Filament Formation Drives Catalysis of Glutaminase

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Glutamine metabolism, which satisfies the unique metabolic requirements of aggressive cancers, is initiated by the mitochondrial glutaminase enzymes, which catalyze the hydrolysis of glutamine to glutamate and thus represent potential therapeutic targets. However, the mechanisms responsible for the regulation and catalytic activity of these enzymes are not well understood. Recent studies suggested that glutaminases form filament-like structures that are essential for their activation. In this study, we present the first cryo-EM structures of the full-length human glutaminase isozyme GLS2 at 3.1 Å, and also present the structural evidence that the glutaminase enzymes form filaments upon substrate binding, which provides an unprecedented view of the mechanism responsible for catalyzing glutamine hydrolysis. The activation loop of GLS2 assumes a unique conformation and, together with a lid that closes over the active site, locks in the substrate glutamine. In the activated GLS2 filament structure, the cation-π interaction between tyrosine 251 of the activation loop and lysine 222 in the active site is critical for catalysis by enabling Serine 219, a key catalytic residue to undergo deprotonation for nucleophilic attack on the substrate. The results indicate that allosteric inhibitors of glutaminase disrupt, while activators like inorganic phosphate promote, the essential interactions required for catalysis. The GLS2 structures also reveal the regulatory role of ankyrin repeats in different glutaminase isozymes.