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A heterogeneous tag-attachment to the homodimeric type 1 photosynthetic reaction center core protein in the green sulfur bacterium *Chlorobaculum tepidum*

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ABSTRACT

The *6xHis-tag-pscA* gene, which was genetically engineered to express N-terminally histidine (His)-tagged PscA, was inserted into a coding region of the *recA* gene in the green sulfur bacterium *Chlorobaculum tepidum* (*C. tepidum*). Although the inactivation of the *recA* gene strongly suppressed a homologous recombination in *C. tepidum* genomic DNA, the mutant grew well under normal photosynthetic conditions. The His-tagged reaction center (RC) complex could be obtained simply by Ni^{2+}-affinity chromatography after detergent solubilization of chlorosome-containing membranes. The complex consisted of three subunits, PscA, PscB, and PscC, in addition to the Fenna–Matthews–Olson protein, but there was no PscD. Low-temperature EPR spectroscopic studies in combination with transient absorption measurements indicated that the complex contained all intrinsic electron transfer cofactors as detected in the wild-type strain. Furthermore, the LC/MS/MS analysis revealed that the core protein consisted of a mixture of a His-/His-tagged PscA homodimer and a non-/His-tagged PscA heterodimer. The development of the *pscA* gene duplication method presented here, thus, enables not only a quick and large-scale preparation of the RC complex from *C. tepidum* but also site-directed mutagenesis experiments on the artificially incorporated *6xHis-tag-pscA* gene itself, since the expression of the authentic PscA/PscA homodimeric RC complex could complement any defect in mutated His-tagged PscA. This method would provide an invaluable tool for structural and functional analyses of the homodimeric type 1 RC complex.

1. Introduction

Green sulfur bacteria are obligatory anaerobic phototrophs that inhabit anoxic and sulfur-rich water, such as bottom sediments or deep layers of a water column, and deep-sea hydrothermal vents in the Pacific Ocean [1]. To survive under extremely low-light intensity, these bacteria have developed large light-harvesting apparatuses attached to the inner membrane surface, called "chlorosomes," which contain a very large amount of bacteriochlorophyll (BChl) *c*, *d*, and *e* molecules [2]. Green sulfur bacteria grow photoautotrophically utilizing reduced sulfur compounds, such as sulfide, elemental sulfur, and/or thiosulfate, as electron sources [3,4]. The solar energy captured by chlorosomes is finally converted to a highly reducing power, reduced ferredoxin, through a series of redox reactions in a homodimeric type 1 reaction center (RC), in which iron–sulfur (Fe–S) clusters serve as terminal electron acceptors, as in the case of photosystem (PS) I RC of oxygenic phototrophs, including cyanobacteria.

The green sulfur bacterial RC complex consists of four subunits, PscA, PscB, PscC, and PscD, and is additionally associated with the BChl *a*-containing Fenna-Matthews-Olson (FMO) protein [5]. It has a much simpler architecture than that of the PS I RC complex, which consists of more than 12 different protein subunits [6]. A pair of PscA makes up a homodimeric core protein that houses most electron transfer (ET) cofactors: P840, a special dimer of BChls *a* as the primary electron donor; A_0, a derivative of Chl *a*, Chl a_{PD}, as the primary electron acceptor; and F_X, a [4Fe–4S] center, as the secondary or tertiary electron acceptor. PscB is a functional homologue of PsaC in PS I, holding two [4Fe–4S] centers that serve as the terminal electron acceptors, F_A and F_B [7]. PscC, which is also called cytochrome (cyt) c_z, is a unique subunit of the green sulfur bacterial RC complex. Two molecules of cyt c_z are tightly bound to the RC complex and serve as the direct electron donor to the photooxidized $P840^+$ [8–10]. The ET rate from cyt c_z to $P840^+$ has unusual dependence on solvent viscosity due to the fluctuation nature of its C-terminal domain [11–13]. The FMO protein has a trimeric form *in vivo* and transfers excitation energy from chlorosomes to the RC core protein [14–16]. PscD is involved in its efficient energy transfer but is not essential for photosynthetic growth [17].

Abbreviations: RC, reaction center; PS, photosystem; cyt, cytochrome; ET, electron transfer; (B)Chl, (bacterio)chlorophyll; FMO, Fenna–Matthews–Olson; β-OG, n-octyl-β-D-glucopyranoside; ESP, Electron spin-polarization

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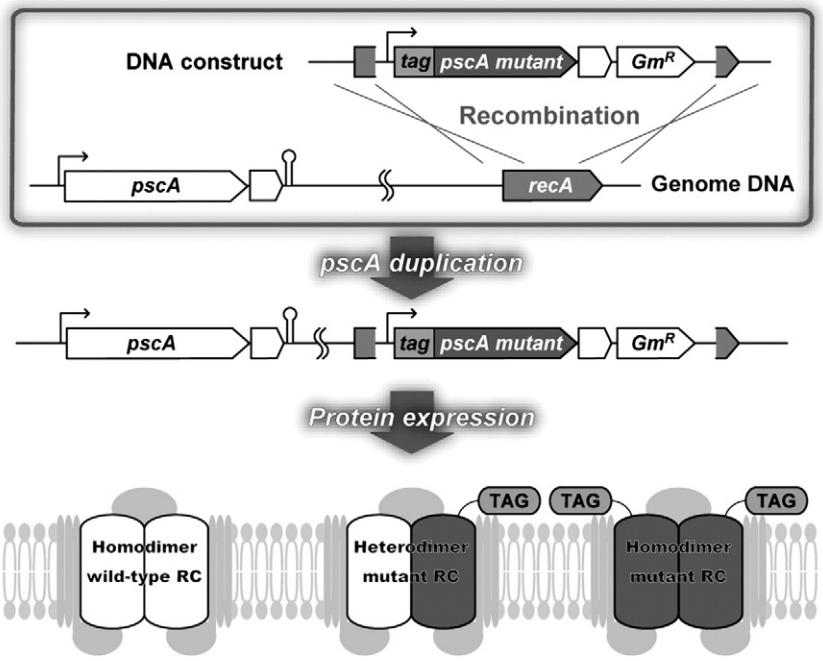

Fig. 1. Conceptual representation for the construction of the mutated PscA in *C. tepidum*. The *recA* gene is inactivated by an insertion of the mutated *tag-pscA* gene together with a selection marker (e.g., a gentamicin resistance cassette). The RC complex with the affinity tag attached to the mutated PscA could be isolated with conventional affinity chromatography. The authentic *pscA* gene is expected to express the wild-type RC complex and would complement any mutant defect in photosynthetic growth.

As to the ET pathway, whether the electron is transferred from A_0 directly to F_X without any involvement of the quinone molecule remains controversial [5,18]. Although menaquinone has been shown to be present in some RC preparations [19–21], no direct spectroscopic evidence for its function as a secondary electron acceptor (A_1) has been obtained [20,22,23]. At present, there is no three-dimensional structure of the green sulfur bacterial RC. The X-ray crystal structures of RCs so far determined, i.e., type 2 RCs and PS I RC, have demonstrated that they share common folding motifs of membrane-spanning α-helices of core proteins as well as the spatial configurations of ET cofactors [24]. Therefore, it would be reasonable to conclude that the structure modeling of the green sulfur bacterial RC, especially its central region, which coordinates ET cofactors, is quite similar to that of PS I RC [18]. However, the possible quinone-binding pocket is very hydrophilic compared to that in PS I RC and seems to loosely interact with a quinone molecule even if present [5,18]. The same situation is also applicable to the case for another homodimeric type 1 RC of heliobacteria [25], although the transient electron paramagnetic resonance (EPR) signal ascribable to the $P800^+A_1^-$ radical pair has recently been observed in it [26]. Moreover, the ET reaction is considered to proceed equivalently in two branches within the homodimeric RC, although the information on its symmetrical ET pathways is very scarce.

A molecular genetic study to modify the RC core protein would be the most promising approach for the elucidation of its detailed molecular constructions and physicochemical properties. Many mutagenesis experiments have already been executed in heterodimeric RCs of purple bacteria as well as oxygenic phototrophs, especially cyanobacteria (reviewed in Refs. [27–29]). Amino acid residues around cofactors were substituted intensively and systematically to explore their energy and ET processes. The recent work on the mutant PS I of a green alga, *Chlamydomonas reinhardtii*, has also revealed the bidirectional ET to quinone molecules in two branches and slightly different ET rates to the next cofactor F_X due to energetically different redox potentials [30,31].

A meso-thermophilic green sulfur bacterium, *Chlorobaculum* (*C.*) *tepidum*, is amenable to a genetic manipulation, and the information of its genome sequences is available [32–34]. However, there has been no practical method to examine amino acid substitution variants of the PscA core protein so far, since any substitution unfavorable for photosynthetic growth would be lethal or could easily be replaced or modified, resulting in the generation of a spontaneous revertant. We, here, propose a new strategy to obtain mutants in the core protein: "the *pscA* gene duplication method." The *6xHis-tag-pscA* gene encoding the PscA core protein with an affinity tag attached to its N-terminus is inserted into the *recA* locus, which causes the disruption of the *recA* gene concomitantly with a duplication of the *pscA* gene (see Fig. 1). This strategy incorporates three different ideas at once.

First, an affinity-tag attachment to the PscA subunit would make it possible to perform a brief and large-scale preparation of the RC complex [35–44]. Second, since the *recA* gene is responsible for homologous DNA recombination and repair [45], its disruption mutant could be a suitable host for the expression of the mutated *6xHis-tag-pscA* gene incorporated into the genome. Finally, the authentic *pscA* gene ensures the expression of the wild-type RC in any mutant. Any mutagenesis on the *6xHis-tag-pscA* gene has no critical influence on photoautotrophic growth. It would be even possible, in principle, to construct an artificial heterodimeric RC core protein consisting of the wild-type PscA and the His-tagged PscA with any given mutation on it (Fig. 1, lower).

In this study, we investigated the availability of this *pscA* gene duplication method without any mutagenesis on the *6xHis-tag-pscA* gene. The His-tagged RC complex was expressed in the *recA*$^-$ mutant that grew photoautotrophically under ordinary cultural conditions. Its spectroscopic and biochemical properties were studied in detail by EPR and LC/MS/MS analyses.

2. Materials and methods

2.1. Strains and growth conditions

The strain WT2321 [46] of *C. tepidum* was used as the wild-type and host strain for transformation. All *C. tepidum* strains were grown anaerobically in liquid CL media or solid CP media [32], basically as previously described [3]. Gentamicin (30 μg ml^{-1}) was added to