Cyanobacterial Non-mevalonate Pathway

(\textit{E})-4-HYDROXY-3-METHYLBUT-2-ENYL DIPHOSPHATE SYNTHASE INTERACTS WITH FERRodoxIN IN \textit{THERMOSYNECHOCOCCUS ELONGATUS} BP-1\textsuperscript{*}

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(\textit{E})-4-Hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE), which catalyzes the conversion of 2-C-methyl-D-erythritol cyclophosphate (MEcPP) into (\textit{E})-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP), is an essential enzyme of the non-mevalonate (2-C-methyl-D-erythritol-4-phosphate (MEP)) pathway for isoprenoid biosynthesis. The terminal steps of the MEP pathway are still not fully understood, although this pathway is necessary for survival in various organisms such as cyanobacteria, plastids of algae and higher plants, and the apicoplast of human malaria parasites. To determine the efficient redox partner for thermophilic cyanobacterial GcpE, we have expressed the 	extit{gcpE} and 	extit{petF} genes in \textit{Escherichia coli} and studied the protein-protein interaction of GcpE protein with ferredoxin I (PetF) from the thermophilic cyanobacterium \textit{T. elongatus} BP-1. Recombinant GcpE protein was purified by an N-terminal His\textsubscript{6} tag and reconstituted as a [4Fe-4S]\textsuperscript{2\+} metalloprotein. GcpE was shown to interact strongly with PetF via the bacterial two-hybrid system designed to detect protein-protein interactions. Moreover, a direct protein-protein interaction between PetF and GcpE was confirmed in an \textit{in vitro} glutathione S-transferase (GST) pull-down assay. To investigate electron transfer activity from PetF to GcpE, we also constructed a NADPH-dependent reducing shuttle system with purified recombinant ferredoxin-NADP\textsuperscript{+} oxidoreductase (PetH) and PetF. The result demonstrated that PetF has the ability to transfer electrons to GcpE. Thus, the combined data provide the first evidence that GcpE is a ferredoxin-dependent enzyme in \textit{T. elongatus} BP-1.

Metabolites derived from isoprenoids play important roles in systems such as electron transport, photosynthesis, plant defense responses, hormonal regulation of development, and membrane fluidity and are essential in various organisms such as eubacteria, higher plants (1), and protozoan parasites of the phylum Apicomplexa (2, 3). Isoprenoids are synthesized ubiquitously through condensation of two isomeric five-carbon (C\textsubscript{5}) building blocks, isopentenyl diphosphate and dimethylallyl diphosphate (4). In a cytosolic pathway of higher plants, two distinct biosynthetic routes to isopentenyl diphosphate and dimethylallyl diphosphate, which start from acetyl-CoA and proceed through the intermediate mevalonate, provide the precursors for sterols and ubiquinone. By contrast, in cyanobacteria (5–7), the plastids of algae and higher plants (1, 4) and the relict plastid (apicoplast) of the Apicomplexa (2), isopentenyl diphosphate and dimethylallyl diphosphate are synthesized via the 2-C-methyl-D-erythritol-4-phosphate (MEP)\textsuperscript{1} pathway, which involves a condensation of pyruvate and glyceraldehyde 3-phosphate via 1-deoxy-D-xylulose 5-phosphate as the first intermediate (7–10).

The first five enzymatic steps of the MEP pathway have been well established, but the terminal steps are still not fully understood (9–11). Recent data has shown that (\textit{E})-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) and LytB proteins are iron-sulfur proteins containing a [4Fe-4S]\textsuperscript{2\+} cluster after reconstitution of the purified protein (12–14). GcpE catalyzes the reduction of MEcPP into HMBPP via two successive one-electron transfers (13, 14). The last step of the MEP pathway is catalyzed by IspH (or LytB), which converts HMBPP into isopentenyl diphosphate or dimethylallyl diphosphate via two successive one-electron transfers (15). These reactions were followed using flavodoxin/flavodoxin reductase/NADPH or sodium dithionite as a reductant (11–13, 15). In contrast to the bacterial GcpE enzyme, which utilizes flavodoxin/flavodoxin reductase/NADPH as a reducing shuttle system, the plant GcpE enzyme could not use this reduction system (14). Yet, there have been no reports of an efficient redox partner for GcpE or LytB protein in cyanobacteria, the plastid of higher plants, or the relict plastid of human malaria parasite.

Here we report the interaction between GcpE and ferredoxin I (PetF), enabling transfer of electrons from photosystem I to ferredoxin-dependent enzymes, in the thermophilic cyanobacterium \textit{T. elongatus} BP-1. GcpE protein was shown to interact strongly with PetF via a bacterial two-hybrid system designed to detect protein-protein interactions. Moreover, a direct protein-protein interaction between PetF and GcpE was confirmed using an \textit{in vitro} GST pull-down assay. We also constructed an NADPH-dependent reduction system with purified recombinant ferredoxin-NADP\textsuperscript{+} oxidoreductase (PetH), PetF, and GcpE and investigated that electron transfer activity of PetF to GcpE. From the reductive titration of PetF with reconstituted GcpE, the dissociation constant \(K_d\) for the electron transfer of PetF to GcpE was estimated as \(\sim 10 \mu M\). Therefore, We propose that GcpE is a ferredoxin-dependent enzyme in \textit{T. elongatus} BP-1.

\textsuperscript{1} The abbreviations used are: MEP, 2-C-methyl-D-erythritol-4-phosphate; HMBPP, (\textit{E})-4-hydroxy-3-methylbut-2-enyl diphosphate; MEcPP, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate; PetF, ferredoxin I; PetH, ferredoxin-NAD\textsuperscript{+} oxidoreductase; PcyA, phycocyanobilin: ferredoxin oxidoreductase; HO1, heme oxygenase 1; GST, glutathione S-transferase; DTT, dithiothreitol; B2H, bacterial two-hybrid; 3-AT, 3-amino-1,2,4-triazole.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Enzymes for DNA manipulation were obtained from New England Biolabs. Agar and organic nutrients for LB were obtained from Difeo, and other chemicals were from Sigma, BD Biosciences, Clontech, and Qiagen.

**Cloning of Relevant T. elongatus BP-1 Genes**—Standard procedures were used for most DNA manipulations. Gene sequences were obtained from the Kazusa DNA Research Institute CyanoBase (16, 17), and all accession numbers given below refer to that data base. Using primers designed below, all genes were amplified from *T. elongatus* BP-1 genomic DNA by PCR. The fidelity of all PCR-generated fragments was verified by direct DNA sequencing.

**Cloning of the Gene Encoding *T. elongatus* BP-1 PetF**—Primers used to amplify the *gcfE* gene (tl0996) were forward primer TeGcpENdeI and reverse primer TeGcpESacI (Table 1, also other primers used below). The resulting 1.2-kb product was digested with restriction enzymes NdeI and SacI and cloned into NdeI- and SacI-digested *pET28b*-containing plasmid. The *gcfE* gene fragment was PCR-amplified using the forward primer TeGcpEBamHI and reverse primer TeGcpESacI. The product was digested with restriction enzymes BamHI and SacI and cloned into BamHI- and SacI-restricted *pET24b* (Novagen), giving the plasmid pET24b-TePetH-ld.

**Cloning of the Gene Encoding *T. elongatus* BP-1 PetH**—Primers used to amplify the *petH* gene (tl1211) were forward primer TePetH-NdeI and reverse primer TePetHXhol. The resulting 0.9-kb PCR fragment was digested with restriction enzymes NdeI and Xhol and cloned into NdeI- and Xhol-digested *pET28b* (Novagen), giving the plasmid pET28b-TePetH. This construct lacked CpcD-like rod linker domain fused at 5′-end to the gene coding for RNAPα gene, giving the plasmid pTRG-TePcyA.

**Cloning of the Gene Encoding *T. elongatus* BP-1 Heme Oxygenase 1**—Primers used to amplify the *hoxA* gene were forward primer TeHO1NotI and reverse primer TeHO1XhoI. The resulting 0.29-kb PCR fragment was digested with restriction enzymes NotI and XhoI and cloned into NotI and XhoI restriction sites of the *pTRG-target* vector (Stratagene) in-frame with the *RNAPα* gene, giving the plasmid pBT-TePetF. cDNA encoding the *petF* gene was PCR-amplified using the forward primer TePetFNcoI and reverse primer TePetFXhol. The product was digested with restriction enzymes NcoI and XhoI and cloned into NcoI- and XhoI-digested *pET42b*-containing plasmid, giving the plasmid pET42b-TePetF. The resulting 0.29-kb PCR fragment was digested with restriction enzymes NdeI and XhoI and cloned into the NotI and XhoI restriction sites of the *pTRG-target* vector in-frame with the *RNAPα* gene, giving the plasmid pTRG-TeHO1.

**Cloning of the Gene Encoding *T. elongatus* BP-1 Phycocyanobilin: Ferredoxin Oxidoreductase (PcyA)**—Primers used to amplify the *pcyA* gene (tl2308) were forward primer TePcyANotI and reverse primer TePcyAXhol. The resulting 0.71-kb product was digested with restriction enzymes NotI and Xhol and cloned into the NotI and Xhol restriction sites of the plasmid pTRG-TeHO1.

**Expression and Purification of Recombinant Proteins**—The plasmid *pET28b*-TeGcpE was transformed into *Escherichia coli* strain BLR (DE3) (Novagen). A fresh single colony of *E. coli* BLR (DE3) was transformed with the plasmid expressing the His-TeGcpE fusion protein was cultured overnight at 37 °C in 100 ml of Luria-Bertani medium containing 1% glucose. 80 ml of this culture was incubated overnight and used to inoculate 8 liters of Luria-Bertani medium. The cells were grown at 37 °C to mid-log phase, and then His-TeGcpE was induced by adding 0.2 mM isopropyl β-D-thiogalactoside (IPTG) to 20 °C. Cells were harvested after overnight induction and lysed by sonication in binding buffer (50 mM sodium phosphate, pH 7.4) containing 10 mM β-mercaptoethanol, 300 mM NaCl, and 5 mM imidazole for 30 s on ice. The lysate was centrifuged at 50,000 × g for 30 min, and the supernatant was applied to a BD TALON Superflow metal affinity column (1.5 cm × 5 cm BD Biosciences Clontech). The His-TeGcpE fusion protein was purified, according to the manufacturer’s instructions (BD Biosciences Clontech). The peak fraction was concentrated to 3.7 ml using an Amicon Ultra-15 unit with a 30-kDa cut-off (Millipore). Final purification was carried out by gel filtration using an XK26/100 Sephacryl S-200HR column (Amersham Biosciences) equilibrated with buffer A (50 mM HEPES-NaOH, pH 7.5) containing 1 mM NaCl and 5 mM dithiothreitol (DTT). The main fraction was concentrated to 18 mg ml⁻¹ and rebuffered in buffer A containing 100 mM NaCl and 5 mM DTT, using prepacked Sephadex G-25 gel filtration columns NAP-10 (Amersham Biosciences). The plasmid *pET24b*-TePetH-ld containing the *petH* gene and the plasmid *pET21b*-TePetF containing the *petF* gene were expressed in *E. coli* BL21 (DE3) (Novagen) and purified essentially as described previously except that the plasmid *pET21b*-TePetF containing the *petF* gene fused at the 5′-end to the gene encoding for *Schistosoma japonicum* GST was constructed and transformed into *E. coli* strain HMS174 (DE3) (Novagen). A fresh single colony of *E. coli* HMS174 (DE3) was transformed with the plasmid expressing the GST-TePetF fusion protein and cultured overnight at 37 °C in 50 ml of Luria-Bertani medium containing 1% glucose, according to the manufacturer’s instructions (Novagen). 10 ml of the overnight culture was used to inoculate 1 liter of Luria-Bertani medium. The cells were grown at 37 °C to mid-log phase, and then GST-TePetF was induced by adding 1 mM isopropyl β-D-thiogalactoside at 25 °C. Cells were harvested after overnight induction and lysed by sonication in the binding buffer (phosphate-buffered saline, pH 7.3) containing 1 mM DTT, 140 mM NaCl, and 5 mM imidazole for 30 s on ice. The lysate was centrifuged at 50,000 × g for 30 min, and the supernatant was applied to a GST-glutathione-Sepharose high performance column (5 cm × 5 cm Amersham Biosciences). The GST fusion protein was purified according to the manufacturer’s instructions (Amersham Biosciences).

**Interaction between Ferredoxin and GcpE**—Interaction between ferredoxin and GcpE was studied by preparing 3 μM of the protein and carrying out experiments as described above. To record the UV-visible absorption spectrum, a fraction of the reconstituted sites of the plTRG-target vector in-frame with the *RNAPα* gene, giving the plasmid *pTRG-TePcyA*, was used.

**Reconstitution of the Iron-Sulfur Cluster in GcpE**—Reconstitution of as-isolated GcpE with iron and sulfide was carried out inside an anaerobic chamber with argon-saturated buffers and solutions that were prepared with deoxygenated water. A typical reconstitution reaction contained 200 μM TeGcpE and a 10-fold molar excesses of FeCl₃ and Na₃S in a final volume of 1 ml. The protein was initially treated with a 50-fold molar excess of DTN for 10 min on ice. The FeCl₃ was then added, and a solution of Na₃S was added dropwise over 10 min. After 4 h, the reaction mixture was desalted on a NAP-10 column (Amersham Biosciences) equilibrated with 50 mM HEPES-NaOH buffer (pH 7.5). To record the UV-visible absorption spectrum, a fraction of the reconstituted sites of the plTRG-target vector in-frame with the *RNAPα* gene, giving the plasmid *pTRG-TePcyA*, was used.

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tuted protein was directly transferred into a cuvette, which was closed with a septum before being removed from the anaerobic chamber.

**Bacterial Two-hybrid Assay**—Protein-protein interactions were investigated using the BacterioMatch two-hybrid system (B2H) vector kit and the BacterioMatch II validation reporter strain (Stratagene) (20, 21). The B2H validation reporter cells were made competent by the

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**Fig. 1. Protein sequence comparison of different GcpEs.** A, protein sequence alignment of *Arabidopsis thaliana* and *P. falciparum* 3D7 GcpE with those of cyanobacterium using the MEP pathway. Pf, *P. falciparum* 3D7 (PlasmoDB accession number PF10_0221 or GenBank™ protein identification resource accession number AAN35418); At, *A. thaliana* (GenBank™ protein identification resource accession number AAL91150); Te, *T. elongatus* BP-1 (CyanoBase accession number thr0996 or GenBank™ protein identification resource accession number BAC08548); Syn6803, *Synechocystis* sp. strain PCC6803 (CyanoBase accession number shr2136 or GenBank™ protein identification resource accession number BAA17717). The alignment was carried out by ClustalW. Black and gray outlines indicate identical and similar amino acid residues, respectively. B, *P. falciparum* 3D7 and *A. thaliana* GcpE precursors have heterogeneous N-terminal extensions. *P. falciparum* 3D7 GcpE precursor contains a bipartite apicoplast targeting signal showing N-terminal extensions resembling signal plus transit peptides, and *A. thaliana* GcpE contains an N-terminal extension resembling chloroplast targeting transit peptide. The insertion region indicates sequence insertion of 269 amino acids in the case of *A. thaliana* and of 322 amino acids in the case of *P. falciparum* 3D7 with weak similarities to each other. These four regions are represented with differently colored boxes.
RESULTS

Identification of gcpE Gene in T. elongatus BP-1—gcpE represents a highly conserved gene identified in a variety of organisms including eubacteria, higher plants (1, 23), and the human malaria parasite Plasmodium falciparum and other protozoan parasites of the phylum Apicomplexa, all of them known to possess the MEP pathway (Fig. 1) (2, 3, 24). Recently, we identified a GcpE homologue in the human malaria parasite T. elongatus BP-1 ge-

A GST Pull-down Assays—Purified GST-TePetF fusion proteins were incubated with glutathione-Sepharose high performance beads in phosphate-buffered saline buffer (pH 7.4) and washed repeatedly with phosphate-buffered saline buffer (pH 7.4). When appropriate, the beads were incubated with reconstituted His-TeGcpE at 4 °C for 15 min, followed by a wash step in phosphate-buffered saline buffer (pH 7.4). The beads were then washed repeatedly, digested at 2 and 12 h with factor Xa (Novagen) and separated from GST according to the manufacturer’s instructions (Novagen). The reaction mixtures were centrifuged at 21,500 × g for 5 min, and the supernatant was boiled for 3 min in 2× SDS sample buffer, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue.

Spectrometric Assay of Electron Transfer Activity—An electron transfer pathway from NADPH to GcpE was reconstituted using PetH and PetF. The assay mixture contained in a final volume of 500 μl 50 mM HEPES-NaOH (pH 7.5), 100 mM NaCl, 5 mM DTT, 50 μM NADPH, 1 mM glucose-6-phosphate, 0.5 units of glucose-6-phosphate dehydrogenase, 7.2 mM PetH-Id, and 75.5 μM reconstituted GcpE, and 0, 5, 10, 20, and 40 μM PetF, respectively. The reaction was initiated by adding PetF at 25 °C. In the assay system, a reduction of the [4Fe-4S] cluster in GcpE before and after reduction. Absorption spectra of the sample as reconstituted in the oxidized form and the reduced form after the addition of 0.5 and 5 mM sodium dithionite were anaerobically recorded at room temperature. Absorbance decreases at 395 and 585 nm and increases at 314 nm are indicated by arrows.

GcpE Is an Iron-Sulfur Protein—GcpE proteins are reported to be unstable, losing activity quickly during purification and, to some extent, even in the cell (11, 14). His-GcpE protein is to be unstable, losing activity quickly during purification and, to some extent, even in the cell (11, 14). His-GcpE protein is derived from GcpE by addition of a tag of six histidines at the N terminus. The protein was obtained by E. coli overexpression and purified aerobically using a BD TALON superflow column that specifically retains proteins containing a cluster of histidines. The enzyme was found by SDS-PAGE to be 99% pure (Fig. 2A). The purified protein has a reddish-brown color in agreement with the light absorption spectrum (Fig. 2B, lower spectrum), and the analysis for labile iron and sulfide suggested the presence of a protein-bound [4Fe-4S] center. However, iron content was substoichiometric with regard to GcpE, and the protein contained sulfide in slight excess with regard to iron, probably as a consequence of loss of the cluster during purification (Fig. 2B, lower spectrum). The as-isolated His-
GcpE protein was therefore reconstituted with a 10-fold excess of ferrous iron and sodium sulfide under anaerobic conditions as described under “Experimental Procedures.” After anaerobic desalting on a Sephadex G-25 column, the protein was intensely brown. The UV-visible spectrum of the reconstituted protein is also shown in Fig. 2B (upper spectrum). The electronic absorption spectrum of the as reconstituted His-GcpE displays absorption bands, including a shoulder at 305 and 585 nm and a hump at around 395 nm, more consistent with a \([4\text{Fe}-4\text{S}]\) cluster (Fig. 2B, upper spectrum). During anaerobic reduction of reconstituted His-GcpE with 0.5 and 5 mM sodium dithionite, bleaching of the solution and a loss of the visible absorption bands were observed (Fig. 3).

**Bacterial Two-hybrid Assay**—To examine whether the interactions between PetF and GcpE occurred in *T. elongatus* BP-1, constructs were made to test the direct protein-protein interactions between PetF and ferredoxin-dependent enzymes, HO1, PcyA, and GcpE, via the B2H system. The region encoding the *petF* gene was ligated into the bait vector of the B2H system to produce the construct pBT-TePetF. The entire coding region of the *ho1* and *pcyA* genes was ligated into the target vector to produce the construct pTRG-TeHO1 and pTRG-PcyA, and the entire coding region of *gcpE* gene was ligated into the target vector to produce the pTRG-TeGcpE construct. When the reporter strain was cotransformed with hybrid bait and target proteins. If the proteins interact, the RNA polymerase is recruited to the promoter, activating the detectable transcription of *HIS3*. Growth of the reporter strain on medium lacking histidine and containing 5 mM 3-AT occurs when transcriptional activation increases expression of the *HIS3* gene product.
to levels that are sufficient to overcome competitive inhibition by 3-AT. This allows for positive selection for plasmids encoding interacting proteins on media containing 5 mM 3-AT. Interaction of the HO1 and PcyA proteins with PetF was not detected on medium lacking histidine (Fig. 4). The GcpE protein was shown to interact strongly with PetF, as indicated by the strong growth on medium lacking histidine (HIS3 activation) (Fig. 4) and validated by a resistance to streptomycin (aadA activation) (Fig. 5).

GST Pull-down Assay (Interaction between PetF and GcpE in Vitro)—In vitro interaction between PetF and GcpE was verified using a GST pull-down assay. First, the cDNA coding sequence of PetF was subcloned into the pET42b vector to generate a GST-PetF, and this fusion protein was expressed in the HMS174 (DE3) bacterial strain. The purified His-PetF fusion protein was incubated with affinity-purified GST-PetF fusion protein immobilized on glutathione-Sepharose high performance beads. The GST-PetF fusion protein was digested with factor Xa and separated from GST. After the treatment for 2 and 4 h, the PetF and bound GcpE were then separated by 12.5% SDS-PAGE, and the proteins were detected with Coomassie Brilliant Blue stain. Fig. 6 shows that purified GST-PetF efficiently pulled down GcpE protein (lanes 4 and 5), but no protein was bound by glutathione-Sepharose high performance beads (lanes 6 and 7). Lane 5 shows that GcpE was isolated with PetF; lane 4, naturally released GcpE. Lane 1, protein molecular mass standards; lane 5, factor Xa; lane 9, GSH beads alone. B, comparison of the band intensity of GcpE and PetF proteins. Protein concentrations of GcpE and PetF were determined spectrophotometrically with an extinction coefficient of 16 mM⁻¹ cm⁻¹ at 395 nm and 10 mM⁻¹ cm⁻¹ at 422 nm, respectively. Molar ratios (GcpE:PetF): lane 2, 1:0.5; lane 3, 1:1; lane 4, 1:2; lane 5, 1:3; lane 6, 1:4; lane 7, 1:5. Positions of molecular weight markers are indicated in the left margin. Lane 1, molecular mass markers (M).

Spectrophotometric Assay for Electron Transfer from NADPH to Holo-GcpE Protein—In photosynthetic organisms, a major function of PetF is to transfer electrons from photosystem I to ferredoxin-dependent enzymes. An NADPH-de-
The proposed mechanism of action of GcpE. Modified from Refs. 11 and 13. Fd, ferredoxin.

In this study we described the biochemical characterization of T. elongatus BP-1 GcpE. When overexpressed in E. coli, the isolated GcpE protein, containing an N-terminal His6 tag, contained small amounts of iron and sulfide and displayed a weak UV-visible spectrum in the 300–700-nm region consistent with the presence of a [4Fe-4S] cluster. Anaerobic treatment of the protein with FeCl3 and Na2S in the presence of DTT resulted in the uptake of substantial amounts of iron and sulfide, as well as a dramatic increase in the activity of the protein (13). Hypothetical mechanisms for the GcpE-mediated reaction suggest that enzymatic conversion of MEcPP to HMBPP by GcpE is dependent on a [4Fe-4S] cluster as a cofactor, which is sensitive to dioxygen, and can be reduced by 5-deazaflavin or sodium dithionite as an artificial one-electron donor (11, 13, 14). To our knowledge, this is the first report characterizing GcpE in detail in terms of protein-protein interactions with PetF. Bacterial two-hybrid analysis of GcpE and ferredoxin-dependent enzymes (HO1 and PcyA) with PetF from T. elongatus BP-1 indicated that GcpE and PetF interact strongly and that PetF and the ferredoxin-dependent enzymes (HO1 and PcyA) interact, but less strongly, in this system. Moreover, a direct protein-protein interaction between PetF and GcpE was confirmed in an in vitro GST pull-down assay. This raises the interesting possibility of the formation of a functional complex between PetF and GcpE. Such a complex might serve as a system for electron donations to GcpE in vivo. We also constructed an NADPH-dependent reduction system with purified recombinant PetH, PetF, and GcpE and demonstrated that PetF had the ability to transfer electrons to GcpE (Fig. 7). From the reductive titration of PetF with reconstituted GcpE, the dissociation constant value (Kd) for the electron transfer of PetF to GcpE was determined to be around 10 μM (Fig. 7B). The data show that all components, NADPH, PetH, and PetF, were required for reduction of GcpE (Fig. 7A). Time-dependent absorbance changes associated with the reductive titration of PetF to reconstituted GcpE are shown in Fig. 7B. From this reductive titration of PetF to GcpE, the dissociation constant value (Kd) for the electron transfer of PetF with GcpE was estimated as ~10 μM. Therefore, the present work reveals that PetF has the ability to transfer electrons to GcpE.

To further clarify the molecular mechanism of GcpE catalysis, we must establish an assay system using the electron transfer ability of PetF to GcpE.

Recent studies have not shown that GcpE directly and/or indirectly interacts with flavodoxin (13). In this study, PetF has been identified as an interacting partner of GcpE. The results show that TeGcpE protein interacted with PetF. PetF may function as an efficient electron donor for GcpE in thermophilic cyanobacteria. Other electron carrier proteins are probably unable to function as efficient redox partners for this GcpE protein in T. elongatus BP-1, because other small electron carrier proteins, such as flavodoxin (isiB gene), that could support the reduction of GcpE are absent from the genome (16). At present, we do not know what kind of reduction system is operating for GcpE other than PetF in T. elongatus BP-1. It is possible that such flavoproteins may support the reduction of GcpE. In conclusion, we propose that the GcpE catalytic reaction of enzymatic conversion of MEcPP to HMBPP is dependent on ferredoxin as a one-electron carrier protein (Fig. 8).

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Interaction between Ferredoxin and GcpE

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