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Homology modeling of Chromobacterium violaceum and Thiobacillus ferrooxidans atypical ArsR proteins

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Short Abstract: The 3D structures of ArsR proteins from Chromobacterium violaceum and Thiobacillus ferrooxidans were investigated by homology modeling. The models have the same secondary structure seen in the template SmtB protein. We identified a putative C-terminal arsenite/antimonite binding site in these atypical but functional ArsR proteins.

Long Abstract:
The ArsR/SmtB protein family compasses five groups of negative transcriptional regulators that controls heavy metal stress responses in bacterial and archaeal cells, each one having different metal selectivity and affinity. All these proteins have in common an Helix-Turn-Helix, a dimerization interface and the metal/metalloid binding sites. The ArsR/SmtB proteins are classified according to the metal binding site. Some proteins have a site in the alpha-helix 3, others have a site in the dimerization interface (alpha-helix 5) and others have the both sites. Despite ArsR/SmtB protein family has been studied for almost two decades, there is no crystallographic information about some members like the arsenic responsible ArsR proteins. Unlike the others regulators, the ArsR proteins binding specifically to oxyanions compounds (arsenite and antimonite). Typical arsenic responsive proteins have a conserved metal binding site in the alpha-helix 3 formed by the ELCVCD sequence, while atypical proteins have not this site. In the first case, arsenite/antimonite binds to the site and triggers a conformational change in the protein, making it release to the operator. In the atypical case, the metalloids bind to an unknown site and trigger the same response. A putative site placed in the C-terminal region has been suggested recently, but little molecular evidences have been given for its function. This site is formed by a conserved ENCC sequence and by some cystein residues placed in variable regions. Here we report the homology modeling study of two functional atypical ArsR proteins. The modeled sequences were from Chromobacterium violaceum and Thiobacillus ferroxidans ars operons. We are studying the ars operon in C .violaceum and our results show that its ArsR protein is functional in response to the arsenite concentration. The T. ferrooxidans protein was chosen because it was the first atypical proteins characterized. Models were done with the Synechococcus sp. SmtB crystallographic structure deposited in the Protein Data Bank. The ArsR and SmtB sequences were automatic aligned using ClustalW program and manually using BioEdit program. The Swiss-Pdb Viewer program was used to make a modeling job with the alignments and the SmtB structure. The jobs were submitted to automatic modeling in the Swiss-Model server. The stereochemistry

qualities of the Models were analyzed with the Procheck program. Although the target and the template sequences have low identity, the resultant models were of good quality (more than 90 % of the residues in most favorable region). The resultant models were dimer with the same SmtB secondary structure. In T. ferrooxidans protein, the beta-sheet and the alpha-1 regions were smaller than in SmtB, while in C. violaceum they were of the same size. In the two proteins, the helix-turn-helix domain was well conserved, but significant differences were seen in the recognition helix (alpha-4 region). The acid and basic amino acid distribution in this helix varies between the three proteins. The T. ferrooxidans partner is almost identical to C. violaceum, accept that the former have not a negative aa placed in the N-terminal end of the recognition helix as seen before. The high identity in this helix suggests a similar operator region recognized, but these two operators have not been found yet. A blast search for sequences with similarity to Thiobacillus and Chromobacterium ArsR proteins shows that the two C-terminal vicinal cysteine residues well conserved in most found proteins. In T. ferrooxidans model, the cysteine residues were placed in the C-terminal alpha-helix 5 end, with side chains turned to the external region. The same is seen in the C. violaceum model, but that the second cysteine is not in the alpha-helix region. Some ArsR/SmtB members have an alpha-5 binding site formed by residues from the helix 5 of the two monomers. When the metal binds to this site, the conformational change this helix interferes with the dimer structure and reduces the protein-DNA affinity. The same mechanism may exist in atypical ArsR proteins. Arsenite and antimonite are highly thiophilic compounds, therefore the allosteric regulation by these oxyanions may occur through interactions with cysteine residues. Arsenite/antimonite generally form a trigonal-pyramidal structure in the site reported. In our two models, there are in cysteines proximity many residues like serine, histidine and arginine which would be part of the putative site. We are not able to conclude which residue is the third in this coordination geometry. In the next steps, molecular dynamics calculations will be applied to investigate the metalloids interactions in this putative binding site.

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