FIRST PRINCIPLES STUDY OF INTEIN REACTION MECHANISMS

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ABSTRACT

Protein splicing is an autocatalytic reaction where two flanking sequences (exteins) are excised and ligated. The enzymatic protein sequence that lies between the exteins, known as the intein, is extremely efficient at protein splicing and has been utilized for biotechnological applications. The characterization of intein reaction mechanisms is important for understanding how and why certain mutations may be used to control the splicing and cleavage reactions, as well as tuning the reaction rate without affecting the mechanism. In combination with crystallographic structures as well as both site-directed and random mutagenesis, we have studied the reaction mechanisms for intein splicing as well as for cleavage at the C-terminus using first principle quantum mechanical simulations. Previous experimental studies have shown that mutation at a critical N-terminal residue of the intein resulted in splicing inhibition. Despite this inhibition of the overall splicing reaction, peptide bond cleavage isolated at the C-terminal may still occur independently. With an aspartate to glycine mutation, the "cleavage mutant" was found to react more rapidly in a low pH environment. We have characterized the pH dependent C-terminal cleavage reaction and studied the effect of mutation on the energy barrier, and have provided for the first time an atomic level understanding of this important process. Next, we have extended our computational study to address the overall intein splicing mechanism. The splicing reaction is a highly synchronized chemical process where the effect of mutation can accelerate, decelerate, or partially or completely inhibit steps along the reaction. We have focused our study on the energetic effect of mutation on the reaction profile and corresponding protein structure. From this, we have made a prediction for the splicing mechanism that utilizes the highly conserved amino acids and explicitly describes the behavior of protons. An explanation for the experimental inhibition of splicing with the aspartate to glycine mutation is also presented. In summary, with a series of quantum mechanical calculations ranging from gas phase, to an implicit solvent scheme, to combined quantum/classical simulations, we have provided insight into some of the key steps of intein reactions. These studies may be

exploited for many applications involving inteins including molecular switches and sensors as well as controlled drug delivery.