

Structural basis for recruitment of tandem hotdog domains in acyl-CoA thioesterase 7 and its role in inflammation

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As part of our program on high-throughput crystallography of macrophage proteins, we have carried out a comprehensive structural and functional characterisation of mouse acyl-CoA thioesterase 7 (Acot7). Acots catalyse the hydrolysis of fatty acyl-CoA to free fatty acid and coenzyme A and thereby regulate lipid metabolism and cellular signalling. While prokaryotic homologues possess a single thioesterase domain, mammalian Acot7 contains a pair of domains in tandem. We determined the crystal structures of both the N- and C-terminal domains of the mouse enzyme, and the structure of the full-length enzyme using a combination of chemical crosslinking, mass spectrometry, and molecular modelling. The novel quaternary arrangement features a trimer of hotdog fold dimers. We show that both domains of Acot7 are required for activity, that only one of two possible active sites in the dimer is functional, and identify Asn24 and Asp213 (from N- and C-domains, respectively) as the catalytic residues through site-directed mutagenesis. We also designed an enzyme with higher activity than wild-type Acot7 by mutating the residues in the non-functional active site. Because Acot7 shows the highest activity towards arachidonoyl-CoA (a precursor of eicosanoids), is highly expressed in macrophages and upregulated by pro-inflammatory factors, and its over-expression in macrophages alters the production of prostaglandins D2 and E2, we propose a role in inflammatory processes. Together, our results provide a foundation to relate the molecular and cellular functions of Acot7 in macrophages and other mammalian tissues.