Abstract for an Invited Paper
for the MAR12 Meeting of
The American Physical Society

Novel Aspects of Hydrogen Bonding in Protein Function: Active Site Ionic Hydrogen Bonds

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We use photoactive yellow protein (PYP), a bacterial photoreceptor, to explore novel aspects of the role of hydrogen bonding in protein function. PYP exhibits photochemical activity based on its ionized p-coumaric acid (pCA) chromophore, which is hydrogen bonded to Tyr42 and Glu46. We report that these active site ionic hydrogen bonding interactions cause unexpected molecular and functional properties of PYP. First, we describe a novel spectroscopic isotope effect (SIE) in which dissolving PYP in D2O causes a red-shift in its electronic absorbance spectrum. We assign this SIE to the ionic hydrogen bond between pCA and Glu46, which–in contrast to standard hydrogen bonds–is weakened upon H/D exchange. These findings extend the effects of H/D exchange from kinetic isotope effects to include shifts in absorbance spectrum, and illustrate the biological relevance of ionic hydrogen bonding to protein active sites. Secondly, we examine how the protein environment achieves the unusual strong preference of the pCA to remain ionized in the protein interior. We use the rescue of the Y42F mutant of PYP by incorporation of a trans-locked analog of pCA to dissect the contributions of active site hydrogen bonding to the large down-shift in the pKa of the pCA. Together, the Tyr42 and Glu46 hydrogen bonds to the pCA account for $\sim$80% of this shift, which can be quantitatively explained by the loss of ionic hydrogen bonding upon pCA protonation from the solvent. Since ionic hydrogen bonds occur in many proteins, this mechanism of pKa tuning is likely to be of general relevance.