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 (FrpD1-271) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein (FrpD21-271) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported FrpD21-271 precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys2 residue, and sorted to the outer bacterial membrane [1]. The biological function of FrpD appears to be linked to the FrpC fold could be expected. We found out that the full version of FrpD protein couldn’t be crystallized. Therefore, we performed a specific truncation of 21 amino acid residues from N-terminus of FrpD protein. The native and Se-Met substituted variants of recombinant, truncated version (lacking the first 21 amino acid residues from N-terminus) FrpD21-271 protein were prepared and crystallized using the sitting-drop vapour-diffusion method. The crystals of native FrpD21-271 protein belong to the primitive orthorhombic space group P2_12_1_2 [2]. Crystal structure of Se-Met substituted FrpD21-271 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 FrpD21-271 protein. Currently, the analysis of the Se-Met substituted FrpD21-271 structure and comparison with calculated native FrpD21-271 protein structure are underway.


Keywords: biocrystallization, lipoprotein, iron-regulated protein

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 possess 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 catalyse reaction: S-methyl-5’-thioadenosine + phosphate = adenine + S-methyl-5-hio-a-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 (Clonotech). 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 2XTY 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_12_1. The first in complex with adenine (MTAP-Ade), has cell dimensions of a=81.31Å, b=82.60Å, c=150.30Å and γ=101.5°. The second in the form of apo-enzyme (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 ICBO). 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(merge)(%) = 19.8 and a RFREE(%) = 24.6 and for the MTAP-apo is R(merge)(%) = 18.0 and a RFREE(%) = 23.5. The active site of 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,N 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-helices are displaced toward the ligand.

Supported by Fapesp and CNPq.

Keywords: phosphorylase; MTAP; Schistosoma mansoni