UNDERSTANDING INTEIN-MEDIATED PROTEIN SPLICING

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ABSTRACT

An intein is a genetic intervening sequence that is removed post-translationally *via* protein splicing, a process consisting of four precisely-coordinated steps. The intein is autocatalytic and mediates the first three steps of protein splicing: the N-X acyl rearrangement, transesterification, and asparagine cyclization. The general mechanism for these reactions is known, but the atomistic details are not fully understood. The primary objective of our research is to gain insight into the details of intein activity with the goal of engineering inteins for applications. In our study, we have investigated the chemical and physical aspects of the steps of protein splicing using mini-derivatives of the *Mycobacterium tuberculosis* (*Mtu*) *recA* intein as our model. We carried out site-directed mutagenesis on residues suspected of playing roles in intein activity. We expressed these intein variants within fusion proteins in *Escherichia coli* and resolved the protein products through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). By evaluating the amounts of intein-related products, we analyzed how mutating these residues affects each of the intein-catalyzed reactions and how it affects protein splicing as a whole.

In our research, we investigated primarily Asp422, but we also studied residues Asp24, Val67, His73, Glu424, His429, and His439. We first isolated the different steps of protein splicing in the *Mtu recA* mini-inteins by incorporating combinations of mutations to the terminal residues. Our analyses of the N-X acyl rearrangement and transesterification reveal that Asp422 plays a catalytic role in at least one of these reactions. Variants with either the D422A or D422G mutation do not undergo the N-X acyl rearrangement. The same residue, 422, also affects asparagine cyclization. Our results indicate that residue 422 physically attenuates asparagine cyclization. Furthermore, we demonstrate that Glu424, His429, and His439 play important roles in asparagine cyclization. We then examined the overall protein splicing mechanism and found that the protein splicing results are consistent with the results derived from the studies of the constituent reactions. Using the insights gained from our research, we propose a mechanistic pathway for protein splicing.

Packing of the active site, which includes Asp422, attenuates asparagine cyclization due to some physical constraint. A negatively-charged amino acid at 422 is capable of catalyzing the N-X acyl rearrangement and transesterification so that the branched intermediate forms. Formation of this intermediate disrupts the active site packing such that asparagine cyclization is no longer inhibited; splicing proceeds. Further work must be performed to test and validate this hypothesis.