Poster Presentation

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Structure and specificity of novel aminopeptidase from marine sediment Archaea

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The Earth's microbial diversity remained largely unexplored until recent developments in DNA sequencing. Novel methods enabled us to access genomic information of uncultured microbial organisms and create hypotheses about their metabolic capabilities. These predictions primarily rely on the sequence similarity between a novel protein and characterized proteins. Such an approach introduces a "culture" bias: the well-understood proteins come from a set of laboratory-grown bacteria, while novel microbial proteins are obtained from a variety of environments, including the most extreme. One such niche is ocean sediment – an unexplored ecosystem that plays important roles in geochemical cycles. Single-cell genomics targeting sedimentary populations identified four new archaeons encoding putative intra- and extra-cellular proteases [1]. This discovery suggests that heterotrophic marine Archaea evolved to degrade detrital proteins and might contribute to global carbon cycling. The novel proteases share some sequence similarity with well-known protein-degrading enzymes, but generally are distant homologs. Thus, functional screening is necessary to validate sequence-based predictions. One of the proteases shares sequence similarity with S15 peptidases, cocaine esterases and α-amino acid ester hydrolases (AEH). Phylogeny indicates that the gene is of bacterial origin. Enzymatic assays reveal α-aminopeptidase activity towards dipeptides with a preference for a small, L-configured hydrophobic residue at the N-terminus. The crystal structure shows a homotetrameric, self-compartmentalizing enzyme with four independent active sites localized inside the oligomeric assembly accessible from the internal channel. The active site contains a serine protease triad (Ser-His-Asp) and a cluster of negatively charged residues that bind the N-terminal NH3+ group of the substrate molecule. Therefore, the observed activity suggests that the enzyme (designated as AP TA1) may act on di- or tri-peptides produced during extracellular degradation and subsequently imported to the cell. As a close homolog of AEHs, it is also possible that AP TA1 might participate in the synthesis of yet-to-be-discovered secondary metabolites. Supported by NIH GM094585, DOE/BER DE-AC02-06CH11357 & C-DEBI 36202823 & 157595.

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