Molecular Dynamics Simulation of the D102A Variant of Chymotrypsin Supports Asp-His Hydrogen Bonding Conformational Restraint

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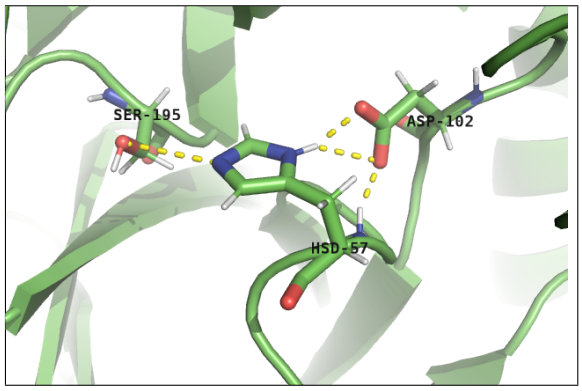
December 14, 2017

1 Introduction

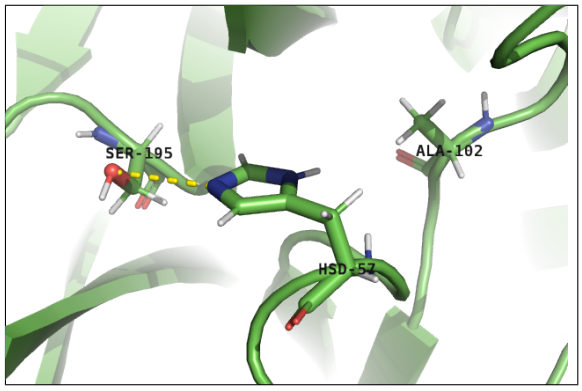
Almost one third of all proteases can be classified as serine proteases [1]. These proteins are named for the nucleophilic Ser residue at the active site. Serine proteases fall into two broad categories based on their structure: chymotrypsin-like (trypsin-like) or subtilisin-like [2]. Chymotrypsin-like proteases are the most abundant in nature and can be found in eukaryotes, prokaryotes, archae, and viruses. They are involved in many critical physiological processes, such as hemostasis, apoptosis, digestion, and reproduction [1].

In order to hydrolyze a peptide bond, these well-studied enzymes must overcome three major mechanistic barriers: (i) amide bonds are extremely stable due to electron donation from the amide nitrogen to the carbonyl; (ii) water is a poor nucleophile and proteases always activate water via a general base; (iii) and amines are poor leaving groups because proteases protonate the amine prior to expulsion [1].

To overcome these three reaction barriers, serine proteases contain a group of three residues called the catalytic triad that use hydrogen bonding to increase reaction favorability. In chymotrypsin, the triad is composed of serine, histidine, and aspartate, and is part of a larger hydrogen bonding network [1].



(a) Wild-Type



(b) D102A variant

Figure 1: Conformation of catalytic triad structure in wild-type and D102A variant of chymotrypsin.

Mutagenesis experiments of catalytic triad residues show decreased catalytic activity; substitution of Ser-195 or His-57 with alanine effectively disables the triad [1]. For this project, we test

the hypothesis that the Asp-His hydrogen bond restrains the conformational flexibility of the His ring so that it can more strongly hydrogen bond to serine, thus activating serine for a nucleophilic attack.

To test this, two all-atom molecular dynamics simulations of two chymotrypsin series variants were carried out: the wild-type sequence, and with Asp-102 substituted for alanine (mutation D102A). This enabled us to determine rotamer distributions of the His side chain and the time-dependent frequencies of His-Ser bond formations.

2 Methods

A chymotrypsin from *Bos taurus* (RCSB PDB ID: 1GGD) was used as the starting structure, with an additional calcium atom added to stabilize the enzyme. The charged form of aspartate (ASP) and the neutral form of histidine (HSD) were used when building the system with VMD 1.9.3. After stabilizing, VMD was used to neutralize the system with water and 0.2M KCl. The D102A system was created using the same parameters, solvation, and neutralization as the wild-type system but the catalytic triad aspartate was mutated to an alanine residue.

After these steps, the wild-type system contained 16142 atoms and the D102A system contained 16138 atoms. Both systems were centered about the origin and the B-factor of the protein atoms was set to 1.00. The minimum and maximum coordinates of the system were then calculated in order to set the bounding box for periodic boundary conditions with side length of 60Å in all 3 coordinate directions.

The systems were simulated in an isobaric ensemble (NPT conditions), with a temperature of 298.15 K and pressure of 1 atmosphere. To control temperature, Langevin dynamics were used with a coupling coefficient $\gamma = 1\text{ps}^{-1}$. To maintain constant pressure, a Langevin piston Nosé-Hoover method was used in NAMD with a piston period of 100 fs and a piston decay of 50 fs [3]. Finally, the CHARMM force field was used to calculate interatomic forces with a cutoff distance of 12Å.

Both simulations were run through three equilibration runs and one production run. The first equilibration run had a 1 fs time-step and was run for 10000 steps (10 ps simulation), and water molecules were free to move around the fixed protein. The second equilibration run had a 1 fs time-step and was run for 10000 steps (10 ps simulation), but in this case both water and protein were free to move. The third and final equilibration run had a 2 fs time-step and was run for 2500000 steps (5 ns simulation), and again, both water and protein were free to move. The production run used a 2 fs time-step and was run for 5000000 steps (10 ns simulation) with a frame capture every 2 ps for a total of 5000 frames in the resulting dcd trajectory file.

Simulations were conducted on the JHU Biophysics kirin cluster running Ubuntu 12.04 with 12 computation nodes. Analysis of results was done using VMD 1.9.3 and Python 2.7.13 (NumPy, SciPy, and matplotlib packages).

3 Results

Figure 1 shows the catalytic triads of the wild-type and mutated chymotrypsin. Comparing figure 1a and figure 1b shows the loss of three Asp-His hydrogen bonds in the mutant. These hydrogen bonds are the ones that were hypothesized to conformationally restrain the histidine.

The root-mean-square deviation (RMSD) of atomic positions of the D102A variant was determined to be slightly higher than that of the wild-type variant, with an average of 2.09Å compared to 2.00Å. As seen in Figure 2, after about 8 ns of simulation, the D102A RMSD sharply increases to around 2.5Å, indicative of a loop flexing out, which can be confirmed from visual inspection of the trajectory.

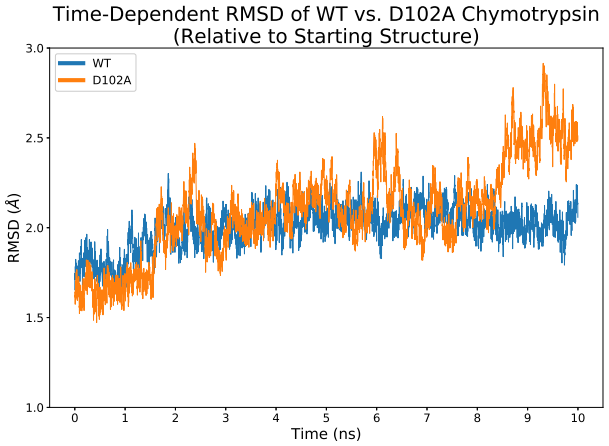


Figure 2: RMSD of wild-type (WT) and D102A simulations.

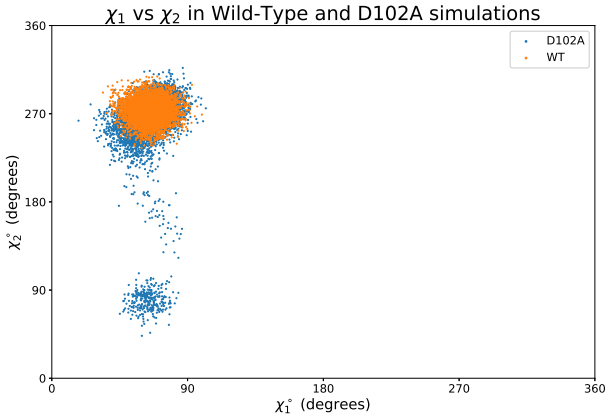


Figure 3: Dihedral bond angle distribution for Histidine Side Chains in WT and D102A simulations reveals bimodal distribution in D102A mutant.

The dihedral angle between H59 α-carbon and β-carbon (χ_1) and the dihedral angle between H59 β-carbon and γ-carbon (χ_2) were measured for each frame in the WT and D102A simulations. As seen in Figure 3, the wild-type chymotrypsin stayed in one region centered around $(\chi_1, \chi_2) \approx (70°, 270°)$. In contrast, the D102A variant showed significantly higher variability in dihedral angles, where the distribution appeared to be bimodal with two peaks centered around $(\chi_1, \chi_2) \approx (70°, 90°)$ and $(\chi_1, \chi_2) \approx (70°, 270°)$. Even in the region centered around $(\chi_1, \chi_2) \approx (70°, 270°)$, the mutant samples a wider range of bond angles.

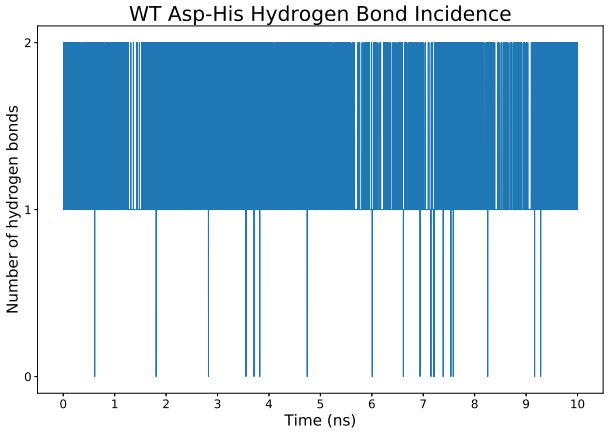


Figure 4: Rapid Conversion between 1 and 2 Asp-His hydrogen bonds of wild-type chymotrypsin indicates persistent bond.

For the analysis, hydrogen bonds were defined as a maximum distance of 3.5Å between donors and accepters with a maximum angle of 45°. Hydrogen bond incidence can then be plotted versus simulation time – see Figures 4, 5, 6. Figure 4 demonstrates wild-type Asp-His hydrogen bonds are relatively stable, terminating infrequently over the entire simulation. The fluctuations are mostly between 1 and 2 hydrogen bonds (an Asp-His hydrogen bond exists in 99.6% of the sampled frames), indicating that the hydrogen in the histidine was forming and breaking bonds with the oxygen in the aspartate rapidly.

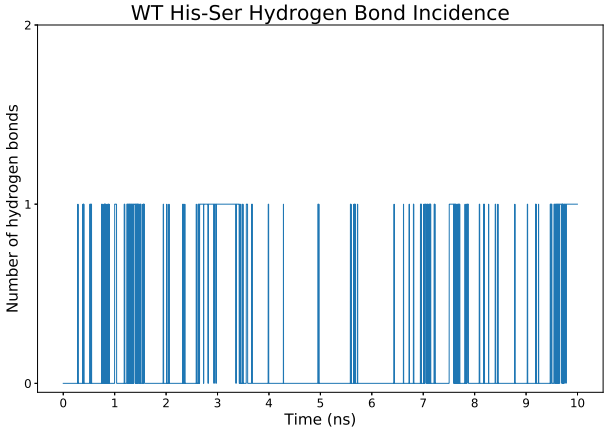


Figure 5: Conformations containing a His-Ser hydrogen bond are more infrequently sampled than those with a Asp-His hydrogen bond. 304 bonds shown.

Figure 5 shows that conformations with the His-Ser hydrogen bond are more infrequently sampled over the simulation compared to conformations with a Asp-His hydrogen bond. Quantitatively, 20.3% of frames contained a His-Ser hydrogen bond.

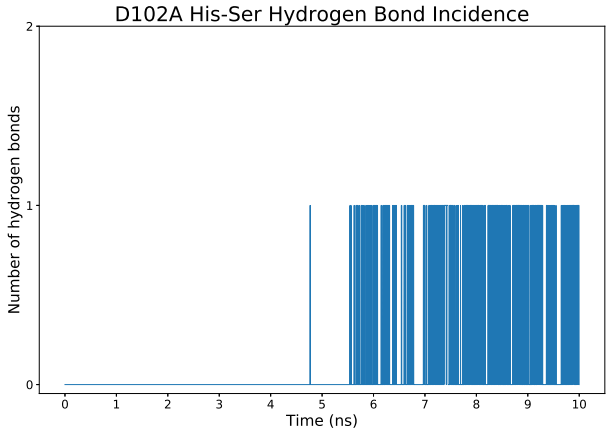
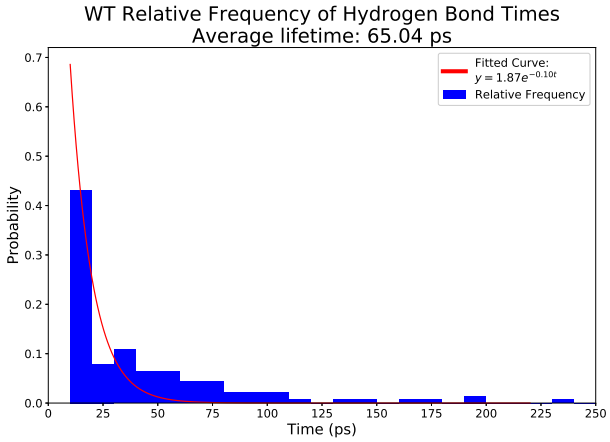


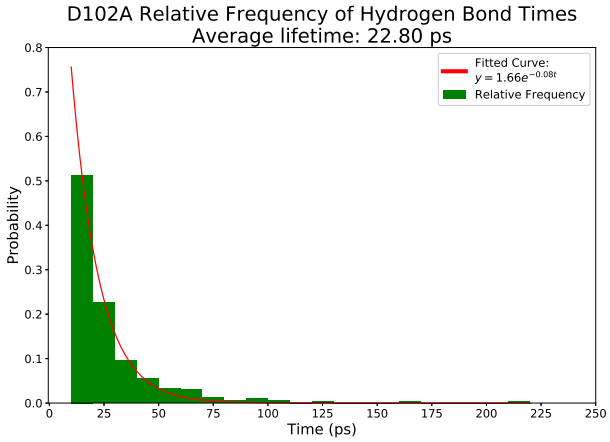
Figure 6: D102A simulation initially samples conformations without hydrogen bonding, but suddenly samples such conformations. 139 bonds shown.

Interestingly, in Figure 6 the mutated variant, the histidine residue initially does not sample conformations with any hydrogen bonds, but ends up sampling such conformations somewhat frequently. In the first half of the D102A simulation, only one frame has a His-Ser hydrogen bond. However, in the second half of the simulation, 27.7% of the frames contain a His-Ser hydrogen bond. It seems that the histidine rotated into position and stayed in position even without aspartate to stabilize it with hydrogen bonds.

His-Ser hydrogen bond lifetimes were also calculated with VMD, and analysis of the results was done in Python. The wild-type chymotrypsin had an average bond lifetime of 65.04 ps, while the mutated variant had an average lifetime of 22.80 ps. The wild-type also had higher maximum bond lifetimes (6 instances of bond lifetimes over 200 ps, with a maximum of 1930 ps) than the D102A variant (maximum bond lifetime of 210 ps). Furthermore, an exponential best-fit line can be fit to the histogram. Analysis code can be found in Appendix A.



(a) Wild-type, 304 bonds measured



(b) D102A, 139 bonds measured

Figure 7: Hydrogen bond lifetimes in simulations of (a) Wild-type chymotrypsin and (b) D102A variant

4 Conclusion

The data supports the hypothesis that Asp-102 conformationally restrains His-57 to better enable hydrogen bonding between His-57 and Ser-195. First, the higher RMSD for the D102A from the simulation is evidence of a greater range of motion and increased flexibility within the D102A variant. One explanation for this is the lack of two hydrogen bonds at the active site between residues 57 and 102. However, this isn't strong direct evidence for the hypothesis because the RMSD is a measure of *all* deviations of the displacement between the mutant and the starting structure over the course of the simulation, and is not specific to the active site. Furthermore, the difference between RMSD is rather small (only 0.09Å), and up until the sudden jump at 8 ns, the RMSD of the D102A simulation is larger than the RMSD of the WT simulation by just 0.01Å! Clearly this is not strong enough evidence to support acceptance or rejection of the hypothesis.

We can infer more specific information about the active site from the dihedral angle distributions in Figure 3. The wild-type chymotrypsin has lower variance in sampled bond angles in the presence of Asp-102, while the mutated chymotrypsin samples a wide range of dihedral angles, and samples the wild-type angle combinations infrequently throughout the simulation. This is much stronger evidence that the Asp-His hydrogen bonds conformationally restrain the His-57 residue.

The role of Asp-102 is evident when we consider the formation and lifetime of the His-Ser hydrogen bond. The average bond lifetime in the wild-type simulation was 65.04 ps, compared to an average lifetime of 22.80 ps in the mutant simulation. In the wild-type simulation, 51.07% of bonds had lifetimes longer than 20 ps, while in the D102A simulation, 26.00% of bonds had lifetimes longer than 20 ps. Of course, it must be noted that this data is imprecise due to the short length of the simulation, but suggests that Asp-102 has an effect in maintaining the His-Ser bond.

If we also consider the percentage of time a hydrogen bond existed in each simulation, we see that the wild-type had the His-Ser hydrogen bond in 20.28% of frames, while the D102A variant had the bond in only 13.86% of frames, even though there were only 139 bonds in the WT simulation compared to 304 in the D102A variant simulation. This is discrepancy in number of bonds and time with a hydrogen bond is explained by the lifetime of bonds discussed earlier. Again, even though our data is somewhat imprecise, it suggests that Asp-102 is important for forming the His-Ser hydrogen bond.

While the results from averaging the data seem conclusive, given the short simulation time, it is hard to tell whether the sudden change in behavior in the D102A simulation after 5 ns is anomalistic or typical, or if even the lack of bonding at the beginning was atypical. As such, it's difficult to draw meaningful conclusions from the bond incidence data.

Overall, the data are imprecise and the effects of variance still play a role, but as a whole, all the different approaches to the hypothesis from the different data we collected all indicate that Asp-102 is clearly involved in forming and maintaining the His-Ser bond by conformationally restraining His-57, and suggests acceptance of the hypothesis.

As mentioned earlier, these simulations suffered from lack of running time. Because of the short simulation time, odd behaviors such as the sharp increase in RMSD in the mutant and the sudden formation of hydrogen bonds in the mutant affected large portions of the simulation. Although the RMSD data were still reliable for the first 8 ns of simulation, the significance of the His-Ser bond incidence was doubtful. With longer simulation time, we would have a more precise comparison when averaging over the WT and D102A simulations. In order to determine the length of time to run our simulations, we could run one *very* long simulation as a control and compare it to smaller simulations of variable length and see at which time the shorter simulations approximate the results of the control simulation closely.

Acknowledgements

I would like to thank Dr. Fleming for allowing me to take this class and for being so helpful when my lack of knowledge in biology and chemistry arises. I definitely learned quite a bit about biology from this class and really enjoyed it!

References

[1] Hedstrom L. Serine protease mechanism and specificity. Chemical reviews. 2002 Dec 11; 102(12):4501–24.

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[3] Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. Journal of computational chemistry. 2005 Dec 1;26(16):1781–802.

A Hydrogen Bond Lifetime Code

```
from matplotlib import pyplot as plt
from __future__ import division
from scipy.optimize import curve_fit
import numpy as np

"""
Hydrogen bonds involving last frame are intentionally omitted
since their duration cannot be calculated
"""

#prev tracks if hbond in previous frame
#hbond is bond status
#count is bond duration
prev = hbond = count = 0
sizes = [] #list of bond durations

#Read file and compute hbond lifetimes
with open("../D102A/hbonds_his_ser.dat") as f:
    for line in f:
        line = line.split() #splits line by whitespace
        hbond = int(line[1])
        if hbond == 1:
            count += 1
            prev = 1
        elif hbond == 0 and prev == 1:
            #if the hbond terminates
            sizes.append(count)
            count = prev = 0
        else:
            #hbond == 0 and prev == 0, nothing happens
            continue

sizes = [x*10 for x in sizes]
#weighting for relative frequency
weights = np.ones_like(sizes)/float(len(sizes))
#prints average lifetime
print(sum(sizes)/len(sizes))
#print bond lifetimes and frequencies
print(np.unique(sizes, report_counts=True))


#Plotting & Saving
plt.figure(figsize=(12, 8))

```

```
#Histogram
n, bins, patches = plt.hist(sizes, color='b', weights=weights,\
            bins=range(10, max(sizes)+20, 10), label='Relative Frequency')

#define function to fit to
def func(x, a, b):
    return a * np.exp(-b * x)

#Compute best-fit exponential
bins = [0.5*(bins[i]+bins[i+1]) for i in range(len(bins)-1)]
popt, pcov = curve_fit(func, bins, n, p0=(4, 0.1))
x = np.linspace(10, 220, 2100)
A, B = popt #optimal values of a, b in func()

#Plot best-fit exponential
plt.plot(x, func(x, *popt), 'r-',\
        label='Fitted Curve:\n$y = %0.2f e^{-%0.2f t}$' % (A, B))

#Labeling
plt.title("WT Relative Frequency of Hydrogen Bond Times\n\
        Average lifetime: {:0.2f} ps".format(sum(sizes)/len(sizes)), fontsize=24)
plt.xlabel("Time (ps)", fontsize=18)
plt.ylabel("Probability", fontsize=18)
plt.xlim((0, 250))
plt.xticks(np.arange(0, 251, 25), fontsize=14)
plt.yticks(np.arange(0, 0.71, 0.1), fontsize=14)

#legend formatting
lgnd = plt.legend(prop={'size': 14})
lgnd.legendHandles[0].set_linewidth(5.0)
lgnd.legendHandles[1].set_linewidth(5.0)

#save figure
plt.savefig('../figures/wt_hbond_times.eps',\
        format='eps', dpi=1000, bbox_inches='tight')
```