NMR Study of Intramolecular Hydrogen Bonding in Imidazole-4-Propanoic Acid

Michael J. Kenney, Arizona State University
Dr. John D. Roberts, Dr. William R. Carroll, Department of Chemistry, California Institute of Technology, Pasadena, CA, 91106
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Introduction

The catalytic triad in the serine protease enzyme family has been the subject of much study.[1, 2, 3] Enzymes in this family are able to hydrolyze peptide bonds at a rate that is on the order of a million times faster than ambient conditions.[1] Three amino acid residues make up the catalytic triad: aspartic acid, histidine and serine (Figure 1). These residues form an extensive hydrogen-bonding network that has been shown to be vital for the enzyme to function.[3] Both the careful positioning and local solvent environment around this catalytic triad may be important to the hydrogen bonding network as hydrogen bonds are often disfavored in water. This study investigates the solvent environment that is necessary for the Asp-His hydrogen bond to form, using a simplified model molecule in imidazole-4-propanoic acid (Figure 2).

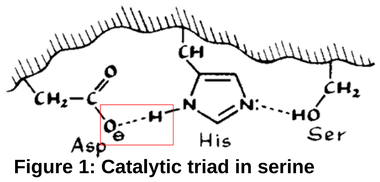

Figure 1: Catalytic triad in serine protease family.

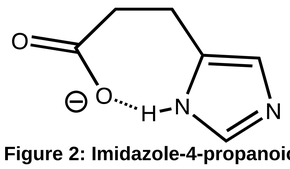

Figure 2: Imidazole-4-propanoic acid.

Previous work has shown that an intramolecular hydrogen bond in imidazole-4-propanoic acid that mimics the Asp-His does not likely form in water but can form in aprotic environments such as acetonitrile. This difference in hydrogen bond behavior is due to ability of water to donate and accept hydrogen bonds from the carboxylate group and imidazole nitrogen in imidazole-4-propanoic acid). Acetonitrile does not interact as strongly with these groups and permits the intramolecular hydrogen bond to form. Nuclear magnetic resonance (NMR) techniques such as ^{15}N and ^{1}H conformational analysis were used in this study to quantitatively verify the presence of the intramolecular hydrogen bond and to measure the amount of water necessary to disrupt the hydrogen bonding interaction in a solution of acetonitrile.

Fraction of Gauche Conformers

NMR spectroscopy can be used to measure the fraction of gauche conformers (Figure 3) present in a sample using coupling constants and the Altoona equation. Statistically, there should be 2 gauche conformers for every 1 trans. When a fraction is observed that shows a gauche preferred greater than 2 to 1, it is indicative of some attractive interaction between two of the substituents. In this study, we were able to use NMR to determine the conformational preference of the CH_2CH_2 segment of imidazole-4-propanoic acid in different solvent environments. Figure 4 shows Newman projections of two of the three possible conformations of imidazole-4-propanoic acid about the C6-C7 bond.

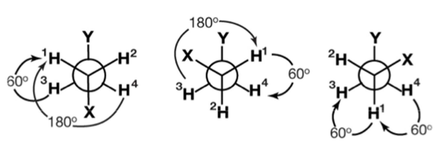

Figure 3: Generic gauche and trans conformers

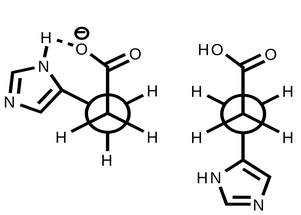

Figure 4: Gauche and trans conformers of imidazole-4-propanoic acid.

Results

^{15}N shifts as a function of pD –

This study showed that as pD is increased and the imidazole compound is taken through its ionization states in D_2O (Figure 6), both N1 and N3 shift downfield (Figure 5). If an intramolecular hydrogen bond were present in this system we would expect N1 to shift but not N3.[2] When one of the imidazole nitrogens is deprotonated in a system with no intramolecular hydrogen bond, a proton exchange occurs between N1 and N3 and two non-equivalent tautomers form. An equilibrium is established that results in the tautomer with the proton on N1 being slightly more prevalent than the corresponding histidine tautomer with the proton on N3.[7] As a result, we observe both nitrogens shifting downfield with N3 shifting slightly more because the proton is spending less time on it.

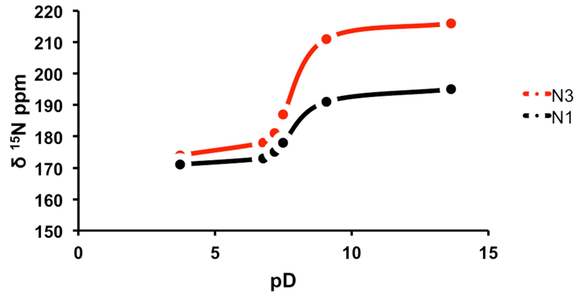

Figure 5: Graph showing ^{15}N shifts vs. pD.

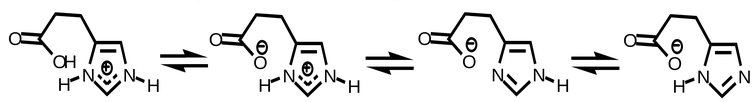

Figure 6: Possible protonation states of imidazole-4-propanoic acid

Acetonitrile-d3/H_2O Mixed Solvent Study –

Successive two microliter increments of water were added to a solution of deprotonated imidazole-4-propanoic acid in acetonitrile-d_3 and the changes in the fraction of gauche conformers were measured using proton NMR. The data accumulated in this study is shown in Figure 7. In Figure 7 we see that a relatively small amount of water, 12:1 mole ratio of water to imidazole-4-propanoic acid eliminates the gauche preference. This shows that water easily outcompetes the interaction between the carboxylate anion and the imidazole proton.

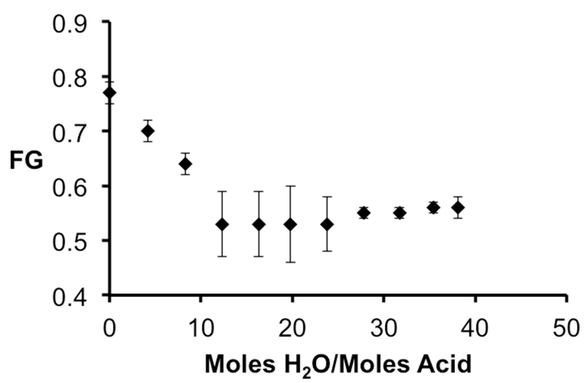

Figure 7: Relationship between fraction of gauche conformers and moles of H_2O

^{15}N NMR Study of 2-Mercaptoimidazole-4-Propanoic Acid –

Due to its poor solubility, nitrogen shifts of imidazole-4-propanoic acid could not be obtained in acetonitrile. To avert this problem, 2-mercaptoimidazole-4-propanoic acid was used because of its higher solubility (Figure 8). Both the hydrogen bonding and non-hydrogen bonding cases were observed in acetonitrile. To create an intramolecular hydrogen bond in 2-mercaptoimidazole-4-propanoic acid, we deprotonate the carboxylic acid using tetrabutylammonium hydroxide. Once the carboxylic acid group is deprotonated, the carboxylate anion becomes a hydrogen bond acceptor and the proton on N3 may act as a hydrogen bond donor. Two solutions were prepared: one with base and one without. The ^{15}N shifts relative to liquid $^{15}NH_3$ of these samples were then obtained and compared (Figure 9). As predicted, when the propanoic acid group is deprotonated, we see a downfield shift of N3 indicating an intramolecular hydrogen bond.

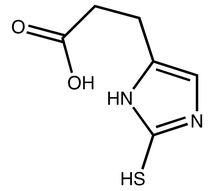

Figure 8: Structure of 2-mercaptoimidazole-4-propanoic acid

	δ ^{15}N (N3)	δ ^{15}N (N1)	δ ^{15}N ($CD_3C^{15}N$
Non-H-Bonding	167.35 ppm	161.89 ppm	244.6 ppm
H-Bonding	176.89 ppm	163.02 ppm	244.6 ppm
Δ	**9.54 ppm**	**1.13 ppm**	**0 ppm**

Figure 9: Table showing ^{15}N shifts of N1 in a hydrogen and non-hydrogen-bonding environment

Conclusions and Future Work

We have shown ^{15}N and ^{1}H NMR data that supports our hypothesis that intramolecular hydrogen bonding in imidazole-4-propanoic acid appears to be unable to take place in water but can likely take place in aprotic organic solvents. This provides experimental evidence for the conventional belief that the dielectric constant in the interior of an enzyme is lower than that of water and provides an empirical measure of the degree to which this effect matters. Having a nonpolar environment at the interior of the enzyme allows it to create an environment that is more similar to an organic solvent and allows it to carry out reactions that would not be possible in pure water (Figure 10). Future work will focus on testing more solvent environments and obtaining the imidazole-4-propanoic acid shifts in acetonitrile.

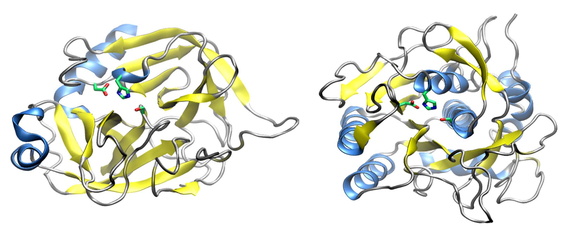

Figure 10: Diagram of two serine protease enzymes.

References

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