Microgravity Crystallization and Neutron Diffraction of Perdeuterated Tryptophan Synthase

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The ubiquitous cofactor, pyridoxal 5'-phosphate (PLP), is present in all forms of life. PLP-dependent enzymes are functionally diverse, catalyzing transamination, racemization, □- and □-elimination, □-decarboxylation, replacement reactions, and phosphorylation. Due to their significance in metabolic pathways and amino acid synthesis, PLP-dependent enzymes are attractive targets for specific inhibitor design. Such developments require atomic-level structural studies to understand how PLP is modulated to perform specific chemistry. Because neutron diffraction provides the ability to directly visualize the position of hydrogens and assign protonation states, it is a favorable technique for studying PLP-dependent enzymes. The bottleneck of neutron diffraction, however, is the growth of large crystals (≥ 0.5 mm3) to overcome the low fluxes of neutron sources. The inclusions and high mosaicity of crystals of tryptophan synthase (TS), a Fold Type II PLP-dependent enzyme, are only amplified when increasing the size, resulting in poor diffraction quality. Microgravity crystallization provides the opportunity to grow large, well-ordered crystals by reducing gravity-driven convection currents that permit variable crystal feeding and impede crystal growth. We developed the Toledo Crystallization Box (TCB), a membrane-barrier capillary-dialysis device, to grow neutron diffraction quality crystals of perdeuterated TS in microgravity. Here, we present the design of the TCB and the results from Center for Advancement of Science in Space (CASIS) supported International Space Station (ISS) Missions Protein Crystal Growth (PCG)-8 and PCG-15. From perdeuterated TS crystals grown on the ISS, we were able to collect a 2.1 Å neutron diffraction data set and solve the joint X-ray/neutron structure.