CztABCD of Haemophilus influenzae is a high affinity Cu++/Zn++ ABC transporter

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Divalent metal ions are essential cofactors in a number of cellular processes. Cu**, Zn** and Mn** play an important role in bacterial survival and proliferation in the environment as well as within the host. One of the classical examples is protection of a pathogen from free radicals generated by host cells. On the other hand, transition metals are highly toxic if accumulated inside the cells, hence uptake and efflux of transition metals in bacterial cells is regulated stringently. The H. influenzae KW20 genome has a total of 138 ABC transporters, among them 44 are assigned for transport of transition metals. In genetic annotation Cu**-ABC transporters are not defined and very little is known about transition metal uptake system in H. influenzae except Fe** and Fe**⁺⁺ sequestration. The ABC transporter system we identified here is a high affinity Cu** and Zn** transporter. The name assigned here Copper Zinc ABC transporter (CztABCD) includes CztA metal ion binding periplasmic protein, CztBC membrane spanning permeases and CztD a cytoplasmic nucleotide binding domain. In our experiments, recombinantly expressed and purified CztA was tested for its binding specificity with transition metals and divalent cations by using fluorescence spectrophotometry. The highest binding affinity was found with Cu** (K_d=0.35 ± 0.1µM) and for Zn** (K_d=25 ± 2.5µM). Other divalent metal ions like Fe** and Fe**⁺⁺ have >60 µM K_d value and likely not to be the ligand for Czt ABC transporter. CztA was modeled by using Swiss-Model automated server, where MntC (Manganese binding protein) from Synechocystis sp. (PDB entry-1xvl) was used as template (52.1% similarity and 67% identity). The active site residues H58, D264, H123, H125 and D189 showed different extent of involvement in ligand coordination.

<table>
<thead>
<tr>
<th>Organism name</th>
<th>Protein</th>
<th>% identity</th>
<th>% similarity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versinia pestis</td>
<td>A6BP7</td>
<td>64</td>
<td>74</td>
<td>Fe** transporter</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Q7CPX8</td>
<td>63.4</td>
<td>77.3</td>
<td>Mn** transporter</td>
</tr>
<tr>
<td>Rhizobium leguminosarum</td>
<td>Q1MG7</td>
<td>62.9</td>
<td>74.2</td>
<td>Mn** transporter</td>
</tr>
<tr>
<td>Escherichia coli (APEC)</td>
<td>Q7TT4</td>
<td>64.1</td>
<td>78.1</td>
<td>Fe** and Mn** transporter</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>Q5S280</td>
<td>52</td>
<td>67</td>
<td>Mn** transporter</td>
</tr>
<tr>
<td>Streptococcus pneumoniae POAAG2</td>
<td>32</td>
<td>54.7</td>
<td>Adhesion and virulence lipoprotein</td>
<td></td>
</tr>
</tbody>
</table>

CztA similarity and identity: Similarity and identity with other known ABC solute binding SBP_Bac_9 family members. It has remarkable similarity with Fe** and Mn** binding proteins.

Conclusions:

Model stereo view of CztA: In the figure CztA (coloured) is superimposed with template MntC (1XVL) of Synechocystis 6803 (gray). The active site residues are shown as sticks in between two lobes.

Model view of CztA active site: The negatively charged side chains of H58, H123, E189 and D264 are involved in ligand binding with different bonding pattern.

Binding of Cu** and Zn** with CztA and variants: Binding was determined by fluorescence spectrophotometry and % fluorescent quenching or % fluorescent increase plotted against ligand concentration. The data were fitted with single site saturation of ligand binding module (SigmaPlot 8.0)

- CztA is a Cu** / Zn** binding protein with high affinity to Cu** (K_d=0.35 ± 0.1µM).
- Cu** is a preferential ligand, but Zn** binds (K_d=25 ± 2.5µM) in a different manner and showed increase in fluorescence instead of quenching.
- Other divalent metal ions like Ni**, Co**, Mn**, Fe**, Fe**⁺⁺, Ca**, Mg** did not interact with CztA, hence CztA is highly selective for Cu** and Zn**.
- Side chains of H58, H123, D264 are important in ligand coordination with Cu**, however E189 is not involved significantly.
- Alanine scanning mutagenesis approach in CztA suggested important understanding about Cu** coordination and will be used in understanding binding more clearly by CztA crystalization.
- Metal binding ABC periplasmic proteins of bacteria (SBP_Bac_9) are significantly similar in their active sites and structures, but specificity is not predictable! Like how CztA is only specific for Cu**?

Reference:
Ruhman V., Anal I., Miled-Mark F.M., Adir N. 2005. The MntC crystal structure suggests that import of Mn2+ in cyanobacteria is redox controlled. Journal of Molecular Biology. 348: 961-969