

Functional characterization of the trigger factor protein PceT of tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* Y51

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Received: 9 February 2009 / Revised: 9 March 2009 / Accepted: 10 March 2009 / Published online: 4 April 2009
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Abstract *Desulfitobacterium hafniense* strain Y51 dechlorinates tetrachloroethene to *cis*-1,2-dichloroethene (*cis*-DCE) via trichloroethene by the action of the PceA reductive dehalogenase encoded by *pceA*. The *pceA* gene constitutes a gene cluster with *pceB*, *pceC*, and *pceT*. However, the gene components, except for *pceA*, still remained to be characterized. In the present study, we characterized the function of PceT. PceT of strain Y51 showed a sequence homology with trigger factor proteins, although it is evolutionally distant from the well-characterized trigger factor protein of *Escherichia coli*. The PceT protein tagged with 6x histidine was expressed as a soluble form in *E. coli*. The recombinant PceT fusion protein exhibited peptidyl-prolyl *cis*–*trans* isomerase activity toward the chromogenic peptide *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide. The PceT fusion protein also exhibited chaperon activity towards the chemically denatured citrate synthase. Immunoprecipitation analysis using antibodies raised against PceA and PceT demonstrated that PceT specifically binds to the precursor form of PceA with an N-terminal twin-arginine translocation (TAT) signal sequence. On the other hand, PceT failed to bind the mature form of PceA that lost the TAT signal sequence. This is the first report in dehalorespiring bacteria,

indicating that PceT is responsible for the correct folding of the precursor PceA.

Keywords *Desulfitobacterium hafniense* · Dehalorespiring bacteria · Tetrachloroethene · PCE genes · Chaperon · Trigger factor

Introduction

Recently, a rapidly increasing number of anaerobic bacteria have been isolated and characterized that are able to couple the reductive dehalogenation to energy conservation (Mohn and Tiedje 1992; Holliger and Schumacher 1994; Wohlfarth and Diekert 1997; Holliger et al. 1998; Damborsky 1999; Smidt et al. 2000; Smidt and de Vos 2004; Villemur et al. 2006; Löffler and Edwards 2006). Thus, some anaerobic bacteria use a specific haloorganic compound as the electron acceptor of their respiratory process. These dehalorespiring bacteria are believed to play an important role in the degradation of chlorinated environmental pollutants. A number of tetrachloroethene (PCE)-dehalorespiring bacteria have been isolated to date. Most of these bacteria reduce PCE or trichloroethene (TCE) to *cis*-DCE, which accumulates as the dead end product. Some strains belonging to *Dehalococcoides* are able to sequentially convert PCE to ethene. Such *Dehalococcoides* strains have a unique dehalogenation activity because they cannot grow without halogenated compounds.

Desulfitobacterium hafniense strain Y51 isolated in our laboratory does not use chloroaromatic compounds, but exhibits a strong dechlorinating activity toward PCE at concentrations as high as 960 μ M (at saturation) and as low as 0.6 μ M, converting it to *cis*-DCE via TCE (Suyama et al. 2001). The PceA reductive dehalogenase of this strain has

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been previously purified and characterized in some detail (Suyama et al. 2002). PceA possesses a corrinoid cofactor, which constitutes the active site, and a twin-arginine translocation (TAT) signal sequence at the N-terminus, which is implicated in the translocation of the PceA protein to periplasm.

Most reductive dehalogenase genes are organized in an operon comprised of at least two genes. In case of strain Y51, the *pceA* gene is flanked by the *pceB* gene coding for a protein containing a three-hydrophobic transmembrane domain (Suyama et al. 2002; Furukawa et al. 2005). Although the function of PceB has not been clarified, this protein has been assumed to act as a membrane anchor protein of PceA on the periplasmic face of the inner membrane. Downstream of *pceB*, the *pceC*, and *pceT* genes are followed in strain Y51. PceC is an unknown protein, but similar to those of the NirI/NosR family membrane-binding transcriptional regulators involved in nitrous oxide (N₂O) respiration (Cuypers et al. 1992; Wunsch and Zumft 2005). PceC contains a six-hydrophobic transmembrane domain, an FMN binding domain, and a C-terminal polyferredoxin-like domain. Moreover, the *pceABC* genes of strain Y51 are co-transcribed, implying that these genes play important roles in the dehalorespiration (Futagami et al. 2006b). On the other hand, PceT is similar to a trigger factor involved in the protein-folding. Based on the Tat translocation system, PceA is considered to be translocated to the periplasm only if the PceA precursor is properly folded with the corrinoid cofactor. Thus, we speculated that PceT might contribute to the correct folding of the PceA precursor protein prior to a TAT secretion process.

In the present paper, we demonstrated for the first time that PceT exhibited the prolyl *cis*–*trans* isomerase activity and chaperon activity in vitro and that PceT specifically interacted with the precursor form of PceA.

Materials and methods

Bacterial strains and culture conditions

The *Escherichia coli* strain XL1-Blue was used for the general propagation of the plasmids. The *E. coli* strain BL21 (DE3) codonplus RIL was used for the expression of the *pceT*. For expression of the *pceT*, *E. coli* cells were grown at 20°C for 8 h to get 0.4 at OD₆₆₀ in Luria–Bertani medium with 50 µg/ml of ampicillin and further grown at 20°C for 5 h in LB in the presence of 1 mM isopropyl-β-D-galactopyranoside and 50 µg/ml ampicillin. *D. hafniense* strain Y51 and its PCE-nondechlorinating variant LD, in which the entire *pceABCT* genes were deleted (Futagami et al. 2006b), were anaerobically grown in MMYPF medium: K₂HPO₄, 8.0 g; KH₂PO₄, 1.2 g; sodium

citrate, 0.5 g; MgSO₄·7H₂O, 0.1 g; yeast extract, 2.0 g; sodium pyruvate, 7.5 g; sodium fumarate, 0.69 g, and resazurin sodium salt, 1.0 mg (all per liter), pH 7.2, as previously described (Suyama et al. 2001).

Construction of the expression plasmid

Genomic DNA was prepared from strain Y51 as already described (Suyama et al. 2002). The *pceT* gene was amplified from the genomic DNA by PCR using the following set of primers, F1-*pceT*; 5'-AACGAGGGAG-GAATCGTGCATATGAAGCAGTTTCGAACTTG-3', in which the *NdeI* site is underlined, and R1-*pceT*; 5'-CAACAAAGAATTCTAGTTTCAATCTTGA-3', in which the *EcoRI* site is underlined. The amplified 1.1 kb DNA fragment was digested with *NdeI* and *EcoRI* and inserted into the corresponding sites of pET22b to yield pET22b-*pceT*. The *E. coli* strain BL21(DE3) codonplus RIL was transformed using pET22b-*pceT*.

Preparation of the recombinant PceT

E. coli cells expressing *pceT* were harvested by centrifugation at 4,000×g for 10 min at 4°C. The cell pellet was resuspended in TE buffer (10 mM TrisCl, 1 mM EDTA, pH8.0) and centrifuged at 4,000×g for 10 min at 4°C. The cell pellet was then resuspended in 20 ml of TE buffer, and the cell was disrupted by a French pressure cell (Ohtake, Japan). The resultant cell extract was centrifuged twice at 14,000×g for 30 min and at 100,000×g for 1 h at 4°C. The supernatant was filtered by a nitrocellulose membrane filter (pore size 0.45 µm). The cell extract was loaded onto HisTrapHP (GE Healthcare, USA) that had been equilibrated with binding buffer (30 mM TrisCl, 500 mM NaCl, pH 8.0). The column was washed with binding buffer and eluted with elution buffer (0–500 mM imidazole, 30 mM TrisCl, 500 mM NaCl, pH 8.0) in a stepwise fashion. The fraction eluted with the elution buffer containing 500 mM imidazole was dialyzed against TE buffer and concentrated by Amicon Ultra-15 (Millipore) and used as the PceT fusion protein.

Measurement of peptidyl-prolyl *cis*–*trans* isomerase activity

The peptidyl-prolyl *cis*–*trans* isomerase (PPI) activity of the PceT fusion protein was measured by a method described by Scholz et al. (Scholz et al. 1997). Briefly, 845 µl of 0.1 M TrisCl, pH 8.0, and 100 µl of 60 mg/ml solution of α-chymotrypsin were mixed in the spectrophotometer cell and preincubated at 15°C for 10 min. Thirty microliters of PceT solution was then added, and after 5 min, the assay was initiated by adding 25 µl of a

15.6 mM solution of peptide *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide in trifluoroethanol that additionally contained 0.47 M LiCl. The *cis*–*trans* isomerization of the Ala-Pro bond, coupled with the chymotryptic cleavage of the *trans* peptide was followed by the increase in absorbance at 410 nm in a spectrophotometer UV2200 (Shimadzu, Japan). As a control experiment, bovine serum albumin was used in place of the PceT fusion protein.

Measurement of chaperon activity

The chaperon activity of the PceT fusion protein was measured using chemically denatured citrate synthase (CS) by light scattering (Buchner et al. 1991). CS (Sigma, USA) was dialyzed against TE buffer using a Microcon Ultracel YM-10 (Millipore). Eight milliliters of 7.5 M guanidine hydrochloride (GndHCl) in 0.1 M TrisCl, pH 8.0, 1 ml of 0.2 M dithiothreitol (DTT), and 1 ml of 300 μ M CS solution were mixed and stored at 25°C for 2 h. Fifty microliters of 12 μ M PceT solution and 1,940 μ l of 50 mM TrisCl, pH 8.0, were added to the fluorometer cell and preincubated with stirring at 25°C for 2 min. The reaction was initiated by adding 10 μ l of 30 μ M GndHCl-denatured CS solution and done with stirring for 10 min at 25°C. The light scattering was measured using a Hitachi F-7000 fluorometer with excitation and emission at 500 nm. The spectral band width was 2.0 nm for both the excitation and emission. As a control experiment, bovine serum albumin was used in place of the PceT fusion protein.

Preparation of anti-PceA and anti-PceT antibodies

An anti-PceA polyclonal antibody was prepared as previously described (Suyama et al. 2002). PceT fused with a histidine tag was purified using a HisTrapHQ column and subjected to SDS-PAGE. The purified PceT fusion protein was used to raise the anti-PceT polyclonal antibody against rabbit.

Immunoprecipitation of PceT and PceA

The Y51 cells were harvested from a 400 ml culture by centrifugation. The cells were washed once with PBS buffer (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂PO₄, and 1.5 mM KH₂PO₄) and centrifuged. The cell pellet was resuspended in 1 ml of lysis buffer [25 mM TrisCl, pH 7.5, 0.1 M NaCl, 2 mM EDTA, 0.5% (*w/v*) Triton X-100, 10 mM DTT, and 4 mM 4-(2-aminoethyl) benzene sulfonyl fluoride hydrochloride] and sonicated. The cell lysate was centrifuged at 20,000 \times g for 15 min, and the supernatant containing the PceA and PceT proteins was used for the immunoprecipitation. As a control, the cell lysate from the mutant strain LD that lost all the *pce* genes was also prepared in the same fashion. To remove nonspecific

proteins, 1 ml of the cell extracts and 20 μ l of a slurry of 50% (*v/v*) ProteinA–Sepharose in the lysis buffer were mixed and incubated at 4°C for 1 h with shaking. The mixture was centrifuged at 8,000 \times g for 1 min at 4°C, and 0.8 ml of the supernatant was transferred to a new microtube. Five to ten micrograms of the anti-PceA or anti-PceT antibodies was added to the tube and incubated for 1 h at 4°C with shaking. Fifty microliters of a slurry of 50% (*v/v*) ProteinA–Sepharose in lysis buffer was added to the mixture and incubated for 1 h. The mixture was centrifuged at 8,000 \times g for 1 min at 4°C, and the supernatant was discarded. The precipitated ProteinA–Sepharose was washed five times with 1 ml of lysis buffer, followed by centrifugation and precipitation. Twenty microliters of the SDS-PAGE buffer (62.5 mM TrisCl, pH 6.8, 2% SDS, 5% (*v/v*) 2-mercaptoethanol, 0.005% (*w/v*) BPB, and 7% (*v/v*) glycerol) was added and incubated in a boiling water bath to elute the complex with the antibody from the ProteinA–Sepharose. The supernatant was loaded onto the SDS-PAGE gel. Proteins were transferred from the gel to the PVDF membrane. Immunoblotting using the anti-PceA or anti-PceT antibodies were done as previously described (Suyama et al. 2002).

Results

Sequence analysis of PceT

A *pce* gene cluster is comprised of *pceA*, *pceB*, *pceC*, and *pceT* in *D. hafniense* Y51. The BLAST search analysis revealed that PceT (PceT_Y51, AAW80326) shares a sequence similarity to the trigger factor (TF) proteins. The ribosome-associated TF is one of the major chaperons implicated in the folding process of the newly synthesized proteins. TF is an ATP-independent chaperon and displays chaperon and peptidyl-prolyl *cis/trans* isomerase (PPIase) activities *in vitro*. It is composed of at least three domains, an N-terminal domain, which mediates association with the large ribosomal subunit (Hesterkamp et al. 1997); a central substrate binding and PPIase domain with homology to the FK506 binding protein (FKBP; Stoller et al. 1995); and a C-terminal domain, which acts as the central module of the TF chaperone activity (Merz et al. 2006). In addition, the interaction between the structurally associated N- and C-terminal domains and the nascent peptide chains during the translation was revealed (Lakshmiathy et al. 2007).

PceT_Y51 encodes 316 amino acids with the calculated molecular mass of 37,050. Amino acids 3–34 and 43–130 in PceT_Y51 showed insignificant Pfam matches with the bacterial TF N-terminus (*E* value, 0.016) and FKBP (*E* value, 0.027), respectively. Amino acids 131–311 in PceT_Y51 showed significant Pfam matches with the TF

C-terminus (E value, $2.5e^{-17}$). The TF N-terminal domain of PceT_Y51 is characteristically shorter than that of the TF of *E. coli*. PceT showed a 99% amino acid sequence identity to the uncharacterized proteins from *Dehalobacter restrictus* PER-K23 (CAG70348) and *Dehalobacter dichloroeliminans* DCA1 (CAJ75433) among the protein databank. PceT_Y51 showed a 43% sequence identity to the putative trigger factor protein from *D. hafniense* DCB-2 (ACL18763). While PceT_Y51 showed a relatively low sequence identity (17–21%) to the TF proteins from *E. coli* (AAA62791), *Bacillus subtilis* (CAA99536), and *Streptococcus pyogenes* (AAC82391; Guthrie and Wickner 1990; Göthel et al. 1997; Lyon et al. 1998). Based on the genome information of *D. hafniense* Y51 (Nonaka et al. 2006), PceT_Y51 possesses at least two paralogues such as DSY3205 (20.3% homology) and DSY3514 (29.2% homology) in the genome of strain Y51. Unlike the *pceT* gene, both genes encoding DSY3205 and DSY3514 locate in the vicinity of the genes that are not related with PCE-dechlorination. DSY3205 and DSY3514 showed a higher homology with the TF protein of *E. coli* than that of PceT_Y51. Accordingly, both proteins showed significant Pfam matches with the bacterial TF N-terminus, FKBP, and TF C-terminus. According to the phylogenetic tree, the putative trigger factor proteins of dechlorinating bacteria such as PceT_Y51, CAG70348, CAJ75433, and ACL18763 have an evolutionarily close relationship, but are distant from the putative TF proteins of other nondechlorinating bacteria, DSY3205 and DSY3514 (Fig. 1).

Expression of PceT

To clarify the function of PceT, the *pceT* gene of strain Y51 was cloned and expressed as a fusion protein with 6x

His-tag in *E. coli*. The PceT fusion protein was produced as an insoluble protein at 37°C, but the decreased cultivation temperature resulted in the increased yield of a soluble PceT fusion protein. Thus, we expressed the *pceT* in *E. coli* at 20°C. The recombinant PceT fusion protein has a molecular mass of 38.8 kDa that corresponds to the expected molecular mass (Fig. 2).

Peptidyl-prolyl *cis*–*trans* isomerase activity

The PPI catalyzes a reaction from the *cis/trans* isomerization of the amino acid–proline peptide bond. PceT possesses the FKBP domain responsible for the PPI activity. To assess the PPI activity of the PceT fusion protein, we used the chromogenic peptide *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide as the substrate. In trifluoroethanol, approximately 88% of this compound has a *trans* conformation. Under the *trans* conformation of the Ala-Pro peptide, α -chymotrypsin can cleave the C-terminal side of Phe to liberate nitroanilide. During the *in vitro* PPI assay, the addition of the recombinant PceT fusion protein enhanced the release of nitroanilide in a dose-dependent manner (Fig. 3), indicating that the PceT fusion protein catalyzed the reaction that converts the substrate molecule from *cis* to *trans*.

Chaperon activity

In addition to the FKBP domain, PceT has a C-terminal conserved domain based on the amino acid sequence analysis. We determined the *in vitro* chaperon activity of the recombinant PceT protein. For this purpose, we chose citrate synthase (CS) as a chemically denatured substrate because CS, which tends to self-aggregate, is a well-

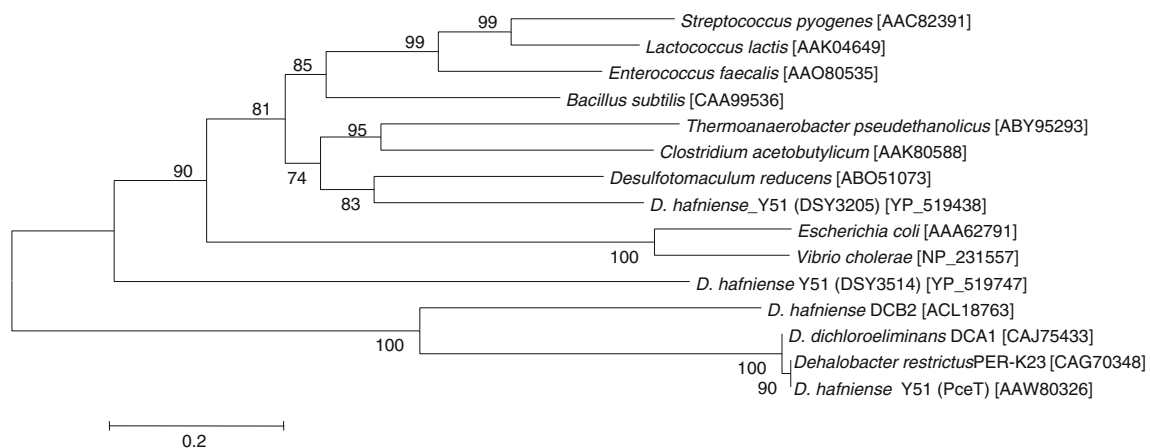


Fig. 1 Phylogenetic analysis of trigger factor proteins in bacteria. Amino acid sequences were obtained from the GenBank, RefSeq, and EMBL databases. Multiple alignment was performed using the ClustalW program in MEGA version 4.0 (Thompson et al. 1994;

Tamura et al. 2007). Unrooted tree was prepared according to the neighbor-joining method (Saitou and Nei 1987) using MEGA version 4.0. The scale bar represents a 20% sequence divergence. Numbers at the nodes represent bootstrap support values

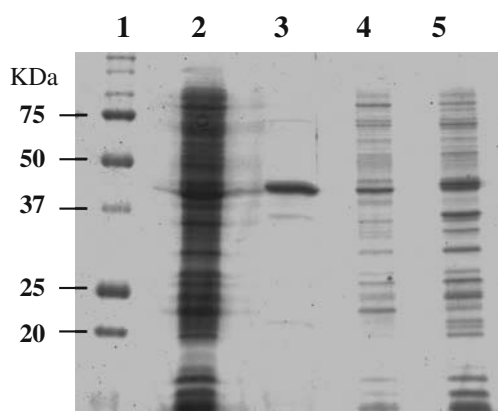


Fig. 2 SDS-PAGE of PceT fusion protein. Proteins were separated on 12.5% polyacrylamide gel and stained with Coomassie Brilliant Blue. Lane 1 mass marker proteins (Precision Plus Protein standards, Biorad, USA), lane 2 soluble fraction from *E. coli* with pET22b-pceT, lane 3 PceT fusion protein after purification by a His-trap column, lane 4 insoluble fraction from *E. coli* with pET22b-pceT, lane 5 total proteins from *E. coli* with pET22b

established substrate for various chaperons. Light scattering of the GndHCl-denatured CS solution in the absence or presence of the PceT fusion protein was monitored (Fig. 4). In the absence of the PceT fusion protein, the light-scattering intensities of the CS rapidly increased and reached a plateau within 100 s. BSA as a control showed a similar result, indicating that without PceT, the denatured CS was converted to the aggregated form within 100 s. On the contrary, the addition of PceT fusion protein resulted in a decreased intensity of the

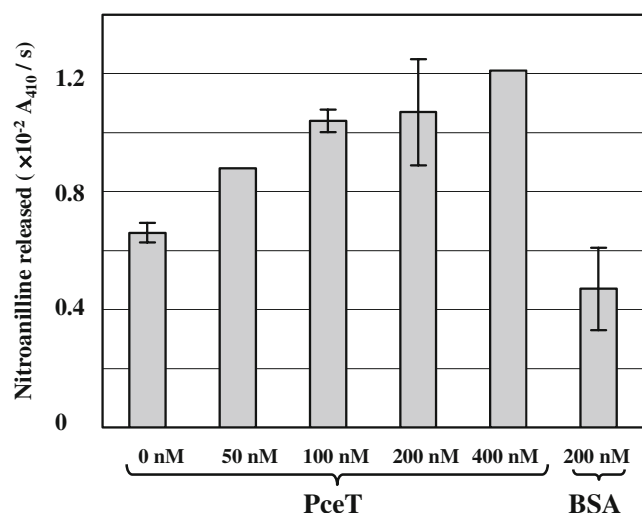


Fig. 3 Peptidyl-prolyl *cis-trans* isomerase activity. α -Chymotrypsin were preincubated in the spectrophotometer cell at 15°C for 10 min. The PceT fusion protein solution was then added, and after 5 min, the assay was initiated by adding peptide *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide solution in trifluorethanol. The *cis-trans* isomerization of the Ala-Pro bond, coupled with the chymotryptic cleavage of the *trans* peptide, was followed by the increase in absorbance at 410 nm

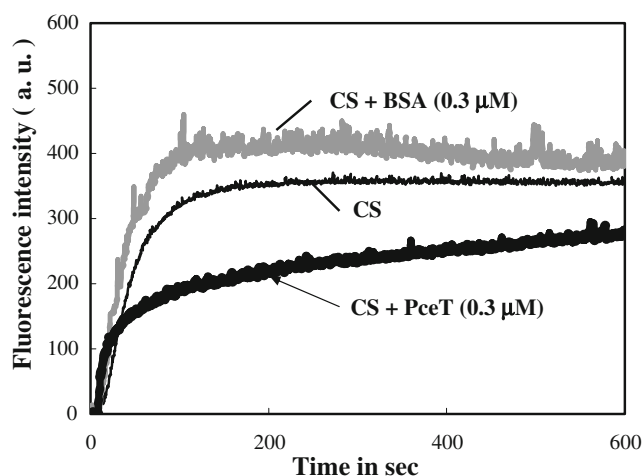


Fig. 4 Measurement of chaperon activity. The chaperon activity of the PceT fusion protein was measured using the chemically denatured citrate synthase (CS) by light scattering. CS was denatured with 6 M guanidine hydrochloride. The reaction was initiated by adding 3.0 μ M PceT to the denatured CS that had been diluted 200 times. Aggregation of the chemically denatured CS (0.15 μ M) was monitored as an increase in the light-scattering signal at 500 nm. As the control experiment, bovine serum albumin was used instead of the PceT fusion protein

light scattering of CS, and the fluorescence intensity gradually increased under the experimental conditions. Thus, the PceT fusion protein exhibited a chaperon activity *in vitro* toward the chemically denatured CS.

Immunoprecipitation of PceT and PceA

PCE dehalogenase PceA possesses a TAT signal sequence (SS) at the N-terminus and a corrinoid molecule as a cofactor (Suyama et al. 2002). PceA is localized in the periplasmic space after secretion via a TAT system. The TAT system requires correct folding of the proteins and incorporation of a cofactor in the cytoplasm (Lee et al. 2006). We investigated if PceT interacts with PceA and exerts a chaperon activity. We did immunoprecipitation experiments using the anti-PceA and anti-PceT antibodies. We prepared the cell extracts of strain Y51 to obtain the cytoplasmic precursor PceA (prePceA) with a TAT-SS and periplasmic mature PceA (mPceA) without the TAT-SS (Suyama et al. 2002). We also used the LD variant that lost all the *pce* genes as a control (Futagami et al. 2006b).

First, we confirmed that both the prePceA and mPceA (Fig. 5a and c, lanes 1) and PceT (Fig. 5b and d, lanes 1) were present in the cell extracts of the wt strain Y51 using respective antibodies. Also confirmed was the absence of all these proteins in the cell extracts of the LD variants (Fig. 5a–d, lanes 2 and 4). When the anti-PceA antibody was used to immunoprecipitate the wt Y51 cell extract, both prePceA and mPceA were detected by using the same antibody (Fig. 5a, lane 3). Interestingly, PceT was also

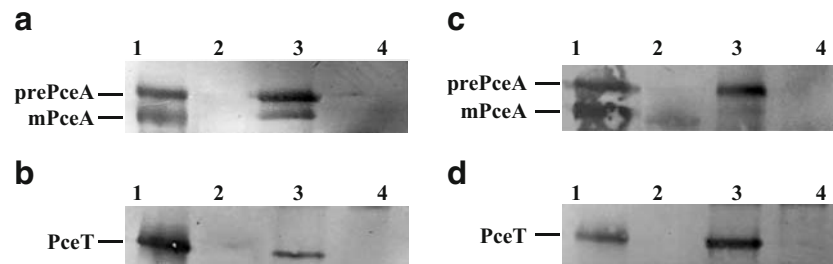


Fig. 5 Immunoprecipitation assay of PceA and PceT proteins The total cell extracts from the *D. hafniense* strains Y51 and LD were immunoprecipitated with the anti-PceA (**a** and **b**) antibody and anti-PceT antibody (**c** and **d**). In **a** and **c**, the anti-PceA antibody was used, and in those of **b** and **d**, the anti-PceT antibody was used, respectively. Lanes 1 and 2 represent the total cell extracts from wt strain Y51 and

strain LD without immunoprecipitation, respectively. Lanes 3 and 4 depict the total cell extracts from wt strains Y51 and LD after immunoprecipitation with the antibody–Sepharose, respectively. The cell extracts from wt strain Y51 contains both the precursor form (prePceA) and mature form (mPceA) of PceA. However, those from strain LD, as the negative control, contain neither PceA nor PceT

detected by the anti-PceT antibody for the immunoprecipitate fraction using the anti-PceA antibody (Fig. 5b, lane 3), indicating that PceT interacts with PceA to be pulled down by the anti-PceA antibody. To further verify this, we alternatively used the anti-PceT antibody to immunoprecipitate the wt Y51 cell extract. Naturally, PceT was detected (Fig. 5d, lane 3). More interestingly, prePceA, but not mPceA, was detected by the anti-PceA antibody (Fig. 5c, lane 3) for the same cell extract, indicating that only prePceA interacts with PceT to be pulled down by the anti-PceT antibody. These results imply that PceT specifically interacts with the precursor form of PceA in the cytoplasm and that PceT may bind to the TAT-SS of the nascent PceA protein.

Discussion

D. hafniense Y51 possesses at least three putative TF proteins such as PceT, DSY3205, and DSY3514. Among them, only the PceT-encoding gene is located in the *pce* gene cluster, and only PceT is structurally different from the TF protein of *E. coli* of which the function and structure are well characterized. The structural feature of PceT is characterized by the presence of a short N-terminal domain and by the fact that both the bacterial TF N-terminus and FKBP domains are less conserved than those of the other well-characterized TF. In the present paper, we successfully expressed the *pceT* gene in *E. coli* as a soluble form. Although the bacterial TF N-terminus and FKBP domains of PceT are less conserved, the recombinant PceT fusion protein exhibited PPI activity as well as chaperon activity in vitro, indicating that PceT could function as the trigger factor. This is the first report that the function of the *pce* gene cluster components, except for PceA, was experimentally demonstrated. We also demonstrated that PceT forms a stable complex with the precursor form of PceA present in the cytoplasm, but not with the mature form of PceA localized in the periplasm. PceA possesses a corrinoid

cofactor, which constitutes the active site of the PceA and the TAT signal sequence at the N-terminus, which is implicated in the translocation of the PceA protein to the periplasm. It is well understood that the TAT secretion system requires proper folding of the proteins and incorporation of the cofactor molecules prior to translocation of the membrane (Lee et al. 2006). This result allows us to suggest that PceT acts on prePceA through interaction with the TAT signal sequence before the cofactor incorporation.

In *E. coli*, TorA, a member of the dimethyl sulfoxide reductase family, is the main respiratory enzyme responsible for the trimethylamine oxide reduction under anaerobic conditions. TorA is located in the periplasm and receives electrons from TorC, a pentahemic *c*-type cytochrome. TorA and TorC are encoded by the *torCAD* operon. TorA crosses the inner membrane by the TAT machinery in the folded state. TorD is the specific chaperone of TorA. TorD interacts with apoTorA and allows it to become competent to receive the molybdenum cofactor. A second role was also attributed to TorD during the translocation process of TorA by the TAT translocon to prevent export of the immature TorA. During this proofreading mechanism, TorD binds the signal peptide of TorA and exhibits a quality control activity to ensure that only mature TorA is addressed to the TAT translocase. These two functions of TorD appeared independent (Ilbert et al. 2003; Hatzixanthis et al., 2005).

Similar to the specific interaction of TorD with TorA, PceT specifically interacts with prePceA because *pceA* and *pceT* are present in the same gene cluster similar to the *torCAD* operon. In strain Y51, the *pce* gene cluster is surrounded by the two nearly identical *ISDesp* sequences and situates within the complex transposon. Thus, *pceT* in the *pce* gene cluster is transposed together with *pceA*. The gene arrangement of these IS regions also frequently occurs (Futagami et al. 2006a,b). The TF proteins from the nondechlorinating bacteria, including DSY3205 and DSY3214, are evolutionarily distant from PceT. These TF proteins contain TF with a N-terminal domain longer

than that of PceT. On the contrary, PceT is very similar to other putative TF proteins found in the dechlorinating gene cluster from *Dehalobacter restrictus* PER-K23, *D. dichloroeliminans* DCA1, and *D. hafniense* DCB2. These facts are in agreement with the suggestion that PceT is a specific trigger factor for PceA.

The heterologous expression and production of the active PceA, PceB, and PceC have been unsuccessful to date. Recombinant technology of the dechlorinating bacteria has also been under development. Further biochemical and genetic analyses of the unknown PceB and PceC allow an in-depth understanding of the dehalorespiration mechanism mediated by the *pce* gene cluster in *D. hafniense* Y51.

Acknowledgments We thank Drs. Shinya Sugimoto, Fuminori Yoneyama, and Kenji Sonomoto for light-scattering analysis. This work was supported in part by a Grant-in-aid (Hazardous Chemicals) from the Ministry of Agriculture, Forestry, and Fisheries of Japan (HC-04-2321-1).

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