Poster# K-773

The Role of the Tetraheme Cytochrome c_3 in *Desulfovibrio vulgaris* Hildenborough Metabolism

Elizabeth S. Semkiw, Grant M. Zane, and Judy D. Wall

1. Lawrence Berkeley National Lab, 2. University of California - Berkeley, 3. University of Missouri--Columbia

Contact:
The University of Missouri, Department of Biochemistry
211 Schweitzer Hall
Columbia MO 65211
Telephone: (573) 882-9771; Fax: (573) 882-5635

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Abstract

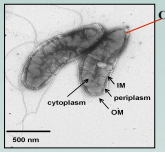
The role of tetraheme cytochrome c_3 (CycA) in the metabolism of the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (DvH) was investigated by deletion of the *cycA* gene using a marker-exchange deletion strategy. A highly abundant periplasmic cytochrome, CycA has the important function of transferring electrons from periplasmic hydrogenases (Hyd, Hyn, Hys) to transmembrane complexes which transport the electrons to the cytoplasm where sulfate is reduced. Previous studies have indicated that during its interaction with periplasmic hydrogenases, CycA is also involved in the reduction of toxic metals.

Growth of the cycA mutant strain on lactate as the electron donor and sulfate as the terminal electron acceptor showed that, despite its abundance, CycA is not essential for DvH growth. However, the rate of growth of the mutant strain was significantly lower, and the extent of growth less, than rates and extents of growth of the wild type and complement strains on lactate/sulfate medium. This indicates that a portion of the electrons generated from cytoplasmic lactate oxidation are transported by CycA for energy production, possibly in a hydrogen cycling mechanism employed to generate ATP. Failure of the mutant strain to grow on either formate or H_2, with sulfate or sulfite as electron acceptors, further indicated that CycA may be the only redox partner of periplasmic hydrogenases.

The cycA mutant strain also did not grow as well as either the wild type or complement strains on medium supplemented with pyruvate/sulfate. Final growth on pyruvate/sulfate was comparable, but the mutant grew more slowly than the wild type and complement strains. Interestingly, the mutant grew better than the wild type or complement strains on pyruvate alone, possibly due to the release of H_2 and/or CO_2 in concentrations which may be somewhat inhibitory to wild type growth.

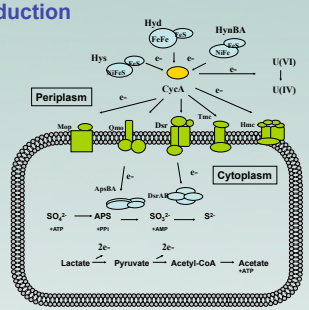
Introduction

Sulfate-reducing Bacterium



Tetraheme cytochrome c_3 (CycA) is a highly abundant electron carrier protein in the periplasm of *Desulfovibrio* species.

Deletion of the *cycA* gene in *D. vulgaris* Hildenborough (DvH) is allowing continued investigation of the role of CycA in the reduction of sulfate as well as toxic metals including uranium.

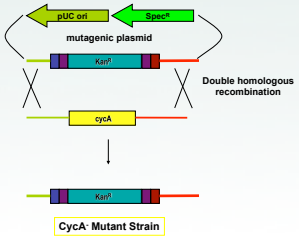


Potential pathways of electron transport in DvH involving CycA

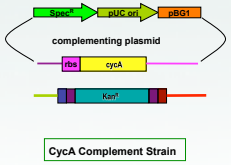
CycA⁻ Mutant and Complement Strain Construction

Predicted cycA operon *www.microbesonline.org

A marker-exchange deletion strategy was used to construct the CycA⁻ mutant strain lacking DVU3171



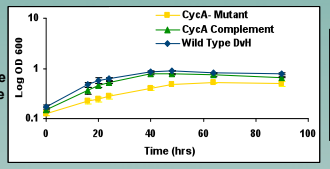
Transformation of the CycA⁻ mutant strain with a stable complementing plasmid reintroduced DVU3171 (*cycA*)

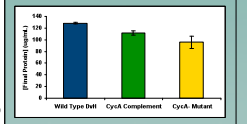


CycA Complement Strain

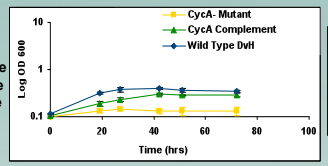
Results of Growth Studies

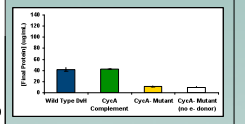
60mM Lactate
30mM Sulfate



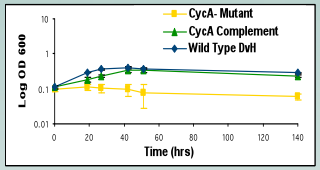


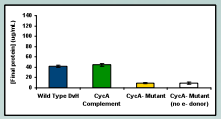
50mM Formate
20mM Acetate
30mM Sulfate





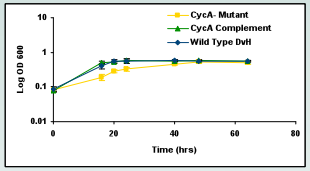
100% H_2 (atm)
20mM Acetate
30mM Sulfate

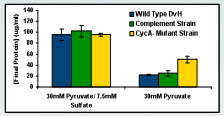




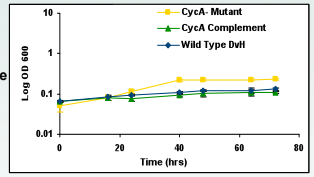
CycA transports a portion of electrons generated by lactate oxidation but, because the CycA⁻ mutant strain does not appear to grow on H_2 or formate, CycA may be the only redox partner for periplasmic hydrogenases.

30mM Pyruvate
7.5mM Sulfate





30mM Pyruvate

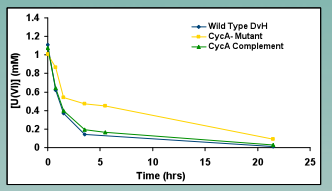


CycA deletion affects the rate but not the extent of growth on pyruvate/sulfate.

Interestingly, better fermentative growth on pyruvate is observed with deletion of *cycA*. This may be the result of the release of H_2 or CO_2 in concentrations inhibitory to wild type growth.

CycA Involved in Uranium Reduction

1mM Uranium(VI)
20mM Lactate



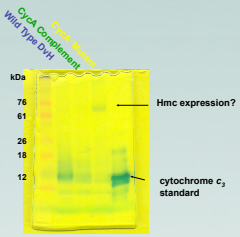
Data points are the average of duplicates

U(VI) Reduction Rates (umols/mg protein/hr) with Organic Acid Electron Donors		
	Lactate	Pyruvate
Wild Type DvH	0.61	0.52
CycA Complement	0.63	0.65
CycA⁻ Mutant	0.38	0.06

Initial rates determined at 3.5 hrs.

Uranium (VI) reduction assays were performed with washed whole cells grown on lactate/sulfate. A kinetic phosphorescence analyzer (KPA) was used to quantify U(VI) (as uranyl acetate).

CycA Deletion Confirmed by Heme Stain



For confirmation of CycA deletion in the mutant strain, total proteins of whole cell extracts were separated using SDS-PAGE, and heme-containing proteins were stained.

When grown on lactate and sulfate, the CycA⁻ mutant appears to express a high molecular weight cytochrome – possibly Hmc (65kDa), a transmembrane complex.

Conclusions

- CycA is important, but not essential, for DvH growth
- CycA can be circumvented in sulfate reduction when organic acids supply electrons
- Hmc transmembrane complex may be expressed to compensate for the loss of CycA
- CycA may be the only redox partner of periplasmic hydrogenases
- CycA participates in uranium reduction but can also be bypassed when organic acids supply electrons

ACKNOWLEDGEMENTS

ENIGMA is a Scientific Focus Area Program supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Foundational Science through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.