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Structural Studies on Human Calpain-3 Protease Core

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Skeletal muscle-specific protein calpain-3 (CAPN3/p94) is a member of the calcium-dependent cysteine protease family. CAPN3 is indispensable for the maintenance and survival of functional skeletal muscles, and defects in the CAPN3 gene that cause loss of enzyme activity lead to the development of limb-girdle muscular dystrophy type 2A. The CAPN3 protease core (domains PC1 and 2) is particularly rich in these inactivating mutations. The whole enzyme, a homodimer of CAPN3, undergoes very rapid autoproteolysis. It has two insertion sequences, IS1 and IS2 not found in other calpains, and a long N-terminal region, NS. IS1 is present in the protease core. Previous biochemistry studies from our lab have shown that IS1 contributes to the instability of CAPN3 by acting as a readily cleavable internal pro-peptide that occupies the active site of the protease core. Here we report the 2.3Å resolution crystal structure of CAPN3 (ΔNS, C129S) protease core with Ca2+ ions present. Although the architecture of this protease core is similar to those of the CAPN1 and CAPN2, with two Ca2+ bound, the crystal structure of the CAPN3 core exhibits several novel features. The N-terminal anchor is more flexible than that of CAPN1 and CAPN2 even with the N-terminal 45 residues removed. Residues in the mutation-rich region of PC1 (207 to 234) form van der Waals and H-bond interactions with residues of both IS1 and helix10. The active site is blocked by part of IS1 that forms H-bonds through the OH groups of Tyr274 and Tyr322 to Trp360 from the active site, as well as a hydrophobic interaction between Tyr274 of IS1 to His334 of the catalytic triad. This aromatic stacking will hinder substrates and inhibitors from entering the active site. The crystal structure further confirms that IS1 is a critical region governing CAPN3 activation and the binding of substrates and inhibitors. Supported by funds from CIHR and the CRC program.

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