John Clizer & Greg Ralph

Crystal Structure of the Subtilisin Carlsberg: OMTKY3 Complex

The turkey ovomucoid third domain (OMTKY3) is considered to be one of the most studied protein inhibitors.[1] Ovomucin is found in raw egg white and it is responsible for the gel properties of fresh egg white.[2] Protein inhibitors of serine proteases generally are not exceedingly specific; they usually are promiscuous and form strong inactive 1:1 complexes with many different serine proteases. The turkey ovomucoid third domain is so extensively studied that it was chosen as the wild type for various relative studies of natural and recombinant variants of bird ovomucoid third domains. There is a vast amount of structural evidence and many recent publications showing that the reactive site is always intact. This is due to the standard mechanism that complexes are made from an enzyme and either the intact or hydrolysed inhibitor is the same substance. Thus, the reactive-site peptide bond is resynthesized upon complex formation with hydrolysed inhibitor. This makes the biological content of the turkey protein inhibitor OMTKY3 very useful to use and study (Fig. 1).

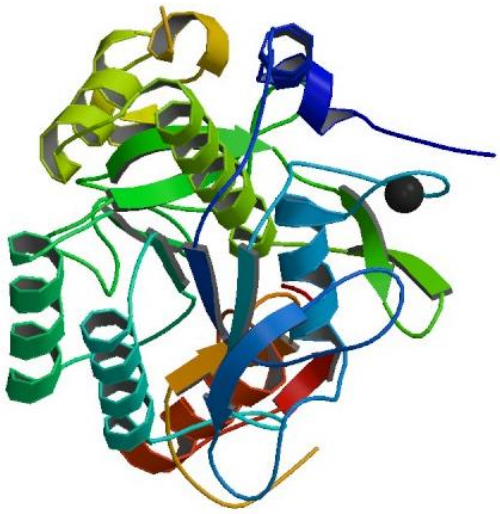


Figure 1. Subtilisin Carlsberg-OMTKY3 complex complete with alpha-helices and beta-sheets.

The crystal structure of the turkey ovomucoid OMTKY3 is known to be bound to subtilisin Carlsberg (Fig. 2). The structure contains a central seven-strand parallel beta-sheet with helices crammed on both

sides of the sheet. The active-site residues are located near the C-termini of the strands. The beta-sheet is lined on one side by two alpha-helices and on the other by an alignment of four alpha-helices that contain active-site residues His64 and Ser221; while Asp32 is on the C-terminus (Fig. 3). The structure observed for the complex between subtilisin and the turkey ovomucoid inhibitor is very similar to other known CARL structures. The OMTKY3 complexes were made from hydrolysed turkey ovomucoid third domains and eight different serine proteases. In all eight cases, when the complexes were subjected to kinetically controlled disassociation, the main products were the free enzyme and the intact inhibitor, proving that the Leu18I-Glu19I peptide bond serves as the reactive site in the eight serine proteases.

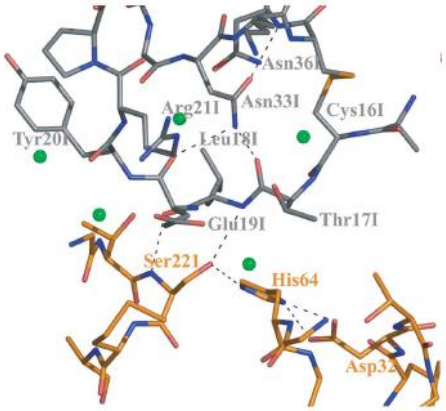


Figure 2. Turkey ovomucoid third domain (OMTKY3 complex) in grey, bound to the Subtilisin Carlsberg in orange, with active site residues at Asp32, His64 and Ser221. Bond length of Leu18I O atom to Ser221 N atom is 3.21Å.

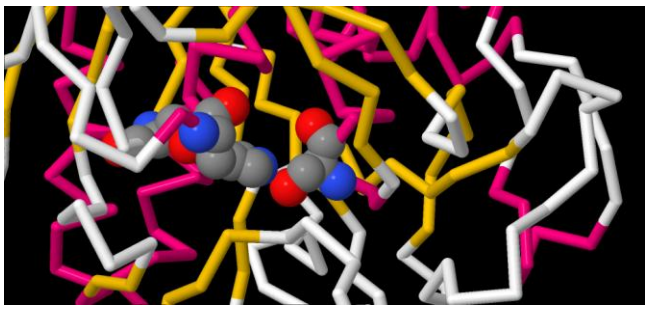
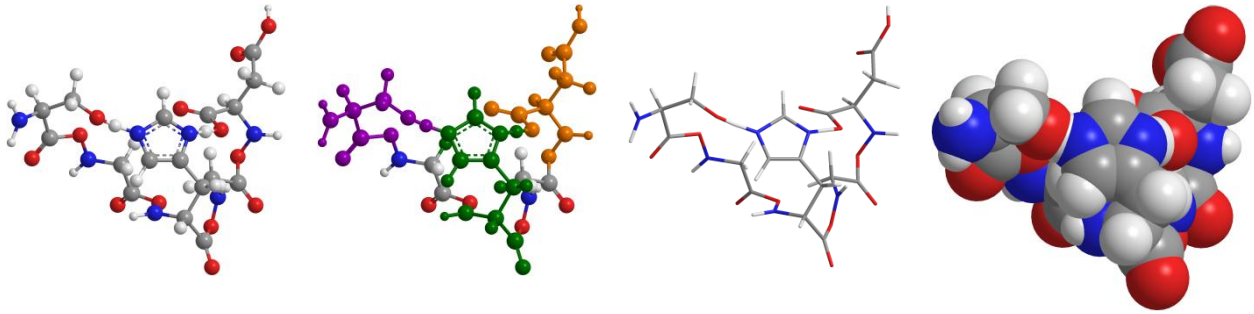


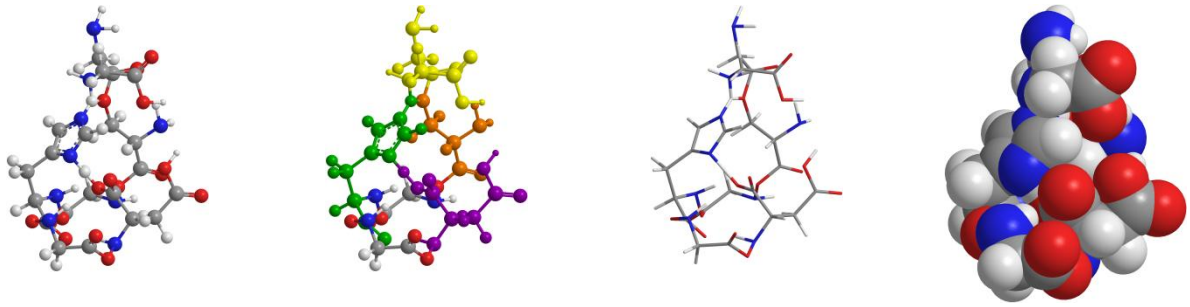
Figure 3. Close up model of the active site residue for Subtilisin Carlsberg; with Asp32 to the far right, His64 in the middle, and Ser221 to the far left. The carbonyl O atom of Leu18I also interacts with two enzyme residues: the backbone N atom of Ser221 (3.14 Å) and the N2 of Asn155 (2.74 Å).

The active site of subtilisin Carlsberg-OMTKY3 consists of histidine, asparagine, and serine. The models shown in Fig. 4 are simple representations of the active site with the residues histidine, asparagine and serine bonded to a simple peptide. In a real protein such as OMTKY3 the amino acids would not be next to each other on the peptide chain as shown in these models. In the color model it is possible to clearly distinguish the three amino acids histidine (green), asparagine (purple) and serine (orange). In Fig. 5, the same peptide model is shown bonded to a simple dipeptide, in his case glycylglycine (yellow). In the interaction between the active site and the dipeptide the dipeptide is initially drawn to the active site through electrostatic forces and is then severed through a hydrolysis reaction. This reaction is shown in detail in the last section.



Ball and Stick Model Color Stick Model Space Filling Model

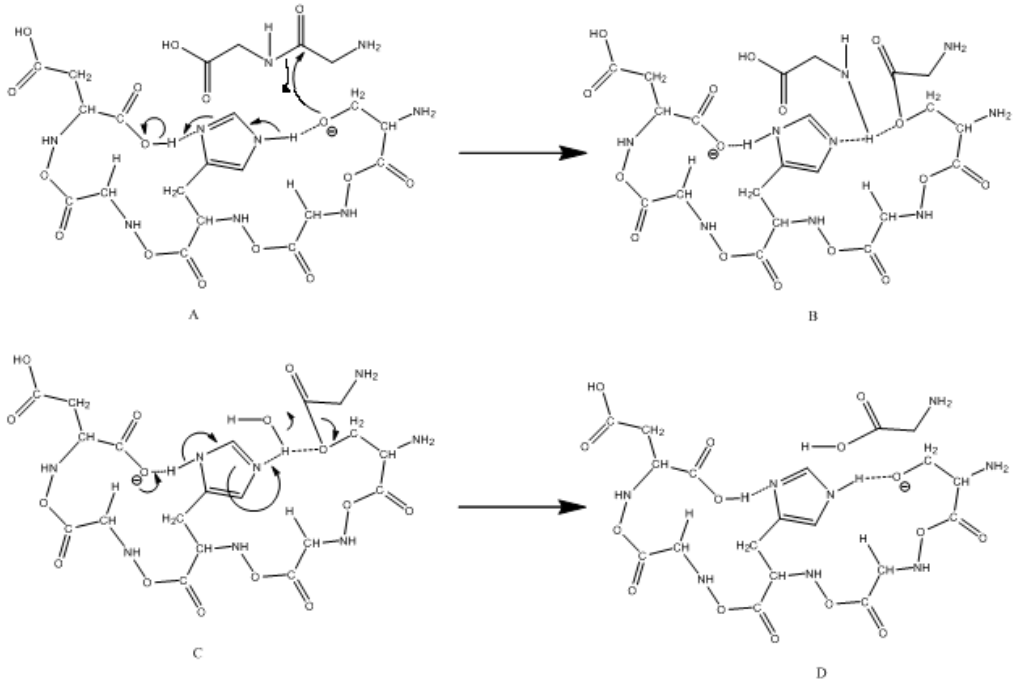
Figure 4. Active Site Model and Major Structural Parameters: The OMTKY3 molecules bound to chymotrypsin and now CARL differ typically by a 1 Å. Thr47I O in one inhibitor molecule hydrogen bonds to Ser44I O of the other inhibitor and vice versa; both bonds are 2.67 Å in length.



Ball and Stick Model Color Stick Model Space Filing Model

Figure 5. Active Site - Dipeptide Model and Major Structural Parameters: Glu19I is positioned to have a moderate electrostatic hydrogen-bonded interaction with Arg21I (2.99 Å). Leu18I C and Gly154 C are 3.73 Å apart. The typical value for residue in bound OMTKY3 is 140*, whereas the corresponding values in the subtilisin complexes are approximately 90*. The structural changes that these alterations cause can be seen in the alignment of the ovomucoids as causing the N-terminal coil region of the inhibitor to turn away from the enzyme and is similar to the previously determined CARL–OMTKY3 complex.

The purposed mechanism of protein hydrolysis in the histidine-asparagine-serine active site is shown in Scheme 1.[3] Before hydrolysis can occur it is necessary for the hydrogen on the serine side chain to be deprotonated. This is accomplished by the nearby histidine which acts as a base. With the serine deprotonated it is possible for acylation to occur (**A**). This is accomplished when the now reactive alcoholate adds to the amide carbonyl-carbon and leads to amide bond cleavage. The histidine loses its hydrogen bond and undergoes an interior shift while claiming a proton from the carboxylic side chain of asparagine. The newly formed amine (shown in **B**) leaves and is replaced with a water molecule (**C**). With the water molecule present the reverse of the original acylation occurs. The newly formed hydroxyl radical forms a bound with the carbonyl of the original dipeptide causing it to return its electrons to the serine reforming the oxygen radical and leaving the active site. The now severed protein can be seen in **D** with the active site returned to its original form.



Scheme 1. Mechanism of protein hydrolysis.

References

1 Maynes, J. T.; Cherney, M. M.; Qasim, M. A.; Laskowski, Jr., M.; James, M. N. Subtilisin Carlsberg–OMTKY3 complex. *Acta Cryst.* **2005**, *D61,* 580–588.

2 Wikipedia http://en.wikipedia.org/wiki/Ovomucin (accessed March 19, 2012).

3 Neitzel, J. J. Enzyme Catalysis: The Serine Proteases. *Nature Education* **2010**, *3*, 21.