Structure-function analysis of the neutron crystallographic structure of Inorganic Pyrophosphatase determined from microgravity-grown crystals

Inorganic Pyrophosphatase (IPPase) from *Thermococcus thioreducens* catalyzes the hydrolysis of inorganic pyrophosphate (PP_i) to form orthophosphate (P_i). The action of this enzyme shifts the overall equilibrium in favor of synthesis during a number of ATP-dependent cellular processes such as in the polymerization of nucleic acids, production of coenzymes and proteins and sulfate assimilation pathways. IPPase was crystallized by counter-diffusion crystallization in the Granada Crystallization Facility (GCF) as a Center for the Advancement of Science in Space (CASIS) payload on board the International Space Station (ISS). The protein was allowed to crystallize for 6 months, the longest duration of time for any protein crystal growth experiment using the counter-diffusion technique. To-date, the largest IPPase crystals were obtained in the experiments performed on board the ISS. Crystals with the largest volume (> $6mm^3$) were subjected to neutron diffraction studies using the Macromolecular Neutron Diffractometer (MaNDi line) at Oakridge National laboratories. The neutron crystallographic structure was determined to 2.3Å with final R_{free} and R_{work} to be 23.8 % and 25.2 % respectively. We report here the structure-function analysis of IPPase as revealed by the neutron crystallographic structure coupled to previously determined X-ray crystallographic structures, including that of the holo *Thermococcus thioreducens* IPPase. The structural comparisons between the apo IPPase (neutron) and holo (X-ray) structures revealed different side chain conformations at the active site resulting from hydrogen alterations from proximal water molecules and magnesium coordination.

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