Computational Studies on Tautomers of Fluorinated-Histidine and Fluorinated-Imidazole

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Abstract. Fluorinated amino acids are playing an important role in understanding protein-protein interactions, protein folding and protein structure function. Since histidine contains an imidazole ring, we considered the N1-H and N3-H tautomers of 2F-histidine and 4F-histidine, 2F-imidazole and 4F-imidazole, and calculated free energies and 19F NMR shifts in water and acetonitrile. The calculations were performed by the COSMO solvation model with B3LYP method 6-31++G(d) using Gaussian09 software. The calculations showed that the relative stabilities of the four isomers of fluorohistidine are consistent in both the solvents. The same trend is observed for fluoroimidazole isomers.The 19F NMR calculations showed different chemical shifts from zwitterionic/neutral to protonated forms for N1-H and N3-H tautomers of fluorohistidine and fluoroimidazole.

1. Introduction:

Fluorinated amino acids gained widespread importance as non-canonical building blocks of peptides and proteins. Recent studies on fluorinated amino acids suggest new opportunities for the construction of hyper stable protein folds and specific protein-protein interactions. The high electronegativity of fluorine affects the pH of neighboring groups, which causes an overall change in stability and reactivity of a molecule and the effects of fluorinated amino acids on protein structure-function are still being explored.

Histidine is an essential amino acid, present in around one-half of all enzyme active sites. The imidazole ring of histidine exists in two tautomeric forms (N1-H) & (N3-H) in aqueous solutions [2] and it has a pKa value of ~6, which is nearest to physiological pH among all amino acids. In contrast, the imidazole rings of 2-fluorohistidine [3] and 4-fluorohistidine [1] have much lower pK_a values of ~1.22 and ~1.76 respectively. In pH dependent biological processes, these differences in pK_a in the fluorinated analogues assist in exploring the role of histidine on protein structure function [4].

2. Methods

Optimized geometries, ^{19}F NMR chemical shifts, and energies for protonated and zwitterionic (neutral) forms of 2F-histidine, 4F-histidine, 2F-imidazole and 4F-imidazole, including each tautomer (N1-H and N3-H) were calculated in solvents, water and acetonitrile. We performed the calculations using DFT methods with B3LYP functional and the 6-31++G(d) basis set, using Gaussian09 software. All these calculations were performed using the COSMO solvation model, in which the solvent is considered as a dielectric continuum, which interacts with the molecular surface.

3. Calculation Summary:

^{19}F NMR calculations:

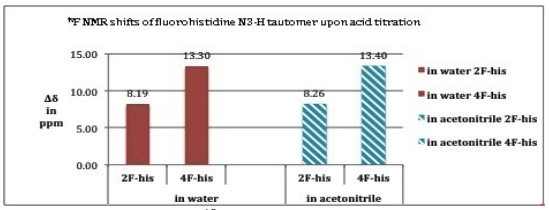


Figure 1: Calculated ^{19}F NMR shifts for fluorohistidine N3-H tautomers in different solvents

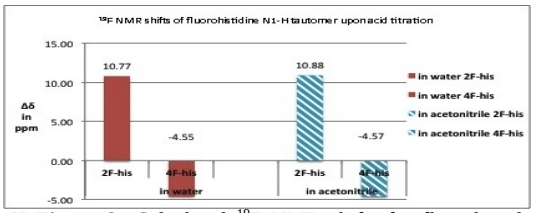
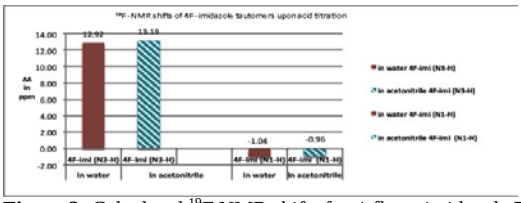


Figure 2: Calculated ^{19}F NMR shifts for fluorohistidine N1-H tautomers in different solvents tautomers in different solvents



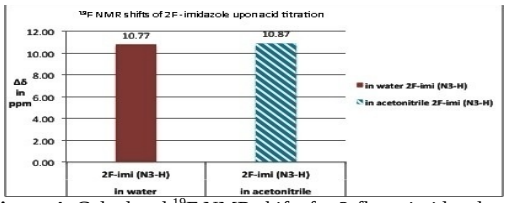


Figure 3: Calculated ^{19}F NMR shifts for 4-fluoroimidazole **Figure 4:** Calculated ^{19}F NMR shifts for 2-fluoroimidazole tautomers (N1-H & N3-H) in different solvents in different solvents

Results and Discussion:

Figures 1 and 2 give ^{19}F-NMR shifts for N3-H and N1-H tautomers of fluoro histidine, respectively upon protonation. From figure 1 in N3-H tautomeric form, fluorohistidine isomers showed an up field shift upon protonation, in both the solvents. Whereas from figure 2, in N1-H tautomeric form, 2F-histidine showed an upfield shift and 4F-histidine showed downfield shift in both the solvents upon protonation. Figure 3 and figure 4 shows the ^{19}F NMR shifts for 4F-imidazole and 2F-imidazole respectively. Note that in figure 4, 2F-imidazole does not have any tautomers because of its plane of symmetry and it shows an upfield shift upon protonation in both the solvents.

Free energy calculations:

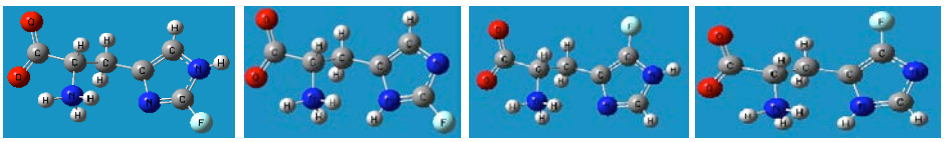
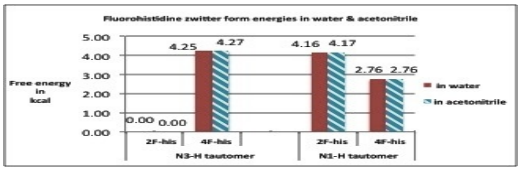


Image 1: 2F-his of N3-H tautomer **Image 2**: 2F-his of N1-H tautomer **Image 3**: 4F-his of N3-H tautomer **Image 4:** 4F-his of N1-H tautomer



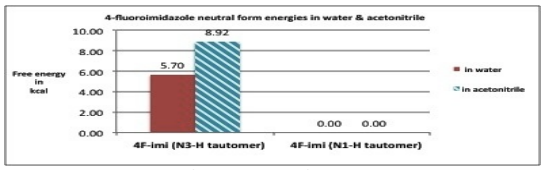


Figure 5: Calculated free energies for fluorohistidine zwitter **Figure 6:** Calculated free energies for 4F-imidazole neutral form tautomers in water and acetonitrile form tautomers in water and acetonitrile

Results and Discussion

Figure 5 gives the free energy differences for all of the histidine isomers, relative to the most stable form: the N3-H tautomer of 2F-histidine. The results in figure 5 suggest that in solution, N3 is protonated for 2-fluorohistidine, whereas N1 is protonated for 4-fluorohistidine. In contrast, Figure 6 shows that the N1 tautomer of 4F-imidazole is more stable. Because of symmetry there are no tautomers for 2F-imidazole, but the energy of 2F-imidazole was calculated to be 1.17 kcal/mol higher than the 4F-imidazole N1-H tautomer in water (4.4 kcal/mol in acetonitrile).

4. Summary and Outlook

The free energies and ^{19}F NMR shifts of isomers of fluorohistidine and fluoroimidazole were calculated to understand the stability of isomers and solvent effect. Combining calculated relative stabilities and predicted NMR shifts, we can predict that among fluorohistidine isomers, 2F-his is more stable in water and acetonitrile, whereas among fluoroimidazole isomers, 4F-imidazole is more stable. Future experiments will include experimental ^{19}F NMR calculations for comparative studies.

5. Acknowledgements

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6. References

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[2] Akira Toyama, Kunio Ono, Shinji Hashimoto, and Hideo Takeuchi. *J. Phys. Chem. A*, 2002, **106**, 3403-3412.
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