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Behind the Scene Role of Conserved Threonine in Intein Splicing ALBERT DEARDEN, Rensselaer Polytechnic Institute, BRIAN CALLAHAN, MARLENE BELFORT, University at Albany, SAROJ NAYAK, Rensselaer Polytechnic Institute — Protein splicing is an autocatalytic process where an "intein" self-cleaves from a precursor protein and catalyzes ligation of the flanking fragments. Inteins occur in all domains of life and have myriad uses in biotechnology. While reaction steps of intein splicing are known, mechanistic details remain incomplete. Here, we investigate the possible role of a highly conserved active-site Threonine residue in bringing about the initial step of splicing: peptide bond rearrangement at a conserved Glycine-Cysteine motif. We report that although not part of the active transition state in this reaction, Threonine plays an important role in reducing the energy barrier through charge screening of active residues in the transition state. Interestingly, Threonine-Glycine hydrogen bonding makes sulfur of the attacking Cysteine less nucleophilic, thereby minimizing Coulomb repulsion in the transition state. These non-intuitive results are obtained through a combination of crystal structure, quantum mechanical simulations, and mutagenesis data. Our results further predict that the sluggish reaction rates observed with intein mutants harboring Threonine-Alanine substitutions can be accelerated in the presence of non-aqueous solvents.

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