ABSTRACT

Protein splicing is a self-catalyzed post-translational modification. This process involves the excision of the intein (intervening protein) from a precursor protein, resulting in the ligation of the flanking exteins (external proteins) into a single polypeptide chain. Inteins are found in unicellular organisms and occur in all three domains of life: bacteria, archaea and eukaryota. More than 600 intein genes have been reported, but only a few have been thoroughly characterized. The goal is to understand the structural and dynamic basis of their catalysis to engineer intein enzymes with novel applications.

Structure of a 136-residue DnaX intein enzyme, derived from the cyanobacterium *Spirulina platensis* (abbreviated as *Spl* DnaX intein), was determined by solution NMR spectroscopy. Chemical shift-based torsion angle restraints and nuclear Overhauser effect (NOE)-derived distance restraints were used to calculate the three-dimensional structure of the intein. In the structure 13 β-strands, one 3_{10}-helix, and one α-helix are arranged in a symmetric horseshoe-shape, typically found in the HINT (Hedgehog/INTein) domain superfamily. The NMR structural ensemble has a backbone RMSD of 0.27 Å and heavy atom RMSD of 0.52 Å for all ordered residues (RCSB PDB entry: 7CFV). The *Spl* DnaX intein has a very stable core as determined by NMR-based hydrogen exchange experiments. Backbone ^{15}N-dynamics experiments (R_1, R_2 and {^{15}N-^{1}H} heteronuclear NOE) showed the presence of conserved motions in symmetric positions in the intein structure, which is most likely a result of the symmetrical fold of the protein. Western blot based *in-cell* splicing assays revealed *Spl* DnaX intein to be a highly active enzyme. The precursor protein was not detected at any timepoint of the assay. Apart from the splicing reaction, catalytic cleavage at the N- and C-termini of the precursor protein was also observed.

Overall, this work showed that the *Spl* DnaX intein is a robust, stable and catalytically highly active enzyme. It is found to be highly tolerant to most of the extein residues that regulate the catalytic function by restricting the near-attack conformations of the active site residues.

This work provides important insights into this enzyme and should help in the engineering of promiscuous intein enzymes for novel applications with less dependence on extein residues.

Key words: NMR spectroscopy, protein structure, splicing reaction, molecular dynamics simulation, sidechain rotamers, near-attack conformation, western blotting.