Characterization of Cystathionine Beta Synthase in *Cyclospora Cayetanensis* with Comparative Species Analysis

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Abstract

Cyclospora cayetanensis is an apicomplexan protozoan parasite causing a food-borne diarrheal disease called cyclosporiasis. Molecular mechanisms underlying the pathogenesis of the disease, and mechanisms related to development and environmental survival of the parasite, are not known. Protozoan parasites rely on cysteine residues to construct the active site of cysteine proteases. Cysteine proteases contribute to the pathogenesis of protozoan parasites. Cysteine is a product of the transsulfuration pathway. This metabolic pathway begins with cystathionine beta synthase (CBS) catalyzing the condensation of homocysteine to cystathionine, a substrate later converted into cysteine by Cystathionine gamma-lyase (CS). The presence of the CBS protein has been confirmed in *Cyclospora cayetanensis*. While the CBS sequence and structure are well defined in higher eukaryotes, further analysis is required to understand the function of this protein in protozoan parasites, including *C. cayetanensis*. We performed a multiple sequence alignment of CBS protein sequences among *C. cayetanensis* and other protozoan parasites, including *Eimeria tenella, Toxoplasma gondii, Leishmania braziliensis, Leishmania major, and Tryposanoma cruzi*, to search for conserved domains/residues along the CBS protein sequence, and found that the *C. cayetanensis* CBS protein is divided into two regions: the Pyridoxal phosphate (PLP) catalytic domain and the Bateman module. These regions are well conserved among higher and lower eukaryotes. We searched for solvent accessible heme-binding motifs along the CBS protein using Hemoquest and RaptorX programs. These motifs were absent in *C. cayetanensis*, as well as in other lower eukaryotes, suggesting that the regulation of the function of *C. cayetanensis* CBS protein was independent of heme as well as the modulation of the oxidoreductase motif. This study demonstrates that the *C. cayetanensis* CBS protein may function similarly to other protozoan parasites.

Methods

The *C. cayetanensis* reference CBS sequence was accessed from the NCBI public database(Genbank Accession No. OEH77947.1). Conserved domains were confirmed using Genome workbench to construct a multiple sequence alignment of the CBS sequences of both higher and lower eukaryotes, including *Cyclospora cayetanensis*, *Eimeria tenella*, *Homo sapiens*, *Leishmania braziliensis*, *Leishmania major*, *Saccharomyces cerevisiae*, *Tryposanoma cruzi*, and *Toxoplasma gondii*. Predicted *C. cayetanensis* CBS ligand binding sites, including heme binding motifs, were identified with Hemoquest and confirmed with the returned RaptorX solvent accessibility values. Known residues and motifs related to CS activity, ubiquitination, binding, and oxido-reductase confirmed with Genome Workbench multiple sequence alignment. Phylogenetic tree of CBS enzymes belonging to organisms of interest were constructed using Genome Workbench.

Results: Multiple Sequence Alignment

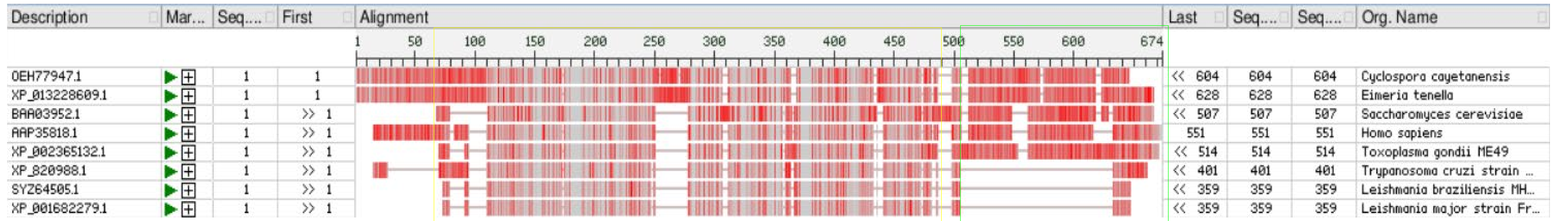

Figure 1: Genome Workbench MUSCLE alignment of CBS proteins belonging to higher and lower eukaryotes. Species listed are as follows: *C. cayetanensis*(top*), E. tenella, S. cerevisiae, H. sapiens, T. gondii, T. cruzi, L. braziliensis, and L. major*(bottom). PLP catalytic domain annotated by the yellow box on the left. Bateman module annotated by green box to the right.

Results: Hemoquest and Solvent Accesibility

Hemoquest Motif	Solvent Accessibility Value	K_D Value(μM)	Potential Heme Binding Motif
PSDRYFMRL	33.7% Exposed 39.3% Medium 27.1% Buried	0.13 μM	-
ERAIHAIKK	42.8% Exposed 23.6% Medium 33.6% Buried	0.62 μM	-
RSKMCIKKL	42.9% Exposed 30.8% Medium 26.2% Buried	0.38 μM	-

Figure 2: Returned Hemoquest motifs within the *C. cayetanensis* CBS sequence. Potential heme-binding motifs evaluated based on K_D value and solvent accessibility.

Results: RaptorX

RaptorX Solvent Accessibility Values:
Buried Medium Exposed

Motif Solvent Accessibility Distribution:

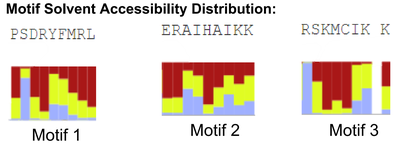

Figure 3: RaptorX residue solvent accessibility distribution for figure 2 potential heme-binding motifs. Percent area of exposed, medium, and buried used to assess motif's binding potential in figure 1.

Results: Conserved Residues

Conserved Residues	Presence	Location
CS Activity Lysine Residue 1	+	Lys-142
CS Activity Lysine Residue 2	+	Lys-327
CS Activity Lysine Residue 4 (PLP Binding)	+	Lys-153
Pyridoxal Phosphate-binding Motif (PXXSVKDR)	+	Gly-314 Thr-315 Gly-316 Gly-317
Oxido-reductase Motif (Cys-X-X-Cys)	-	Leu-330 X X Cys-333
Ubiquitination Lysine Residue 1	+	Lys-91
Ubiquitination Lysine Residue 2	-	Thr-588
Heme Binding Cysteine Residue	-	Ile-67
Heme Binding Histidine Residue	-	Arg-84

Figure 4: MUSCLE alignment comparison of known conserved CBS residues and *C. cayetanensis* CBS residues. Presence and location of *C. cayetanensis* residues confirmed in columns two and three, respectively.

Results: Phylogeny

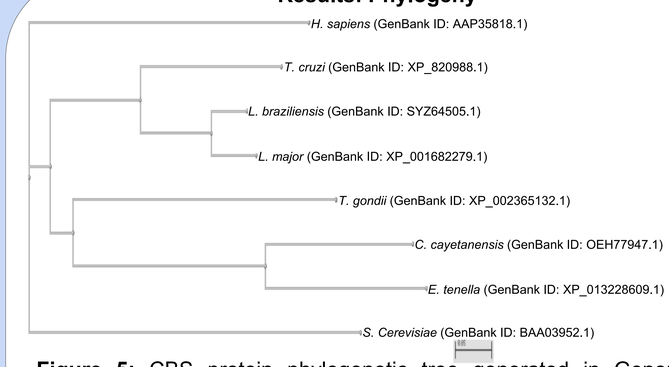

Figure 5: CBS protein phylogenetic tree generated in Genome Workbench. Organisms include protozoan parasites(*C. cayetanensis, E. tenella, T. gondii, L. major, L. braziliensis, and T. cruzi*), higher eukaryotes(*H. sapiens*), and unicellular fungi(*S. cerevisiae)*

Conclusions

- The *C. cayetanensis* CBS sequence is divided into two regions: the PLP catalytic domain and the Bateman module.
- *C. cayetanensis* lacks a heme binding motif at the N-terminal of the CBS sequence. *C. cayetanensis* CBS enzyme does not rely on heme-dependent regulation during redox conditions.
- Further experimental analysis, including a coupled-coupled assay should be performed to determine changes in enzymatic activity in the presence of Heme and SAM, which acts as an allosteric activator in the Bateman module of higher eukaryotes.
- Similarly to other lower eukaryotes, *C. cayetanensis* lacks the oxidoreductase motif, C-X-X-C, indicating CBS enzyme downregulation does not depend on the modulation of the oxidoreductase motif.
- Lysine residues required for CS activity in the transsulfuration pathway to convert cystathionine into cysteine and α-ketobutyrate.

References

Acknowledgements
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Public Disclosure Abstract: *Cyclospora cayetanensis* is a parasite responsible for large outbreaks in the US. Despite public health importance, disease pathogenesis of the organism is poorly understood. We used publicly available data and analysis tools to characterize *C. cayetanensis* Cystathionine Beta Synthase, which is involved in the development and pathogenesis of related parasites.