Poster Presentation

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Crystal structures of human dihydroxyacetone kinase

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Dihydroxyacetone (Dha), also known as glycerone, is a metabolically active carbohydrate. Dha kinase (DAK) is highly conserved enzyme that utilizes mainly Dha as substrate. In bacteria, DAK converts dihydroxyacetone to dihydroxyacetone phosphate in the glycerol fermentation pathway. In higher eukaryotes, DAK can accomplish two enzymatic activities, namely, ATP-dependent dihydroxyacetone phosphorylation and FMN cyclization. The biological significance of cFMN is still not identified. Possible speculations of the functions are as signaling molecule, minor redox flavocoenzyme or intermediate of a degradative pathway for FAD. In order to understand the mechanisms of the FMN cyclizing reaction, we here present the crystal structures of hDAK soaked with FAD and its apo state. The crystals are diffracted up to 2.0 Å in space group C2 with a dimer in each asymmetric unit. hDAK contains 2 domains: L domain and K domain, joined together by a linker region. In the FAD and Mn2+ soaked DAK structure, AMP is seen on K domain. The second cyclization product, cFMN, however is diffused during soaking and cannot be seen in the density map. Mammalian DAK is also shown to be a negative regulator of MDA5. MDA5 is a cytoplasmic protein that has important role in viral RNA detection and initiate innate immune response. Specifically, MDA5 undergoes conformational change upon viral RNA binding and allows its N terminal CARD domain to expose and bind to the CARD domain of the downstream signaling protein IPS-1. IPS-1 is essential for the activation of proinflammatory cytokines and type 1 interferon. Pulldown assays indicated that MDA5 CARD domain is responsible for the interaction of IPS-1 CARD as well as hDAK. The competition and the exact mechanisms are still unknown. We shall apply biophysical techniques such as ITC and SPR to quantify the binding parameters. Last but not least, to crystallize the complex in order to provide a clear molecular detail of the interaction between MDA5 and its binding partners.

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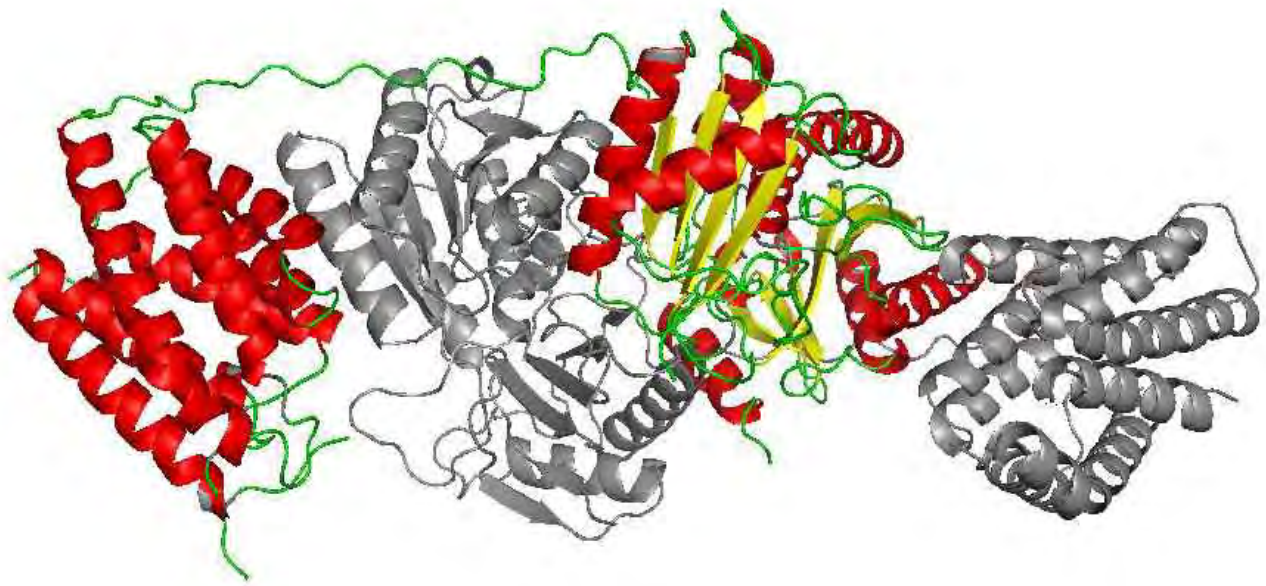

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