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Q_A-depletion and reconstitution of a reaction center preparation from the photosynthetic bacterium *Rhodopseudomonas viridis*

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Isolated RC's from *Rps. viridis* were reversibly depleted of both UQ (Q_B) and MK (Q_A). The photochemical activity was eliminated and restored upon the depletion and reconstitution of the Q_A. Four methods for depleting Q_A from the RC's were studied and their results were compared. The best way allowed us for the first time to prepare Q_A-depleted RC's from *Rps. viridis* that retained only 10% of primary photochemical activity, which could be restored up to 85% by addition of vitamin K_1.

Menaquinone; Ubiquinone; Reaction center; Depletion; Reconstitution; *Rps. viridis*

1. INTRODUCTION

RC's from the purple non-sulfur photosynthetic bacterium, *Rhodopseudomonas viridis* differ in their composition from those of *Rb. sphaeroides* in that the latter contain Bchl-a, Bph-a and two UQ, while the former contain Bchl-b, Bph-b, both UQ (Q_B) and MK (Q_A) and also an additional bound cytochrome. Except for these differences, their structures are very similar to each other.

In 1975, Okamura et al. [1] first found an effective way to reversibly remove both Q_A and Q_B from RC's of *Rb. sphaeroides* R-26. Since then a lot of work has been done to remove the native quinone (Q_A) from these RC's and replacing it with other quinones, with different structures and/or redox potentials [2-18]. These studies have yielded fruitful results on the mechanism of quinone oxidation/reduction in reaction centers of *Rb. sphaeroides* R-26. On the other hand, only little related work, confined to the extraction of quinones, has been done for reaction centers of *Rps. viridis* [19-20]. A few years ago, Shopes and Wraight [21] reported that the native Q_A activity was uniquely provided by MK and UQ was not involved in RC's from *Rps. viridis*. Although they also reported removal of Q_B from these RC's, until now no one has suceeded to remove Q_A from these RC's. We have studied 4 methods to deplete the Q_A from RC's of *Rps. viridis*, the best one allowing us for the first time to prepare Q_A-depleted RC's from *Rps. viridis*. A preliminary report of this work was presented at the VIIIth International Congress on Photosynthesis [22].

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Abbreviations: Q_A and Q_B, primary and secondary electron acceptors; Vitamin K_1, 2-methyl-3-phytyl-1.4-naphthoquinone; UQ, ubiquinone; MK, menaquinone; Bchl-a and Bchl-b, bacteriochlorophyll a and bacteriochlorophyll b; Bph-a and Bph-b, bacteriopheophytin a and bacteriopheophytin b; LDAO, lauryldimethylamine *N*-oxide; PEG, polyethylene glycol

2. MATERIALS AND METHODS

Reaction centers from *Rps. viridis* were prepared essentially as described by Den Blanken and Hoff [23], except that we started from $OD_{1015nm}^{1cm} = 50$ instead of $OD_{1015nm}^{1cm} = 25$, and used 6% LDAO instead of 5% LDAO for incubation.

Q_B-depleted RC's from *Rps. viridis* were prepared following the method of Okamura et al. [11]. LDAO in the Q_B-depleted RC's was removed by dialyzing against TTX buffer (10 mM Tris HCl, 0.1% Triton X-100 and 1 mM EDTA, pH = 8) or by using a DE-52 column, which was washed and eluted with TTX buffer.

Q_A-depleted RC's from *Rps. viridis* were prepared by the following four methods:

Method 1. Q_B-depleted RC's in TL buffer (10 mM Tris HCl, 0.1% LDAO, 1 mM EDTA pH = 8) (or in TTX buffer) were absorbed to a DE-52 column, which was pretreated with 1 M NaCl or a solution of 1 mg/ml BSA, the column was then washed at 27-30°C with 4-5% LDAO (or 5% Triton X-100) in the presence of *o*-phenanthroline.

Method 2. The Q_A in the Q_B-depleted RC's was prereduced by adding $Na_2S_2O_4$, followed by dialyzing to remove the excess of $Na_2S_2O_4$. 100 ml Q_B-depleted, Q_A-reduced RC's in TTX, $OD_{830}^{1cm} = 0.5$ was incubated at room temperature for 2 h with the same volume of 9% Triton X-100, 20-30 mM *o*-phenanthroline, 20 mM Tris HCl and 2 mM dithiothreitol, pH = 8. After incubation the mixture was diluted to lower the concentration of Triton X-100 to less than 2% and then the RC's were precipitated onto a column of Celete 545 by adding 23% ammonium sulfate. The column was washed with 10 mM Tris HCl, 1.5% Triton X-100 in the presence of 23% ammonium sulfate for 0.5 h and eluted with TTX buffer. The procedure was repeated if necessary. The raw Q_A-depleted RC's were purified on a column of DE-52.

Method 3. The incubation procedure was the same as described in method 2. After incubation the mixture was layered on top of a linear 0.1-1.0 M sucrose gradient in 10 mM Tris-HCl buffer, pH = 8, con-

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taining 1 mM EDTA and 0.1% Triton X-100 and centrifuged for 16 h at 175 000 × g. The brown band was collected and purified on a DE-52 column.

Method 4. 2 ml Q_B-depleted RC's in TTX, $OD_{830}^{1cm} = 35$ was incubated at 4°C for 24 h with 600 ml hexane/petro-ether (1/1-3, v/v) in the presence of 20 mM *o*-phenanthroline, 0.2 ml DMSO and 0.5 ml PEG 400, pH = 8. The organic solution was divided by 4 and added every 6 h. After extraction the water layer was put onto the DE-52 column for purification.

Reconstitution of Q_A-depleted RC's with vitamin K_1 was accomplished by the method as described by Marinetti et al. [24].

The content of quinone in RC's was determined chemically by its extraction and purification, followed by difference spectroscopy assay. For the extraction of quinone, the method of Gast et al. [25] was followed with some modifications: 0.3 ml of 0.1 M $FeCl_3$ was added to 1.5 ml RC's, $OD_{830}^{1cm} = 25$ and stirred for 1 or 2 min, then 15 ml of aceton/methanol (1/1, v/v) was added and stirred vigorously for 5 min. After that the mixture was extracted by 45 ml diethyl ether, repeating the diethyl ether extraction two times. The organic layers were combined and first washed two times with 15 ml methanol/water (9/1, v/v), then two times with water. (Ag_2O was added and stirred for 6 h, if the Q_A was prereduced.) Diethyl ether was removed by gassing with N_2, the residue was redissolved in 0.5 ml of ethanol and brought to TLC isolation, using a silica gel with luminescer (254 nm). The plate was first developed with 4/1 (v/v) of cloroform/cyclohexane and then with 3/2 (v/v) of cloroform/cyclohexane. The yellow bands corresponding to UQ and MK were removed separately and dissolved in ethanol, the gel was removed by centrifugation. The ethanol was evaporated by N_2 to a volume of 0.6 ml. The difference spectrum was taken by adding KBH_4, repeating the adding of KBH_4 until a maximum absorbance was observed. The quinone content was determined by comparing the height of the peak at 268 nm for UQ and at 246 nm for MK for the native and Q-depleted RC's. The photochemical activity was determined by measuring the bleaching at 960 nm. For the experiment, a combination of two BG18 and two CalflexC was used as filters for the actinic light; a filter of RG9 was used for the photomultiplier. The Q_A was reoxidized before measurement when Q_A had been prereduced for depletion.

3. RESULTS AND DISCUSSION

3.1. *Removal of ubiquinone (Q_B)*

The quinone content in our purified RC's was found to be close to 0.9 UQ + 1.0 MK/RC. The UQ was easily removed by the method described by Okamura et al. [1]. After treatment, the UQ content was found to be reduced by 96% as compared with that of native RC's (see Table I). Reaction centers treated as such are referred to as Q_B-depleted RC's, which still retain 1MK/RC and full primary photochemical activity as compared with that of native RC's (see Table I and Fig. 1). This result indicates that the primary electron acceptor (MK) is not affected by the removal of the secondary electron acceptor (UQ).

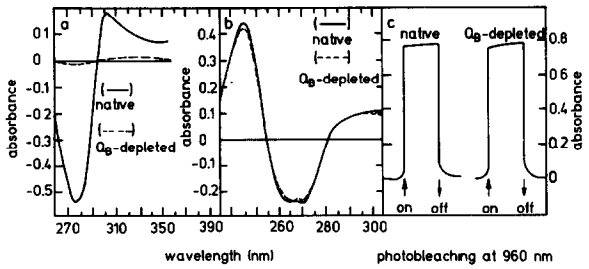

Fig. 1. (a) Difference absorption of UQ. (b) Difference absorption of MK. (c) Photobleaching at 960 nm in the Q_B-depleted RC's and native RC's from *Rps. viridis*.

3.2. *Removal of menaquinone (Q_A)*

We have studied four possible methods to remove MK (Q_A) from the Q_B-depleted RC's of *Rps. viridis*.

Method 1. We first tried to follow the method of Okamura et al. [1] for removing the Q_A from RC's of *Rb. sphaeroides* R-26 to remove the MK from Q_B-depleted RC's of *Rps. viridis*. We did not succeed because the Q_B-depleted RC's did not absorb to the DE-52 column when high concentration of LDAO was used. Therefore we tried some other detergents (such as Triton X-100, etc.) and other column materials. All these attempts were unsuccessful. We could not remove the Q_A (MK) from RC's of *Rps. viridis* by using the Okamura method, although it is very successful in RC's from *Rb. sphaeroides* R-26.

Methods 2 and 3. After Q_A was prereduced by $Na_2S_2O_4$, we incubated the Q_B-depleted RC's from *Rps. viridis* with high concentration of detergent (5% Triton X-100) in the presence of *o*-phenanthroline, followed by ether precipitation (in method 2) or sucrose gradient centrifugation (in method 3) to get rid of the MK, which was dissolved in high concentration of detergent. We found that RC's treated twice under the most severe conditions (5% Triton X-100, 15 mM *o*-phenanthroline, 15 mM Tris-HCl and 1 mM dithiothreitol, pH = 8, $OD_{830}^{1cm} = 0.5$, 3.5 h, 30°C) were depleted of their Q_A (MK), containing only about 15% of MK and retaining about the same percentage of the primary photochemical activity (see Table I), as compared with that of the native RC's. We also used LDAO for incubation, but the result was not as good as that of using Triton X-100 (data not shown). We believe that this is because MK is better soluble in Triton X-100 than that in LDAO. We emphasize that the RC's did

Table I

Comparing Q_A-reconstituted RC's prepared with different methods (see text)

RC's	UQ (Q_B)	MK (Q_A)	Activity (%)	Recovery (%) (+ vitamin K_1)
native RC's	0.9UQ/RC	1.0MK/RC	100	–
Q_B-depleted	0.04UQ/RC	1.0MK/RC	100	–
Q_A-depleted (2)	0UQ/RC	0.15MK/RC	15	80
Q_A-depleted (3)	0UQ/RC	0.15MK/RC	16	80
Q_A-depleted (4)	0UQ/RC	0.10MK/RC	10	85

not absorb to the DE-52 column in the presence of high concentration of detergent (>1.5%). The ammonium sulfate precipitation or sucrose gradient centrifugation was essentially necessary to remove the MK, which had been dissolved in high concentration of detergent. The reducing condition was used for the following two reasons, (i) because the reduced MK is much better soluble in Triton X-100; (ii) because the reduced MK is only loosely bound to the RC's. In Q_A-depleted RC's, the primary photochemistry was quantitatively reduced in correlation with the removal of MK, the spectrum remaining essentially the same as that of the native RC's. Results obtained from methods 2 and 3 were not very different (see Table I), indicating that the MK had already dissolved in the detergent after incubation.

Method 4. In method 4, we depleted Q_A by a mixture of hexane and petro-ether instead of by detergent. Q_B-depleted RC's were extracted by hexane/petro-ether (1/3, v/v), containing *o*-phenanthroline, DMSO and PEG 400 (a phase transfer catalyst) for 24 h at 4°C. The RC's were found to be depleted of the MK to 90% (Fig. 2a); they retained only about 10% of the primary photochemical activity (Fig. 2b). It was necessary to add the organic solution 4 times for maximal efficiency. The presence of DMSO and PEG 400 is very important, because they can form a speical 'inter-layer' between the buffer and the organic solvent. This protects the RC's from damage by the organic solvent. The PEG 400 also catalyzes the depletion process, because it can transfer the MK from RC's to the organic solvent. The spectrum of the hexane/petro-ether extracted RC's is essentially the same as that of the native RC's, indicating that the organic solvent does not damage the RC's. The reducing condition was also used here, but the result was not much different compared with that for the non-reducing condition. We believe this is because MK dissolves well in organic solvent.

Reconstitution of Q_A-depleted RC's with vitamin K_1 was necessary to determine whether changes observed upon depletion of Q_A resulted from its absence or from irreversible denaturation accompanying its removal. The reconstitution with vitamin K_1 has been done in Q_A-depleted RC's prepared with the different methods. The results (Table I and Fig. 2c) rule out the latter possibility, because readdition of vitamin K_1 results in 80–85% recovery of the primary photochemistry in the three preparations.

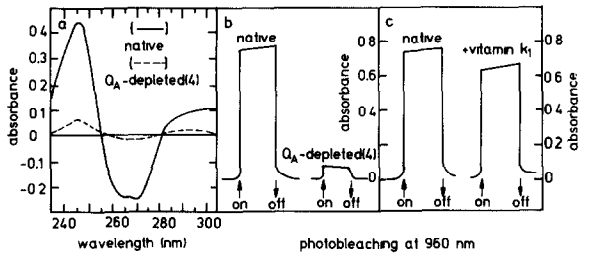

Fig. 2. (a) Difference absorption of MK in the Q_A-depleted RC's (from method 4) and in native RC's. (b) Bleaching at 960 nm of Q_A-depleted (from method 4) and native RC's. (c) Bleaching at 960 nm of vitamin K_1 reconstituted and native RC's from *Rps. viridis*.

Having found that the method of Okamura et al. [1] is not suitable for removing the Q_A from RC's of *Rps. viridis*, we developed three new methods to remove Q_A from these RC's. Method 3 is related to method 2, their results are very similar. Method 4 uses an organic solvent instead of detergent to remove Q_A; it is simpler and gives better results than methods 2 and 3 (see Table I). It allowed us for the first time to prepare Q_A-depleted RC's from *Rps. viridis*, which retained only about 10% of MK and about the same percentage of primary photochemistry. The latter could be restored up to 85% upon the readdtion of vitamin K_1. We conclude that there is no fundamental difference between RC's of *Rb. sphaeroides* and *Rps. viridis* with regard to Q_A extraction and reconstitution, so that it should now be possible to carry out on *Rps. viridis* similar studies on the thermodynamics of quinone electron transport as have been done on *Rb. sphaeroides*.

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