Structure and Function Studies of Membrane and Soluble Proteins

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Introduction

Asparagine-linked N-Glycosylation of proteins occurs within the lumen of rough endoplasmic reticulum (RER). Oligosaccharyltransferase (OST), a heterooligomeric membrane associated enzyme, catalyzes N-glycosylation, an essential and highly conserved protein modification reaction that occurs in all eukaryotic and some prokaryotic organisms. This modification is essential for many secreted and membrane proteins and ensures their proper folding, providing resistance to proteases, dictating intracellular trafficking and plays a role in growth regulation. Genetic defects in this modification pathway in humans cause a class of disorders known as congenital disorders of glycosylation (CDG). These conditions affect multiple organs with severe clinical manifestations including mental retardation, developmental delay, hypoglycemia, liver dysfunction, gastrointestinal disorders, dysmorphic features, and anorexia. Complete loss of N-glycosylation is lethal in all organisms. In yeast, *Saccharomyces cerevisiae*, OST is composed of nine non-identical membrane protein subunits. The long-term goal of this proposal is to understand the molecular details of the integrated action of this membrane-associated enzyme. Along with this project, NMR data were collected on a soluble protein project involving pheromone-binding protein (PBP) from the Lepidopteran male moth. PBPs play an important role in pheromone perception. Structure and function studies of PBPs are in progress with the long-term goal of designing inhibitors to block pheromone perception in invasive pest species.

Experimental

We collected several pairs of 3D heteronuclear NMR experiments for the backbone, side chain and NOE assignments of a membrane protein, Ost4p (a subunit of yeast OST) in DPC micelles and two different soluble proteins involved in moth olfaction. All proteins were uniformly labeled with N-15 and C-13 isotopes.

Results and Discussion

Both Ost4p and its mutant Ost4V23D have been sequence-specifically assigned. NOESY assignments (Fig. 1) is in progress. The OfurPBP2 (PBP of an invasive pest) sequence-specific backbone assignments are in progress. Preliminary NMR investigation along with other biophysical data suggest that OfurPBP2 may bind and release pheromone with a novel mechanism.

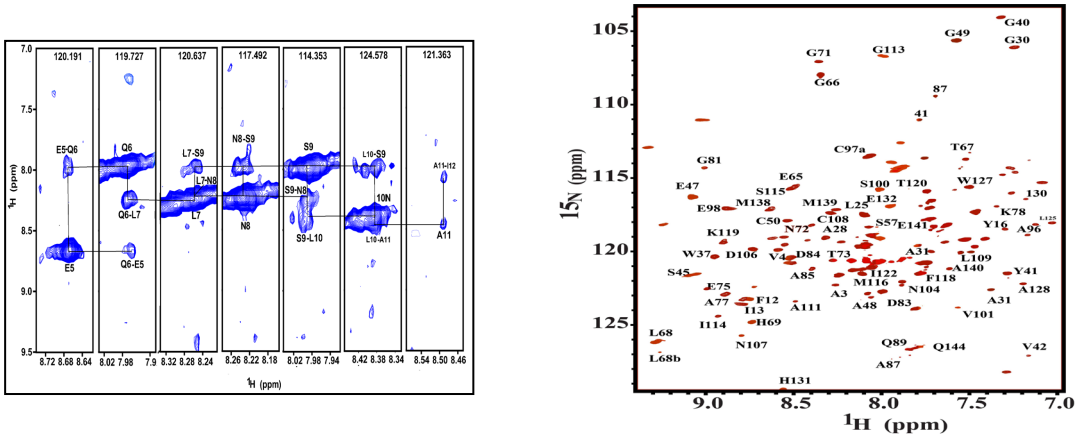

Fig. 1: (Left) NOESY Assignment of Ost4p in DPC micelles (Right) 2D HSQC assignment of OfurPBP2 in phosphate buffer, pH 6.5.

Conclusions

Our results suggest that mutation of Val23 to Asp impacts the structure of Ost4p. For OfurPBP2, NMR data suggest that it may have a novel mechanism of pheromone binding and release. This work was published recently [1].

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Reference

[1] Mazumder, S., *et al*., Scientific Reports, **8**, 17105 (2018).