Chemical Shift Anisotropy Determination of Fluorinated Amino Acids

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Introduction

NMR spectroscopy using labeled proteins (protein-observed) was the first biophysical method used for establishing the field of FBDD.[1] Fragments are characterized as low molecular weight, low complexity small molecules that typically bind to their protein target with low affinity (mid micromolar to millimolar dissociation constants) which can be readily detected using NMR.[2] ^{19}F NMR is an attractive approach for fragment screening. ^{19}F is an ideal background-free NMR-active nucleus for protein binding studies. ^{19}F chemical shifts are hyperresponsive to changes in the molecular environment and are thus sensitive probes for fragment binding events.[3] In the case of fluorine-labeled proteins, the environmental sensitivity of fluorine nuclei typically results in well-resolved 1D ^{19}F NMR spectra of proteins whose labeled side-chains are observed at micromolar concentrations (e.g. 25–100 μM). [3] We recently showed that protein-observed ^{19}F NMR (PrOF NMR) is both 6-20 fold more responsive than protein-observed ^{1}H NMR,[4] and in some cases 2-4 fold faster to acquire.[5]

Due to the enrichment of aromatic amino acids at PPI interfaces,[6] fluorinated aromatic amino acids such as 4-fluorophenylalanine, 5-fluorotryptophan, and 3-fluorotyrosine can be sequence selectively incorporated at protein interfaces.[5] Although a variety of fluorinated aromatic amino acids have been well-characterized by PrOF NMR,[7,8] 2-fluorotyrosine (**Figure 1**, **6**) remains understudied. One specific aspect of this amino acid that has not been determined is the chemical shift anisotropy (CSA) and its responsiveness to changes in chemical environment. These data will inform researchers about its suitability for incorporating into large proteins.

Experimental

^{19}F NMR spectra were acquired on a 600 MHz solid state NMR magnet with sample spinning at 12.5 and kHz and 71.4 kHz proton decoupling field. Both samples were referenced to 2-fluorobenzoic acid. At -108 ppm.

Results and Discussion

Prior studies had already characterized the chemical shift responsiveness of amino acids to different chemical environments (grant preliminary data) The next set of experiments carried out measured the T_2* of the various small molecules in an 80% glycerol solution to simulate a 12 kDa protein,[8] where longer T_2* values would lead to narrower linewidths in larger proteins. Consistent with studies by Ulrich et al. 5-fluoroindole, **3**, had a longer T2* than 6-fluoroindole **4**, (473 ms vs 460 ms), whereas 2-fluorocresol, **1**, had the shortest T_2* of 138 ms. Alternatively, the 2-fluorotyrosine equivalent, 3-fluorocresol, **2**, had an intermediate T_2* of 240 ms, indicating this amino acid may be more suitable for incorporating into larger proteins. However, due to multiple factors influencing T_2*, CSA determination and comparison of 2-fluorotyrosine and 3-fluorotyrosine was decided on as the more rigorous way to determine this effect.

To determine the CSA of amino acids **5** and **6,** solid state ^{19}F NMR experiments were conducted to determine the chemical shift tensors and CSA. CSA of **5** was previously determined by Ulrich and co-workers to be -74.6.[8] We determined a similar value of -77.6. For **6** we determined an anisotropy of -69.7, significantly smaller than **6** based on an approximate 1 ppm linewdith. These results are thus consistent with our prior finding based on the T2* measurements with **1** and **2**.

Conclusions

These studies evaluated the NMR properties of fluorinated amino acids for PrOF NMR experiments. Based on the chemical shift responsiveness of **1**, proteins with the corresponding amino acid **5** are anticipated to be more sensitive to drug binding events. However, based on the lower CSA value of **6** this amino acid is anticipated to yield narrower linewidth, both at higher field strengths and higher protein molecular weights.

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