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CRYSTALLOGRAPHIC AND NMR EVIDENCE FOR FLEXIBILITY IN OLIGOSACCHARYLTRANSFERASES AND ITS CATALYTIC SIGNIFICANCE

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Abstract

Oligosaccharyltransferase (OST) is a membrane bound enzyme that catalyzes the transfer of an oligosaccharide to the asparagine residue in the sequon, Asn-X-Thr/Ser. Eukaryotic OST protein complex consists eight non identical subunits, and among them STT3 possesses the transferase activity. The equivalent to STT3 is a single subunit protein called PglB in Eubacteria, and AglB in Archaea. The primary sequences (600 to 1,000 residues) of the STT3/AglB/PglB proteins share a common architecture. The N-terminal part forms a multi-span transmembrane region and the C-terminal part forms a soluble, globular domain which contains a well-conserved, five-residue motif, WWDYG. Structural comparison of the crystal structures of the C-terminal globular domain of AglB from *Pyrococcus furiosus* and of PglB from *Campylobacter jejuni* revealed different conformations of the segment containing the WWDYG motif, raising a question about what was the true conformation of the segment without any crystal packing effects. As part of this research work, crystal structures of the C-terminal globular domain of AglB's from distant as well as close related organisms to *P.furiosus* were determined. Relevant to this study one close homolog, *Pyrococcus horikoshii* AglB, with sequence identity of about 70%, and one distant homolog, *Archaeoglobus fulgidus* AglB, with sequence identity of about 30% were selected. Comparison of the crystal structures with emphasis on the highly flexible region of the WWDYG motif was performed, and found a superimposable conformation of the WWDYG motif between the most distant pair: *A.fulgidus* AglB-S2 and *C.jejuni* PglB, even with a sequence overall similarity of less than 30%. ^{15}N NMR relaxation analysis studies to characterize the dynamic nature of OST using *A.fulgidus* AglB-S2 were performed. Intriguingly, the mobile region contains the binding pocket for +2 Ser/Thr residue in the N-glycosylation sequon. In agreement, the restriction of the flexibility forced by an engineered disulfide crosslink abolished the enzymatic activity, and its cleavage fully reversed the inactivation. These results suggest the multiple catalytic cycle and the essential involvement of a transient conformation in the reaction. It could be that the dynamic property of the Ser/Thr pocket facilitates the efficient scanning of N-glycosylation sequons along nascent polypeptide chains.