

Subtilisin Carlsberg: A Serine Protease

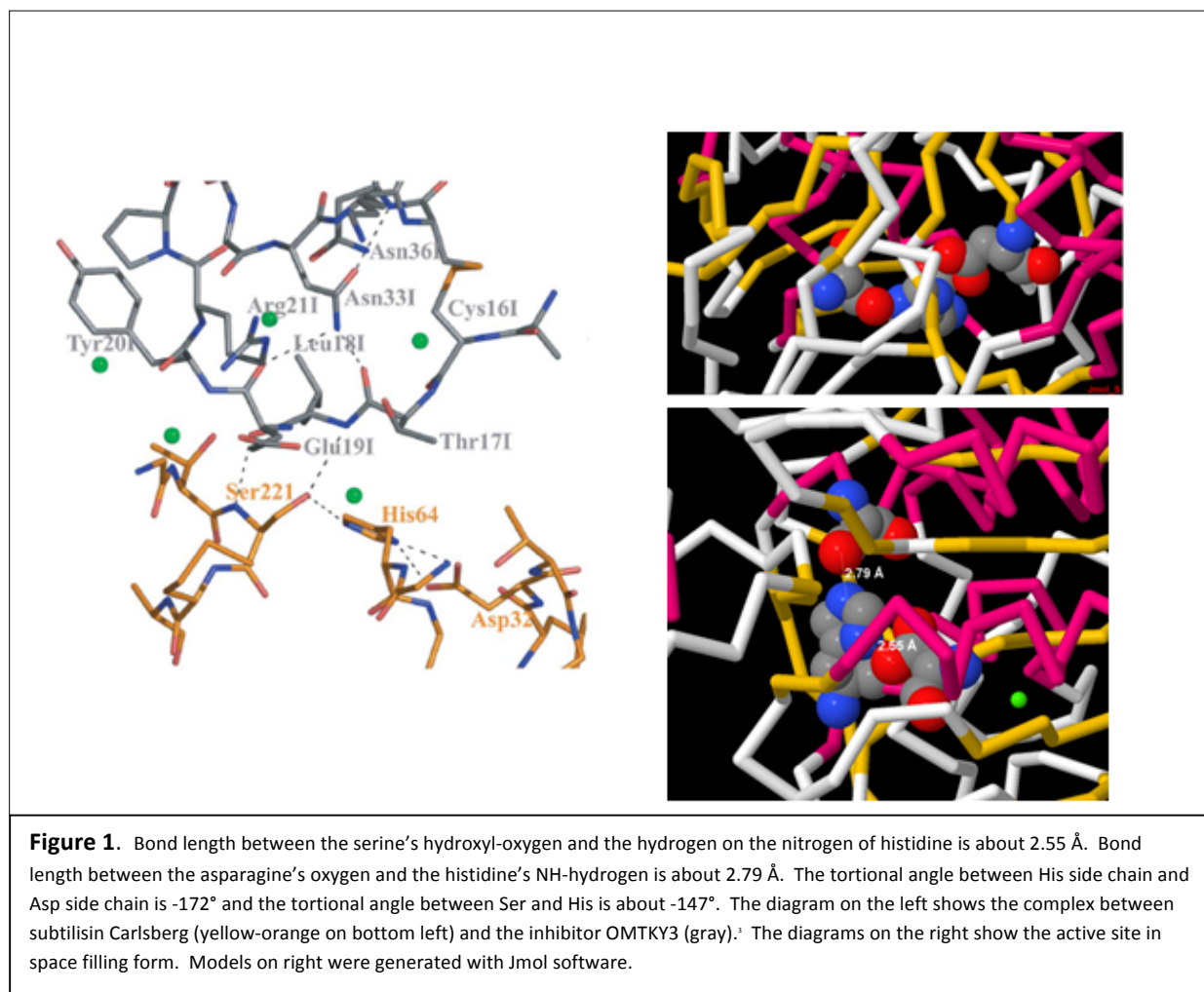
(a) Function of Subtilisin and Other Serine Proteases

Enzymes are protein catalysts, and by definition, a catalyst speeds up the rate of a reaction without itself being consumed by the reaction.¹ Proteases are enzymes that cleave amide bonds in peptides.¹ The amide cleavage is accomplished by addition of a water molecule and, hence, this protease is an example of a hydrolase.¹ Without the protease present, it would take years for the bonds to break down.¹ The way that scientists can figure out the specific action of an enzyme as well as the amino acids involved in the active site is by use of inhibitors, which effectively “poison” the enzyme by forming stable covalent bonds with it.¹ By using experiments with inhibitors such as nerve gas with the serine protease chymotrypsin, scientists discovered that serine played an essential role in the function of the enzyme.¹ Chymotrypsin has the same catalytic mechanisms as the bacterial protease subtilisin (although the amino acid sequence is different), which is the protease that has been researched and modeled in this paper.¹ A specific subtilisin, subtilisin Carlsberg (CARL), has been used in experiments with the well-studied protein proteinase inhibitor, the turkey ovomucoid third domain, OMTKY3.³ The use of the inhibitor has enabled scientists to determine the function of the active site as well as to discover a level of inhibitor plasticity.³ A triad of three specific amino acids makes up the active site for CARL: aspartic acid, histidine, and serine (Figure 1).¹ The specificity pocket is lined with hydrophobic amino acids, so substrate proteins that have hydrophobic amino acids will bind tightly in the correct orientation for the amino acids in the active site to function.¹ Serine proteases are involved in digestion, blood clotting, and the immune system.¹ Subtilisin is an alkaline protease that is used as an additive in commercial laundry products, and it significantly increases the removal of protein-based stains from soiled fabric.²

(b) Structural Analysis of Subtilisin's Active Site

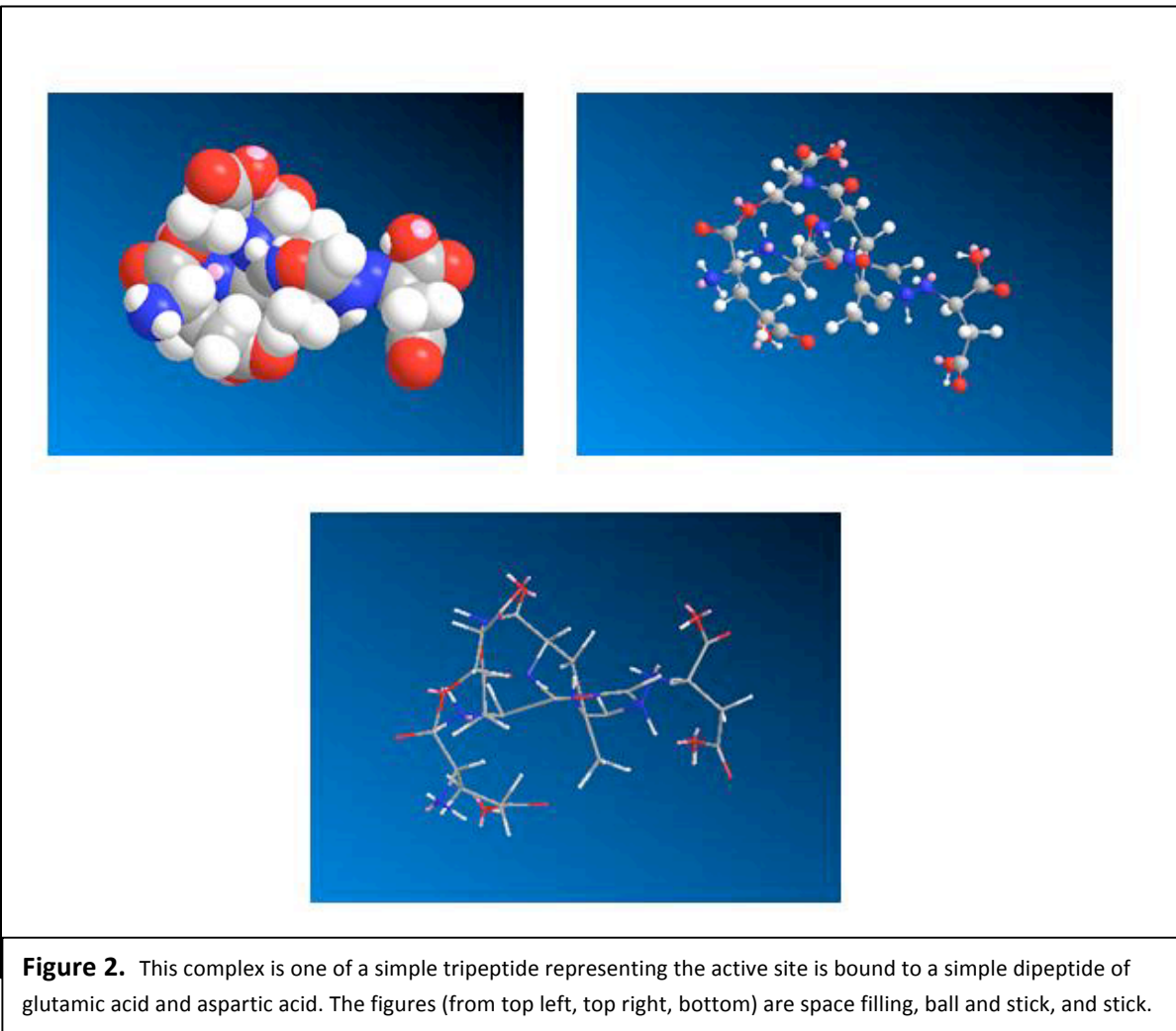
The active site of the protease subtilisin from the bacteria *Bacillus licheniformis* is diagramed below.³

This is the structure of the subtilisin Carlsberg-OMTKY3 complex and it is labeled protein YU6 in the protein database.³ OMTKY3 is a well-studied protease inhibitor known as the turkey ovomucoid third domain.³ The structure of the protein's active site includes the catalytic triad mentioned in part (a), which is the serine at position 221, the histidine at position 64 and the aspartic acid at position 32 (Figure 1).³ The protease folds in such a way that these amino acids can interact with each other in a way that allows for the catalysis of different substrates. The histidine can act as a proton acceptor, or a base, and remove the hydrogen from the hydroxyl group of serine, thus making serine much more reactive to form a new bond with the carbon atom in the peptide bond of the substrate.¹ The carboxylic acid group of aspartic acid has a negative charge that can then stabilize the positively charged side chain of histidine.¹ In this instance, however, the molecule to which the subtilisin is bound is an inhibitor, which binds covalently to the active site, preventing catalysis from happening, but allowing us to discover the function of the active site.



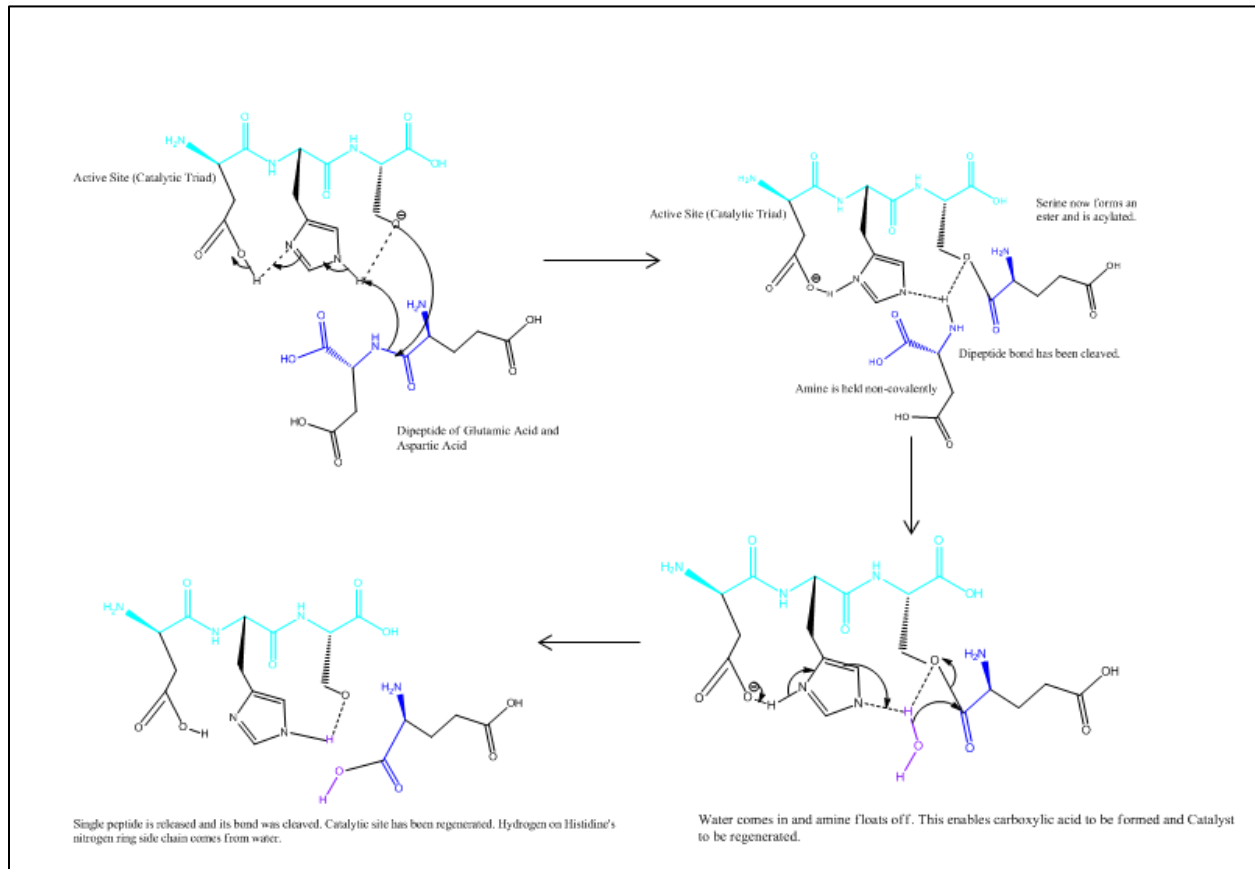
(c) Molecular Model of Protein Hydrolysis

As mentioned before, subtilisin is a serine protease. The active site forms a pocket of specificity, and when a peptide that matches the pocket with a certain level of specificity enters, it can then be hydrolyzed by the catalytic mechanism of subtilisin. If a polypeptide comes into the active site, then the negatively charged oxygen on serine acts as a nucleophile attacking the carbonyl carbon that participates in a peptide bond and thus causes the carbon-nitrogen peptide bond to be cleaved.¹ To exemplify the function of subtilisin, the simple dipeptide Glu-Asp is modeled in the simplified active site model shown in Figure 2. The cleavage of the peptide bond causes serine to be acylated by the carbonyl of the amino acid, and the histidine then hydrogen bonds with the amine that resulted from the cleaved peptide bond.³ The peptide cleavage has occurred and leaves the peptide's acid moiety attached to the active site, and the last section will further explore the mechanism by which protein hydrolysis occurs.



(d) Mechanism of Protein Hydrolysis

Subtilisin is a protease that is a great example of a hydrolase, since part of its mechanism uses water to cleave peptide bonds (Scheme 1).¹ Going back to what was mentioned and diagramed in section c, the polypeptide (dipeptide in this case) comes into the active site, and then undergoes nucleophilic attack by the oxygen on serine's side chain. The serine is then acylated by the carbonyl of one of the amino acids (the carbonyl involved in the peptide bond), and the NH-hydrogen of the reacting peptide bond engages in hydrogen bonding to the acylated serine side chain and to the nitrogen on the histidine side chain. This newly formed amino end of one amino acid that is non-covalently attached between the histidine and serine can then be replaced by water as shown in Scheme 1 while the amino-fragment of the peptide essentially floats off. A reaction then occurs in which the electrons in the oxygen-hydrogen bond attack the carbonyl carbon on the serine ester thus creating a carboxy terminal end on a single amino acid and regenerating the active site. The dipeptide has thus been catalytically cleaved by means of the active site of the protease subtilisin and water.



Scheme 1. Mechanism of protein hydrolysis. For the purpose of tracking what is being cleaved and added, the backbone of active site is color coded in light blue, the backbone of the dipeptide is color coded in dark blue, and the reactive water is shown in purple. Hydrogen bonds are shown by dashes.

References

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- (2) (a) Durham, D. R. "Utility of Subtilisin GX as a Detergent Additive." *J. Appl. Microbiol.* **1987**, 63 (5), 381-86. (b) *Wiley Online Library*. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.1987.tb04858.x/abstract> (Accessed March 19, 2012).
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