Abstract

The docking complex of the peroxisomal protein import machinery consists of at least Pex13p and Pex14p. The C-terminal SH3 domain of Pex13p interacts with the non-PXXP-ligand of Pex5p and the type II SH3-ligand of Pex14p.

The *Sc*Pex13p (*Saccharomyces cerevisiae*) SH3 domain was heterologously overexpressed in *E. coli* and purified in large scale for NMR studies.

The solution structure of the Pex13p SH3 domain comprising 62 amino acids from residues 309 to 370 was determined by NMR spectroscopy. This NMR structure was compared with the computer model illustrated in this work. The structure consists of five β-strand regions and three loops. One of these loops, the n-Src loop, is longer than that in other SH3 domains but appears in all the known Pex13p SH3 domains of different species.

The novel binding pocket for Pex5p which binds an α-helical non-PXXP-peptide and the binding pocket for Pex14p harboring a type II of PXXP motif were identified by the NMR studies. Chemical shift assays revealed both binding pockets for the conventional and non-conventional ligands to be distinct. Pex5p- and Pex14p-peptides can bind simultaneously to the SH3 domain; an access of Pex5p dissociates the Pex14p bound on the SH3 domain.

The binding sites of the Pex13p SH3 domain in Pex5p and Pex14p were also located by combination of two hybrid assays and peptide scanning. The binding site in Pex14p was mapped to a classical PXXP motif (type II), which locates between amino acids 86 and 92 in Pex14p; the binding site in Pex5p was mapped to a non classical peptide comprising amino acids 202-215.

The interactions among three proteins, Pex5p, Pex13p (SH3 domain) and Pex14p were characterized by NMR studies and surface plasmone resonance studies.

These studies indicate that the Pex13p is involved in the dynamics of the peroxisomal protein import process.