Taking a position on intramembrane proteolysis

DOI 10.1074/jbc.H118.002210

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Edited by Karen G. Fleming

Decades of work have contributed to our in-depth mechanistic understanding of soluble proteases, but much less is known about the catalytic mechanism of intramembrane proteolysis due to inherent difficulties in both preparing and analyzing integral membrane enzymes and transmembrane substrates. New work from Naing et al. tackles this challenge by examining the catalytic parameters of an aspartyl intramembrane protease homologous to the enzyme that cleaves amyloid precursor protein, finding that both chemistry and register contribute to specificity in substrate cleavage.

Proteases are crucial regulators of signaling in cells, influencing various aspects of health and disease (1). Most research in the field has focused on soluble proteases, establishing the rules by which these enzymes cleave peptide bonds of proteins in a sequence-selective manner. However, proteolysis also occurs within the membrane, performed by intramembrane proteases; these enzymes similarly carry out key functions influencing development, lipid metabolism, and bacterial growth. Our current knowledge of the active sites of intramembrane aspartyl proteases suggest their chemistry may be similar to their soluble counterparts, but extension of other concepts has met with several hurdles. For example, since the catalytic residues of intramembrane proteases reside within the hydrophobic lipid bilayer, it is not clear whether the established positional relationships between soluble proteases and their substrates that define specificity translate to the membrane environment. The intramembrane aspartyl protease (IAP) (2) presenilin, the enzymatic component of the gamma secretase complex known to cleave amyloid precursor protein, has 90 different known substrates that share no homology or consensus recognition motif (2), suggesting other factors are at play in determining substrate specificity and cleavage. A new study by Naing et al. (3) provides insights into these possible factors in a detailed exploration of catalytic parameters, discovering both chemistry and positioning of substrate matters for hydrolysis.

Thus far, four classes of intramembrane proteases have been identified: aspartyl, serine, metallo, and glutamyl (4). One of the best studied groups is the rhomboid family, serine intramembrane proteases that play roles in signaling events, which make them frequent points of comparison for new studies. Most studies of IAPs have focused on presenilin, but the γ-secretase complex is a tetrameric heterologous oligomer, adding further challenges to a difficult system! Naing et al. (3) instead focus their attention on the microbial IAP (mIAP) from Methanoculleus marisnigri, a presenilin homolog that acts with no known protein co-factors, providing a simplistic view of a still complicated system. The authors employ two different substrates: a presenilin substrate derived from amyloid precursor protein (APP) and a renin peptide, which is a soluble model substrate that is fortuitously cleaved by mIAP. For those studying intramembrane proteases, soluble model substrates provide a good “quality control” assessment, as they are divorced from any issues that might arise with more hydrophobic substrates.

To identify residues important for catalysis, the authors examined variants of mIAP with both a FRET-based assay and discontinuous gel–based assay. Mutation of the conserved YD…GxGD motif containing the catalytic aspartate resulted in conservation of structure yet loss of catalytic efficiency and processivity as tested with various renin peptide substrates. Notably, a tyrosine to alanine switch in the first motif was catalytically impaired yet a phenylalanine mutation behaved similarly to WT, revealing the importance of the aromatic ring in substrate recognition. Mutation of the substrate-gating motif from the mIAP AGL sequence to presenilin’s PAL sequence substantially disrupted catalysis. This indicates that each IAP likely recognizes distinct substrates.

The authors next tested APP as a more physiologically relevant substrate. APP is a single-pass transmembrane protein cleaved by several proteases in several places, terminating with cleavage by presenilin in the transmembrane region. The products of this final cleavage event are known to be responsible for neurofibrillary tangles in Alzheimer’s disease patients, lending increased urgency to understanding IAP processing. In this case, a fragment of the APP transmembrane region was labeled with a fluorophore and a quencher to generate a FRET-C100 substrate (Fig. 1), which was cleaved faster than the renin substrate. The authors also tested a more physiologically relevant context by comparing experiments conducted in detergent micelles with bicelles, which better mimic the membrane environment. Although a more favorable $K_m$ was measured in the bicelle (lipid) environment, similar catalytic efficiencies were observed in both detergent and bicelles. Additionally, the APP cleavage site detected in both detergent and bicelles surprisingly was in agreement, suggesting that although kinetic assays with intramembrane proteases conducted in detergent systems result in slower efficiencies, they still can mimic the in vivo environment.

The author declares that she has no conflicts of interest with the contents of this article.

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2. The abbreviations used are: IAP, intramembrane aspartyl protease; mIAP, microbial IAP; APP, amyloid precursor protein.
Finally, the authors wanted to explore the basis for substrate specificity. They observed a preference for threonine residues at the scissile bond in all substrates tested, although other positions were also cleaved as a result of mutations to the YD…GxGD and AGL motifs, revealing that in mIAP, these motifs influence processivity. Interestingly, with the renin model substrate, the original cleavage site was preferred even in the absence of threonine, demonstrating that position matters in addition to chemistry. Threonine scanning of the APP peptide further revealed that, despite the preference for threonine, introduction of threonine in other locations does not alter cleavage preferences, revealing strong positional cues for the enzyme acting on this substrate.

The work from Naing et al. (3) provides several new insights into IAPs and highlights similarities and differences to other intramembrane proteases. For example, positioning of the substrate recognition motif was less important for rhomboid proteases: Cleavage could even be shifted to positions outside of the transmembrane region by altering the location of the recognition motif (5). Studies with rhomboid proteases found, similar to mIAP, substrate specificity was detected with transmembrane but not soluble model substrates (6). Furthermore, studies in liposomes with rhomboid proteases showed the importance of lipids in kinetic analysis (7). However, with kinetic studies in liposomes, absolute kinetic values cannot be calculated. The Lieberman group uses bicelles to circumvent kinetic studies in liposomes, but small-angle neutron scattering, that the structure of detergent solubilized mIAP is more compact compared to the crystal structure of mIAP or the cryoEM structure of presenilin (10).

Importantly, a structure with substrate is lacking for this class of intramembrane protease, which could reveal how IAPs cut processively at multiple sites and trim transmembrane substrates. Therefore, for substrate recognition during intramembrane proteolysis, for the aspartyl intramembrane protease family, whereas chemistry and position matters, many unanswered questions remain. More study is needed to attain a level of understanding similar to soluble proteases.

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