

NATIONAL HIGH MAGNETIC FIELD LABORATORY 2017 ANNUAL RESEARCH REPORT

Mechanism of Yeast *N*-glycosylation: Structural Characterization of Ost4p and its Mutant

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Introduction

The co-translational transfer of a high mannose oligosaccharide group to the side chain of an asparagine in Asn-X-Thr/Ser (X \neq Pro) sequon in nascent polypeptide is referred as N-linked glycosylation. Genetic defects in N-linked glycosylation in humans lead to a series of diseases, which includes but not limited to mental retardation, developmental delay, hypoglycemia, lever dysfunction etc., known as congenital disorders of glycosylation (CDG) (1). The enzyme responsible for catalyzing the N-glycosylation process is membrane-bound Oligosachharyl transferase (OST) (2). In yeast, eight of the nine non-identical membrane proteins forms OST complex. Three subunits, Stt3p, Ost4p and Ost3p form a sub-complex (3) with Ost4p serving as a bridge (4). We are trying to solve the 3D structure of Ost4p in detergent micelles and in bilayer to compare and contrast to the structure determined previously in mixed organic solvents (5).

Experimental

We expressed and purified ¹³C,¹⁵N labeled wild type Ost4p and reconstituted in 100 mM DPC micelle for collecting NMR data. We also expressed, purified and reconstituted Ost4pV23D mutant in 100 mM PDC. We collected ¹⁵N HSQC, 3D NOESY-HSQC and 3D TOCSY-HSQC on Ost4p and all 3D experiments for both backbone, side chain and NOESY of Ost4V23D at NHMFL. Sequential resonance assignments achieved for Os4p is shown below. Assignments for Ost4V23D is not shown here. Structure calculation for Ost4p is currently in progress.

Results and Discussion

The ¹⁵N HSQC (Figure 1) of Ost4p in 100 mM DPC micelle shows well-dispersed peaks suggesting that the protein has a well-folded structure under the experimental conditions. The 3D TOCSY-HSQC data (Figure 2) was used to assign the side chain resonances and 3D NOESY-HSQC (Figure 3) was very useful for both resonance assignments and for providing distance constrains for 3D structure calculation, which is in progress.

Conclusions

The NMR data collected at NHMFL 800 MHz spectrometer helped to carry out the assignment of the integral membrane protein,Ost4p, a subunit of yeast OST.

Acknowledgements

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References

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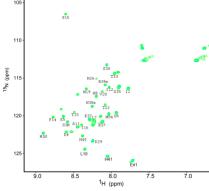
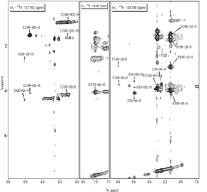


Figure 1: 15N HSQC of Ost4



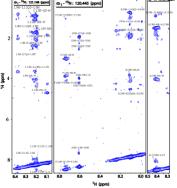


Figure 2: 3DTOCSY HSQC stripes

Figure 3:3D NOESY HSQC plot