Biophysical insights into the mechanism of protein splicing

by

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A Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the degree of

MASTER OF SCIENCE

MAJOR: CHEMICAL ENGINEERING

Approved:

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Rensselaer Polytechnic Institute

Troy, New York

December, 2007

ABSTRACT

Protein splicing is one of those phenomena in nature that has not been completely understood. Though the reactions constituting the protein splicing pathway have been known for quiet some time now, a single theory still cannot explain why and how the reactions occur in the first place. Understanding such a complex problem requires research spanning a variety of branches of science and through this work, we have tried to present the insights we could gain by being part of such collaboration. We have tried to propose and prove the pKa shift hypothesis behind protein splicing through Nuclear Magnetic Resonance spectroscopy. This involved determining the pKa of the key amino residues which are thought to have mechanistic importance in protein splicing. Specifically, we have determined the pKa of residue D422 in the precursor to be 6.6, which is higher than the pKa (6.2) of the same residue in the post splicing product. This result points towards the mechanistic importance of this residue in the protein splicing pathway.

A major accomplishment was to isolate one of the first precursors to protein splicing. This required the development of chromatography and process scale up. In addition, the stability difference with respect to temperature between a wild type intein and a single mutant clone by Circular Dichroism Spectroscopy. This validates previous results from our research group. A difference of 6°C between the melting points of the wild type and mutant intein was obtained. The research also involved extensive collaboration with physicists, molecular biologists and researchers from other disciplines. Such a multidisciplinary approach adopted by our group enabled the exchange of ideas and data resulting from the collaboration. By isolating the

precursor and by utilizing various biophysical methods, we have gained insight into the fascinating yet complex world of protein splicing.