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Characterization of an LM unit purified by affinity chromatography from *Rhodobacter sphaeroides* reaction centers and interactions with the H subunit

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Reaction centers from wild-type *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*) were separated into two components: the LM complex and H subunit. LM was isolated after brief treatment of reaction centers with SDS by affinity chromatography with cytochrome *c* as ligand. A stable H preparation was obtained after dissociation of reaction centers with lithium perchlorate. LM was depleted of the transition metal, Mn, which interacts with Q_A and Q_B in native reaction centers. It retained only 30% of primary photochemistry which could be restored to 50–80% by addition of Q_6, Q_{10} or other quinones. A stable semiquinone radical Q_A^- could be flash-induced in LM. Its absorption properties are similar to those of Q_A^- in native reaction centers. The quantum yield of photochemistry in an LM unit reconstituted with Q_6 is the same as in intact reaction center and in LM in the presence of H. This result was confirmed by the rapid electron-transfer rate between I^- and Q_A in LM + H ($\tau \approx 0.45$ ns). Ubiquinone in LM incubated with H becomes tightly bound at the Q_A site. Flash production of a Q^{2-} species was not detected in LM and LM + H. We conclude that the depletion of the reaction center both of the H subunit and of the metal does not necessarily lower the quantum yield of the primary reaction or greatly modify the rate of electron transfer from I^- to Q_A. These results contrast with observations of others that seemed to demonstrate that the metal is essential for high-rate electron transfer between I^- and Q_A (Debus, R.J., Feher, G. and Okamura, M.Y. (1986) Biochemistry 25, 2276–2287). In our experiments, secondary electron transfer to Q_B was not restored in LM + H, unlike in reconstitution experiments reported with R26 *Rb. sphaeroides* reaction centers (Debus, R.J., Feher, G. and Okamura, M.Y. (1985) Biochemistry 24, 2488–2500). Apparently, interactions between H and LM were too weak for restoring Q_B activity.

Introduction

In the last few years much progress has been made in the study of the properties of photochemical RCs from purple non sulfur bacteria. The results of X-ray crystallographic studies of *Rhodopseudomonas viridis* RC [1] have informed us in a detailed way about the location and protein binding sites of the chromophores involved in the active electron transport chain. This recent structural information agrees well with the conclusions of earlier kinetic studies. Light induces electron transfer from the primary donor, the bacteriochlorophyll dimer P-870, to an acceptor chain consisting of bacteriopheophytin I, primary

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Abbreviations: RC, reaction center; LDAO, lauryldimethylamine *N*-oxide; TMPD, *N,N,N′,N′*-tetramethyl-1,4-phenylenediamine; BPh, bacteriopheophytin; Cyt, cytochrome.

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quinone Q_A and secondary quinone Q_B [2].

The reaction center is a complex of three subunits: L, M and H. L and M bind all the pigments and a metal-ion cofactor [3,4]. Debus et al. [4] have shown that the presence of the H subunit is required for Q_B acting as a secondary electron acceptor.

In the present paper we describe the results of attempts to reconstitute an LM preparation devoid of metal with a H preparation. Both were derived from *Rb. sphaeroides* wild-type reaction centers. The interaction between the two partners resulted in total recovery of photoreduction of Q_A, but not of electron transfer to the secondary quinone. The results disagree with those of Debus et al. [5] concerning the role of the metal co-factor.

Methods

To isolate LM, purified reaction centers from *Rb. sphaeroides* Y, prepared as described [6], were incubated for 5 min at 20°C (3 ml, $A_{800\ nm} = 10$) in 50 mM Tris-HCl/1 mM EDTA (pH 8.0), containing 1% SDS and 0.03% lauryldimethylamine *N*-oxide (Onyx Chemical). After dilution with 4 vols. of 10 mM NaCl/0.1% LDAO, the sample was rapidly chromatographed at 15°C on a small column (approx. 4 ml bed volume) of cross-linked Cyt *c* Sepharose gel, prepared as described by Gaudinot and Gautheron [7] and equilibrated with 10 mM Tris-HCl/10 mM NaCl/0.1% LDAO (pH 8.0). Unadsorbed protein and SDS were eluted in two fractions by washing at 40 $\text{ml} \cdot \text{h}^{-1}$ with 10 bed volumes of equilibration buffer. The column was then washed with 10 mM Tris-HCl/10 mM NaCl (pH 8.0) buffer, containing 0.1% Deriphat 160 (disodium *N*-lauryliminodipropionate; from General Mills). Elution of adsorbed LM units at 20 $\text{ml} \cdot \text{h}^{-1}$ was done by raising the NaCl concentration of the 0.1% Deriphat buffer to 0.5 M. The purity of the fractions was checked by measuring the ratio of absorbance at 280 nm vs. 800 nm; this ratio varied within the eluted peak between 1.05 and 1.15; fractions with higher ratios were discarded. Pooled fractions were dialyzed overnight at 5°C against 10 mM Tris-HCl/10 mM NaCl/0.1% Deriphat 160 (pH 8.0), and were kept at 5°C. The LM concentration of the fractions was determined from $A_{800\,\text{nm}}$, adopting $\varepsilon_{800\,\text{nm}} = 288\ \text{mM}^{-1}$ [8]. We also tried other detergents: elution and dialysis of LM with Brij 35 or LDAO, instead of Deriphat led to its rapid degradation. If sodium cholate was used instead of Deriphat, LM remained bound on the column. We did not store our LM preparations in frozen form: unlike intact RCs, a freeze and thaw treatment led to pigment degradation.

To isolate H, purified reaction centers were incubated for 20 min at 20°C in 30 mM Tris HCl (pH 8.0) containing 1 M $LiClO_4$ and 0.06% LDAO ($7 < A_{800\,\text{nm}} < 15$). Precipitated H was recovered as a pellet after a low-speed centrifugation (4 min, 4000 × *g*) and washed with 10 mM Tris-HCl/10 mM NaCl (pH 8.0). H was redissolved by a 30 min incubation at 30°C in 10 mM Tris-HCl/10 mM NaCl/1% Deriphat 160 (final volume equals 5 times that of the initial reaction center sample), then dialyzed for 24 h at 5°C against a suitable buffer (see Results), usually 10 mM Tris-HCl/10 mM NaCl/0.1% Deriphat 160 (pH 8.0). H samples were kept at 5°C; their protein content was measured by the method of Peterson [9], taking into account by appropriate blanks the interference by Deriphat 160. Molar concentrations were estimated by using an approx. M_r of 30000.

The purity of the LM and H samples was checked by discontinuous buffer SDS-polyacrylamide gel electrophoresis [10]. Samples containing Deriphat 160 or lauryl dimethylamine *N*-oxide were precipitated at 0°C by acetone/methanol 7/2 (v/v), dissolved in 2% SDS/40 mM dithiothreitol/62 mM Tris-HCl (pH 6.85), and heated for 30 min at 65°C. Q_B-less RCs were prepared by the method of Okamura et al. [11].

Reconstitution of LM with quinone was performed by addition of a concentrated ethanolic solution of Q_6 in a molar excess of 20–40 followed by dialysis against an appropriate buffer (see below). Alternatively, Q_{10} was added as a sonicated solution in 10% cholate or 10% LDAO. To enable reassociation between H and LM (reconstituted with quinone) we mixed the two preparations at various molar ratios (in most experiments we used a slight excess of H, 1.6–2 mol H per mol LM) and dialyzed the mixture at 5°C for 24 h against 10 mM Tris-HCl/10 mM NaCl (pH 8.0) containing a low amount of detergent: 0.04% LDAO, or 0.05% Deriphat 160, or 0.025% sodium cholate. The final concentration of LM in the (LM + H) mixture was between 3 and 6 μM. Controls of LM without H were dialyzed in the same way.

In order to determine if any stable association between LM and H took place, agarose gel electrophoresis under non-denaturing conditions was performed on the (LM + H) samples, using as controls reaction centers, LM, and H preparations. We used for the gels, the samples, and the reservoirs the same buffer as described by Debus et al. [4]: 50 mM Tris-HCl/0.1% LDAO/0.025% sodium cholate/1 mM EDTA (pH 8.0). 2 mm thick gels containing 1% agarose (LKB) were cast on Gelbond sheets (LKB, 85 × 90 mm); 5 μl samples were applied and electrophoresed at 10°C and 10 $V \cdot cm^{-1}$ in a LKB multiphor unit, then fixed, stained with Coomassie blue and air dried.

Metal analysis was performed with an atomic absorption spectrophotometer (Perkin-Elmer 2380); we used standard solutions containing the same detergent as the samples.

Flash induced absorbance changes in the millisecond range were measured with a single beam spectrometer. The continuous analytical beam was provided by a 100 W halogen lamp and a Bausch & Lomb High Intensity monochromator with 5 nm band pass. The actinic light was provided by an Applied Photophysics flash apparatus operating at variable power 15 J maximally, and passed through red filters Corning 2-64 or Wratten 89 B. Blue Corning filters (4-76 or 4-96) were put in front of the PM to block scattered excited light. When absorbance changes were measured in the infrared, the position of the red and blue filters was reversed. Maximum intensity flashes were 70 or 80% saturating. The amplified signal changes were sent to a digital DL905 transient recorder. The data were read out to a chart recorder or minicomputer for analysis. The method of Marquardt [12] was used for the deconvolution of exponential kinetics. Measurements of quantum yield (see Fig. 5) were performed on a single beam spectrometer built by Dr. B.R. Velthuys.

Picosecond absorbance measurements were performed with the experimental set-up described elsewhere [13].

Results

Properties of LM, H and LM + H preparations

The properties of the LM preparation described in the present paper were improved relative to a preparation that we described earlier [6]. The amplitude of flash-induced absorbance changes of LM at 20°C was only 30% of that of control RCs but was increased to 50–80%, upon addition of various quinones especially Q_6 or Q_{10}. We point out that the photochemical activity of LM becomes smaller at pH above 9.

We have compared the amount of the transition metal present in the RC preparation with that present in three different LM fractions, and found: 0.9 Mn and 0.47 Fe per RC; 0.01 Mn and 0.32 ± 0.04 Fe per LM. Therefore, manganese, which is the major metal cofactor in this RC [6,14] was completely removed in LM; contaminating iron, on the other hand, was only partially removed.

We tried to reconstitute this LM preparation with H. We did not succeed in isolated H from the Cyt *c* affinity column because it eluted in the same fractions as unbound (denaturated) RCs and SDS. We isolated H as a precipitate after a short incubation of RCs at 20°C in 1 M $LiClO_4$/0.06% LDAO (pH 8.0); we checked its stability by letting it rest as a precipitate in $LiClO_4$ 1 M at 20°C for several hours, or as a solution in 1% Deriphat for 24 h at 20°C; SDS-polyacrylamide gel electrophoresis runs on these samples identified them as consisting of undegraded H polypeptide.

In most reconstitution experiments we dialyzed H first against 10 mM Tris-HCl buffer (pH 8), 0.1% Deriphat and then combined it with LM in a molar ratio LM : H = 1 : 2. This mixture was immediately dialyzed against Tris buffer containing different detergents, most frequently 0.04% LDAO.

Flash-induced activity of LM and LM + H preparations was measured at pH 8 in the presence of an excess of Q_6. We monitored absorbance at 430 and 450 nm where both P^+ and Q^- absorbances contribute to the light-induced changes.

Table I presents the results of four representative reconstitution experiments. Only the nature of the detergent was varied. It can be seen that H modified LM's properties, as follows.

(a) The photoinduced P^+ signal was reproducibly enhanced and reached the amplitude observed

TABLE I

PHOTOCHEMICAL ACTIVITY, MEAN TIME τ OF THE FAST PHASE IN $P^+Q_A^-$ RECOMBINATION AND ITS RELATIVE PROPORTION α AND Q_A^- LIFETIME OF LM, AND LM+H IN THE PRESENCE OF AN EXCESS OF Q_6 AND OF VARIOUS DETERGENTS

The flash intensity was 70% saturating. To cholate buffer 0.2 M NaCl was added. Q_A^- decay was measured in the presence of 0.5 mM $DADH_2$. The samples were prepared as described in Materials and Methods.

Sample	Detergent	$\frac{\Delta A_{430\,nm}}{A_{800\,nm}}$	P^+ fast decay		Q_A^- decay τ (s)
			τ (s)	α	
LM	0.04% LDAO	0.028	0.22	0.66	3.6
LM+H	0.04% LDAO	0.054	0.22	1	11.5
LM	0.04% LDAO	0.041	0.21	0.60	2.1
LM+H	0.04% LDAO	0.06	0.21	0.90	5.6
LM	0.05% Deriphat 160	0.047	0.170	0.68	1.1
LM+H	0.05% Deriphat 160	0.063±0.005	0.170	0.80-83	2.8
LM	0.025% cholate	0.042±0.02	0.165	0.70	1.3
LM+H	0.025% cholate	0.063	0.130	0.70	3.1

with native RC (see Table I and Fig. 1).

(b) The decay of P^+ in LM was about three times slower than in intact Q_B-less RCs and could be resolved into two exponential phases, the major one being the fastest. In presence of H, the fast phase of P^+ decay ($170\ ms < \tau < 200\ ms$) became predominant (see Fig. 1 and Table I).

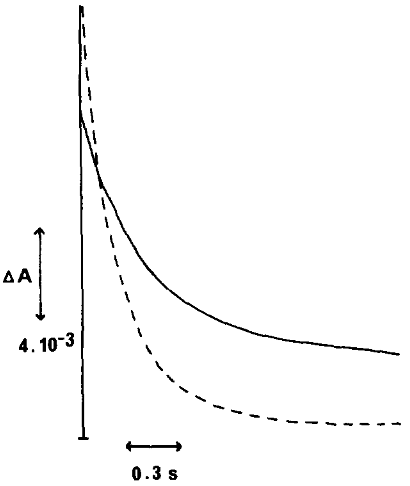

Fig. 1. Kinetics of charge recombination after a 70% saturating flash in 2.5 μM LM (———) and 1.87 μM LM+H (– – –). Both samples were dialyzed 24 h at 4°C with 0.5 mM Q_6 against 10 mM Tris-HCl (pH 8)/0.04% LDAO. Measuring wavelength, 450 nm. Temperature, 20°C. Optical path length, 1 cm. Deconvolution of the kinetics: for LM, $\tau = 0.51$ s with $\tau_1 = 0.25$ s (60%) and $\tau_2 = 2.3$ s (40%); for LM+H, $\tau = 0.21$ s.

For several reconstitution experiments in which LM and LM + H in the presence of a large excess of Q_6 were dialyzed against 0.04% LDAO in Tris buffer, the deconvolution of the decay curves in two exponentials yielded the following values for the half-times τ and weights w of the phases (N is the number of preparations):

LM $\tau_1 = 0.21 \pm 0.02 s$, $w_1 = 62 \pm 8\%$
($N = 7$) $\tau_2 = 1.5 \pm 0.7 s$, $w_2 = 39 \pm 8\%$
LM+H $\tau_1 = 0.2 \pm 0.03 s$, $w_1 = 90 \pm 14\%$
($N = 5$) $\tau_2 = 1.2 \pm 0.8 s$, $w_2 = 10 \pm 0\%$

Therefore, such a rapid relaxation of P^+ in LM + H preparations ($\tau = 0.2$ s) indicates that no Q_B reconstitution occurred in the presence of a H subunit. Indeed in a native or fully reconstituted RC the back electron flow from Q_B^- to P^+ proceeds with $1\ s < \tau < 1.3\ s$ [4].

We suppose that the fast phase represents the back-reaction of P^+ with Q_A^-, whereas the slow phase is due to centers in which Q_A^- has initially been oxidized by exogenous Q_6. This last reaction would retard the return of the electron to P^+. This assumption is supported by the fact that (1) the proportion of the slow phase of P^+ decay was less important when the amount of external Q_6 was small and (2) markedly increases when LM is illuminated with multiple close-spaced flashes.

An increasing proportion of slow phase with the concentration of Q_{10} was also observed on LM-containing iron [4].

(c) In the presence of efficient electron donors to P^+ like $DADH_2$ or TMPD, a PQ_A^- state could be trapped in LM and was stable during several seconds (see Fig. 2 continuous trace, and Table I).

We noticed that the Q_A^- lifetime in LM depends on both concentration and on reduction state of the external electron acceptors including the pool of Q_6. In the presence of a low amount of ubiquinone (LM: $Q_6 = 1:5$) Q_A^- reoxidation was slowed down by a factor of about 10 relative to that in the presence of an excess of Q_6 (LM : Q_6 = 1 : 20–30).

The shape of the characteristic absorption band of Q_A^- (not shown) in the blue spectral region as well as the infrared electrochromic bandshift of BPh induced by the Q_A^- negative charge (see Fig. 3) were very similar to those already observed with R26 RCs by Verméglio and Clayton [15]. This indicates that ubiquinone binds in LM to the Q_A site in a proper position with respect to the BPh molecules. In LM + H mixtures the rate of oxidation of Q_A^- by $TMPD^+$ or by diaminodurol was markedly decreased (2–5-fold) (see Fig. 2 (dashed trace)).

Repeated flashing (20–30 flashes, 200 ms apart) of LM and LM + H in the presence of a diaminodurol-ascorbate mixture and small or large excess of Q_6 did not lead to a diminution of Q^- absorbance at 450 nm. Moreover LM illuminated with continuous light in the presence of reduced Cyt *c* and an excess of Q_6 displayed at low temperature a large EPR signal characteristic of a semiquinone free radical with a *g*-value of about 2.0045 (A.W. Rutherford, unpublished results).

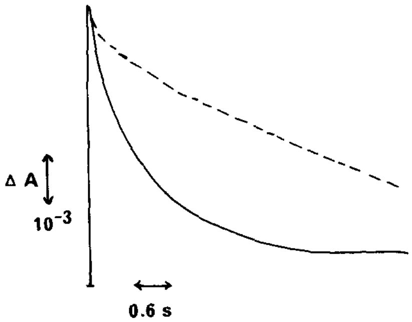

Fig. 2. Kinetics of the decay of Q_A^- after a flash in the presence of 500 μM $DADH_2$ in LM (———) and in LM + H (- - -). For a better comparison the amplitude of the absorbance change for LM was normalized to that of LM + H. Other conditions as for Fig. 1.

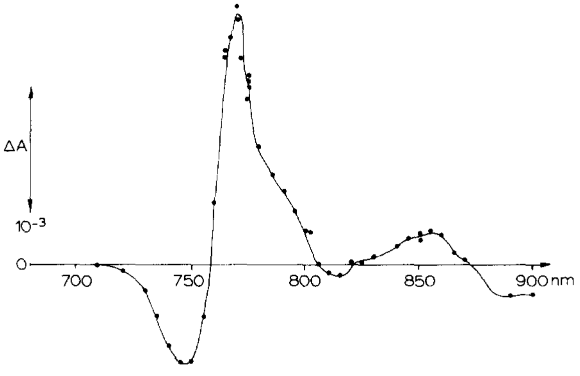

Fig. 3. Electrochromic absorbance changes induced by flash-produced Q_A^- in LM (2.46 μM) reconstituted with Q_6. 10 mM Tris-HCl (pH 8)/0.03% LDAO/500 μM $DADH_2$/500 μM sodium ascorbate; optical path length, 0.2 cm. The absorbance was measured 80 ms after the flash.

Therefore, Q_A^- was not converted into QH_2 in our LM or LM + H samples, in contrast to experiments of Debus et al. [5] on iron-depleted R26 RCs.

The fact that no binary oscillations of Q^- were observed in LM + H preparations is another indication of Q_B absence.

As a control to check if the action of H upon LM was specific, we mimicked reconstitution experiments using bovine serum albumin instead of H; this treatment did not change the LM properties at all.

Quantum yield in LM and reaction centers

In order to know the origin of the smaller flash induced absorbance changes in LM with respect to those in RC and LM + H mixture, we tried to determine the quantum yield of these changes in all three preparations. Besides, metal depletion of RCs was reported to lead to a considerable (approx. 40%) diminution of the quantum yield of photochemistry [5].

A drop in quantum yield can be detected by measuring flash-yields as a function of flash intensity [16]. We tested an LM which displayed 70% of the flash induced signal of Q_A^- (in the presence of diaminodurol/ascorbate) in control RCs and

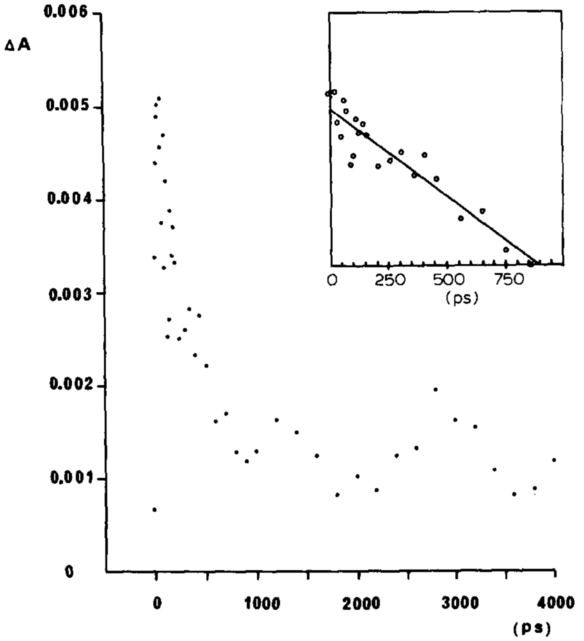

Fig. 4. Kinetics of absorbance changes at 680 nm of LM+H reconstituted with Q_6. The actinic flash was a 35 ps laser flash at 532 nm with incident excitation energy of about 2.5 mJ/cm^2. Optical path length, 0.2 cm; interval between actinic pulses, 1.5 s; 3 μM LM+H in 10 mM Tris-HCl/0.04% LDAO/0.4 mM Q_6/1.3 mM sodium ascorbate. Inset: semilogarithmic plot of the decay kinetics. The solid line is a computer fit of the data to a single exponential.

another one which displayed only 30%. A measurement of this type comparing Q reduction at two flash intensities with RCs and LM is illustrated in Fig. 5 and analyzed in Table II. The data show that the quantum yield of functional LM centers is equal within 10% to that of the RCs. The same results (not shown) were obtained with LM + H, LM and RCs measuring single-flash P oxidation instead of Q reduction. Despite the similar quantum efficiencies, the multiflash patterns of Fig. 5 differ for LM and RCs. We ascribe this to inconstancy of the number of functional LM centers. In contrast to RCs, LM centers need the addition of a large excess of Q in order to become functional, i.e., they bind Q relatively weakly. In case of an insufficient excess of Q only a small fraction of LM is functional (approx. 30% in experience of Fig. 5). Obviously, reduction of Q may change its affinity. The multiflash results of Fig. 5 indicate that when bound Q is converted into Q^- its unbinding is slowed down, and as a consequence the number of Q^- containing LM centers increases between flashes.

Rate of electron transfer from I^- to Q_A in reaction center and (LM + H)

It seemed worthwhile to measure the rate of electron transfer between I^- and Q_A in our metal-depleted preparations. The transfer time in LM + H was $\tau = 0.45$ ns (Fig. 4): a value slightly longer than that we found for control RCs (approx. 0.17 ns); this last value is in the range of values reported earlier [17–19]. The spectrum of the P^F state ($P^+I^- - PI$) measured at 200 ps after

TABLE II

COMPARISON OF THE MEASURED FLASH YIELDS WITH THEORETICAL FLASH YIELD

The measure dvalues were derived from the data of Fig. 5. The estimated precision (for LM) refers to noise on the traces. The theoretical values assume quantum efficiencies of less than 100% in RCs, and are calculated using the equations:

flash yield = $\sum_{n=1}^{\infty} P(n)(1-m^n)$; quantum efficiency = $1-m$

$P(n) = \frac{\bar{n}^n}{n!}e^{-\bar{n}}$ (Poisson's formula)

	Quantum efficiency	A = yield of strong flash	B = yield of weak flash	B/A
RC	100%	0.84	0.142	0.169
LM	?	?	?	0.165 (±0.01)
Calculated values	90%	0.81	0.129	0.159
	80%	0.77	0.115	0.150
	70%	0.72	0.102	0.141
	60%	0.67	0.088	0.132

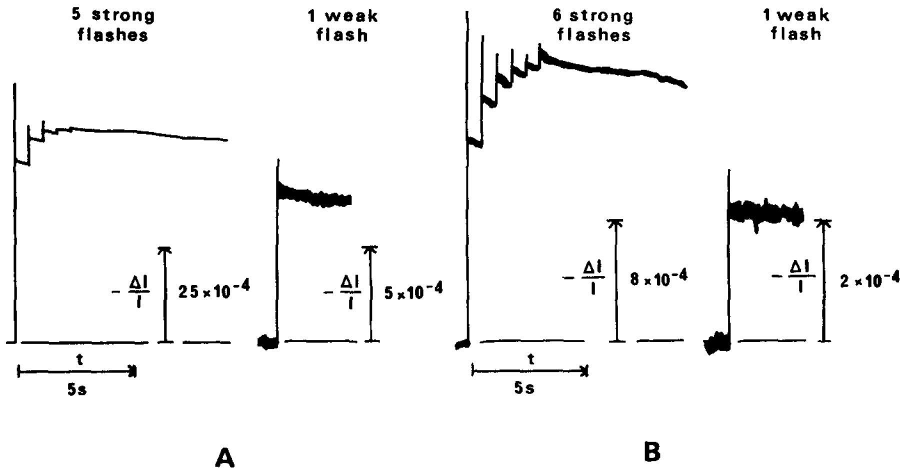

Fig. 5. A series of strong flashes and a single weak flash inducing Q_A^- formation in RC and LM preparations. Measuring wavelength, 450 nm; optical pathlength, 0.2 cm. (A) 2.8 μM RCs in 10 mM Tris-HCl (pH 8)/0.03% LDAO/terbutryn 20 μM/500 μM $DADH_2$ sodium ascorbate 10 mM. (B) 2.8 μM LM dialyzed against 10 mM Tris/0.03% LDAO buffer containing 15 μM UQ_6. Other conditions as in (A).

the actinic flash in LM (containing only residual Q_A) was very similar to that obtained by Rockley et al. [17] with intact RCs, especially the 680 nm band where the contribution of the I^- species is predominant. Therefore the transient acceptor I seemed rather unperturbed in our preparations.

Effect of herbicides

Herbicides as *ortho*-phenanthroline (5 mM), ametryn and terbutryn (approx. 200 μM) added to various LM preparations in presence of an excess of Q resulted mainly in (1) the diminution of the slow phase proportion of the flash-induced P^+ recovery (2) in case of *ortho*-phenanthroline, a decrease by 30–60% of P^+ signal. LM + H preparations are less affected by herbicides; ortho-phenanthroline induces only a small diminution of P^+ amplitude, i.e., 10–20%.

Strength of association between LM and H

Agarose electrophoresis under non denaturating conditions has been used in the case of R26 RCs to demonstrate a true reassociation of LM (still containing the metal) and H [4]. In slab gel electrophoresis performed with the same detergents and buffer as in [4], wild-type RC and LM migrated as single bands with R_f values of 0.85 and 0.78, respectively. For H we observed a band migrating slower ($R_f = 0.40$) than LM and RC, as in the case of R26 *Rb. sphaeroides* [4]; part of the sample often did not move, however, indicating some aggregation. Patterns observed for LM + H samples were merely composite of LM and H, and a band corresponding to native RCs was not present.

Discussion

In the absence of H, a fraction of LM did not bind ubiquinone at the primary acceptor site. However, we ascertained that in the photochemically active LM fraction, the quantum yield of the charge separation was not reduced relative to control RCs. Presence of H resulted in 100% binding of Q_6. In addition the fast phase of the back reaction became predominant and Q_A^- was stabilized towards oxidation by external reagents. These effects must originate from a stronger binding of UQ to the Q_A site in the presence of H. However, the rate of the fast back reaction is still slower than in native RC. This kinetic difference is probably due to a loose interaction of LM with H; indeed when tight reassociation takes place between the two proteins, this kinetic difference disappears [4]. We have shown that in the LM + H sample, the electron-transfer rate from I^- to Q_A is slower than in native RC, being about 450 ps. Likewise, it was observed (Sebban and Lindqvist, unpublished results) that the recombination time of P^+I^- (P^F) radical pair in our LM preparations is longer, being 20 ns instead of 10 ns as determined in intact RC [19].

Presumably, these small modifications in the electron-transfer rates between early acceptors in LM samples could reflect local conformational changes of the protein resulting from the initial detergent treatment during LM preparation.

In our hands H was unable to restore the secondary activity. Apart from trivial strain difference various alternative suggestions can be made to explain this: (a) the Q_B site, located on the LM unit, may have denatured during the initial SDS treatment; (b) the absence of metal, (c) a normal conformation of the Q_B site (of which H is probably a part) may require stronger binding of H than the binding we obtained. Indeed, electrophoresis of the LM + H mixture showed that under an electric field and in 0.1% LDAO no LMH complex is present; and (d) the aggregation state of LM or H or both may prevent full reconstitution of the two proteins.

We have observed that herbicides induced the diminution of the slow phase of the back reaction in LM. We interpret this effect as a nonspecific hydrophobic interaction of these reagents with the protein conferring to LM unit a more compact structure in which Q_A^- is less exposed to the oxidation by external UQ. The partial loss of photochemistry induced by *ortho*-phenanthroline in LM even in the presence of an excess of Q_6 is likely due to the displacement of Q_A by the inhibitor and relevant for a looser binding of Q_A than in intact RC. Removal of Q_A by *ortho*-phenanthroline in Q_B less R26 RCs has been mentioned by C.A. Wraight [20]. The herbicides have weaker effect on LM + H preparations; ortho-phenanthroline is less effective in replacing Q_A.

Atomic absorption and EPR spectroscopy have shown that the present LM preparation was devoid of Mn, the specific transition metal bound between the two quinones in RCs of this particular strain of *Rb. sphaeroides* [14]. It is well known that in RCs treated with SDS or chaotropic agents, the iron [16,21,22] or manganese [6] is lost.

Moreover, in the same LM the light-induced semiquinone was stable against its further reduction to quinol as shown by EPR and multiflash absorption experiments. Therefore, the absence of metal in LM and LM + H preparations does not seem to affect the normal electron transfer between early acceptors and Q_A.

Our results agree with the observations of Blankenship and Parson [16]: the quantum yield of photochemistry as well as the rate of electron transfer between I^- and Q_A were unperturbed in $LiClO_4$ or SDS treated R26 RCs. In such treated material Fe was lost and LM was probably separated from the H subunit.

Our results are in disagreement with those recently reported on R26 RCs depleted of Fe [5]. In these RCs the quantum yield of charge separation was reduced by 40%, consistent with a very slow electron transfer between I^- and Q_A (about 5–10 ns). Moreover, Q_A^- was unstable and could be reduced to Q_A^{2-}. These changes disappeared when the transition metal was put back in place.

From these experiments we assume that the quinone binding sites may undergo different conformational changes depending on the way the metal was removed. Apparently in our case, the release of Mn does not induce a specific alteration, whereas in the experiments of Debus et al. [5] the depletion of metal greatly modifies the photochemical properties of R26 RCs.

The attempts described in the present work to reconstitute an LM unit depleted of metal with the H subunit have shown that some specific interactions could be reestablished between them, and that their effects are restricted to the Q_A binding site. This was unexpected, as according to X-ray crystallographic data on *Rps. viridis* RCs the Q_A site would be shielded from the H subunit [1].

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