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Protein Sci. 2008 17: 2023-2037; originally published online Sep 29, 2008;
Access the most recent version at doi:[10.1110/ps.035436.108](https://doi.org/10.1110/ps.035436.108)

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REVIEW

Unconventional serine proteases: Variations on the catalytic Ser/His/Asp triad configuration

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(RECEIVED March 18, 2008; FINAL REVISION September 17, 2008; ACCEPTED September 17, 2008)

Abstract

Serine proteases comprise nearly one-third of all known proteases identified to date and play crucial roles in a wide variety of cellular as well as extracellular functions, including the process of blood clotting, protein digestion, cell signaling, inflammation, and protein processing. Their hallmark is that they contain the so-called “classical” catalytic Ser/His/Asp triad. Although the classical serine proteases are the most widespread in nature, there exist a variety of “nonclassical” serine proteases where variations to the catalytic triad are observed. Such variations include the triads Ser/His/Glu, Ser/His/His, and Ser/Glu/Asp, and include the dyads Ser/Lys and Ser/His. Other variations are seen with certain serine and threonine peptidases of the Ntn hydrolase superfamily that carry out catalysis with a single active site residue. This work discusses the structure and function of these novel serine proteases and threonine proteases and how their catalytic machinery differs from the prototypic serine protease class.

Keywords: enzymes; active sites; structure/function studies; protein turnover; structure; serine proteases; threonine proteases

Proteases play indispensable functions in all living cells. In mammalian cells, proteases function in angiogenesis, apoptosis, differentiation, immune response, matrix remodeling, and protein activation. They are found in every organelle and compartment of most, if not all, eukaryotic cells. There are over 500 proteases in human cells as determined by sequencing of the human genome (Puente et al. 2005). They are associated with a number of diseases, including cardiovascular and Alzheimer’s disease, cancer, autoimmune diseases, inflammation, and hypertension.

Proteases are typically grouped into four mechanistic classes: the cysteine, serine proteases, metallo, and

aspartic acid proteases. In 2004, the protease field was honored by a Nobel Prize in chemistry presented to Avram Hershko, Aaron Ciechanover, and Irwin Rose for their pioneering work on the ubiquitin-proteasome system in which the proteasome is a novel threonine protease. The proteasome, like the proteases in the other four classes, is a target for drug discovery programs. It is essential to understand the specificity, catalytic mechanism, and structure of proteases in order to facilitate drug design efforts.

The best-known class of proteases is the serine protease class (E.C. 3.4.21) that uses the classical Ser/His/Asp catalytic triad mechanism, where serine is the nucleophile, histidine is the general base and acid, and the aspartate helps orient the histidine residue and neutralize the charge that develops on the histidine during the transition states. Well-studied members of this class are chymotrypsin, trypsin, elastase, and subtilisin. The three-dimensional structure of chymotrypsin was first solved in

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Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.035436.108>.

1967 (Matthews et al. 1967), and the first trypsin structure was solved in 1974 (Huber et al. 1974; Stroud et al. 1974).

John Northrop made a major advance in the serine protease area in the 1930s by successfully crystallizing proteases, including trypsin and chymotrypsin (Northrop and Kunitz 1931). Other early important discoveries included the identification of the specific amino acid that functioned as the nucleophile by modification with DFP (disopropyl fluorophosphates) in both trypsin and chymotrypsin (Dixon et al. 1958). The determination of the amino acid sequence of trypsinogen (Walsh and Neurath 1964) and chymotrypsinogen (Hartley 1964) revealed they are homologous proteases. In 1969, David Blow proposed the famous charge-relay mechanism where two proton exchanges were thought to occur (from Ser195 to His57 and from His57 to Asp102) (Blow et al. 1969). Later NMR data (Robillard and Shulman 1974; Bachovchin 1985) and neutron diffraction structural data (Kossiakoff and Spencer 1981) suggest a single proton exchange from Ser195 O_γ to the His57N_{ε2}. More recently, ultra-high-resolution structures of serine protease intermediates have been performed to provide mechanistic insights into catalysis (Fodor et al. 2006).

In addition to the Ser/His/Asp serine proteases, there are serine proteases that use catalytic residue arrangements other than the canonical triad (Fig. 1; Table 1). These atypical serine proteases use novel triads such as Ser/His/Glu, Ser/His/His, or Ser/Glu/Asp, dyads such as Ser/Lys or Ser/His, or a single Ser catalytic residue. There are also proteases in which the nucleophilic hydroxyl is derived from threonine rather than a serine residue.

In this work, we describe serine proteases with non-canonical active site arrangements. We will discuss and compare atypical serine proteases with the classical serine protease class in terms of the structure–function and catalytic mechanism. Also, we will present some ideas about why there are different types of active site configurations within serine/threonine proteases.

Different clans of Ser/Thr proteases

Serine/threonine proteases have been classified into clans and families (see Merops database, <http://merops.sanger.ac.uk>) (Rawlings et al. 2006). Members of the same clan are proteases that have evolved from a common ancestor and share a common protein fold. Proteases in the same family are related based on the sequence homology of their amino acid sequences. Most, but not all, clans consist of one active site arrangement. The prefix “S” is used for clans formed entirely from families which are serine peptidases, whereas “P” is used for clans in which the homologous families are of several catalytic types (e.g., serine and cysteine), despite the fact that they have

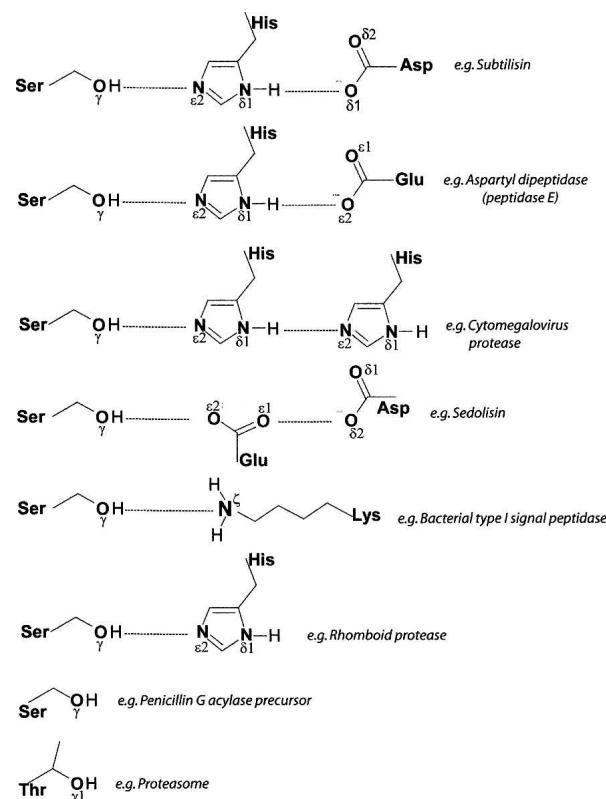


Figure 1. The catalytic residues used in serine and threonine proteases.

diverged from a common ancestor. Table 1 shows that proteases with a Ser/His/Asp triad fall within four of these clans (PA, SB, SC, and SK), while the Ser/Lys proteases fall within five clans (SE, SF, SJ, SK, and SR). This shows that different ancestors can converge on the same Ser/His/Asp or Ser/Lys mechanism.

Since some members of the same clan can use different active site architectures, this indicates that the tertiary structure is not always related to the active site configuration. For example, clan PB has family members that use a Ser/His/Glu active site configuration or Ser- or Thr-only active site architecture (Table 1). Why do different serine/threonine proteases use different active site configurations? The different active site arrangements may allow for activity in a different cellular environment. For instance, the proteases with Ser/Lys active sites typically carry out catalysis with a pH optimum that is higher than Ser/His/Asp proteases. In contrast, the pH optimum is lower for serine proteases with Ser/Glu/Asp active sites than those with Ser/His/Asp active sites. In large part, the different pH optimums reflect differences in the pK_a values of the different general base residues that are employed in catalysis. In addition, variations in the active site architecture of proteases may influence what cellular inhibitor they are susceptible to. There are other possibilities as well that we discuss below.

Table 1. Clans and families of serine and threonine proteases with representative PDB accession codes and active site residues

Protease	PDB	Nucleophile	General base	Other active site residues
Clan PA				
Family S1				
<i>Bos taurus</i> chymotrypsin A	2gmt	Ser195	His57	Asp102
Clan PB				
Family S45				
<i>Escherichia coli</i> penicillin G acylase precursor	1e3a	Ser264		
Family T1				
Archaeal (<i>Thermoplasma acidophilum</i>) 20S proteasome	1pma	Thr1		
Family T2				
<i>Homo sapiens</i> glycosylasparaginase precursor	1apy	Thr206		
Family T3				
<i>Escherichia coli</i> γ -glutamyltranspeptidase precursor	2e0w	Thr391		
Clan PC				
Family S51				
<i>Salmonella typhimurium</i> aspartyl dipeptidase	1fy6	Ser120	His157	Glu192
Clan SB				
Family S8				
<i>Bacillus licheniformis</i> subtilisin Carlsberg	1scn	Ser221	His64	Asp32
Family S53				
<i>Pseudomonas</i> sp. 101; sedolisin	1ga4	Ser287	Glu80	Asp84
Clan SC				
Family S10				
<i>Saccharomyces cerevisiae</i> carboxypeptidase Y	1cpy	Ser257	His508	Asp449
Clan SE				
Family S12				
<i>Streptomyces</i> sp. D-Ala-D-Ala carboxypeptidase B	3pte	Ser93	Lys96	Tyr190
Clan SF				
Family S24				
<i>Escherichia coli</i> UmuD	1umu	Ser60	Lys97	
<i>Escherichia coli</i> LexA repressor	1jhf	Ser119	Lys156	
Family S26				
<i>Escherichia coli</i> signal peptidase I	1b12	Ser90	Lys145	
Clan SH				
Family S21				
Cytomegalovirus protease (human herpesvirus 5)	1cmv	Ser132	His63	His157
Clan SJ				
Family S16				
<i>Escherichia coli</i> Lon-A peptidase	1rr9	Ser679	Lys722	
Family S50				
IPNV VP4 protease (infectious pancreatic necrosis virus)	2pnl	Ser633	Lys674	
Clan SK				
Family S14				
<i>Escherichia coli</i> peptidase ClpP	1tyf	Ser97	His122	Asp171
Family S41				
<i>Scenedesmus obliquus</i> C-terminal processing peptidase-1	1fc6	Ser372	Lys397	
Family S49				
<i>Escherichia coli</i> signal peptide peptidase A	3bf0	Ser409	Lys209	
Clan SP				
Family S59				
<i>Homo sapiens</i> nucleoporin 98	1ko6	Ser864	His862	
Clan SQ				
Family S58				
<i>Ochrobactrum anthropi</i> aminopeptidase DmpA	1b65	Ser250		
Clan SR				
Family S60				
<i>Homo sapiens</i> lactoferrin	1lct	Ser279	Lys93	
Clan SS				
Family S66				
<i>Pseudomonas aeruginosa</i> LD-carboxypeptidase	1zrs	Ser115	His285	Glu217
Clan ST				
Family S54				
<i>Escherichia coli</i> Rhomboid-1	2ic8	Ser201	His254	

Ser/His/Asp triad

Chymotrypsin- and trypsin-like proteases

Two of the best-known serine proteases that utilize the Ser/His/Asp triad are chymotrypsin and trypsin. They share the same protein fold and have their catalytic residues in the order of His/Asp/Ser from the N to C terminus. Their active site regions are composed of (1) the substrate binding groove where nonspecific main-chain hydrogen bond interactions occur between the enzyme and substrate, (2) the substrate specificity binding pockets, (3) the catalytic Ser/His/Asp triad, and (4) the oxyanion hole (for conventions regarding peptides, proteases, protease nomenclature, see <http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/intro.html#EC34>) (for a comprehensive review on the catalytic mechanism of classical serine proteases, see Hedstrom [2002]).

The substrate binding pocket of the protease allows the protease to bind its substrates and determines the substrate specificity of the enzyme. For example, trypsin cleaves its substrates at the C-terminal side of arginine or lysine (P1 position, Schechter and Berger nomenclature) (Schechter and Berger 1967). This specificity for a P1 basic side chain of substrates is stabilized by an acidic residue near the bottom of the sterically complementary deep S1 binding pocket. In some proteases, the specificity can go to the opposite side of the cleavage site (scissile bond) as well, P' side, or also further down the chain of the substrate to the P3, P4, P5 site, etc. The substrate region near the cleavage site needs to be in an extended conformation (not protected by hydrogen bonds within the substrate itself) to bind to the protease in order for the carbonyl of the scissile bond to be attacked by the serine hydroxyl nucleophile of the protease.

Chymotrypsin numbering is conventionally used for sequence and structure comparisons within clan PA such that the catalytic nucleophile is Ser195, the general base is His57, and the aspartate residue is Asp102 (Fig. 2). The backbone amide nitrogens of Gly193 and Ser195 form the oxyanion hole. The serine attacks the trigonal planar scissile carbonyl carbon (Fig. 2A), forming an oxyanion tetrahedral intermediate (Fig. 2B). The tetrahedral intermediate then collapses, which is followed by the loss of the C-terminal peptide. This gives rise to an acyl-enzyme intermediate (Fig. 2C). The acyl intermediate is then attacked by an activated water molecule (the same active site histidine again functioning as the general base), giving rise to the second tetrahedral intermediate (Fig. 2D). This intermediate then collapses leading to the release of the N-terminal peptide.

In the catalytic mechanism, hydrogen bond interactions have been proposed to play important roles. The nucleophilic Ser195 O γ H hydrogen bonds to His57 N ϵ 2 and

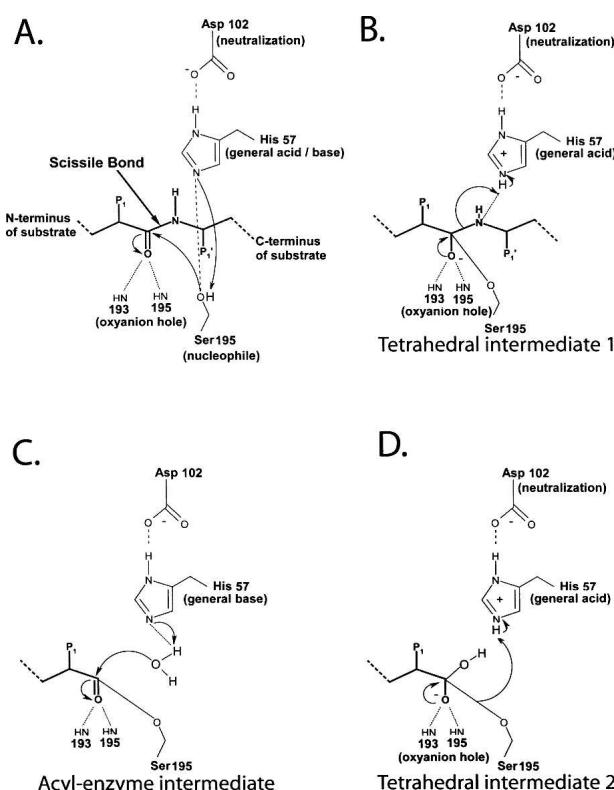


Figure 2. The proteolytic mechanism used by the “classical” Ser/His/Asp serine proteases. The steps in the “classical” Ser/His/Asp serine protease catalytic mechanism includes: (A) Michaelis complex, (B) tetrahedral intermediate 1, (C) acyl-enzyme intermediate, and (D) tetrahedral intermediate 2.

transfers its proton from its hydroxyl group (O γ H) to the electrons on the His57 side-chain nitrogen (N ϵ 2). The His57 N δ 1/Asp102 O δ 1 hydrogen bond could be classified as a low-barrier hydrogen bond (Frey et al. 1994), although its exact role is still controversial. These hydrogen bonds are present throughout the catalytic cycle, as seen from the hydrogen-bonding network in noncovalent complexes, acyl-enzyme complexes, and transition state analog complexes. In addition, a C–H–O hydrogen bond has been proposed between the Ser214 main-chain carbonyl oxygen of chymotrypsin-like proteases and the hydrogen on the ϵ 1 carbon of the His57 side chain (Derewenda et al. 1994; Ash et al. 2000). It has been postulated that this interaction may function to prevent the localization of the positive charge on N δ 1 of His57.

High-resolution crystal structures (at 1.2 Å and better) have provided considerable information about serine protease mechanism and enzyme mechanism in general. Katona et al. (2002) solved the structure of the porcine pancreatic elastase in complex with a peptide at 0.95 Å resolution. This high-resolution structure revealed that the ester bond between the carbonyl bond of the peptide substrate and the O γ of the nucleophilic serine has a planar geometry. This observation refined the previous theory of Warshel and

Russell (1986), who proposed that the H-bonds between the ester carbonyl oxygen and the oxyanion hole induced strain on the ester bond and distorted it toward sp^3 hybridization, which would enhance the formation rate of the second tetrahedral intermediate. A recent comparison of the acyl-enzyme structure and a high-resolution Michaelis complex structure suggests that the β -strand H-bond type interactions between the enzyme binding site and substrate become shorter during the transition from the Michaelis complex to the acyl-enzyme complex (Fodor et al. 2006). This result suggests that the enzyme may be able to utilize the energy released from the shorter, stronger hydrogen bonds to help overcome the energy barrier in the nucleophilic attack steps.

Mutagenesis studies on the related trypsin protein revealed that Ser195 and His57 contribute ~ 1 million-fold toward the rate of catalysis for trypsin (Corey et al. 1992). The Asp102 contributes $\sim 10,000$ -fold as determined by mutagenesis experiments (Craik et al. 1987; Corey et al. 1992).

Subtilisin-like proteases

Like chymotrypsin (Fig. 3A) and trypsin, subtilisin (Fig. 3B) utilizes a Ser/His/Asp triad (Fig. 3B), but it has no sequence similarity to chymotrypsin-like proteases. In fact, it adopts the α/β -twisted open sheet structure (Wright et al. 1969) rather than the double β -barrel structure seen in the chymotrypsin-like proteases. Subtilisin and chymotrypsin provide excellent examples of convergent evolution (Wallace et al. 1996). The protein folds of these proteases are completely different, although they both converged on a similar Ser/His/Asp mechanism to carry out proteolysis. In the subtilisin-like proteases, the order of the active site residues is Asp/His/Ser in the primary sequence, from N to C terminus, which is different than the chymotrypsin-like proteases (His/Asp/Ser). The serine and histidine residues contribute 10^6 -fold toward catalysis, and the aspartic acid contributes 10^4 -fold residue (Carter and Wells 1988). The active site region of subtilisin is shown in Figure 3B. The subtilisin Asn155, which forms a hydrogen bond to the oxyanion intermediate, contributes ~ 100 - to 1000-fold (Wells et al. 1986; Pantoliano et al. 1987). Subtilisin is engineered for thermal and pH stability for use in laundry detergents. These subtilisin mutants were among the first patents granted for engineered proteins.

Other Ser/His/Asp proteases with different folds than the chymotrypsin- and subtilisin-like proteases include carboxypeptidase Y (Jung et al. 1999) and ClpP (Wang et al. 1997) proteases (Table 1). Interestingly, the ClpP protease is within the SK clan that has members that possess alternative active site arrangements such as the Ser/Lys dyad configuration.

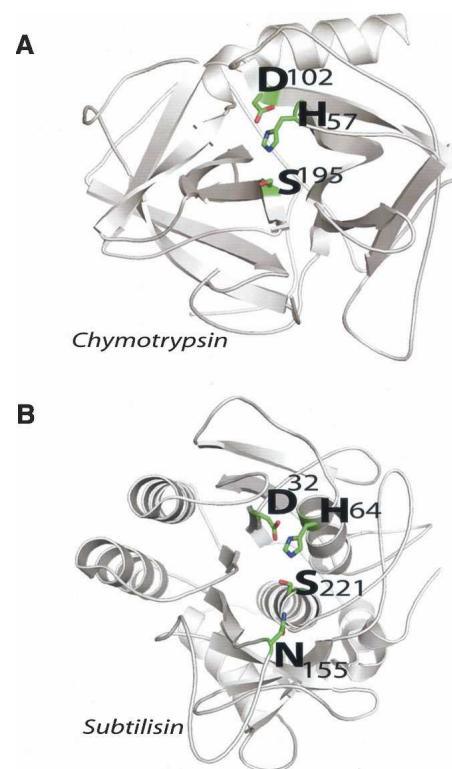


Figure 3. Structures of the “classical” Ser/His/Asp serine proteases. (A) Chymotrypsin from *Bos taurus* (PDB 2gmt). (B) Subtilisin Carlsberg from *Bacillus licheniformis* (PDB 1scn). The subtilisin protease family is different from the chymotrypsin protease family in protein fold, and it utilizes the side chain of an asparagine in its oxyanion hole (Asn155). The proteases are shown in standard orientation. The “standard orientation” presents the enzyme such that the reader is looking down onto the substrate binding groove so that the catalytic residues (the Ser-His-Asp catalytic triad) are located on the right side of the figure. The N-terminal side of the substrate would bind on the left-hand side of the binding groove, and the C-terminal side of the substrate (P1/P1' residues, the scissile bond) would bind the right side of the binding groove, nearest the catalytic residues. The length of the substrate binding groove varies from protease to protease.

Ser/His/Glu triad

Aspartyl dipeptidase

A variation of the classical Ser/His/Asp triad is found in the aspartyl dipeptidase protease, where the aspartate is substituted by a glutamate residue (Rawlings and Barrett 1999). Aspartyl dipeptidase is not inactivated by the conventional serine protease inhibitor DFP or PMSF (phenyl methane sulfonyl fluoride) (Conlin et al. 1994). While initial site-directed mutagenesis studies suggested that aspartyl dipeptidase was a classical serine protease, the structure of aspartyl dipeptidase from *Salmonella typhimurium* revealed that it in fact belongs to a new serine peptidase family with a Ser/His/Glu triad (Hakansson et al. 2000). The Ser120 O γ atom is 2.7 Å away from the His157 N $\epsilon 2$ atom. The

Glu192 O_ε2 is within 2.7 Å of the His N_δ1 atom. This glutamate carboxylate/histidine imidazole hydrogen bond adopts an unusual *anti*-conformation rather than the *syn*-conformation usually seen with other serine proteases (Ippolito et al. 1990).

Mutagenesis of the glutamate residue of the *S. typhimurium* aspartyl dipeptidase resulted in a 100-fold drop in activity. Typically, mutagenesis of the aspartate residue, the third member of the catalytic triad (Ser/His/Asp), leads to a 10,000-fold loss in activity (Craik et al. 1987; Carter and Wells 1988; Corey et al. 1992). Interestingly, the aspartyl dipeptidase has a similar protein fold as the cysteine proteases γ -glutamyl hydrolase and PfpI peptidase, suggesting they evolved from a common ancestor.

LD-carboxypeptidase

The Ser/His/Glu active site arrangement has evolved more than once. The LD-carboxypeptidase from *Pseudomonas aeruginosa* that functions in peptidoglycan recycling uses a Ser/His/Glu triad (Fig. 4A). The crystal structure of the LD-carboxypeptidase from *P. aeruginosa* was solved to 1.5 Å resolution (Korza and Bochtler 2005). Its protein fold consists of an N-terminal β -sheet and a C-terminal β -barrel domain that is different from the aspartyl dipeptidase fold. This indicates that convergent evolution has taken place to give rise to the Ser/His/Glu triad. It is not clear why most serine proteases utilize an aspartate rather than the glutamate observed with the LD-carboxypeptidase. Notably, the Ser/His/Glu active site is observed in lipases (Schrag et al. 1991), and in some esterases as well (Nachon et al. 2005).

Ser/His/His triad: Cytomegalovirus protease

Another unconventional active site triad, Ser/His/His, is found in herpes virus proteases that plays a key role in the maturation of the assembly protein. The cytomegalovirus protease is inactivated by DFP (Stevens et al. 1994). Ser132 was identified as the active site serine of the human cytomegalovirus protease as it was modified by the inhibitor. The invariant His63 is critical for activity as determined by site-directed mutagenesis (Welch et al. 1993).

The Ser/His/His catalytic triad was validated by the crystal structure of the cytomegalovirus protease (Qiu et al. 1996; Shieh et al. 1996; Tong et al. 1996). The structure revealed that the protein has a unique fold with the Ser132 O_γ atom and bridging His63 N_ε2 nitrogen being within hydrogen-bonding distance (Fig. 4B). In addition, the His63 N_δ1 is within hydrogen-bonding distance to the His157 N_ε2 that replaces the aspartate residue within the classic serine proteases.

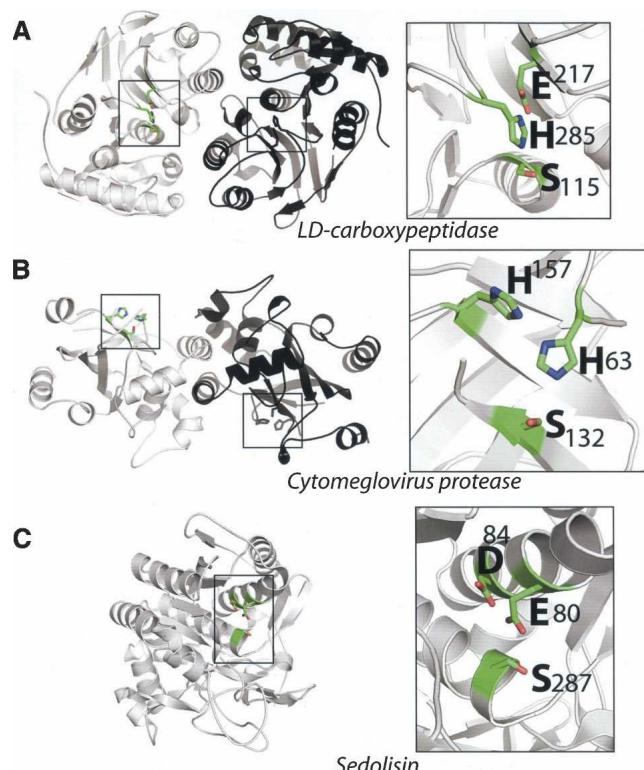


Figure 4. Structures of serine proteases that use variations on the active site triad. (A) LD-carboxypeptidase from *Pseudomonas aeruginosa* (PDB 1zrs) utilizes a Ser/His/Glu triad. (B) Cytomegalovirus protease (PDB 1cmv) utilizes a Ser/His/His triad. (C) Sedolisin from *Pseudomonas* sp. (PDB 1ga4) uses a Ser/Glu/Asp triad. A zoomed-in view of the catalytic residues is shown to the right of each structure. The side chains are shown as sticks with nitrogens in blue, oxygens in red, and carbons in green. LD-carboxypeptidase (A) and cytomegalovirus protease (B) exist as homodimers in solution; therefore, one monomer is shown in white and the other monomer is shown in black.

Site-directed mutagenesis studies showed the non-bridging histidine residue (His157) of the triad contributes only \sim 10-fold to the catalytic rate of the enzyme (Khayat et al. 2001) in comparison to 1000- to 10,000-fold for the contribution of aspartate in the classical triad protease (Carter and Wells 1988; Corey et al. 1992). Therefore, the cytomegalovirus protease functions more like a Ser/His dyad. Herpes virus proteases, in comparison to Ser/His/Asp proteases such as trypsin and subtilisin, are kinetically less efficient enzymes. This suggests that this alternate active site is a way to tune the catalytic activity of the serine protease.

Ser/Glu/Asp triad: Sedolisin proteases

The sedolisin proteases carry out catalysis using a novel Ser/Glu/Asp triad (Wlodawer et al. 2001a). These proteases take their name from their catalytic groups serine (S), glutamate (E), and aspartate (D). Due to the low pK_a

of the carboxylate that plays the role of the general base, these proteases are active at low pH, which is unusual for serine proteases. Proteases in this clan include kumamolysin that is found in acidic hot springs and human tripeptidyl peptidase that is found in the low pH environment of the lysosome. This is an excellent demonstration of how the different active site architectures allow the protease to be active in different cellular environments.

Because they operate at low pH, these proteases were thought to be aspartic acid proteases. However, these proteases are not inactivated by pepstatin, which is a natural inhibitor of the aspartic acid class of proteases. Rather they are inactivated by inhibitors with aldehyde functional groups such as chymostatin, which typically modify the serine nucleophile within serine proteases (Wlodawer et al. 2001b).

The X-ray structure of the sedolisin protease from *Pseudomonas* revealed that these proteases have the subtilisin protein fold (Wlodawer et al. 2001a). Structures of the enzyme with the iodotyrosatin inhibitor revealed that Ser287 is the catalytic residue. Ser287 is covalently attached to the inhibitor and forms a hemiacetal linkage to the Ser278 O γ side chain. Within hydrogen-bonding distance of the Ser278 side chain is the side-chain carboxylate of Glu80 (Fig. 4C), suggesting that the acidic residue acts as the general base in catalysis. The third member of the triad is Asp84, which interacts with the Glu80 side chain. It is the Glu80/Asp84 pair that allows this protease class to have optimum activity at acidic pH. The active site geometry was also confirmed in a 1.4 Å X-ray structure of kumamolysin, a serine-carboxyl-type protease from *Bacillus* (Comellas-Bigler et al. 2002). Site-directed mutagenesis studies confirmed the importance of these active site serine, glutamate, and aspartate residues (Oyama et al. 1999, 2005).

Ser/Lys dyad

Type I signal peptidases

The prototypic protease of the Ser/Lys class is the type I signal peptidase found in the SF clan (Table 1). These proteases function to proteolytically remove N-terminal signal peptides from exported proteins after they have been translocated across the membrane (for review, see Paetzel et al. 2002b). Signal peptidases are integral membrane proteins with their catalytic domain on the extracytoplasmic side of the membrane.

Early studies pointed to the fact that signal peptidases were novel proteases. First, these proteases failed to be inhibited by inhibitors against the aspartic acid, metallo, serine, and cysteine protease groups (Zwizinski et al. 1981; Black et al. 1992; Kuo et al. 1993). Second, the

Escherichia coli signal peptidase had a critical serine (Ser90) and lysine (Lys145) residue (Sung and Dalbey 1992; Black 1993; Tschantz et al. 1993) but no critical histidine residue (Sung and Dalbey 1992), and both residues are absolutely conserved in evolution within the bacterial, chloroplast, and mitochondrial signal peptidase family members (Paetzel et al. 2002b).

The 1.9 Å X-ray structure of the catalytic domain of the *E. coli* signal peptidase with a covalently bound inhibitor provided direct proof of a Ser/Lys dyad operating within the signal peptidase family (Paetzel et al. 1998). The Ser90 was covalently linked to the 5S-penem inhibitor, indicating that the Ser90 is the nucleophile. The Lys145 was within 2.8 Å of the Ser90 (Fig. 5A), supporting the idea that it does indeed function as the general base. No other ionizable groups were located within 7.5 Å of the Ser90 O γ residue. The Lys145 is completely buried within the active site, which presumably lowers the lysine's pK_a, explaining how the lysine can serve as a general base at biological pH. Interestingly, Ser278 O γ forms a hydrogen bond to Lys145 N ζ , helping to orient the lysine residue. The structure of the signal peptidase apoenzyme is consistent with Ser88 O γ participating in the formation of the oxyanion hole (Paetzel et al. 2002a).

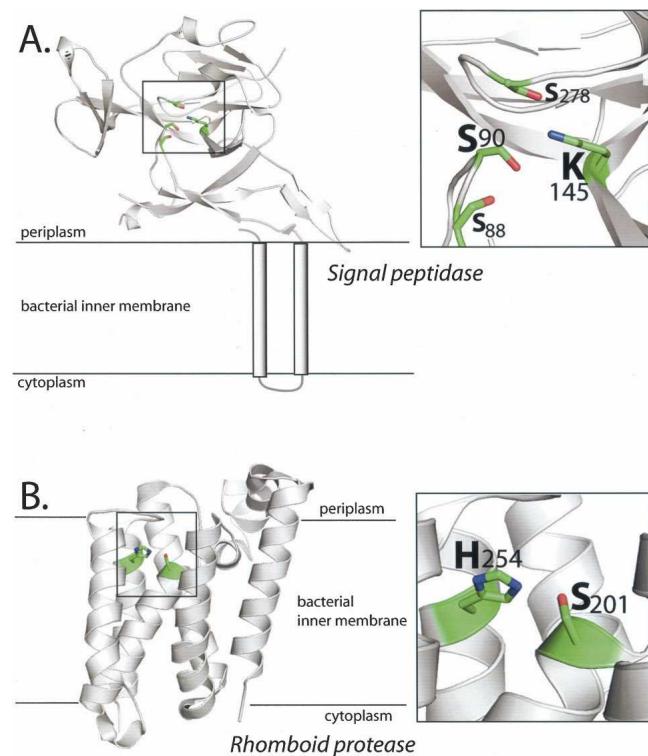


Figure 5. Structures of serine proteases that use active site dyads. (A) The Type I signal peptidase from *E. coli* (PDB 1b12) uses a Ser/Lys catalytic dyad. (B) The rhomboid protease GlpG from *E. coli* (PDB 2ic8) uses a Ser/His dyad.

Mutagenesis studies have shown Ser88 contributes \sim 1000-fold to catalysis by stabilizing the oxyanion intermediate (Carlos et al. 2000).

The active site lysine residue has an apparent pK_a of 8.7 as determined from the pH rate profile of the *E. coli* signal peptidase (Paetzel et al. 1997). Typically, Ser/Lys proteases show optimum activity at a higher pH such as 9 compared with Ser/His/Asp proteases that have optimum activity near pH 7.

The UmuD family of peptidases

Other Ser/Lys dyad proteases are found within the UmuD family of peptidases that include UmuD, LexA, and the λ repressor (Slilaty and Little 1987; Little 2004; Paetzel and Woodgate 2004). These proteases are involved in the SOS response and function as repressors. These proteins undergo autoproteolysis *in vivo* in a manner dependent on RecA, a protein involved in homologous recombination. The biological function of autocleavage is to inactivate these proteins (Mustard and Little 2000).

UmuD was the first protease in the UmuD family whose structure was solved to high resolution. Its protein fold consists of a β -sheet structure (Peat et al. 1996), and the critical serine and lysine residues that were implicated in autocatalysis (McDonald et al. 1998) are within hydrogen-bonding distance. The likely role of the RecA binding in the *in vivo* autoproteolysis reaction is to decrease the pK_a of the general base by burying the ϵ -amino group of the lysine in a hydrophobic environment.

LexA protease is proposed to use an active site Ser/Lys dyad that is not inactivated by a classical protease inhibitor (Slilaty and Little 1987). Autodigestion of LexA is pH-dependent. In the absence of RecA, a basic residue with a pK_a of 10 is necessary for autodigestion (Slilaty et al. 1986). A likely candidate for this basic group is a lysine residue. Further evidence came from structural studies. Briefly, the structure of LexA was solved at 2.0 \AA with two conformations of the protein present (Luo et al. 2001). One of the conformations, the “cleavable form (C),” revealed the scissile bond of LexA within the active site region. The other conformation, the “noncleavable form (NC)” of the protein had the scissile bond of LexA located \sim 20 \AA from the Ser/Lys dyad. A 1.8 \AA resolution crystal structure revealed that the catalytic serine and lysine are within hydrogen-bonding distance. Consistent with the lysine functioning as a general base in the proteolytic mechanism, the lysine side chain is completely buried in a hydrophobic pocket in the cleaved form. Similar to signal peptidase, this hydrophobic environment is expected to lower the pK_a of the catalytic lysine. The residues that contribute to the oxyanion hole are the main-chain amide nitrogens of Met118 and Ser119.

The λ repressor, which also has a Ser/Lys dyad, has a Thr190 residue that may also help orient the catalytic Lys192 residue as it is hydrogen bonded to this residue (Bell et al. 2000). Most of the Ser/Lys proteases (such as UmuD, LexA, Lon, VP4, signal peptidase I, and signal peptide peptidase SppA) have a second (non-nucleophilic) hydroxyl group coordinating to the lysine, suggesting this interaction has a functional role (Paetzel et al. 2002a). This seems to be a common theme with Ser/Lys proteases, in general.

Comparison of the active site regions of the signal peptidase and UmuD family proteases reveals that the orientation of the serine and lysine are quite similar (Paetzel and Strynadka 1999; Paetzel et al. 2002b). In these proteases, the serine hydroxyl groups attack the *si*-face of the scissile amide bond. In contrast, serine proteases that contain the Ser/His/Asp triad attack the *re*-face of the substrate (James 1994).

Lon protease

Another Ser/Lys dyad serine protease is the Lon protease, which is involved in quality control and degradation of misfolded proteins. It has a peptidase and an AAA domain. The latter domain uses the energy of ATP hydrolysis to actively unfold proteins (Chung 2004). However, ATP hydrolysis is not required for cleavage of short peptides.

In 2003, the *E. coli* Lon protease was shown to contain an essential serine and lysine residue (Rotanova et al. 2003). Like many Ser/Lys proteases (Tschantz and Dalbey 1994), the Lon protease is not inhibited by the classical serine protease inhibitors such as PMSF or DFP. The X-ray crystal structure of the peptidase domain was achieved using a mutant with the catalytic serine residue mutated to an alanine. Modeling studies where the Ala679 was replaced with Ser679 are consistent with a Ser/Lys dyad mechanism and revealed that the ϵ -amino group of Lys722 is in position to hydrogen bond to the catalytic Ser679 residue. In addition, the conserved Thr704 residue could hydrogen bond to the Lys722 side chain, helping to position the general base. The protein fold of the *E. coli* Lon protease domain is completely different from that found in the signal peptidase and UmuD family members and contain 10 β -strands and six α -helices (Botos et al. 2004).

C-terminal processing peptidases

The Ser/Lys dyad C-terminal processing peptidases (Cpps) cleave precursor proteins in eubacteria and plants and show no sequence homology with signal peptidases and UmuD members or to the D-Ala-D-Ala

carboxypeptidases. *E. coli* tail specific protease (Tsp) exhibits C-terminal processing activity and is implicated in the degradation of truncated proteins that are tagged by the SsrA pathway (Keiler et al. 1996). Tsp is located in the periplasm of Gram-negative bacteria and in the extracellular/cell wall region of Gram-positive bacteria.

The *E. coli* Tsp, as seen with the *E. coli* signal peptidase, is not inactivated by inhibitors against the four groups of proteases (Silber et al. 1992). Mutagenesis studies showed that the protein had a critical serine and lysine residue (Keiler and Sauer 1995). Sequence alignment studies of Cpp members revealed that these residues are absolutely conserved (Keiler and Sauer 2001). Interestingly, N-ethylmaleimide could inactivate Tsp when the critical serine is changed to a cysteine, supporting the notion that the serine is an active site residue (Keiler and Sauer 1995). Tsp has activity over a broad pH range, from pH 5 to 9.

Currently, the only Cpp whose structure is solved is the photosystem II D1 C-terminal processing protease from the green algae *Scenedesmus obliquus* (Liao et al. 2000), which is essential for the assembly of the manganese cluster involved in water oxidation (Taylor et al. 1988). The structure reveals the C-terminal protease domain with the active site Ser372 and Lys397 residues, a middle PDZ-like domain, and a mainly α -helical N-terminal domain. The active site Lys397 is hydrogen bonded to a threonine residue. The active site Ser372 interacts with several water molecules. In order for Lys397 to act as a general base, a conformational change has to take place upon substrate binding. In addition, substrate binding most likely would cause the Lys397 to become buried in a hydrophobic environment so that its pK_a is lowered.

Other Ser/Lys peptidases include the penicillin binding proteins 5 D-Ala-D-Ala carboxypeptidase B (Davies et al. 2001), the viral VP4 protease of the Birnaviridae family (Feldman et al. 2006; Lee et al. 2007), signal peptide peptidase A (Kim et al. 2007), and Lactoferrin (Table 1; Anderson et al. 1989). The Ser/Lys dyad active site arrangement has arisen many times during evolution.

Ser/His Dyad

Rhombopeptidase

Rhombopeptidases have a Ser/His active site dyad. These proteases play roles in signal transduction and participate in regulated intramembrane proteolysis (Selkoe and Kopan 2003; Wolfe and Kopan 2004). They have six transmembrane helices, and their catalytic groups are localized within the membrane plane (Wolfe and Kopan 2004; Maegawa et al. 2005). Initial site-directed mutagenesis studies suggested that the rhombopeptidase utilized a Ser/His/Asn catalytic triad (Urban et al. 2001).

However, further studies showed that the asparagine, the third member of the proposed triad, is not always necessary for catalysis (Lemberg et al. 2001; Maegawa et al. 2005).

Recently, the X-ray structure of the *E. coli* and *Haemophilus influenzae* rhombopeptidase GlpG was solved (Wang et al. 2006; Wu et al. 2006; Ben-Shem et al. 2007; Lemieux et al. 2007)). The *E. coli* structure revealed that the protease does use a Ser/His dyad in catalysis where Ser201 and His254 are within hydrogen-bonding distance (Fig. 5B). The structure showed that its catalytic groups are in a hydrophilic environment, \sim 10 Å below the periplasmic membrane surface. In addition, the GlpG protease has a proposed gate that controls access to the hydrophilic active site region of GlpG (Baker et al. 2007; Wang and Ha 2007). Although the gating mechanism is controversial because none of the structures contain an extended substrate, this mechanism allows for the substrate to enter the substrate binding site of the protease laterally from the lipid phase.

Nucleoporin

Another Ser/His peptidase is the nucleoporin proteins that undergo autoprocessing to produce a dimer. The crystal structure of the human nucleoporin Nup98 revealed an active site Ser/His dyad (Hodel et al. 2002). The structure was determined using a catalytic deficient mutant where the serine nucleophile was substituted with an alanine. The overall structure revealed a β -sandwich-like protein fold that is half open. Modeling studies where the catalytic Ser864 hydroxyl side chain is modeled from the solved mutant Ser864Ala structure show that it can form a hydrogen bond to the His862. Surprisingly, mutagenesis of the His862 to alanine only slightly inhibits the enzyme with over 50% activity remaining. This is very unusual for serine proteases as a similar mutation in subtilisin causes a million-fold inhibition (Hodel et al. 2002).

“Ser-only” configuration

L-aminopeptidase D-Ala-esterase/amidase

The simplest active site arrangement uses a “Ser-only” configuration. Proteases that use this configuration are made in a precursor form and undergo autoprocessing to generate the active site residue at position 1 in the mature protein. This active site arrangement is found in the *Ochrobactrum anthropi* L-aminopeptidase D-Ala-esterase/amidase. After autocleavage, Ser250 becomes the first residue of the mature protein and the α -amino group of the newly generated N terminus of the protein functions as the general base in catalysis abstracting a proton from the

Ser250 hydroxyl group. The protein fold of this protease is novel and characterized by a four-layered $\alpha\beta\beta\alpha$ topology (Bompard-Gilles et al. 2000) called the N-terminal nucleophile hydrolase fold (Brannigan et al. 1995).

Penicillin G acylase precursor

The penicillin G acylase precursor uses a “Ser-only” catalytic configuration to undergo autoproteolysis. It undergoes self-processing using Ser264 as a nucleophile (Fig. 6A) in order to produce the enzymatically active mature acylase. The autoproteolysis generates a dimeric enzyme with the serine nucleophile located at the N terminus of one of the subunits (Ser264 becomes Ser1 in one of the newly generated protein subunits). This penicillin G acylase protein has an $\alpha\beta\beta\alpha$ -core protein fold (Duggleby et al. 1995), similar to the L-amino-peptidase D-Ala-esterase/amidase L. This fold is also found in the proteasome and glutamine PRPP amidotransferase.

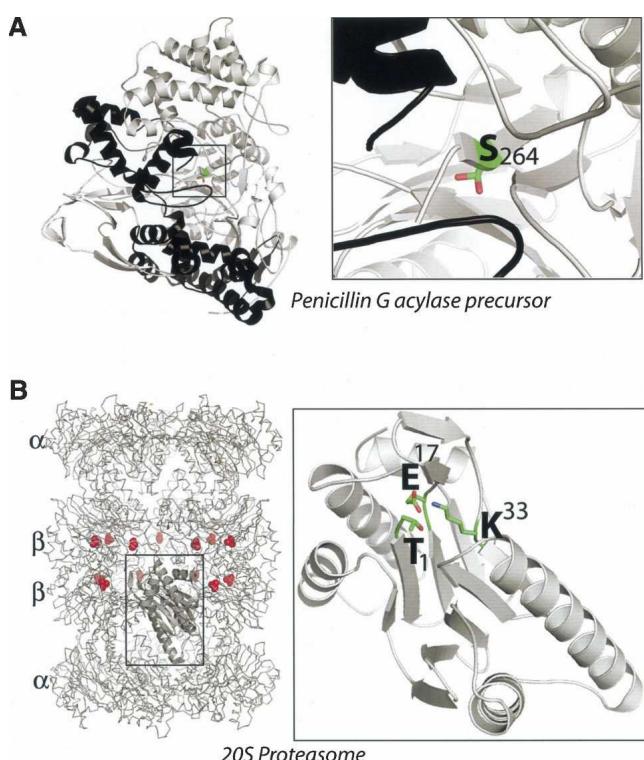


Figure 6. Structures of serine and threonine proteases that use single active site residues. (A) Serine-only peptidase. The penicillin acylase from *E. coli*. The electron density for the nucleophilic Ser264 revealed alternate conformations for the side-chain hydroxyl. One of the conformations directs the $O\gamma$ toward the scissile carbonyl between residues 263 and 264. (PDB 1e3a) (Hewitt et al. 2000). Chain A is shown in black, chain B is in white. (B) “Thr-only” peptidase. The 20S proteasome from the Archaeon *thermoplasma acidophilum* (PDB 1pma). The active site threonine for each β -subunit is shown as red spheres. For clarity, one β -subunit is highlighted as a ribbon diagram; all other protein chains are rendered as $C\alpha$ trace.

transferase that contain a catalytic threonine located at residue 1 in the polypeptide chain (Brannigan et al. 1995; Lowe et al. 1995; Oinonen and Rouvinen 2000). In the glutamine PRPP enzyme, a cysteine nucleophile is found at this position (Smith et al. 1994). Therefore, proteins that have the N-terminal nucleophile hydrolase fold always carry out catalysis with an N-terminal active site residue (Brannigan et al. 1995), although the specific active site residue (serine, threonine, or cysteine) can vary.

Insights into the catalytic mechanism of these peptidases emerged from the structure of the penicillin G acylase precursor from *E. coli* (Hewitt et al. 2000). The structure revealed two conformations of the active site. One of the conformations revealed that Ser264 in the active site is positioned such that its $O\gamma$ group could attack the appropriate carbonyl carbon atom of the scissile bond. Moreover, this active site Ser264 is absolutely essential for activity (Choi et al. 1992). Strikingly, from the structure, there was no obvious candidate for the general base in the proteolytic reaction (Hewitt et al. 2000).

Glutaryl 7-aminocephalosporanic acid acylase precursor (GCA precursor)

There is another member of the acylase family called GCA, glutaryl 7-aminocephalosporanic acid acylase with a Ser-only catalytic configuration. The structure of the precursor GCA at 2.5 Å resolution suggests that a water molecule is the general base that activates the active site serine residue (Kim et al. 2002; Yoon et al. 2004). The water molecule is coordinated by the main-chain NHs of Thr69 and Gly168 and by the amide side chain of Asn244.

“Thr-only” configuration

Proteasome

The proteasome is a threonine protease that plays a key role in protein degradation and quality control. Proteolysis involves a Thr/ α -amine configuration. In archaea and prokaryotes, the proteasome forms a 20S structure comprised of four 7-membered rings ($\alpha\beta\beta\alpha\beta\beta\alpha\beta\alpha$) (for review, see Baumeister et al. 1998; Voges et al. 1999). The two outer rings are comprised of identical α -subunits and the two inner rings contain the identical β -subunits. The β -subunits contain the active site. In eukaryotic cells, the 20S proteolytic core complex associates with a regulatory domain complex, forming a 26S proteinase particle. This 26S particle functions in the ubiquitin-dependent degradation pathway. It uses ATP hydrolysis to unfold protein substrates and promoting translocation of the substrate peptide chain into the protease degradation chamber.

In contrast to eubacterial and archaeal proteasomes, the eukaryotic enzyme contains two copies each of seven distinct α - and β -subunits. The protease chamber contains 28 subunits that form the 20S particle. The α -subunits are located on the outside of the 20S particle, and the β polypeptides are on the inside. Briefly, of the seven different types of β -subunits, three of them are proteolytically active (for review, see Voges et al. 1999). The eukaryotic proteasome exhibits several distinct substrate specificities cleaving after basic, hydrophobic, or acidic amino acids.

The active site of the proteasome is comprised of an N-terminal threonine residue. To form the proteasome active site, it is necessary for the β -subunit to be autoprocessed such that the N-terminal propeptide is removed from the precursor polypeptide as the 20S proteasome particle is being assembled (Seemuller et al. 2001). Evidence that the active site threonine is involved in catalysis comes from site-directed mutagenesis and protease inhibitor studies. Mutagenesis of the threonine to an alanine residue blocks processing of the archaeal (Seemuller et al. 1995) and yeast proteasome (Chen and Hochstrasser 1996; Arendt and Hochstrasser 1997; Heinemeyer et al. 1997). Modification of the threonine residue of the proteasome by protease inhibitors completely inactivates the protease (Fenteany et al. 1995; Bogyo et al. 1997; Groll et al. 1997).

The crystal structure of the *Thermoplasma acidophilum* proteasome showed that the catalytic machinery is composed of the Thr1, Glu17, and Lys33 (Fig. 6B; Lowe et al. 1995). The structure was solved in the presence of the calpain inhibitor I, acetyl-Leu-Leu-norleucinal (LLnL). The structure revealed that the Thr1 hydroxyl group is localized very close to the aldehyde functional group of LLnL and may form a hemiacetal. Thus, the structure supports the notion that the Thr1 $\text{O}\gamma$ residue is the nucleophile involved in catalysis. Also in proximity to the Thr1 residue is the amino group of Lys33 and the Glu17 carboxylate. The Lys33 ϵ -amino group is coordinated to the Glu17 carboxylate and the threonine side-chain oxygen. Based on the coordination partners, it is likely that the Lys33 is positively charged. Initially, the amino group of Thr1 was proposed to function as the general base. However, a bound water molecule, located in between the Thr1 amino group and its alcohol side-chain hydroxyl most likely acts as the general base to deprotonate the Thr1 hydroxyl group. The N terminus is coordinated to the carbonyl groups of Arg19 and Ala168 residues. A similar active site architecture is seen within the enzymatically active β subunit of the yeast proteasome solved at 2.4 \AA resolution (Groll et al. 1997).

Proteasome precursor

A “Thr-only” catalytic mechanism is involved in autoproteolysis to generate mature proteasome (Seemuller

et al. 1996). A water molecule bound to the proteasome precursor β -subunit is proposed to function as a general base in activating the threonine hydroxyl group (Ditzel et al. 1998; for review, see Seemuller et al. 2001).

Glycosylasparaginase and γ -glutamyltransferase I protein precursors

Other “Thr-only” active site peptidases are the glycosylasparaginase and γ -glutamyltransferase I protein in which a threonine residue is the catalytic nucleophile involved in autocleavage. A threonine residue is implicated in autoproteolysis of the glycosylasparaginase (Aronson 2004). The inhibitor 5-diazo-4-oxonorvaline has been shown to react with the N-terminal threonine amino acid of the β subunit (Kaartinen et al. 1991). The γ -glutamyltransferase I protein (Sakai et al. 1996), which undergoes self processing, also requires a threonine residue (Suzuki and Kumagai 2002; Okada et al. 2007). Both the glycosylasparaginase and γ -glutamyltransferase I protein have the N-terminal nucleophile hydrolase fold (Brannigan et al. 1995; Suzuki and Kumagai 2002; Okada et al. 2007). To date, the structural studies did not clearly determine the general base that activates the threonine hydroxyl group in the precursor.

Variation on a theme: Ser/Ser/Lys triad

A general base lysine residue is not only used in serine proteases but also employed in certain amidases that contain a Ser/Ser/Lys triad active site. The amidases are found in archaea, eubacteria, fungi, plants, and mammals and rival the number of proteins in the Ser/His/Asp serine protease families (for review, see McKinney and Cravatt 2005). The signature domain is \sim 130 residues in length and includes the conserved motif with the active site Ser/Ser/Lys residues. Initially, it was unclear what type of mechanism was used by the amidases since they were inactivated by both serine and cysteine types of protease inhibitors (Ueda et al. 1995; Patterson et al. 1996).

By using the inhibitor ethoxy oleoyl fluorophosphonate, it was shown that serine is the nucleophilic residue in this amidase family (Patricelli et al. 1999). Ser241 was established to be the active site nucleophile of the fatty acid amide hydrolase (FAAH). While no histidine residues were found to be required for activity, the invariant Ser217, Ser241, and Lys142 residues were discovered to be indispensable for catalysis (Patricelli and Cravatt 1999; Patricelli et al. 1999).

The pH rate profiles for the FAAH enzyme indicated an increase in activity going from pH 5 to 9, revealing a titratable group with a pK_a of \sim 7.9. This titratable group most likely corresponds to the Lys142. Mutation

of the lysine creates altered pH rate profiles that are similar to those of histidine mutants in classical Ser/His/Asp proteases (Patricelli et al. 1999). The combined data suggested that the amidase family employed a Ser/Lys dyad mechanism (Patricelli and Cravatt 2000).

The Ser/Ser/Lys triad active site was established by the crystal structures of several amidases (Fig. 7; Bracey et al. 2002; Shin et al. 2002). The structure of the malonamidase E2 demonstrated that the catalytic serine residue was separated from the lysine by a bridging serine residue (Shin et al. 2002). The bridging serine was in an unusual conformation, in which the serine hydroxyl side chain and the amide nitrogen polarize the catalytic serine. The lysine ϵ -amino group was hydrogen bonded to the bridging serine residue hydroxyl. The Ser/Ser/Lys triad was also seen in the structure of the FAAH enzyme (Bracey et al. 2002). The Ser241 was covalently attached to a phosphonate inhibitor while the bridging Ser217 was hydrogen bonded to Lys142.

The catalytic mechanism of the FAAH amidases is unusual. In addition to using a lysine as a general base, the residue (Ser217) that normally removes the proton from the catalytic nucleophile (Ser241) is not the “catalytic base.” It is Lys217 that interacts only with the bridging serine that initiates the entire proton transfer process (McKinney and Cravatt 2003).

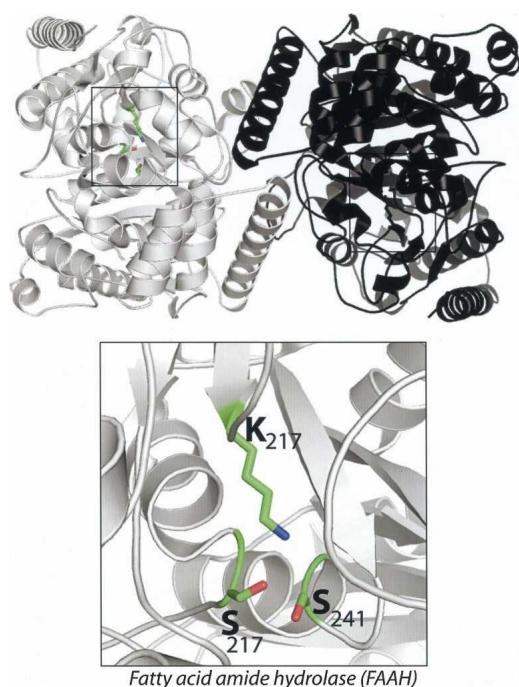


Figure 7. Structure of the Ser/Ser/Lys amidase FAAH from *Rattus norvegicus* (PDB 1mt5). This enzyme exists as homodimers in solution; therefore, one monomer is shown in white and the other monomer is shown in black.

Conclusion

The results collected over the past two decades indicate that there are a wide variety of active site configurations in serine and threonine proteases. Variations are observed in each of the residues that comprise the Ser/His/Asp triad. Serine can be substituted by a threonine residue; histidine can be replaced with glutamic acid and lysine; and the aspartic acid can be substituted by a glutamic acid or a histidine residue or in some cases eliminated all together.

Ser/Glu/Asp, Ser/His/Glu, and Ser/His/His triads are found in serine proteases, and a Ser/Ser/Lys triad is found in the amidase family. Glutamate and even serine (activated by a lysine) can function as a general base residue within serine proteases/amidases. Furthermore, the third member of the triad can include a histidine, glutamate, or a lysine residue. Here histidine and glutamate are proposed to function in the orientation of the catalytic general base similar to the way aspartic acid orients histidine in the conventional serine proteases. However, in the amidases, the general base lysine is believed to function via a bridging serine residue to activate the serine hydroxyl oxygen nucleophile. The lysine also assists in the protonation step of the substrate, allowing it to break down and release one of the products.

In addition to catalytic triads, active site dyads are found to function in serine and threonine proteases. The most common dyad is the Ser/Lys dyad, where lysine functions as a general base. Some of these proteases also contain a third residue (usually a serine or threonine) that helps to orient the lysine side chain such that it can function properly as a general base.

The simplest catalytic center that was discovered among serine/threonine peptidases utilizes a single catalytic residue. A Thr/ α -amine configuration, which is generated by autoproteolysis, is utilized in the proteasome. In the proteasome, a water molecule that is activated by the amine may function as the general base. A serine-only catalytic residue is found in the glutaryl 7-aminocephalosporanic acid acylase precursor. The serine is the nucleophile, and a bound water is believed to be the general base. The mechanism is not fully understood. A similar single catalytic residue is found in threonine protease precursors that undergo self processing. The active site reveals a threonine catalytic residue with no candidate general base protein residue except for a bound water. How the threonine nucleophile is activated such that it can catalyze the reaction is not yet certain.

So far, serine and threonine peptidases have been found in 15 clans. Some of these clans contain mixed catalytic types. The mixed catalytic types include the PB clan with serine or threonine proteases, the PC clan with serine and cysteine peptidases, and the PA clan that contain the serine, cysteine, or threonine peptidases. All peptidase

members of a clan have similar protein folds. While many of the clans contain peptidase families that carry out catalysis using the same active site catalytic residues, some family members within a clan use different active site residues. This indicates that there is not necessarily a connection between clans and the catalytic residues.

Why are different active site arrangements used in serine proteases across the three kingdoms of life? One possibility is that they allow these proteases to work in different environments as seen with the sedolisin protease with the Ser/Glu/Asp triad that can function at low pH. Also, alternate active site geometries might allow the cell to regulate proteases that use specific types of active site geometries without interfering with other configurations. Future studies will provide insight into the reasons why alternative active site constellations have arisen during evolution.

Acknowledgments

We thank Alan Barrett for discussions of clans. This work was supported by the National Sciences Foundation Grant MCB-0316670 (to R.E.D.), a Canadian Institute of Health Research operating grant (to M.P.), National Science and Engineering Research Council of Canada discovery grants (to M.P.), and a Michael Smith Foundation for Health Research Senior Scholar award (to M.P.).

References

Anderson, B.F., Baker, H.M., Norris, G.E., Rice, D.W., and Baker, E.N. 1989. Structure of human lactoferrin: Crystallographic structure analysis and refinement at 2.8 Å resolution. *J. Mol. Biol.* **209**: 711–734.

Arendt, C.S. and Hochstrasser, M. 1997. Identification of the yeast 20S proteasome catalytic centers and subunit interactions required for active-site formation. *Proc. Natl. Acad. Sci.* **94**: 7156–7161.

Aronson, N.N. 2004. Glycosylasparaginase precursor and other self-processing N-terminal nucleophile amidohydrolases. In *Handbook of proteolytic enzymes*, 2nd ed. (eds. A. Barret et al.), pp. 2086–2089. Elsevier, London, UK.

Ash, E.L., Sudmeier, J.L., Day, R.M., Vincent, M., Torchilin, E.V., Haddad, K.C., Bradshaw, E.M., Sanford, D.G., and Bachovchin, W.W. 2000. Unusual ¹H NMR chemical shifts support (His) C^{ε1}—H—O—C H-bond: Proposal for reaction-driven ring flip mechanism in serine protease catalysis. *Proc. Natl. Acad. Sci.* **97**: 10371–10376.

Bachovchin, W.W. 1985. Confirmation of the assignment of the low-field proton resonance of serine proteases by using specifically nitrogen-15 labeled enzyme. *Proc. Natl. Acad. Sci.* **82**: 7948–7951.

Baker, R.P., Young, K., Feng, L., Shi, Y., and Urban, S. 2007. Enzymatic analysis of a rhomboid intramembrane protease implicates transmembrane helix 5 as the lateral substrate gate. *Proc. Natl. Acad. Sci.* **104**: 8257–8262.

Baumeister, W., Walz, J., Zuhl, F., and Seemüller, E. 1998. The proteasome: Paradigm of a self-compartmentalizing protease. *Cell* **92**: 367–380.

Bell, C.E., Frescura, P., Hochschild, A., and Lewis, M. 2000. Crystal structure of the λ repressor C-terminal domain provides a model for cooperative operator binding. *Cell* **101**: 801–811.

Ben-Shem, A., Fass, D., and Bibi, E. 2007. Structural basis for intramembrane proteolysis by rhomboid serine proteases. *Proc. Natl. Acad. Sci.* **104**: 462–466.

Black, M.T. 1993. Evidence that the catalytic activity of prokaryote leader peptidase depends upon the operation of a serine-lysine catalytic dyad. *J. Bacteriol.* **175**: 4957–4961.

Black, M.T., Munn, J.G., and Allsop, A.E. 1992. On the catalytic mechanism of prokaryotic leader peptidase 1. *Biochem. J.* **282**: 539–543.

Blow, D.M., Birktoft, J.J., and Hartley, B.S. 1969. Role of a buried acid group in the mechanism of action of chymotrypsin. *Nature* **221**: 337–340.

Bogyo, M., McMaster, J.S., Gaczynska, M., Tortorella, D., Goldberg, A.L., and Ploegh, H. 1997. Covalent modification of the active site threonine of proteasomal β subunits and the *Escherichia coli* homolog HslV by a new class of inhibitors. *Proc. Natl. Acad. Sci.* **94**: 6629–6634.

Bompard-Gilles, C., Villeret, V., Davies, G.J., Fanuel, L., Joris, B., Frere, J.M., and Van Beeumen, J. 2000. A new variant of the Ntn hydrolase fold revealed by the crystal structure of L-aminopeptidase D-ala-esterase/amidase from *Ochrobactrum anthropi*. *Structure* **8**: 153–162.

Botos, I., Melnikov, E.E., Cherry, S., Tropea, J.E., Khalatova, A.G., Rasulova, F., Dauter, Z., Maurizi, M.R., Rotanova, T.V., Wlodawer, A., et al. 2004. The catalytic domain of *Escherichia coli* Lon protease has a unique fold and a Ser-Lys dyad in the active site. *J. Biol. Chem.* **279**: 8140–8148.

Bracey, M.H., Hanson, M.A., Masuda, K.R., Stevens, R.C., and Cravatt, B.F. 2002. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* **298**: 1793–1796.

Brannigan, J.A., Dodson, G., Duggleby, H.J., Moody, P.C., Smith, J.L., Tomchick, D.R., and Murzin, A.G. 1995. A protein catalytic framework with an N-terminal nucleophile is capable of self-activation. *Nature* **378**: 416–419.

Carlos, J.L., Klenotic, P.A., Paetzel, M., Strynadka, N.C., and Dalbey, R.E. 2000. Mutational evidence of transition state stabilization by serine 88 in *Escherichia coli* type I signal peptidase. *Biochemistry* **39**: 7276–7283.

Carter, P. and Wells, J.A. 1988. Dissecting the catalytic triad of a serine protease. *Nature* **332**: 564–568.

Chen, P. and Hochstrasser, M. 1996. Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly. *Cell* **86**: 961–972.

Choi, K.S., Kim, J.A., and Kang, H.S. 1992. Effects of site-directed mutations on processing and activities of penicillin G acylase from *Escherichia coli* ATCC 11105. *J. Bacteriol.* **174**: 6270–6276.

Chung, C.H. and Goldberg, A.L. 2004. Endopeptidase La. In *Handbook of proteolytic enzymes*, 2nd ed. (eds. A. Barret et al.), pp. 1998–2002. Elsevier, London, UK.

Comellas-Bigler, M., Fuentes-Prior, P., Maskos, K., Huber, R., Oyama, H., Uchida, K., Dunn, B.M., Oda, K., and Bode, W. 2002. The 1.4 Å crystal structure of kumamolysin: A thermostable serine-carboxyl-type proteinase. *Structure* **10**: 865–876.

Conlin, C.A., Hakansson, K., Liljas, A., and Miller, C.G. 1994. Cloning and nucleotide sequence of the cyclic AMP receptor protein-regulated *Salmonella typhimurium* pepE gene and crystallization of its product, an α-aspartyl dipeptidase. *J. Bacteriol.* **176**: 166–172.

Corey, D., McGrath, M.E., Vasquez, J.R., Fletterick, R.J., and Craik, C.S. 1992. An alternate geometry for the catalytic triad of serine proteases. *J. Am. Chem. Soc.* **114**: 4905–4907.

Craig, C.S., Rocznak, S., Largman, C., and Rutter, W.J. 1987. The catalytic role of the active site aspartic acid in serine proteases. *Science* **237**: 909–913.

Davies, C., White, S.W., and Nicholas, R.A. 2001. Crystal structure of a deacetylation-defective mutant of penicillin-binding protein 5 at 2.3 Å resolution. *J. Biol. Chem.* **276**: 616–623.

Derewenda, Z.S., Derewenda, U., and Kobos, P.M. 1994. (His)C^ε-H—O=C < hydrogen bond in the active sites of serine hydrolases. *J. Mol. Biol.* **241**: 83–93.

Ditzel, L., Huber, R., Mann, K., Heinemeyer, W., Wolf, D.H., and Groll, M. 1998. Conformational constraints for protein self-cleavage in the proteasome. *J. Mol. Biol.* **279**: 1187–1191.

Dixon, G.H., Kauffman, D.L., and Neurath, H. 1958. Amino acid sequence in the region of diisopropylphosphoryl binding in diisopropylphosphoryl trypsin. *J. Biol. Chem.* **233**: 1373–1381.

Duggleby, H.J., Tolley, S.P., Hill, C.P., Dodson, E.J., Dodson, G., and Moody, P.C. 1995. Penicillin acylase has a single-amino-acid catalytic centre. *Nature* **373**: 264–268.

Feldman, A.R., Lee, J., Delmas, B., and Paetzel, M. 2006. Crystal structure of a novel viral protease with a serine/lysine catalytic dyad mechanism. *J. Mol. Biol.* **358**: 1378–1389.

Fenteany, G., Standaert, R.F., Lane, W.S., Choi, S., Corey, E.J., and Schreiber, S.L. 1995. Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science* **268**: 726–731.

Fodor, K., Harmat, V., Neutze, R., Szilagyi, L., Graf, L., and Katona, G. 2006. Enzyme:substrate hydrogen bond shortening during the acylation phase of serine protease catalysis. *Biochemistry* **45**: 2114–2121.

Frey, P.A., Whitt, S.A., and Tobin, J.B. 1994. A low-barrier hydrogen bond in the catalytic triad of serine proteases. *Science* **264**: 1927–1930.

Groll, M., Ditzel, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H.D., and Huber, R. 1997. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* **386**: 463–471.

Hakansson, K., Wang, A.H., and Miller, C.G. 2000. The structure of aspartyl dipeptidase reveals a unique fold with a Ser-His-Glu catalytic triad. *Proc. Natl. Acad. Sci.* **97**: 14097–14102.

Hartley, B.S. 1964. Amino-acid sequence of bovine chymotrypsinogen-A. *Nature* **201**: 1284–1287.

Hedstrom, L. 2002. Serine protease mechanism and specificity. *Chem. Rev.* **102**: 4501–4524.

Heinemeyer, W., Fischer, M., Krimmer, T., Stachon, U., and Wolf, D.H. 1997. The active sites of the eukaryotic 20S proteasome and their involvement in subunit precursor processing. *J. Biol. Chem.* **272**: 25200–25209.

Hewitt, L., Kasche, V., Lummer, K., Lewis, R.J., Mursudow, G.N., Verma, C.S., Dodson, G.G., and Wilson, K.S. 2000. Structure of a slow processing precursor penicillin acylase from *Escherichia coli* reveals the linker peptide blocking the active-site cleft. *J. Mol. Biol.* **302**: 887–898.

Hodel, A.E., Hodel, M.R., Griffis, E.R., Hennig, K.A., Ratner, G.A., Xu, S., and Powers, M.A. 2002. The three-dimensional structure of the autoproteolytic, nuclear pore-targeting domain of the human nucleoporin Nup98. *Mol. Cell* **10**: 347–358.

Huber, R., Kukla, D., Bode, W., Schwager, P., Bartels, K., Deisenhofer, J., and Steigemann, W. 1974. Structure of the complex formed by bovine trypsin and bovine pancreatic trypsin inhibitor. II. Crystallographic refinement at 1.9 Å resolution. *J. Mol. Biol.* **89**: 73–101.

Ippolito, J.A., Alexander, R.S., and Christianson, D.W. 1990. Hydrogen bond stereochemistry in protein structure and function. *J. Mol. Biol.* **215**: 457–471.

James, M.N.G. 1994. *Proteolysis and protein turnover* (eds. J.S. Bond and A.J. Barrett), pp. 1–8. Portland, Brookfield, VT.

Jung, G., Ueno, H., and Hayashi, R. 1999. Carboxypeptidase Y: Structural basis for protein sorting and catalytic triad. *J. Biochem.* **126**: 1–6.

Kaartinen, V., Williams, J.C., Tomich, J., Yates 3rd, J.R., Hood, L.E., and Mononen, I. 1991. Glycosaparaginase from human leukocytes. Inactivation and covalent modification with diazo-oxonorvaline. *J. Biol. Chem.* **266**: 5860–5869.

Katona, G., Wilmouth, R.C., Wright, P.A., Berglund, G.I., Hajdu, J., Neutze, R., and Schofield, C.J. 2002. X-ray structure of a serine protease acyl-enzyme complex at 0.95 Å resolution. *J. Biol. Chem.* **277**: 21962–21970.

Keiler, K.C. and Sauer, R.T. 1995. Identification of active site residues of the Tsp protease. *J. Biol. Chem.* **270**: 28864–28868.

Keiler, K.C. and Sauer, R.T. 2001. Tsp and related C-terminal proteases. In *The enzymes, co- and posttranslational proteolysis of proteins* (eds. R.E. Dalbey and D.S. Sigman), Vol. 22, pp. 373–386. Academic Press, San Diego, CA.

Keiler, K.C., Waller, P.R., and Sauer, R.T. 1996. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* **271**: 990–993.

Khayat, R., Batra, R., Massariol, M.J., Lagace, L., and Tong, L. 2001. Investigating the role of histidine 157 in the catalytic activity of human cytomegalovirus protease. *Biochemistry* **40**: 6344–6351.

Kim, Y., Kim, S., Earnest, T.N., and Hol, W.G. 2002. Precursor structure of cephalosporin acylase. Insights into autoproteolytic activation in a new N-terminal hydrolase family. *J. Biol. Chem.* **277**: 2823–2829.

Kim, A.C., Oliver, D.C., and Paetzel, M. 2007. Crystal structure of a bacterial signal peptide peptidase. *J. Mol. Biol.* **376**: 352–366.

Korza, H.J. and Bochtler, M. 2005. *Pseudomonas aeruginosa* LD-carboxypeptidase, a serine peptidase with a Ser-His-Glu triad and a nucleophilic elbow. *J. Biol. Chem.* **280**: 40802–40812.

Kossiakoff, A.A. and Spencer, S.A. 1981. Direct determination of the protonation states of aspartic acid-102 and histidine-57 in the tetrahedral intermediate of the serine proteases: Neutron structure of trypsin. *Biochemistry* **20**: 6462–6474.

Kuo, D.W., Chan, H.K., Wilson, C.J., Griffin, P.R., Williams, H., and Knight, W.B. 1993. *Escherichia coli* leader peptidase: Production of an active form lacking a requirement for detergent and development of peptide substrates. *Arch. Biochem. Biophys.* **303**: 274–280.

Lee, J., Feldman, A.R., Delmas, B., and Paetzel, M. 2007. Crystal structure of the VP4 protease from infectious pancreatic necrosis virus reveals the acyl-enzyme complex for an intermolecular self-cleavage reaction. *J. Biol. Chem.* **282**: 24928–24937.

Lemberg, M.K., Bland, F.A., Weihofen, A., Braud, V.M., and Martoglio, B. 2001. Intramembrane proteolysis of signal peptides: An essential step in the generation of HLA-E epitopes. *J. Immunol.* **167**: 6441–6446.

Lemieux, M.J., Fischer, S.J., Cherney, M.M., Bateman, K.S., and James, M.N. 2007. The crystal structure of the rhomboid peptidase from *Haemophilus influenzae* provides insight into intramembrane proteolysis. *Proc. Natl. Acad. Sci.* **104**: 750–754.

Liao, D.I., Qian, J., Chisholm, D.A., Jordan, D.B., and Diner, B.A. 2000. Crystal structures of the photosystem II D1 C-terminal processing protease. *Nat. Struct. Biol.* **7**: 749–753.

Little, J.W. 2004. Repressor LexA. In *Handbook of proteolytic enzymes*, 2nd ed. (eds. A. Barret, et al.), pp. 2086–2089. Elsevier Academic Press, London, UK.

Lowe, J., Stock, D., Jap, B., Zwickl, P., Baumeister, W., and Huber, R. 1995. Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 Å resolution. *Science* **268**: 533–539.

Luo, Y., Pfuetzner, R.A., Mosimann, S., Paetzel, M., Frey, E.A., Cherney, M., Kim, B., Little, J.W., and Strynadka, N.C. 2001. Crystal structure of LexA: A conformational switch for regulation of self-cleavage. *Cell* **106**: 585–594.

Maegawa, S., Ito, K., and Akiyama, Y. 2005. Proteolytic action of GlpG, a rhomboid protease in the *Escherichia coli* cytoplasmic membrane. *Biochemistry* **44**: 13543–13552.

Matthews, B.W., Sigler, P.B., Henderson, R., and Blow, D.M. 1967. Three-dimensional structure of tosyl-α-chymotrypsin. *Nature* **214**: 652–656.

McDonald, J.P., Frank, E.G., Levine, A.S., and Woodgate, R. 1998. Intermolecular cleavage by UmuD-like mutagenesis proteins. *Proc. Natl. Acad. Sci.* **95**: 1478–1483.

McKinney, M.K. and Cravatt, B.F. 2003. Evidence for distinct roles in catalysis for residues of the serine-serine-lysine catalytic triad of fatty acid amide hydrolase. *J. Biol. Chem.* **278**: 37393–37399.

McKinney, M.K. and Cravatt, B.F. 2005. Structure and function of fatty acid amide hydrolase. *Annu. Rev. Biochem.* **74**: 411–432.

Mustard, J.A. and Little, J.W. 2000. Analysis of *Escherichia coli* RecA interactions with LexA, λ CI, and UmuD by site-directed mutagenesis of recA. *J. Bacteriol.* **182**: 1659–1670.

Nachon, F., Asojo, O.A., Borgstahl, G.E., Masson, P., and Lockridge, O. 2005. Role of water in aging of human butyrylcholinesterase inhibited by echothiophate: The crystal structure suggests two alternative mechanisms of aging. *Biochemistry* **44**: 1154–1162.

Northrop, J.H. and Kunitz, M. 1931. Isolation of protein crystals possessing trypic activity. *Science* **73**: 262–263.

Onionen, C. and Rouvinen, J. 2000. Structural comparison of Ntn-hydrolases. *Protein Sci.* **9**: 2329–2337.

Okada, T., Suzuki, H., Wada, K., Kumagai, H., and Fukuyama, K. 2007. Crystal structure of the γ-glutamyltranspeptidase precursor protein from *Escherichia coli*. Structural changes upon autocatalytic processing and implications for the maturation mechanism. *J. Biol. Chem.* **282**: 2433–2439.

Oyama, H., Abe, S., Ushiyama, S., Takahashi, S., and Oda, K. 1999. Identification of catalytic residues of pepstatin-insensitive carboxyl proteinases from prokaryotes by site-directed mutagenesis. *J. Biol. Chem.* **274**: 27815–27822.

Oyama, H., Fujisawa, T., Suzuki, T., Dunn, B.M., Wlodawer, A., and Oda, K. 2005. Catalytic residues and substrate specificity of recombinant human tripeptidyl peptidase I (CLN2). *J. Biochem.* **138**: 127–134.

Paetzel, M. and Strynadka, N.C. 1999. Common protein architecture and binding sites in proteases utilizing a Ser/Lys dyad mechanism. *Protein Sci.* **8**: 2533–2536.

Paetzel, M. and Woodgate, R. 2004. UmuD and UmuD' proteins. In *Handbook of proteolytic enzymes*, 2nd ed. (eds. A. Barret, et al.), pp. 1976–1981. Elsevier Academic Press, London, UK.

Paetzel, M., Strynadka, N.C., Tschantz, W.R., Casareno, R., Bullinger, P.R., and Dalbey, R.E. 1997. Use of site-directed chemical modification to study an essential lysine in *Escherichia coli* leader peptidase. *J. Biol. Chem.* **272**: 9994–10003.

Paetzel, M., Dalbey, R.E., and Strynadka, N.C. 1998. Crystal structure of a bacterial signal peptidase in complex with a β-lactam inhibitor. *Nature* **396**: 186–190.

Paetzel, M., Dalbey, R.E., and Strynadka, N.C. 2002a. Crystal structure of a bacterial signal peptidase apoenzyme: Implications for signal peptide binding and the Ser-Lys dyad mechanism. *J. Biol. Chem.* **277**: 9512–9519.

Paetzel, M., Karla, A., Strynadka, N.C., and Dalbey, R.E. 2002b. Signal peptidases. *Chem. Rev.* **102**: 4549–4580.

Pantoliano, M.W., Ladner, R.C., Bryan, P.N., Rollence, M.L., Wood, J.F., and Poulos, T.L. 1987. Protein engineering of subtilisin BPN': Enhanced stabilization through the introduction of two cysteines to form a disulfide bond. *Biochemistry* **26**: 2077–2082.

Patricelli, M.P. and Cravatt, B.F. 1999. Fatty acid amide hydrolase competitively degrades bioactive amides and esters through a nonconventional catalytic mechanism. *Biochemistry* **38**: 14125–14130.

Patricelli, M.P. and Cravatt, B.F. 2000. Clarifying the catalytic roles of conserved residues in the amidase signature family. *J. Biol. Chem.* **275**: 19177–19184.

Patricelli, M.P., Lovato, M.A., and Cravatt, B.F. 1999. Chemical and mutagenic investigations of fatty acid amide hydrolase: Evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* **38**: 9804–9812.

Patterson, J.E., Ollmann, I.R., Cravatt, B.F., Boger, D.L., Wong, C.-H., and Lerner, R.A. 1996. Inhibition of oleamide hydrolase catalyzed hydrolysis of the endogenous sleep-induced lipid *cis*-9-octadecenamide. *J. Am. Chem. Soc.* **118**: 5938–5945.

Peat, T.S., Frank, E.G., McDonald, J.P., Levine, A.S., Woodgate, R., and Hendrickson, W.A. 1996. Structure of the UmuD' protein and its regulation in response to DNA damage. *Nature* **380**: 727–730.

Puente, X.S., Sanchez, L.M., Gutierrez-Fernandez, A., Velasco, G., and Lopez-Otin, C. 2005. A genomic view of the complexity of mammalian proteolytic systems. *Biochem. Soc. Trans.* **33**: 331–334.

Qiu, X., Culp, J.S., DiLella, A.G., Hellmig, B., Hoog, S.S., Janson, C.A., Smith, W.W., and Abdel-Meguid, S.S. 1996. Unique fold and active site in cytomegalovirus protease. *Nature* **383**: 275–279.

Rawlings, N.D. and Barrett, A.J. 1999. MEROPS: The peptidase database. *Nucleic Acids Res.* **27**: 325–331.

Rawlings, N.D., Morton, F.R., and Barrett, A.J. 2006. MEROPS: The peptidase database. *Nucleic Acids Res.* **34**: D270–D272.

Robillard, G. and Shulman, R.G. 1974. High resolution nuclear magnetic resonance studies of the active site of chymotrypsin. II. Polarization of histidine 57 by substrate analogues and competitive inhibitors. *J. Mol. Biol.* **86**: 541–558.

Rotanova, T.V., Mel'nikov, E.E., and Tsirul'nikov, K.B. 2003. Catalytic dyad Ser-Lys at the active site of *Escherichia coli* ATP-dependent Lon-proteinase. *Bioorg. Khim.* **29**: 97–99.

Sakai, H., Sakabe, N., Sasaki, K., Hashimoto, W., Suzuki, H., Tachi, H., Kumagai, H., and Sakabe, K. 1996. A preliminary description of the crystal structure of γ -glutamyltranspeptidase from *E. coli* K-12. *J. Biochem.* **120**: 26–28.

Schechter, I. and Berger, A. 1967. On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **27**: 157–162.

Schrag, J.D., Li, Y.G., Wu, S., and Cygler, M. 1991. Ser-His-Glu triad forms the catalytic site of the lipase from *Geotrichum candidum*. *Nature* **351**: 761–764.

Seemuller, E., Lupas, A., Stock, D., Lowe, J., Huber, R., and Baumeister, W. 1995. Proteasome from *Thermoplasma acidophilum*: A threonine protease. *Science* **268**: 579–582.

Seemuller, E., Lupas, A., and Baumeister, W. 1996. Autocatalytic processing of the 20S proteasome. *Nature* **382**: 468–471.

Seemuller, E., Zwickl, P., and Baumeister, W. 2001. Self-processing of subunits of the proteasome. In *The enzymes*, Vol. 22, 3rd ed. (eds. R.E. Dalbey and D.S. Sigman), pp. 335–371. Academic Press, San Diego, CA.

Selkoe, D. and Kopan, R. 2003. Notch and presenilin: Regulated intramembrane proteolysis links development and degeneration. *Annu. Rev. Neurosci.* **26**: 565–597.

Shieh, H.S., Kurumbail, R.G., Stevens, A.M., Stegeman, R.A., Sturman, E.J., Pak, J.Y., Wittwer, A.J., Palmer, M.O., Wiegand, R.C., Hofwerda, B.C., et al. 1996. Three-dimensional structure of human cytomegalovirus protease. *Nature* **383**: 279–282.

Shin, S., Lee, T.H., Ha, N.C., Koo, H.M., Kim, S.Y., Lee, H.S., Kim, Y.S., and Oh, B.H. 2002. Structure of malonamidase E2 reveals a novel Ser-cisSer-Lys catalytic triad in a new serine hydrolase fold that is prevalent in nature. *EMBO J.* **21**: 2509–2516.

Silber, K.R., Keiler, K.C., and Sauer, R.T. 1992. Tsp: A tail-specific protease that selectively degrades proteins with nonpolar C termini. *Proc. Natl. Acad. Sci.* **89**: 295–299.

Slilaty, S.N. and Little, J.W. 1987. Lysine-156 and serine-119 are required for LexA repressor cleavage: A possible mechanism. *Proc. Natl. Acad. Sci.* **84**: 3987–3991.

Slilaty, S.N., Rupley, J.A., and Little, J.W. 1986. Intramolecular cleavage of LexA and phage λ repressors: Dependence of kinetics on repressor concentration, pH, temperature, and solvent. *Biochemistry* **25**: 6866–6875.

Smith, J.L., Zaluzec, E.J., Wery, J.P., Niu, L., Switzer, R.L., Zalkin, H., and Satow, Y. 1994. Structure of the allosteric regulatory enzyme of purine biosynthesis. *Science* **264**: 1427–1433.

Stevens, J.T., Mapelli, C., Tsao, J., Hail, M., O'Boyle II, D., Weinheimer, S.P., and Dianni, C.L. 1994. In vitro proteolytic activity and active-site identification of the human cytomegalovirus protease. *Eur. J. Biochem.* **226**: 361–367.

Stroud, R.M., Kay, L.M., and Dickerson, R.E. 1974. The structure of bovine trypsin: Electron density maps of the inhibited enzyme at 5 Å and at 2.7 Å resolution. *J. Mol. Biol.* **83**: 185–208.

Sung, M. and Dalbey, R.E. 1992. Identification of potential active-site residues in the *Escherichia coli* leader peptidase. *J. Biol. Chem.* **267**: 13154–13159.

Suzuki, H. and Kumagai, H. 2002. Autocatalytic processing of γ -glutamyl-transpeptidase. *J. Biol. Chem.* **277**: 43536–43543.

Taylor, M.A., Packer, J.C.L., and Bowyer, J.R. 1988. Processing of the D1 polypeptide of the photosystem II reaction centre and photoactivation of a low fluorescence mutant (LF-1) of *Scenedesmus obliquus*. *FEBS Lett.* **237**: 229–233.

Tong, L., Qian, C., Massariol, M.J., Bonneau, P.R., Cordingley, M.G., and Lagace, L. 1996. A new serine-protease fold revealed by the crystal structure of human cytomegalovirus protease. *Nature* **383**: 272–275.

Tschantz, W.R. and Dalbey, R.E. 1994. Bacterial leader peptidase 1. *Methods Enzymol.* **244**: 285–301.

Tschantz, W.R., Sung, M., Delgado-Partin, V.M., and Dalbey, R.E. 1993. A serine and a lysine residue implicated in the catalytic mechanism of the *Escherichia coli* leader peptidase. *J. Biol. Chem.* **268**: 27349–27354.

Ueda, N., Kurahashi, Y., Yamamoto, S., and Tokunaga, T. 1995. Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J. Biol. Chem.* **270**: 23823–23827.

Urban, S., Lee, J.R., and Freeman, M. 2001. *Drosophila* rhomboid-1 defines a family of putative intramembrane serine proteases. *Cell* **107**: 173–182.

Voges, D., Zwickl, P., and Baumeister, W. 1999. The 26S proteasome: A molecular machine designed for controlled proteolysis. *Annu. Rev. Biochem.* **68**: 1015–1068.

Wallace, A.C., Laskowski, R.A., and Thornton, J.M. 1996. Derivation of 3D coordinate templates for searching structural databases: Application to Ser-His-Asp catalytic triads in the serine proteinases and lipases. *Protein Sci.* **5**: 1001–1013.

Walsh, K.A. and Neurath, H. 1964. Trypsinogen and chymotrypsinogen as homologous proteins. *Proc. Natl. Acad. Sci.* **52**: 884–889.

Wang, Y. and Ha, Y. 2007. Open-cap conformation of intramembrane protease GlpG. *Proc. Natl. Acad. Sci.* **104**: 2098–2102.

Wang, J., Hartling, J.A., and Flanagan, J.M. 1997. The structure of ClpP at 2.3 Å resolution suggests a model for ATP-dependent proteolysis. *Cell* **91**: 447–456.

Wang, Y., Zhang, Y., and Ha, Y. 2006. Crystal structure of a rhomboid family intramembrane protease. *Nature* **444**: 179–180.

Warshel, A. and Russell, S. 1986. Theoretical correlation of structure and energetics in the catalytic reaction of trypsin. *J. Am. Chem. Soc.* **108**: 6569–6579.

Welch, A.R., McNally, L.M., Hall, M.R., and Gibson, W. 1993. Herpesvirus proteinase: Site-directed mutagenesis used to study maturational, release, and inactivation cleavage sites of precursor and to identify a possible catalytic site serine and histidine. *J. Virol.* **67**: 7360–7372.

Wells, J.A., Cunningham, D.C., Graycar, T.P., and Estell, D.A. 1986. Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin. *Philos. Trans. R. Soc. Lond. A* **317**: 415–423.

Wlodawer, A., Li, M., Dauter, Z., Gustchina, A., Uchida, K., Oyama, H., Dunn, B.M., and Oda, K. 2001a. Carboxyl proteinase from *Pseudomonas* defines a novel family of subtilisin-like enzymes. *Nat. Struct. Biol.* **8**: 442–446.

Wlodawer, A., Li, M., Gustchina, A., Dauter, Z., Uchida, K., Oyama, H., Goldfarb, N.E., Dunn, B.M., and Oda, K. 2001b. Inhibitor complexes of the *Pseudomonas* serine-carboxyl proteinase. *Biochemistry* **40**: 15602–15611.

Wolfe, M.S. and Kopan, R. 2004. Intramembrane proteolysis: Theme and variations. *Science* **305**: 1119–1123.

Wright, C.S., Alden, R.A., and Kraut, J. 1969. Structure of subtilisin BPN' at 2.5 Å resolution. *Nature* **221**: 235–242.

Wu, Z., Yan, N., Feng, L., Oberstein, A., Yan, H., Baker, R.P., Gu, L., Jeffrey, P.D., Urban, S., and Shi, Y. 2006. Structural analysis of a rhomboid family intramembrane protease reveals a gating mechanism for substrate entry. *Nat. Struct. Mol. Biol.* **13**: 1084–1091.

Yoon, J., Oh, B., Kim, K., Park, J., Han, D., Kim, K.K., Cha, S.S., Lee, D., and Kim, Y. 2004. A bound water molecule is crucial in initiating autocatalytic precursor activation in an N-terminal hydrolase. *J. Biol. Chem.* **279**: 341–347.

Zwizinski, C., Date, T., and Wickner, W. 1981. Leader peptidase is found in both the inner and outer membranes of *Escherichia coli*. *J. Biol. Chem.* **256**: 3593–3597.