Development of new strategies to produce and characterize PceC, a membrane-bound flavoprotein involved in organohalide respiration

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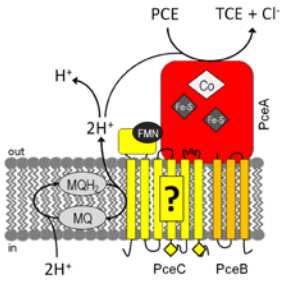
INTRODUCTION

- Organohalide respiration (OHR) is a bacterial anaerobic process in which chlorinated compounds, *e.g.* tetrachloroethene (PCE), are used as terminal electron acceptor (1).
- Our model organisms are *Dehalobacter restrictus* and *Desulfitobacterium hafniense*.
- PceA is the well characterized reductive dehalogenase (RdhA) that catalyzes PCE dechlorination.
- PceC was predicted to be an integral membrane protein with six trans-membrane α-helices, a peripheral domain and two conserved CX_3CP motifs (2).
- The peripheral domain harbours a conserved motif for covalent binding of a flavin mononucleotide (FMN) cofactor.



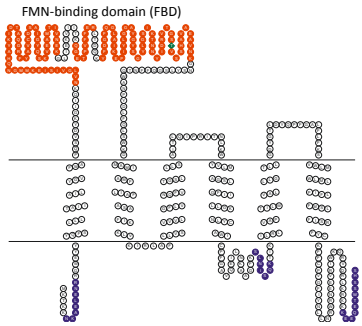
Tentative model of RDH complex in OHR, taken from (2).

Flavin-binding domain (FBD)

- **What is the topology of FBD in the membrane?**
- **What is the redox potential of the flavin in FBD?**

Surfaceome analysis of *D. restrictus* cells with trypsin confirmed the predicted topology of PceC. FBD is exposed to the exocytoplasmic face of the membrane.

This finding is in line with the hypothesis of a possible electron transfer between PceC and PceA.



Topology of PceC protein. Peptides in color were identified by MS analysis: in red, peptides obtained after shaving the cell surface; in blue, peptides exclusively found in lysed cells.

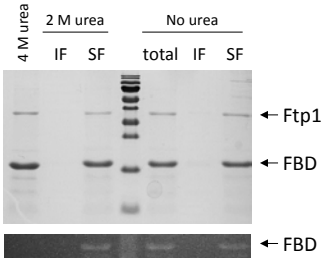
IB + 8 M urea
↓ 2h dialysis
4 M urea denatured FBD
Ftp1 + FAD ↓ 1h30 dialysis
2 M urea denatured FBD
Ftp1 ↓ 1h30 dialysis
No urea

Protocol for the reconstitution of FBD from inclusion bodies (IB).

The production of FBD in *E. coli* and its reconstitution from inclusion bodies (IB) follows a protocol established earlier (2) and refined here.

The use of a flavin transferase (Ftp1) from *D. hafniense* was required to bind FMN covalently to FBD.

The reconstitution of FBD with FMN following the new protocol made it fully soluble. Mass spectrometry analysis showed that 100% of FBD was loaded with FMN (data not shown).



FBD reconstitution. SDS-PAGE gel stained with Coomassie (top panel), and under UV illumination (bottom panel). IF: insoluble fraction; SF: soluble fraction.

The **redox properties** of FMN in FBD will be determined with redox titration and spectroelectrochemistry.

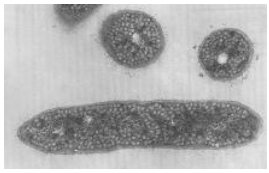
An **assay for electron transfer** between FBD and PceA will be developed using cell extracts of *D. restrictus*.

The complete PceC protein

- **How to produce the complete PceC protein?**
- **Is PceC in stoichiometric amount with PceA?**

Expression of the complete PceC protein in *E. coli* failed so far, most likely due to its toxicity.

Rhodobacter capsulatus represents an alternative host for the expression of PceC, as it produces large amounts of internal membranes (3). The *pceC* gene was recently cloned in two different expression plasmids for *R. capsulatus*.



Rhodobacter capsulatus (courtesy from the Institute of Biotechnology, Grenoble).

Preliminary proteomic analyses in *D. hafniense* revealed that PceC and PceB appear in good stoichiometric relationship, however in largely lower amount than PceA.

Protein	LFQ*
PceA	$1.51 \cdot 10^{11}$
PceB	$6.18 \cdot 10^{9}$
PceC	$3.10 \cdot 10^{9}$

* LFQ: label-free quantification

Analysis of PceC detected peptides indicates several candidates to be used in quantitative proteomic analysis. This technique will be used for each protein of the putative RDH complex.

PceC peptides	# detected spectra
FYAVCDSAIGYQSK	56
TNNYIDR	52
QGETPVFFER	46
NVLGVISEK	41
VTGSTVSSHAVAEAVNK	40
EPIYLGGAYGYSGYLGSIK	38
YFDGFQGLAIK	26
EEQETWSSHS	21
IDTAQGR	20
NKAELENR	15
KEEQETWSSHS	8
VEAMTIVNEK	8
SLNISQK	7
MMGNQHAYK	1

Stoichiometric analysis of PceA, B and C proteins

Quantitative proteomics will be applied to membranes of *D. restrictus* in order to evaluate if PceC builds a possible complex with PceA and PceB.

Identification of a possible PceABC protein complex

Blue-Native PAGE will be used to determine whether PceC participates to a membrane-bound RDH protein complex together with PceA and PceB.

References

(1) Schubert *et al.* 2018, *FEMS Microbiol Ecol* **94**:fiy035 ; (2) Buttet *et al.* 2018, *Front Microbiol* **9**:755; (3) Katzke *et al.* 2010, *Protein Exp Purif* **69**:137.