Crystal structure of the human calpain-5 catalytic core

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Abstract

Calpain-5 (CAPN5) is a calcium-activated cysteine protease that is expressed throughout the central nervous system and retina. Mutations in the catalytic core of CAPN5 make it hyperactive and cause autosomal dominant neovascular inflammatory vitreoretinopathy (ADNIV; OMIM#193235), a severe, blinding eye disease. Although crystal structures have been solved for some classical calpains (e.g. CAPN1 and 2), that of CAPN5, a non-classical calpain, remains unsolved. Recombinant CAPN5 catalytic core (comprised of domains IIa and IIb) was expressed in *E. coli* BL21 (DE3) cells and purified using a combination of affinity and size-exclusion chromatography. Small-angle x-ray scattering (SAXS) performed on the human CAPN5 catalytic core in the absence of calcium, combined with molecular dynamics simulations and modeling, revealed a highly-open conformation in solution compared to other calpains. We next determined the crystal structure of the human CAPN5 catalytic core in the presence of calcium. Data were collected on flash cooled crystals at beamline 4.2.2 at the Advanced Light Source at the Lawrence Berkley National Laboratory. The crystals diffracted to 3.0 Å and structural data were refined to an R-free of 23.3% and R-factor of 31.2%. The structure was solved by molecular replacement using our previously-published CAPN5 homology model as a search model. Due to the presence of calcium in the buffers, the crystal structure adopted a closed conformation compared to the SAXS model. Biophysical studies and higher resolution structures of the ADNIV mutants should illuminate the molecular mechanisms that regulate the calpain family proteins and inform the design of specific inhibitors for ADNIV.