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Characterization of the quinones in purple sulfur bacterium *Thermochromatium tepidum*

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ABSTRACT

Quinone distributions in the thermophilic purple sulfur bacterium *Thermochromatium tepidum* have been investigated at different levels of the photosynthetic apparatus. Here we show that, on average, the intracytoplasmic membrane contains 18 ubiquinones (UQ) and 4 menaquinones (MQ) per reaction center (RC). About one-third of the quinones are retained in the light-harvesting–reaction center core complex (LH1–RC) with a similar ratio of UQ to MQ. The numbers of quinones essentially remains unchanged during crystallization of the LH1–RC. There are 1–2 UQ and 1 MQ associated with the RC-only complex in the purified solution sample. Our results suggest that a large proportion of the quinones are confined to the core complex and at least five UQs remain invisible in the current LH1–RC crystal structure.

1. Introduction

Quinones are membrane-soluble redox molecules found in nearly all living organisms [1]. They mainly exist in photosynthetic and respiratory electron transport chains and function as electron and proton carriers to produce transmembrane proton gradients. Four types of quinone have been reported in anoxygenic photosynthetic bacteria [2]: ubiquinone (UQ), menaquinone (MQ), rhodoquinone (RQ) and chlorobiumquinone (CQ). Both UQ and RQ belong to the benzoquinones that are considered to be evolutionarily younger than the naphthoquinones to which the MQ and CQ belong. UQs are present in all purple phototropic bacteria [3] and have been shown to play a crucial role in the photochemical reactions of bacterial photosynthesis [4–6]. MQs are found in some purple bacteria and are the major quinone compounds in green bacteria and heliobacteria. RQs are present in some purple non-sulfur bacteria, and CQs are only found in the green sulfur bacteria.

Although these quinones have been identified at the levels of cell or photosynthetic membrane, we have only limited and fragmentary knowledge on their distribution and composition in the specific apparatus or protein complexes to which they are associated. In the well studied *Rhodobacter* (*Rba.*) *sphaeroides* that contains solely UQ_{10} (subscript number specifies the number of isoprenoid units in the side chain), about 20–30 UQ_{10} per reaction center (RC) were estimated for the so-called quinone pool in chromatophores based on both biochemical and spectroscopic analyses [7–10]. The stoichiometric ratio decreased to about 10–15 in the light-harvesting–reaction center core complex (LH1–RC, per monomer) [9–11] and further to 1–2 in the RC [6,9]. Two UQ_{10} molecules were confirmed in the crystal structure of the RC [12]. A similar number of 25 quinones/RC was reported for chromatophores from *Phaeospirillum* (*Pha.*) *molischianum* that contains both UQ_9 and MQ_9 [3,13]. However, the diffusion rate of quinone/quinol exchange was found to be 30 times slower than that in

Abbreviations: CQ, chlorobiumquinone; DDM, *n*-dodecyl β-D-maltopyranoside; LDAO, lauryl dimethylamine *N*-oxide; LH, light-harvesting; MALDI TOF, matrix-assisted laser desorption/ionization time-of-flight; MQ, menaquinone; RC, reaction center; RQ, rhodoquinone; THAP, 2,4,6-trihydroxy acetophenone; TFA, trifluoroacetic acid; UQ, ubiquinone

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Rba. sphaeroides. In the purified RC solution of *Blastochloris* (*Blc.*, formerly *Rhodopseudomonas*) *viridis*, one UQ_9 and one MQ_9 per RC were measured, but nearly half of the UQ_9 were lost during crystallization with a final ratio of $UQ_9/MQ_9 = 0.6$ in the RC crystals [14]. The UQ_9 at Q_B site in the crystal structures is characterized by low occupancy and heterogeneity in position [15–18]. In *Allochromatium* (*Alc.*) *vinosum* that contains both UQ_8 and MQ_8 [3,19,20], a smaller number of 5–10 quinones/RC was estimated in the chromatophores [5]. In *Thermochromatium* (*Tch.*) *tepidum*, which is a close relative to the *Alc. vinosum*, a ratio of UQ/MQ = 4.3 was reported for whole cell extracts [21]. The MQs consisted of a mixture of isoprenoid chains of lengths 6, 7 and 8 units in a ratio of 11:4:85, respectively, and only MQ_8 was detected in the isolated RC solution [21]. In the recently published crystal structure of the *Tch. tepidum* LH1–RC core complex at 3.0 Å resolution, we have identified one UQ_8 and one MQ_8 [22]. However, the UQ_8 was not found in a RC-only structure at much higher resolution [23]. Since the LH1–RC core complex is a more natural form than the RC-only complex, the above results prompt us to ask how many quinones could be expected in the *Tch. tepidum* LH1–RC and whether there is a loss during crystallization of the LH1–RC. To answer these questions, we have conducted a quantitative study to investigate the distribution of the quinones in *Tch. tepidum* and to compare their compositions among chromatophore, LH1–RC solution and crystal samples, and the RC-only complex.

2. Materials and methods

2.1. Preparations of chromatophores, LH1–RC and RC complexes

Tch. tepidum cells were cultured for seven days. Chromatophores were prepared as previously reported [24]. The chromatophores were suspended in 0.5 mL of 20 mM Tris–HCl (pH 7.5) at concentration of $A_{850} = 120$, and were then lyophilized. LH1–RC complex was isolated from the chromatophores and purified following the same procedure described before [25]. Crystals of the LH1–RC were the same as those used in the structure determination [22]. About 200 crystals were collected and dissolved in 20 mM Tris–HCl (pH 7.5) buffer containing 0.05% w/v *n*-dodecyl β-D-maltopyranoside (DDM). RC complex was prepared by the same procedure as reported [26]. After recorded the absorption spectra, all samples in the solutions (0.5 mL) were precipitated by adding the same volume of 50% w/v PEG3000, followed by washing the precipitates with 17% w/v PEG3000. The pellets were dried and stored at −80 °C. Absorption spectra of the samples used in this study are shown in Fig. 1.

2.2. Extraction and separation of the quinones

All chemicals used were purchased from Wako Pure Chemical Industries, Ltd. (Japan) and Sigma Chemical Co. (U. S. A), unless otherwise noted. Three methods of quinone extraction were tested: (1) the quinones were extracted with a mixture of 1:1 acetone/methanol; (2) the quinones were first extracted by 1:1 acetone/methanol, followed by petroleum ether (bp. 30–70 °C) extraction [27]; and (3) the quinones were extracted with a mixture of 2:1 chloroform/methanol [21,28]. All extractions were repeated twice and the combined extracts were dried under argon stream. The dried extracts were dissolved in ethanol (0.2 mL) and injected onto a reverse-phase HPLC column (TOSOH, TSKgel ODS-80Ts, 4.6 × 250 mm). The quinones and pigments were eluted isocratically at 25 °C by 7:3 methanol/isopropanol at a flow rate of 0.7 mL/min and were monitored by a multi-wavelength UV detector (ÄKTA Purifier, UV-900) at 270 nm, 370 nm and 500 nm.

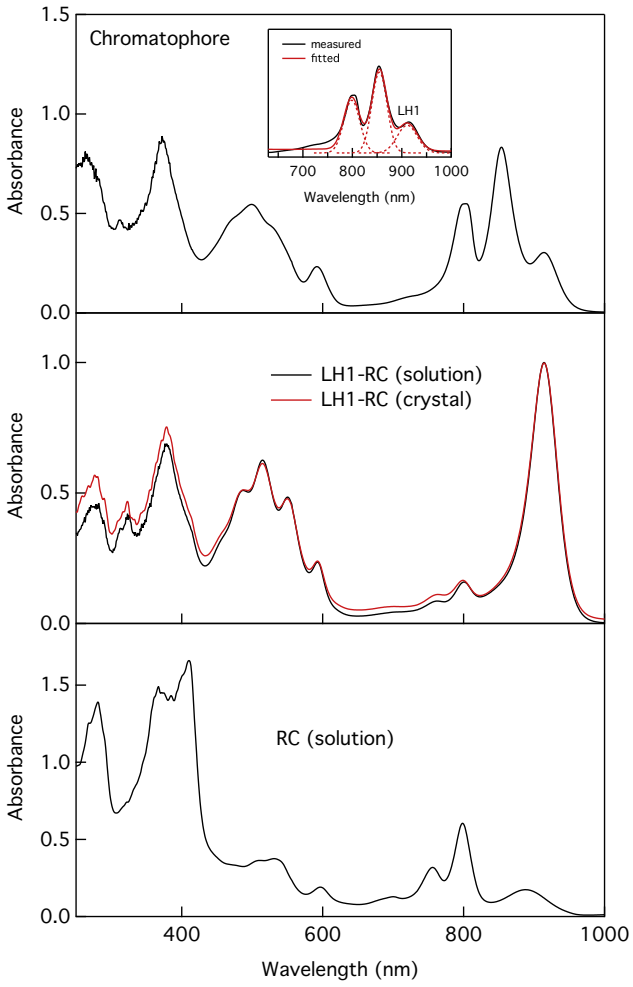

Fig. 1. Absorption spectra of the samples used in this study. Top: chromatophores (inset: deconvoluted region, dotted lines show the components). Middle: LH1–RC solution and crystal samples. Bottom: purified RC.

2.3. Determination of quinone content

The quinone identities were determined by comparing their absorption and mass spectra with those of authentic UQ_{10} and MQ_4 purchased from Sigma–Aldrich (U. S. A). The absorption spectra were recorded on a Agilent 8453 UV–Vis spectrophotometer. The mass spectra were measured on a 4800 Plus MALDI TOF/TOF Analyzer (Applied Biosystems, MDS SCIEX). 2,4,6-Trihydroxy acetophenone (THAP) was used as matrix and was dissolved in 50% acetonitrile solution containing 0.3% TFA with the final concentration of 20 mg/mL. The quinones dissolved in chloroform were mixed with the THAP solution in a ratio of 1:1 (v/v) and then loaded onto the sample stage for co-crystallization. Measurement was performed in positive and reflector modes. The spectra obtained were calibrated externally using the authentic UQ_{10}, MQ_4 and the $[M+H^+]$ ions from five standards: angiotensin I (m/z 1297.51), ACTH (clip 1–17) (m/z 2094.46), ACTH (clip 18–36) (m/z 2466.72), ACTH (clip 7–38) (m/z 3660.19) and insulin (bovine) (m/z 2867.80, $z = 2$). Quantification of the quinone contents was conducted by integrating the quinone peaks in chromatogram based on the calibration using authentic UQ_{10} and MQ_4 as the standards. Following extinction coefficients were used to calculate the number of quinones per RC: 14.7 mM^{-1} cm^{-1} at 275 nm for the UQ in ethanol [11,14], 17.3 mM^{-1} cm^{-1} at 270 nm for the MQ in ethanol [29], 4320 mM^{-1} cm^{-1} at 915 nm for the *Tch. tepidum* LH1–RC