



Graphics Problem Set # 7 (Beese)

Serine Proteinase

(Estimated time for completion: 45 minutes-1 hour)

The questions are based on c11SerPr.kin and Chapter 11 “An Example of Enzyme Catalysis” in Branden and Tooze.

1. Kinemages 2 and 3 use the structure of trypsin complexed with basic pancreatic trypsin inhibitor (BPTI, in yellow tint) to illustrate the features of serine proteinases. Kinemage 2 starts with an overview of the trypsin-BPTI complex. Alpha carbons of the inhibitor are shown in yellow tint, specificity-residue Lys 15 in yellow, an SS bridge that helps stabilize the inhibitory loop in gold, and the bond to be cleaved (called the “scissile bond”) in seagreen. Animation steps from View 1 looking at the complex from the side, to View 2 which looks through the inhibitor down the interface from a “standard” viewpoint, then through a series of close-ups of features of the interaction between inhibitor and enzyme. Details include a close-up of non-specific H-bonding to the inhibitor mainchain (including 2 beta-sheet H-bonds, and one to the Ne of Gln 192. One of these H-bonds is a bit long, and becomes shorter in the tetrahedral transitions state.

Which H-bond is this?

Find the 4 essential structural features of serine proteinase catalysis. In particular, locate the catalytic triad of sidechains (Ser 195, His 57, and Asp 102) with Ser 214 in position under the scissile peptide bond of the inhibitor.

How close is the Ser 195 Og to the carbon in the scissile peptide? _____

Kinemage 3 shows further detail of the trypsin-BPTI active site using patches of dots to show where atoms contact between enzyme and inhibitor (color-coded by atom type). View 1 shows Lys 15 of BPTI in the trypsin specificity pocket. Lysine contacts the backbone along the left side of the pocket and the NH₃ makes H-bonds to the Ser 190 Og.

What is the distance from BPTI Lys 15 Ne to the nearest O of Asp 189? _____

View 2 shows the oxyanion hole that surrounds the CO of the inhibitor scissile bond, and that is set up to do an even better job of binding the tetrahedral oxyanion that forms in the transition state of the reaction. Both blue contact dots and purple H-bonds show that interactions of the O with the mainchain NH groups of residues 193, 195, and (not as good geometry) 194. View 3 symbolizes with a red arrow the attack of Ser 195 on the underside of the scissile peptide.

Kinemage 4 is a superposition of chymotrypsin (green tint), trypsin (white), and subtilisin (pink tint). Animate to switch among them, or turn them on all together or in pairs. View 1 is an overview that shows how similar the Calpha backbones are for trypsin and chymotrypsin and how completely unrelated the fold of subtilisin is to the other two. Choose View 2 and animate again, to see a close-up comparison of the active-site residues, which are quite closely superimposable for all three enzymes; note that the extended piece of backbone (vertical, above His 57, in View 2) which H-bonds with the extended backbone of an inhibitor or substrate, is also superimposable for all three.

Those extended (beta sheet) residues that overlap are:

Trypsin: _____

Subtilisin: _____

View 3 looks along the twisted beta sheet of subtilisin. This contrast of the bacterial vs. the pancreatic serine proteinases is a classic example of convergent evolution on the molecular level.

Kinemage 5 shows the complex of subtilisin with its inhibitor eglin. Click on "ANIMATE" for a tour of the binding and the catalytic site. Subtilisin is in white and eglin in pink tint, with the scissile bond emphasized in hot pink. Animation steps 1 and 2 show Calphas of the entire complex, first from the edge of the subtilisin beta sheet and then from one face of the sheet, with the binding loop of the inhibitor thrust down in the cleft at the top middle of the sheet.

Animation steps 3-6 are close-ups of the four features essential to catalytic activity. 1) Non-specific binding to the substrate or inhibitor backbone is provided by 4 beta-sheet H-bonds to extended strands of subtilisin on either side. 2) The specificity pocket of subtilisin (here occupied by Leu 45 of eglin) is a relatively nonspecific one, surrounded mostly by mainchain exposed by several glycines. 3) The catalytic triad of subtilisin is Ser 221, His 64, and Asp 32, with the Ser poised to attack the scissile bond; the nature and geometry of the triad is the same as in chymotrypsin, but the 3 residues come in a different sequence order and from different types of secondary structure. 4) The oxyanion hole is a set of N atoms (2 mainchain and one from Asn 155, marked in blue) which surround the CO of the scissile bond (marked in red). Those N's H-bond to a substrate or inhibitor, as here, but the H-bond geometry as well as the charge interactions are significantly better for the oxyanion of the tetrahedral transition state, especially for the central H-bond shown dashed. Thus the oxyanion interactions not only bind substrate but improves catalysis by binding better to the transition state.

Measure some of the critical distances:

What are the distances between donor and acceptor atoms of the H-bonds in the catalytic triad?

How close to the Oxygen are the 3 H-bonding nitrogens that will stabilize the oxyanion?

How close is the Ser 221 Og to the carbon in the scissile peptide?
