A.12: Crystal structure of PepQxc enzyme using protein crystallography beamline (PX-BL21) of Indus-2

The PX-BL21 beamline has been recently commissioned by High Pressure & Synchrotron Radiation Physics Division, BARC on the 1.5 T bending magnet source of 2.5 GeV Indus-2 synchrotron at RRCAT. This beamline is designed to carry out monochromatic and anomalous diffraction experiments on single crystal of biological macromolecules (protein, DNA or their complexes). Presently, the beamline can be tuned to desired energy in the range of 5-17 keV using a double crystal monochromator. The experimental station consists of a single axis MARdtb goniometer with robotic cryogenic sample changer, cryogenic sample cooler, Rayonix MX-225 CCD detector and fluorescence detector. A well-equipped biochemical laboratory has been developed adjacent to PX-BL21 beamline. Using these facilities our group has crystallized ten novel proteins and have determined crystal structures of eight of those proteins including a Selenium-single wavelength anomalous diffraction structure. The entire study involving selecting the target proteins, gene cloning, protein overexpression, protein purification, crystallization and diffraction and later the data analysis were carried out using PX-BL21 beamline and its associated facilities. The beamline has also been extensively used by researchers from various universities and research institutes in the country. A close to 100 high-resolution datasets on the different proteins have been collected on the PX-BL21 beamline by the users.

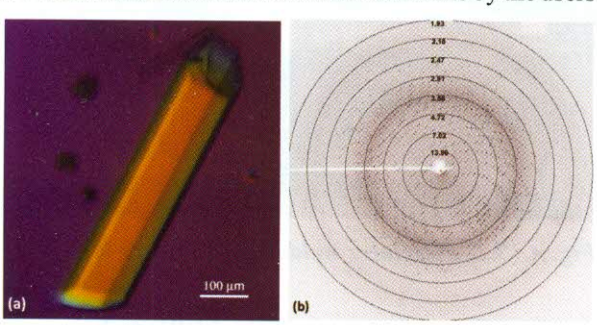

Fig. A.12.1: (a) The protein crystal of PepQxc; it was grown from 40 mM KH_2PO_4, 15 % glycerol, 12% PEG-8000 at 294 K temperature. (b) Diffraction pattern recorded at PX-BL21, Indus-2 on a PepQxc crystal at exposure of 30 s and 1° oscillation.

Here, we are reporting one of the several proteins that have been studied using these facilities; a novel bacterial protein called Xaa-Pro dipeptidase (43 kDa; PepQxc, GenBank accession no., NP_637763). This protein is from a plant pathogenic bacteria, Xanthomonas campestris. Xaa-Pro dipeptidase (EC 3.4.13.9) belongs to M24B family of metallopeptidases and specifically cleave a trans Xaa-Pro iminopeptide bond in dipeptide or polypeptide. The homologs of the protein play important role in the recycling of proline, protein stability and degradation of collagen. These are important commercially in the food and dairy industries for improving flavor and texture of the food. In addition to the dipeptidase activity, the peptidases of M24B family display unexpected activity of degrading toxic organophosphorus (OP) compounds such as pesticides and nerve agents.

The gene encoding PepQxc protein was cloned and expressed and the protein was purified using column chromatography techniques. The diffraction-quality crystals (~0.1 x 0.1 x 0.5 mm^3) were grown using under-oil microbatch method, Fig.A.12.1(a). The diffraction data on the crystal was collected up to ~1.8 Å resolution at the PX-BL21 beamline of Indus-2 synchrotron, Fig.A.12.1(b). The data were processed and analysed. The crystal belonged to $P2_12_12_1$ space group with the unit-cell parameter, $a = 84.32$, $b = 105.5$, $c = 111.4$ Å. The data completeness, average I/sigma(I), overall Rmerge for the processed data were 100%, 18.2 and 7.4% respectively. The structure was solved using molecular replacement (MR) method. The model based on the monomer of homolog from an archaea, Thermococcus sibiricus (PDB entry 4FKC, 29 % sequence identity) was used for the MR solution. The structure was refined up to R_{work}/R_{free} of 18/22% with good stereochemistry using PHENIX and Coot suites (Fig.A.12.2).

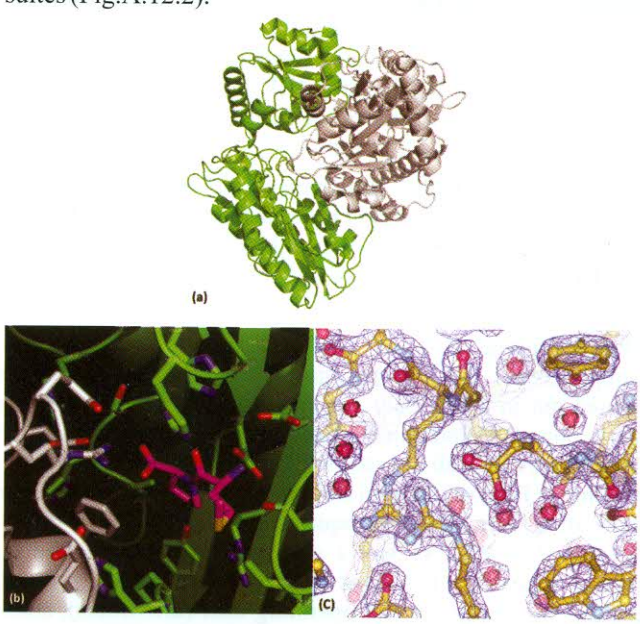

Fig. A.12.2: (a) Crystal structure of PepQxc (cartoon representation), b) Substrate Met-Pro has been docked into the active site; showing extensive interactions from both the protomers of the dimeric protein., c) Electron density map ($2F_o$-F_c at 1.5 sigma) of active site of the protein.

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