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

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**Introduction**

The catalytic triad in the serine protease enzyme family has been the subject of much study. 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. 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. 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).

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.

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**Results**

$^{15}$N shifts as a function of pH

This study showed that as pH is increased and the imidazole compound is taken through its ionization states in D$_2$O (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. When one of the imidazole nitrogens is deprotonated in a system with no intramolecular hydrogen bond, a proton exchange occurs between N1 and N3 (Figure 5). An equilibrium is established that results in the proton on N1 being slightly more prevalent than the corresponding histidine tautomer with the proton on N3. As a result, we observe both of these systems shifting downfield with N3 shifting slightly more because the proton is spending less time on it.

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**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 form in acetonitrile. 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. Future work will focus on testing more solvent environments and obtaining the imidazole-4-propanoic acid shifts in acetonitrile.

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**References**