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RESEARCH NEWS

Rhomboids make do with a weak hydrogen bond

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***JGP* paper explores the strength of the hydrogen bond network at the active site of GlpG.**

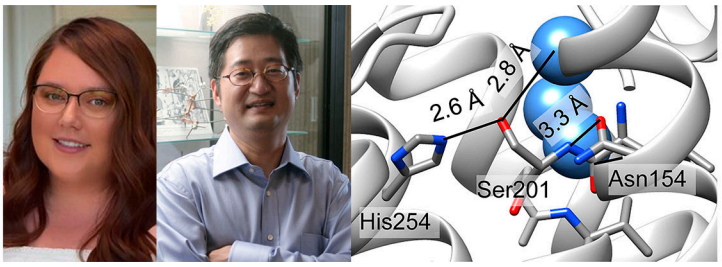
Rhomboids are proteases that specifically cleave membrane proteins, a process that can result in release of various growth factors and transcription factors, and even activation of other enzymes (1). Although rhomboids are related to the larger class of serine proteases, their rate of catalysis is unusually slow (2). In a new *JGP* paper, Gaffney and Hong investigate the bacterial rhomboid protease GlpG, providing important insight into how this class of protease functions (3).

Serine proteases are so named because the region that performs catalysis, the active site, contains the amino acid serine. This amino acid residue carries out a nucleophilic attack that severs the peptide bond making up the backbone of target proteins. Simply possessing serine does not make a protein into an enzyme, though; serine needs help to become capable of nucleophilic attack.

"The first step in proteolysis is the serine residue is activated by certain nearby residues, most often a histidine residue. The histidine's nitrogen atom pulls the hydrogen in serine's hydroxy group strongly so that the oxygen in the serine residue becomes a better nucleophile and can better attack a peptide bond," explains Dr. Heedeok Hong, an associate professor in chemistry at Michigan State University.

The hydrogen bond formed between serine and histidine is therefore crucial for catalysis. Many serine proteases possess a third amino acid in their active site, an aspartate residue that assists with catalysis and participates in forming a tight hydrogen bond network. However, rhomboids only contain a serine–histidine dyad (1, 4). The strength of the hydrogen bond between these residues, and how proteolysis occurs, is unknown.

Hong's laboratory is primarily interested in the process by which membrane proteins fold into their final, functional shape. However, Hong and graduate student Kristen Gaffney realized that tools developed to study protein folding could also be used to probe a rhomboid protease's active site.



Kristin Gaffney (left) and Heedeok Hong (center) studied the hydrogen bond between serine and histidine at the active site of the bacterial rhomboid protease GlpG (ribbon diagram at right; water molecules depicted in blue). Photos courtesy of the authors.

The authors used a method called steric trapping (5) in which a protein is labeled with a pair of small biotin tags that bind bulky streptavidin probes. The tags are placed so that simultaneous binding of two probes only occurs when the protein has become unfolded. This technique allows analysis of protein folding under native conditions and without use of chemical denaturants.

Gaffney and Hong combined this with a mutational approach called double mutant cycle analysis, in which the individual effects of two single mutations are compared with that of having both residues mutated simultaneously. The authors investigated the strength of interaction between GlpG's active site hydrogen bond residues by mutating them to alanine (which cannot participate in hydrogen bonding) and assessing their effect on protein stability via steric trapping. These experiments showed that GlpG's active site hydrogen bond is surprisingly weak: around 1 kilocalorie per mole, compared with 5–7 kilocalories per mole for other serine proteases.

"That may be partly responsible for the slow proteolysis reaction mediated by rhomboid proteases," theorizes Hong.

Gaffney and Hong were also curious about how GlpG's hydrogen bond affects the protein's structure overall. To assess this, they positioned steric trapping affinity tags at different positions within GlpG, then investigated whether mutations in the active site hydrogen propagate to distant sites. The results suggest that mutations at the active site are strongly propagated throughout the protein.

"We conclude that the hydrogen bonding network is not just important for function. Those highly conserved interactions might have evolved for communication between different parts of the protein," says Hong.

Although Gaffney and Hong studied GlpG folding in detergent, Hong thinks steric trapping may enable studies of protein folding within lipid bilayers—a technically challenging problem that few groups have attempted. "We are currently working on that," he says.

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