Solution NMR Studies of Transmembrane Domain of Amyloid Precursor Protein and Discovery of an Intramolecular Disulfide Bond between Catalytic Cysteines in an Intein Precursor

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ABSTRACT

Alzheimer's disease (AD) is the most common type of dementia in elderly people. Senile plaques, a pathologic hallmark of AD, are composed of amyloid β peptide (Aβ). Aβ aggregation produces toxic oligomers and fibrils, causing neuronal dysfunction and memory loss. Aβ is generated from two sequential proteolytic cleavages of a membrane protein, amyloid precursor protein (APP), by β- and γ-secretases. The transmembrane (TM) domain of APP, APPTM, is the substrate of γ-secretase for Aβ production. The interaction between APPTM and γ-secretase determines the production of different species of Aβ. Although numerous experimental and theoretical studies of APPTM structure exits, experimental 3D structure of APPTM has not been obtained at atomic resolution. Using the pETM41 vector, we successfully expressed an MBP-APPTM fusion protein. By combining Ni-NTA chromatography, TEV protease cleavage, and reverse phase HPLC (RP-HPLC), we purified isotoically-labeled APPTM for NMR studies. The reconstitution of APPTM into micelles yielded high quality 2D ^{15}N-^{1}H HSQC spectra. This reliable method for APPTM expression and purification lays a good foundation for future structural studies of APPTM using NMR.

APPTM has been shown to be prone to form a dimer, or even oligomer in micelle and bicelles, due to its two consecutive GXXXG and another GXXXA dimer motif. We proved APPTM dimerizes in DPC micelles via analytical ultracentrifugation (AUC) and paramagnetic relaxation enhancement (PRE) studies. Complete backbone and side chain assignment were obtained. Intermolecular NOEs were exclusively detected using selectively labeled APPTM sample. Residual dipolar couplings (RDC) were acquired in two different alignment media. APPTM dimer structure were calculated by CYANA, and then refined by Xplor using RDC data. Our structure suggests that APPTM forms a head to head dimer, GXXXG and GXXXA are both crucial for the dimerization. The packing of residue V17, I18, and A19 are key residues that mediate the dimer association.

Protein splicing is a self-catalyzed and spontaneous post-translational process in which inteins excise themselves out of precursor proteins while the exteins are ligated

together. We report the first discovery of an intramolecular disulfide bond between the two active-site cysteines, Cys1 and Cys+1, in an intein precursor protein composed of the hyperthermophilic *Pyrococcus abyssi* PolII intein and extein. The existence of this intramolecular disulfide bond is demonstrated by the effect of reducing agents on the precursor protein, mutagenesis, and liquid chromatography-mass spectrometry (LC-MS) with tandem MS (MS/MS) of the tryptic peptide containing the intramolecular disulfide bond. The disulfide bond inhibits proteins splicing, and splicing can be induced by reducing agents such as tris(2-carboxyethyl)phosphine (TCEP). The stability of the intramolecular disulfide bond is enhanced by electrostatic interactions between the N- and C-exteins but is reduced by elevated temperature. The presence of this intramolecular disulfide bond may contribute to the redox control of splicing activity in hypoxia and at low temperature and point to the intriguing possibility that inteins may act as switches to control extein functions.