Crystallographic Study of the Conserved N-Terminal Domain of the Peroxisomal Matrix-Protein-Import Receptor, Pex14p

Peroxisome is a ubiquitous, single-membrane-bound organelle in eukaryotic cells [1]. It functions in various metabolisms such as detoxification using hydrogen peroxide (H_2O_2), and β-oxidation of very long fatty acids. Its deficiency is involved in inherited diseases such as Zellweger syndrome. Peroxisomal matrix proteins synthesized in the cytosol are imported into the peroxisome by a dynamic system consisting of many peroxins (Pex1p~Pex26p). Peroxisomal matrix proteins harbor the peroxisomal targeting signals. The signals are specifically recognized by Pex5p or Pex7p, depending on their types. The Pex5p and Pex7p-Pex5p complex are docked with the peroxisomal matrix-protein-import receptor Pex14p, which is a central component in the peroxisomal protein import system. The conserved N-terminal domain of Pex14p is involved in interactions with Pex5p, Pex13p and Pex19p [2]. The interaction between Pex14p and Pex5p is mediated via the WXXXF/Y motifs located in the N-terminal half of Pex5p. Pex14p is in different oligomeric states according to the interacting partners. However, no structural information on Pex14p is available.

The full-length Pex14p was less likely suitable for structure determination by X-ray crystallography because the protein is a membrane protein with a multi-domain structure. However, it is known that the amino acid sequences of a domain in the N-terminus of Pex14p are highly conserved between eukaryotic species. Limited proteolysis assays and circular dichroism spectroscopy were employed for constructing variants suitable for crystallographic studies. Consequently, we successfully prepared high-quality crystals using a truncation variant (Pex14p(25-70)) of the domain from *Rattus norvegicus* (rat). Native PAGE analysis indicated that Pex14p(25-70) retained binding ability with the WXXXF/Y motif peptides. The crystals belong to the space group I23 with cell parameters of $a=(b=c=)$ 90.6 Å. The structure was determined at 1.8 Å resolution using diffraction data collected at **BL41XU** beamline [3]. The conserved N-terminal domain of Pex14p has three α helices (α1– α3) with a right-handed twist as shown in Fig. 1. In addition, a short 3_{10} helix is located between the α1 and α2 helices. Similar structures are frequently observed in DNA/RNA-binding proteins. The domain is stabilized by the rigid hydrophobic core. Two pockets are formed at a side of the molecule (Fig. 2). The residues of the side are conserved in a high degree. Two phenylalanine residues (Phe35 and Phe52) are exposed to the solvent, while these are highly

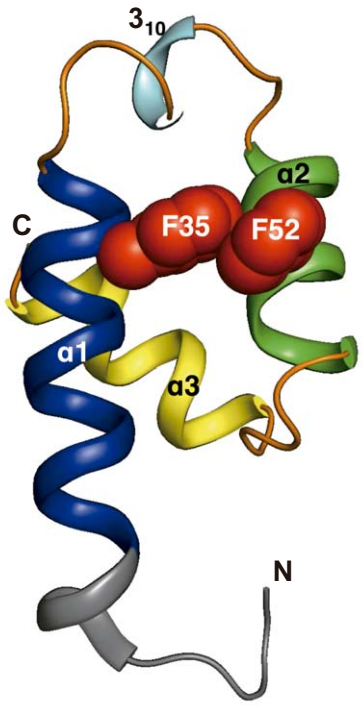

Fig. 1. Overall structure of the conserved N-terminal domain of Pex14p. The completely conserved phenylalanine residues (Phe35 and Phe52) are represented as CPK models in red.

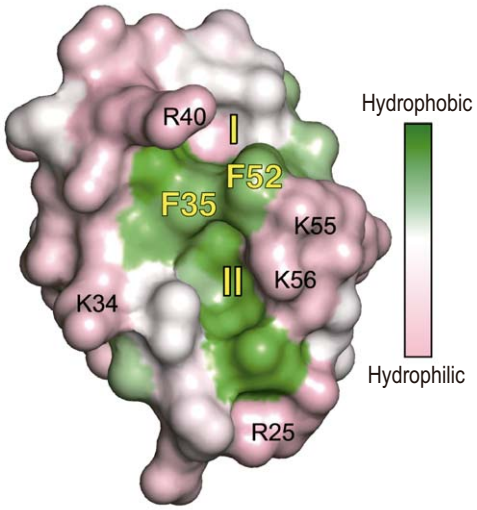

Fig. 2. Hydrophobicity for the hydrophobic side of the conserved N-terminal domain of Pex14p. The molecular surface of the domain is colored in a gradation from pink (hydrophilic) to green (hydrophobic) according to the degree of hydrophobicity. It should be noted that the surface around the two putative binding pockets (I and II) is divided by the hydrophobic phenylalanine residues (Phe35 and Phe52) exposed to the solvent.

hydrophobic. The pockets are surrounded by several basic residues such as Arg25, Lys34, Arg40, Lys55 and Lys56. Consequently, the two pockets are suitable for recognizing the helical WXXXF/Y motif of Pex5p, in which the two conserved aromatic residues of the motif are at the same side of the helix. The aromatic residues of the WXXXF/Y motif can be stabilized by π–π and cation–π interactions in the binding pockets (Fig. 3). Further *in vitro* and *in vivo* assays confirmed that the Phe35 and Phe52 of Pex14p are essential for the interaction between Pex14p and Pex5p [3].

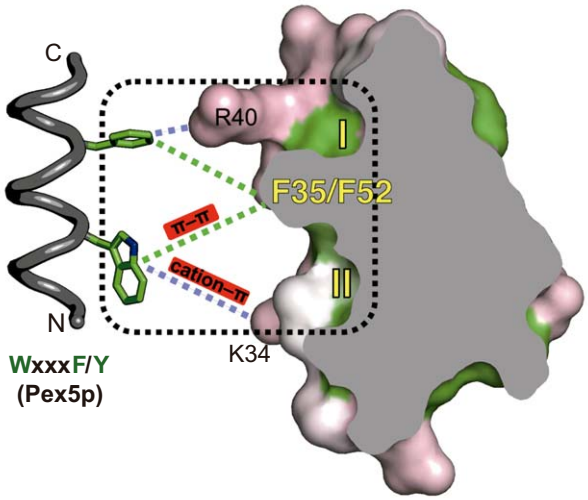

Fig. 3. Molecular model for the interaction between Pex14p and Pex5p. The section view of Pex14p is rotated by 90 degrees from Fig. 2 around the vertical axis. The helical WXXXF/Y motif utilizes its conserved aromatic residues to plug the pockets of Pex14p *via* π–π interactions between aromatic residues. The cation–π interactions may enhance the binding affinity.

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References

[1] S. Subramani *et al.*: Annu. Rev. Biochem. **69** (2000) 399.
[2] R. Itoh and Y. Fujiki: J. Biol. Chem. **281** (2006) 10196.
[3] *J.-R. Su, K. Takeda, S. Tamura, Y. Fujiki and K. Miki: Proc. Natl. Acad. Sci. USA* ***106*** *(2009) 417.*