FUNCTIONAL CHARACTERIZATION OF AUTOCATALYTIC PROTEINS:

GREEN FLOURESCENT PROTEIN AND MYCOBACTERIAL SUFB INTEIN

By

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ABSTRACT

Autocatalytic enzymes are a class of proteins that require no or minimal cofactors for their catalytic activity. Green fluorescent protein (GFP) and inteins are two distinct examples of autocatalytic proteins: in the case of GFP the only cofactor needed for its fluorescence is molecular oxygen while no external substrate is necessary for intein catalysis.

In this work we explore the functional properties of both these proteins as follows. In GFP we explore the capacity of GFP to fold and reconstitute fluorescence from truncated circular permutants, herein called "leave-one-outs" (LOO). The solubility of twelve LOO constructs, each with one complete secondary structure element omitted, was measured using a quantitative *in vivo* solubility assay and *in vivo* reconstitution of fluorescence. Removal of any one of the N-terminal six beta strands or the central helix produces predominantly insoluble proteins that do not reconstitute fluorescence, whereas removal of one of the C-terminal five strands produces proteins that are more soluble and do reconstitute fluorescence. Our results suggest that omitting early folding secondary structure elements leads to increased aggregation, while omitting late folding segments leads to a less aggregation. The results of relative fluorescence (RF) studies and solubility assays correlate well. We have used *in vivo* reconstitution of fluorescence to determine whether the soluble form exists in a natively folded state that can bind to the missing peptide. Our preliminary data also suggests LOO T8 as the possible biosensor candidate with about 50 % of WT fluorescence recovery after in vivo reconstitution.

Inteins are mobile genetic elements or “intervening sequences” that interrupt the coding sequence of a gene that are spliced out at the protein level. *Mycobacterium*

tuberculosis (*Mtu*) contains three genes, *recA, dnaB,* and *sufB*, which are interrupted by inteins, where the proteins become functional through protein splicing process. While RecA and DnaB proteins play important roles in the DNA repair and replication respectively, SufB is a part of Fe-S cluster assembly and is essential for growth. Moreover, protein splicing of SufB is crucial for the functionality of the *Mtu* SUF machinery. In this work, we report the effect of iron on protein splicing of the SufB precursor both *in vivo* and *in vitro*. Addition of increasing concentrations of iron appears to lead to inhibition of protein splicing while adding iron chelator 2, 2'- dipyridyl appears to facilitate protein splicing. Based on a mutagenesis study of critical catalytic residues we suggest that bonding between iron, potentially from Fe-S cluster, and conserved cysteine(s) could be responsible for the subtle iron effect. Our results provide the first study of iron regulation in protein splicing and suggest a novel approach for controlling the functionality of *Mtu* SUF machinery. Taken together our studies provide unique ways of controlling functionality of two classes of autocatalytic proteins.