

Stony Brook University The Graduate School

Doctoral Defense Announcement

Abstract

Biochemical and Structural Characterization of Interactions Mediated by the N and C-terminal Domains of Mouse PNGase and p97

By

Xiaoke Zhou

The inability of N-linked glycoproteins to adopt their native conformations in the endoplasmic reticulum (ER) leads to their retrotranslocation into the cytosol and subsequent degradation by the proteasome. This pathway is termed the ER associated degradation (ERAD) pathway. In this pathway peptide:N-glycanase (PNGase) hydrolytically removes the N-linked glycan chains from denatured glycoproteins in the cytosol. PNGase is highly conserved in eukaryotes and plays an important role in ERAD. In higher eukaryotes, PNGase has an N-terminal and a C-terminal extension in addition to the central catalytic domain, which is structurally and functionally related to transglutaminases. The function of the C-terminal domain has not previously been characterized, whereas the N-terminal domain of PNGase was proposed to be involved in protein-protein interaction. Recently, the N-terminal domain of mouse PNGase has been found to directly interact with p97, a multifunctional AAA ATPase. P97 has been suggested to extract the unfolded ERAD substrates from the ER through the protein dislocating channel located in the ER membrane. P97 associates via its N-terminal domain with various cofactors, which recruit and process ubiquitinated substrates.

In this dissertation, the high resolution crystal structures of the mouse PNGase N-terminal and C-terminal domains were determined by X-ray crystallography. The interaction between p97 and the N-terminal domain of PNGase was further characterized by crystallographic study of this domain in complex with a peptide derived from p97. For the mouse PNGase C-terminal domain, it could be demonstrated that it binds to an oligomannose unit that is an integral part of the N-linked oligosaccharide chains. Biochemical studies revealed that the C-terminal domain of mPNGase enhances the deglycosylation activity of its core domain, presumably through a tighter binding of mPNGase to the glycan chains of misfolded glycoproteins. Finally, the structure of the mPNGase C-terminal domain in complex with mannopentaose defined the interactions in atomic detail and confirmed the biochemical studies.

Date: Aug 6th, 2007

Time: 2pm

Place: LSB, Room No. 038

Program: BSB

Dissertation Advisor: Hermann Schindelin