction from the Q_A as well as Q_B sites in *Rb. sphaeroides* reaction centers in the presence of the detergent LDAO [2]. This finding makes structural sense, in that strong hydrogen bonding has been shown to occur [9] between *o*-phenanthroline and the histidine imidazole pointing toward the Q_B binding pocket in *Rhodopseudomonas viridis* reaction centers. Similar hydrogen bonding can also potentially occur in the Q_A binding pocket with the mirror-image histidine imidazole pointing toward Q_A. Such behavior is likely to be observed in PS II, as well, based on the strong structural homology between the iron-quinone complex in bacterial reaction centers and PS II [9–12,24,25]. Stabilization of Q_A photoreduction by the addition of PQ-9 during Triton incubation is also consistent with reversible quinone binding. While one might argue that such stabilization could occur through binding of the quinone to another site (e.g., Q_B pocket), there is no evidence in Fig. 3 of any rapid electron transfer from Q_A^- to a quinone in the Q_B site. Previous evidence also suggests that this site no longer binds PQ-9 [23].

That the rate of inactivation of Q_A photoreduction is the same (Fig. 1) for reaction centers incubated in the absence of detergent as for those incubated at 250 μM PQ-9 in the presence of 1% TX-100 (2.5 PQ-9 per micelle), suggests that the Q_A site is fully occupied in both cases. The activity loss would be due to some thermal denaturation, independent of the occupancy of the Q_A binding site. At 0.06 and 2 PQ-9 per micelle (6 μM reaction centers in 1% and 0.045% TX-100, respectively, without added PQ-9, Fig. 1) the remaining Q_A photoactivity is 46 and 74%, respectively, after 21 h at 20 °C, compared with the most active centers described above. These results (reproducibility ± 10% in comparing three different series of experiments) are consistent with an approximate dissociation constant for quinone binding to the Q_A site of about 0.05 PQ-9 per micelle. In other words, a micelle containing a reaction center has a 10-fold greater probability of having a PQ-9 present than one that does not. This dissociation constant is an upper limit as centers without quinone bound gradually lose the ability to rebind photoactive quinone to the Q_A site (see below). That UQ-9 does not protect Q_A photoreduction activity as well as PQ-9 may indicate a higher dissociation constant for this quinone or a lower probability of stabilization of charge separation.

The specificity of the Q_A binding pocket in PS II appears to be greater than that of bacterial reaction centers. Attempts at quinone exchange using the aminobenzoquinone, rhodoquinone, and naphthoquinones, vitamin K1 and menaquinone-1 were unsuccessful here, in contrast to *Rb. sphaeroides* centers which have been shown to bind both aminoanthraquinones and naphthoquinones [3].

The temperature is also a critical factor in the exchange process, with the thermal energy of the 20 °C incubation facilitating the escape of PQ-9 from the Q_A pocket. Loss of PQ-9 and consequent exchange with UQ-9 was barely detectable at 4 °C during a 3-day incubation period (not shown).

If PS II reaction centers are treated with 1% Triton X-100 at 15–20 °C in the absence of added quinone and then supplemented with 250 μM PQ-9 at the end of a 24 hour incubation, then no restoration of quinone photoreduction occurs. This observation would suggest that the reaction center exists in at least two states following Q_A extraction. The first, formed following extraction of PQ, is a reactivatable state, RC_{act}, in which immediate quinone rebinding allows the center to regain photoactivity. Should too long a time elapse following extraction, then the center is converted to an inactivatable form, RC_{inact}, which can no longer show Q_A photoreduction. This is either because the quinone can no longer rebind or because, even if rebound, the center can no longer carry out stable charge separation.

$$RC_{act}PQ \leftrightarrow PQ + RC_{act} \rightarrow RC_{inact}$$

The ability to exchange quinone in the Q_A pocket appears to occur in PS II with considerably greater difficulty than in the case of bacterial reaction centers [1–3]. It is possible that the added constraints provided by the additional polypeptides (CP47 and CP43) may limit the conformational motion necessary to free the quinone. Also the stability of the reaction center is considerably lower than that of bacterial reaction centers [2,3], with an 80% irreversible activity loss here after 21 hours of incubation with 1% TX-100 at 20 °C in the absence of exogenous quinone (Fig. 1). It is possible that loss of the quinone and that of CP47 and CP43 are linked. This would be consistent with the observation of Nanba and Satoh [13] that extensive washing with Triton X-100 releases these two polypeptides from PS II-enriched preparations in spinach. Quinone photoreduction is lost in their D1-D2-cytochrome *b*-559 complex and as yet all attempts to restore activity have been unsuccessful. While it is likely that Q_A is bound primarily to the D2 polypeptide of the PS II reaction center [11,12], the presence of CP47 and CP43 may be required for high-affinity quinone binding to the Q_A site. The strong temperature dependence for quinone exchange suggests that there is a considerable energy of activation involved. That this process may require dissociation or unfolding of the CP47 and 43 subunits, exchange of quinone and reassociation of these is a mechanism which requires further elucidation and may provide clues as to how the reaction center assembles.

The experiments described here provide new

avenues for the investigation of quinone function in PS II. These include replacement with quinones having different redox properties for investigating the energetics of stabilization of the charge-separated state and using quinones carrying varied substituents for studying the stereospecificity of binding. Such experiments, which have been carried out successfully in photosynthetic bacteria [2,3], need, however, to be pursued with greater caution in PS II. At least fifty chlorophylls remain per center in PS II core preparations and it is possible to see light-driven electron transfer between chlorophyll and quinones, probably in CP47 and CP43, which do not involve the usual primary reactants. Tests to demonstrate a reaction center-associated photoreaction include: the observation of the appropriate ratio of semiquinone to strictly electrochromic absorbance changes and the similarity of their relaxation rates, and the observation of saturation of quinone photoreduction with increasing flash intensity.

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