Functional analysis of the *Coxiella burnetii* protein Com1

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INTRODUCTION

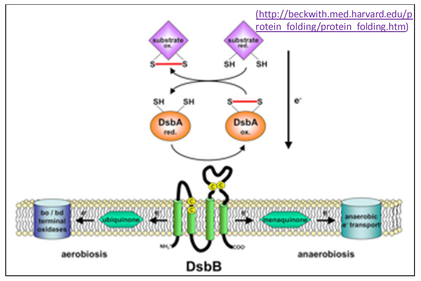
Function of Com1 in *C. burnetii* is unknown

C. burnetii is an obligate intracellular, Gram-negative bacterium and is the causative agent of Q-fever.

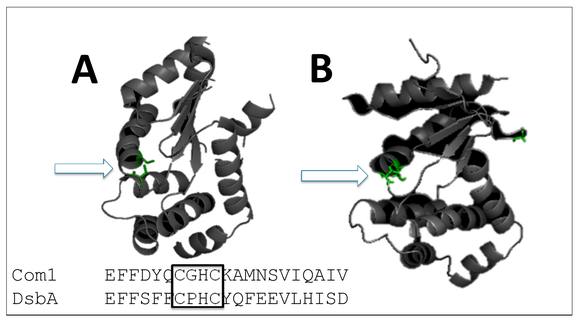
Com1 is an immunoreactive and putative outer membrane protein with a homologous catalytic site to disulfide bond oxidoreductase (DsbA) in *E. coli.*

DsbA-like proteins are associated with pathogen virulence due to vital disulfide bonds in some exotoxins and motility mechanisms.

There is no established protein folding mechanism in *C. burnetii*



Oxidative protein folding mechanism via DsbA in *E. coli*



Catalytic site similarity (CXXC) between the proposed structure of Com1 (**A**) and the structure of DsbA (**B**).

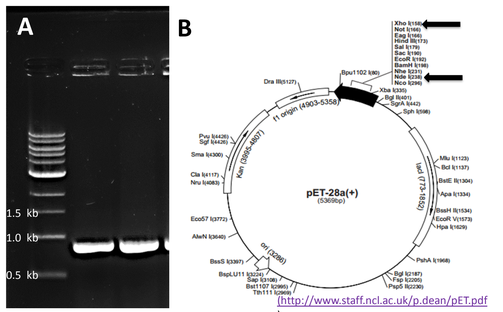
OBJECTIVE

To investigate the function of Com1 in *C. burnetii*

RESULTS

Cloning of com1

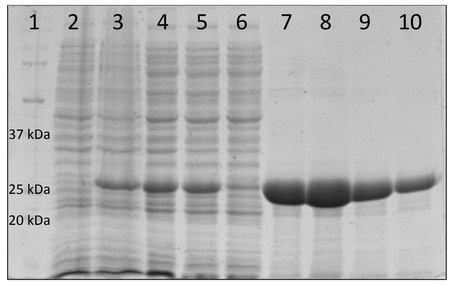
com1 was amplified using PCR and cloned into the pET28a expression vector



Figures
(**A**) Amplified *com1*: *C. burnetii* genomic DNA was used to amplify the *com1* gene, ~0.7 kb.

(**B**) Map of pET28a expression vector: recombinant Com1 was designed with a 6His tag on the C terminus. The final construct was sequenced to verify no presence of mutations.

Purification of Com1



Com1 was purified using an immobilized metal affinity chromatography (IMAC) column

Figure: SDS-PAGE gel of Com1 (27 kDa) purification fractions. Lane 1: Protein standards in kDa; Lane 2: Uninduced culture; Lane 3: Culture induced with IPTG to promote Com1 production; Lane 4: Total sample; Lane 5: Soluble fraction; Lane 6: Flow through Lanes 7-10: elution fractions

Com1 concentration after purification was determined through Bradford assay to be 5.09 mg/mL

Com1 on whole cell *C. burnetii* is Proteinase k sensitive

As Proteinase k concentrations increase, western blot shows amount of Com1 in whole cell *C. burnetii* decreases

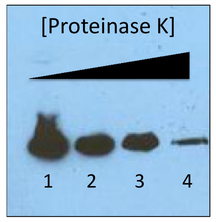


Figure: 30 µL of *C. burnetii* cells (~10^{10} cells) were treated with Proteinase k in presence of 10 mM $MgCl_2$ at 37° C for 2 hours. Cells were pelleted and run on SDS-PAGE gel and western blot was done using anti-Com1 mouse monoclonal antibodies. Proteinase k concentrations- Lane 1: 0 µg/mL; Lane 2: 50 µg/mL; Lane 3: 200 µg/mL; Lane 4: 50 0 µg/mL.

Stipend support was provided by the Department of Veterinary Pathobiology, University of Missouri and project supplies were provided by NIH/NIAID 1RO1AI083364-01 to Guoquan Zhang.

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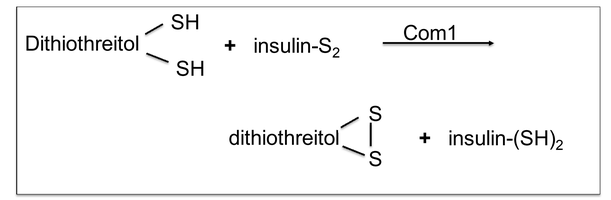
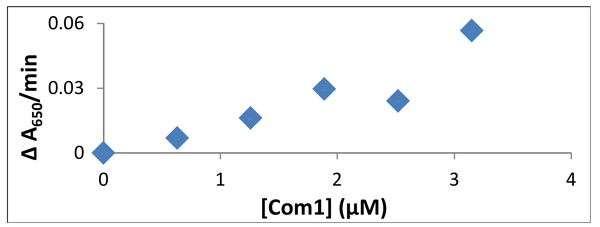
RESULTS

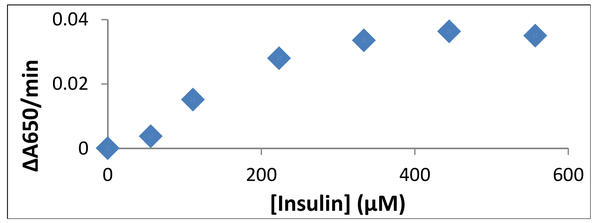
Com1 can function as an oxidative, disulfide forming enzyme



Insulin B chain will precipitate, increasing the A_{650}, in the presence of DTT with a thioredoxin enzyme, we tested Com1 as the enzyme in this reaction.



As concentration of Com1 increases, absorbance at 650 per minute increases. This indicates Com1 can catalyze the precipitation of insulin B chain in this reaction.



As concentration of insulin increases, absorbance at 650 per minute increases. This indicates that the K_m, the substrate concentration at which the reaction reaches half the maximum rate, is approximately 200 uM.

CONCLUSIONS

Com1 is a DsbA-like oxidoreducase

Com1 has enzymatic function and may fold proteins outside of the cell and have a role in the cell's pathogenicity.

Future Studies:
Understanding the role of Com1 *in vivo* through DsbA complementation studies

Detailed characterization of localization of Com1