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Structural study of *Neisseria meningitidis* iron-regulated protein FrpD

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FrpD is a highly conserved lipoprotein of *Neisseria meningitidis* anchored to the bacterial outer membrane. The *frpD* gene sequence contains two translation initiation sites, which give rise to production of the full-length FrpD protein ($FrpD_{271}$) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein ($FrpD_{250}$) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported $FrpD_{271}$ precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys_{25} residue, and sorted to the outer bacterial membrane [1]. The biological function of FrpD appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity (K_d = 0.2 nM) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of any structural information on these proteins. Moreover, the primary amino acid sequence of FrpD does not exhibit any similarity to known protein sequences of other organisms, and therefore, a new type of protein fold could be expected. We found out that the full version of $FrpD_{250}$ protein couldn't be crystallized. Therefore, we performed a specific truncation of 21 amino acid residues from N-terminus of $FrpD_{250}$ protein. The native and Se-Met substituted variants of recombinant, truncated version (lacking the first 21 amino acid residues from N-terminus) $FrpD_{43\text{-}271}$ protein were prepared and crystallized using the sitting-drop vapour-diffusion method. The crystals of native $FrpD_{43\text{-}271}$ protein belong to the hexagonal space group $P6_2$, while the crystals of Se-Met substituted $FrpD_{43\text{-}271}$ protein belong to the primitive orthorhombic space group $P2_12_12_1$ [2]. Crystal structure of Se-Met substituted $FrpD_{43\text{-}271}$ was determined using the single anomalous diffraction (SAD) method. The calculated structure was used as a search model in molecular replacement to determine the structure of native $FrpD_{43\text{-}271}$ protein. Currently, the analysis of the Se-Met substituted $FrpD_{43\text{-}271}$structure and comparison with calculated native $FrpD_{43\text{-}271}$ protein structure are underway.

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Structures of methylthioadenosine phosphorylase (MTAP) from *S. Mansoni*

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The schistosomiasis is a chronic parasitic illness, caused by the parasite *Schistosoma mansoni* that affects approximately 200 million people worldwide. Schistosomiasis is treated by the use of drugs that are not-in fact effective for the eradication of the disease, and although their efficiency cause serious side effects. The *Schistosoma mansoni* parasite does not posses the *de novo* pathway for purine bases biosynthesis and depends entirely on salvage pathways for its purine requirement. Thus this pathway can be identified as a potential target for the development of specific drugs to combat schistosomiasis. The MTAP enzyme (EC 2.4.2.28) is a component of this pathway, using 5'-deoxy-5'-methylthioadenosine (MTA) as it substrate. MTAP catalyze reaction: S-methyl-5'-thioadenosine + phosphate = adenine + S-methyl-5-thio-α-D-ribose 1-phosphate.

The MTAP amplification was performed using a cDNA library from enriched mRNA of the adult worm. The MTAP gene was cloned in pET28a vector. Expression was done using *E. coli* BL21-CodonPlus (DE3) in 2XTY medium, induced with 100μM/mL IPTG. The protein was purified with affinity chromatography technique using Talon resin (Clonetech). The purified protein was dialyzed with 20mM Tris pH 7.4, 200mM NaCl and 5mM β-mercaptoethanol and submitted to a robotic crystallization trials using Honeybee 939 robot. The MTAP was purified in large amounts with a high level of purity as monitored by SDS PAGE. The yielding was 60mg per liter of 2XYT medium. The protein was crystallized in 100mM Bis-tris pH 6.1-6.5 and 14-18% PEG 3350. Diffraction data was obtained in the MX2 beamline of the LNLS, Campinas-Brazil. Two data sets were collected at 2.0Å and 2.1 Å.

The crystals of MTAP belong to the space group $P2_1$. The first in complex with adenine (MTAP-Ade), has cell dimensions of *a*=81.31Å, b=82.60Å, c=150.30Å and γ=100.6°. The second in the form of apoenzyme (MTAP-apo) has cell dimensions of *a*=80.62Å, b=82.40Å, c=150.28Å and γ=101.5°. The MTAP-ade complex was solved by molecular replacement (MR) employing human MTAP as a search model (PDB id 1CBO). The MTAP-apo was solved by molecular replacement (MR) again, but using MTAP-ade as a search model. Both were solving using Phaser and refinement has been carried using the programs Coot and Phenix. The partially refined resulted for MTAP-ade is R_{work}(%) = 19.8 and a R_{free}(%) = 24.6 and for the MTAP-apo is R_{work}(%) = 18.0 and a R_{free}(%) = 23.5.

The active site of each monomer is located near the interface between subunits. The trimeric arrangement of subunits is very similar to that seen in mammalian PNP. In MTAP, the active site of a given subunit is composed entirely of residues from that subunit. The contacts polar in site are between adenine and the residues Asp230 and Asp232. The H_2N of the adenine does interaction with two residues simultaneously. The adenine interact with two water molecules, one of them does polar contact with a Ser188 and Asp232. In the MTAP-apo, the Phe231 occupies the active site, an active site conformational change induced by adenine was observed. The Phe231 residue leaves the site opening space for the adenine. In overlapping structures, is also observed a conformational change in secondary structure, in the MTAP-ade, the fifth and seventh α-helixes are displaced toward the ligand.

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Crystal structure of a metallo-β-lactamase from a multiresistant strain of *A. baumannii*

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