

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

Structural and Biochemical studies of the TCR signaling proteins, Sts-1 and Sts-2

By

Yunting Chen

Recognition of foreign pathogens by T cell receptor (TCR) activates complex signaling cascades to generate an immune response. To ensure that T cells respond appropriately to antigenic stimuli, TCR signaling is subjected to multiple levels of regulation. While the positive regulation of TCR stimulation is well studied, little is known about its negative regulation. Two proteins, Sts-1 and Sts-2, were recently found to negatively regulate signaling pathways downstream of TCR. In an effort to shed light on the role of these proteins, we solved the crystal structures of the C-terminal domains of the Sts proteins. These structures reveal homology to members of the phosphoglycerate mutase/ acid phosphatase (PGM/AcP) family enzymes, with residues known to be important for catalytic activity conserved in sequence and position in the active site. To further ascertain the location of the active site, structures of the complexes with phosphate and tungstate were solved showing the residues involved in catalysis.

Biochemical data show that Sts-1 functions as a phosphatase that can target several tyrosyl-phosphorylated proteins, including the kinase ZAP-70. We further investigated the importance of the conserved active site residues by site-directed mutagenesis and kinetic analysis. Using *p*NPP as a model substrate, analysis of pH effect on the k_{cat}/K_m profiles of Sts-1_{PGM} demonstrated that at least one ionizable group is required for the activity. The dramatic reduction of k_{cat} addressed the importance of H565 and R462 in assisting phosphatase activity by Sts-1_{PGM}. Furthermore, E490Q and E490A mutants exhibited a pH-independent manner in the k_{cat}/K_m profile, indicating that in the native

enzyme E490 serves as general acid and must be protonated for activity. Using mass spectroscopy, we showed that His380, the nucleophilic residue is transiently phosphorylated during hydrolysis.

To understand the cause of weaker catalytic activity of Sts-2_{PGM}, we mutated residues outside of the signature motif to the corresponding residues in Sts-1_{PGM}. The generated mutants have enhanced *in vitro* phosphatase activity, suggesting that Sts-2_{PGM} may have substrate(s) that is/are different from that of Sts-1_{PGM} and the mutated residues may play a role in substrate selection.

Since the substrates of both Sts-1 and Sts-2 are still unknown, we focused on a newly identified silkworm enzyme, ecdysteroid-phosphate phosphatase (EPPase), which shares ~ 40% sequence identity to Sts-1 with known substrate, as a model to understand the mode of action of Sts proteins. The crystal structure of EPPase was determined and showed homology to PGM family members. The presented X-ray, biochemical, and kinetic data provide an initial characterization of a new family of protein tyrosine phosphatase.

Date: Nov 9th, 2007

Time: 2:00 PM

Place: Life Science Building, Room 038

Program: BSB

Dissertation Advisor: Nicolas Nassar