Supplementary Materials for

Structural basis for organohalide respiration

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Materials and Methods

Purification of the tetrachloroethene reductive dehalogenase (PceA)
The enzyme was purified from a *Sulfurospirillum multivorans* mutant strain. The mutant strain produced a PceA variant, which contained a C-terminal Strep-tag (PceA-Strep). *S. multivorans* was cultivated in 30 L pyruvate/PCE-containing medium (9). We obtained 4 mg of PceA-Strep from 3 g cell protein. All steps were conducted under anoxic conditions. The PceA-Strep was isolated from cell extracts via gravity flow using the Strep-Tactin Superflow column material (IBA, Göttingen, Germany). The Strep-Tactin column was equilibrated with buffer A (100 mM Tris-HCl, pH 8). PceA-Strep was eluted from the column material with buffer B (100 mM Tris-HCl, pH 8, 2.5 mM desthiobiotin, 5 mM Tris(2-carboxyethyl)phosphine). The elution buffer was replaced by the storage buffer (30 mM Tris-HCl, pH 7.5, 5 mM TCEP) via repeated concentration and resuspension of PceA-Strep in a Vivaspin 6 (30K) ultrafiltration unit (Sartorius, Göttingen, Germany). The enzyme was stored at -80°C. The PceA enzyme activity in the protein fractions used for crystallization (~1,800 nat/mg protein) was measured with methyl viologen (reduced with Ti(III) citrate) as low potential electron donor (8). When PceA-Strep was incubated under air (18°C), 50% of the enzyme activity was lost after three hours. This process was significantly slowed down by the addition of 5 mM TCEP, which resulted in a half-life time of 19 hours.

Crystallization and incubation with ligands
Crystallization, ligand incubation and flash cooling of crystals were performed under anoxic conditions in a glove box (model B; COY Laboratory Products, Grass Lake, MI) under an atmosphere of 95% N₂/5% H₂. Crystals were grown by the sitting drop vapor diffusion method at room temperature. To obtain the *P*₂₁ crystal form, 12 mg/ml of PceA in 30 mM Tris-HCl, pH 7.5, 5 mM TCEP was mixed at 1:1 ratio with a crystallization solution containing 15% (w/v) PEG 3350 and 0.2 M sodium malonate. The more robust *P*₄₁ crystal form used for substrate soaking experiments was grown by supplementing 2% benzamidine. Crystals were flash cooled in liquid nitrogen after protection in the crystallization solution supplement with 20% (v/v) glycerol and PEG 3350 to bring its
final concentration to 25%. For the iodide soak, 0.5 M NH₄I was added to the cryoprotectant. TCE-containing cryoprotectant not including benzamidine was saturated with trichloroethene (Sigma-Aldrich, St. Louis, MO) by vigorous shaking overnight and crystals were incubated in the solution for 5-10 minutes. Soaking with dibromoethene was performed in two steps: a 30-minute incubation in cryoprotectant without glycerol and a 5-minute soak with glycerol. For this purpose solutions were saturated with the commercially available cis-/trans-dibromoethene mixture (Sigma-Aldrich). Frozen crystals were removed from the anoxic atmosphere and from thereon stored and handled at 100K.

Data collection and refinement
Diffraction data were collected on BL14.1 operated by the Helmholtz-Zentrum Berlin (HZB), BL14.2 operated by the Joint Berlin MX-Laboratory at the BESSY II electron storage ring (Berlin-Adlershof, Germany) (23) and at beamline P11 at the light source PETRA III at DESY (Hamburg, Germany). Data were indexed and integrated with the XDS package (24) and XDSAPP (25), see Table S1. The structure of the P₂₁ crystal form was solved by native Fe,Co-SAD phasing with Phenix AutoSol (26). Restraints for norpseudo-B₁₂ were derived from the publicly available Vitamin B₁₂ dictionary prepared by Dr. Oliver Smart (Global Phasing Inc., Cambridge, UK) using the Grade Server v1.001. Other ligand restraints were generated using eLBOW (27) and the structure built in COOT (28), refined with phenix.refine (29) and validated with Molprobity (30). Residues 1-411 and 431-462 were placed in the P₄₁ and P₂₁ crystal forms, while residues 412-430 were modeled only in chain A of the P₂₁ form. TCE was modeled into the Fₒ-Fc difference map, while cis-dibromoethene and iodide were modeled into the anomalous difference map for the halide. Structural homologues were identified using the Dali server (31).
**Fig. S1.** The PceA fold. Regions are colored throughout as follows: N-terminal unit (blue, residues 1-138), norpseudo-B$_{12}$ binding core (purple, residues 139-163 and 216-323), insertion unit (green, residues 164-215), iron-sulfur cluster binding unit (yellow, residues 324-394) and C-terminal unit (red, residues 395-462), containing helix 15 not observed in all protomers. **A)** Secondary structure representation of a PceA protomer. The norpseudo-B$_{12}$ cofactor (purple) and residues forming the substrate-binding pocket (blue, yellow, purple) are shown as sticks and [4Fe-4S] clusters as ball and stick. **B)**
Representation of the dimer viewed down the non-crystallographic two-fold axis, additionally showing the bound TCE substrate (spheres). C) Topology diagram, norpseudo-B$_{12}$ binding residues are indicated by void circles.
**Fig. S2.** Determination of the apparent molecular mass of PceA-Strep. Size-exclusion chromatography was conducted with the concentrated eluate from the Strep-Tactin affinity chromatography, which was loaded onto a HiLoad 16/60 Superdex 75 prep grade column (GE Healthcare) equilibrated with the following buffer: 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.5 mM DTT, 5 mM TCEP. The apparent molecular mass of the protein (void triangle) was calculated from its retention [elution volume/void volume ($V_e/V_0$)] in comparison with those of standard proteins (filled triangles). The shown data represent the average of two independent experiments with two replicates, respectively. As standard proteins albumin (dimer 134 kDa, monomer 67 kDa) and ovalbumin (dimer 86 kDa, monomer 43 kDa) were used.
Fig. S3. **Structure alignment of the central B$_{12}$-binding core.** Residues 139-163 and 216-323 of PceA (purple) were aligned with A) Human methylmalonic aciduria $cblC$ type with homocystinuria type C protein (cream, PDB: 3SOM, Dali server Z-score: 7.0)
and B) members of the nitroreductase-family: *Escherichia coli* FMN-dependent flavoprotein nitroreductase (PDB: 1DS7, Z: 4.2), *Agrobacterium tumefaciens* FMN-containing oxidoreductase (PDB: 2FRE, Z: 4.1), *Lactococcus lactis* copper-induced nitroreductase CinD (PDB: 2WQF, Z: 4.5) and *Helicobacter pylori* metronidazole activating nitroreductase RdxA (PDB: 3QDL, Z: 4.4), all colored light blue. Other structural elements of PceA as well as the [4Fe4S]-clusters (spheres) are shown as faint outlines.
**Fig. S4. Substrate channel and pocket of PceA.** A) Cut surface representation colored as in Fig. S1, showing the norpseudo-B$_{12}$ cofactor as sticks and TCE as spheres. The top insert shows the entrance to the substrate channel - the letter box - constrained by Thr39,
Phe44, Phe57 (N-terminal), Leu186 and Glu189 (Insertion). Residues lining the substrate pocket and channel are additionally shown as white spheres. The lower insert shows a perimeter fence formed by Arg305, Asn272, Trp376, Phe38 and norspeudo-B₁₂ side chains just above the corrin ring, restricting access to the cobalt to water / small ions. Atoms closest to and in plane with the water ligand are shown as spheres and their distances indicated. B) Van-der-Waals representation of hydrophobic amino acid side chains forming the substrate-binding pocket. TCE and the [4Fe-4S] cluster are shown as spheres. Trp376 was omitted for clarity, but its position is indicated.
Fig. S5. Sequence alignment of known RDases. Regions are colored and residues binding substrate and cofactors indicated in reference to S. multivorans PceA. Selected RDases from the following organohalide-respiring microorganisms were aligned with Clustal Omega (32): PceA Sm - Sulfurospirillum multivorans PCE/TCE RDase
(AF022812), PceA_SmSL2 Sulfurospirillum sp. SL2 PCE-only RDase (AGW23615), PceA_Dres Dehalobacter restrictus DSM 9455 PCE/TCE RDase (AHF10423), PceA_DhY51 Desulfitobacterium hafniense Y51 PCE/TCE RDase (YP519072), RdhA3_Dhaf Desulfitobacterium hafniense DCB-2 dichlorophenol (DCP)/PCE RDase (YP002457196), DcaA_Ddi Desulfitobacterium dichloroeliminans LMG P-21439 dichloroethane (DCA) RDase (CAJ75430), PceA_Dhc195 Dehalococcoides mccartyi 195 PCE/TCE RDase (DET0318), BvcA_DhcBAV Dehalococcoides mccartyi BAV1 TCE/DCE/DCA RDase (AAT48558), VcrA_DhcVS Dehalococcoides mccartyi VS vinylchloride reductase (AAQ94119), CprA_Ddh Desulfitobacterium sp. PCE-1 o-chlorophenol reductive RDase (AF259790). (!) Indicate strictly conserved, (*) partially conserved residues.
Fig. S6

**Fig. S6. Active-site electron density maps**  
**A)** TCE (Two orientations, modeled with 20% and 50% occupancy are overlaid. Of the chloride atoms closest to W56, only the one adjacent to the label is present in each case).  
**B)** cis-DBE,  
**C)** water/empty binding site,  
**D)** iodide. In detail:  
1σ 2mFo−Fc (A, blue, TCE and water),  
4σ mFo−Fc (A, green, TCE),  
8σ anomalous difference (B, purple, cis-DBE),  
1σ 2mFo−Fc (B, blue, cis-DBE),  
1.2σ 2mFo−Fc (C, blue, water),  
5σ anomalous difference (D, purple, iodide),  
1σ 2mFo−Fc (D, blue, iodide). Distances between cobalt and β-ligand are indicated.
Table S1.
Data collection and refinement statistics

<table>
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<tr>
<th>Data collection</th>
<th>6 protomers/ASU</th>
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<th>dibromoethene</th>
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<td>(highest resolution shell)</td>
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<td>99.59 (96.00)</td>
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<td>99.88 (98.84)</td>
<td>99.81 (98.16)</td>
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<td>Mean I/σ(I)</td>
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* Criterion for resolution cut-off: Mean I/σ(I) > 2.
* Redundancy-independent merging R-factor (33)
* Precision-indicating merging R-factor (34)
* Pearson correlation coefficient of half data sets (35)
Values in parentheses refer to the highest resolution shell
References and Notes


7. Materials and methods are available on Science Online


