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Crystal structure of mutant LOX-1, a receptor protein for oxidized LDL

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Introduction

LOX-1, the lectin-like oxidized low-density lipoprotein (LDL) receptor, is the major receptor for oxidized LDL (OxLDL). This enzyme plays a critical role in endothelial dysfunction and injury, leading to initiation and progression of atherosclerosis. LOX-1 belongs to the C-type lectin family, and human LOX-1 constitutes of cytoplasmic domain, transmembrane domain (TM), NECK domain and c-type lectin domain (CTLD), as the order from N-terminal. Crystal structure of LOX-1 ligand binding domain (CTLD-NECK14; C-termini 14 residues of NECK domain and whole CTLD domain, residue range 143-270) was already determined at 1.8 Å resolution by one of the author (ST)'s group [1]. LOX-1 forms homo dimer in physiological condition, and two Trp150s of each molecule are facing to each other, and this residue is expected to play an important role for the dimer formation

In fact, it was found that the mutant W150A loses the ability of dimerization. This dimerization mechanism was approached by using surface plasmon resonance [2], and its dynamics are now progressing by NMR spectroscopy. In this study, in order to elucidate the detailed molecular mechanism, the crystal structure of W150A mutant of CTLD-NECK14 domain was studied.

Results and Discussion

W150A mutant of LOX-1CTLD-NECK14 domain was crystallized based on the wild-type condition. The needle shaped crystal was obtained and its size was 1x0.01x0.01mm. Because the first crystal was obtained in the end of November, and the normal subject application for PF-PAC was already terminated, we offered as a reservation beamtime (2010U002). The crystal was characterized at the beamline BL5A. This crystal belongs to the space group $P4_12_12$, and the crystal parameter was a= b=62.24Å and c=76.67Å, which was different from wild type. As the crystal size was quite small at this stage, diffraction data to 4.0 Å resolution was collected and the R_{merge} was 29%.

After one month, the larger crystals become available. As the opportunity for PF beamtime approach was finished at this time, the diffraction data was collected at SPring-8 (BL38B1), and diffraction data to 2.3Å resolution was collected. The crystal parameters were completely same and isomorphous as the one which was obtained at PF BL5A.

Then, the initial phases are determined by molecular replacement program MolRep, using extracted monomer structure from our wild-type coordinates (1YXK) as a starting model. Although the wild type structure was dimer and contained 2 molecules per asymmetric unit, this mutant is constructed by monomer and this crystal contained only one molecule per asymmetric unit.

The crystallographic refinement is now in progress and current R-factor is converged to 22%. Some loop structures are obviously changed and we are analysing the dimerization factors.

References

[1] 1, Izuru Ohki *et al.* (2005) Crystal structure of Human Lectin-like, Oxidized Low-Density Lipoprotein Receptor 1 Ligand Binding Domain and Its Ligand Recognition Mode to OxLDL. Structure 13:905-917

[2] Izuru Ohki *et al.* (2011), Surface Plasmon resonance study on functional significance of clustered organization of lectin-like oxidized LDL receptor (LOX-1). Biochim. Biophys. Acta. 1814:345-354

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