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Crystallographic study of innate immune receptor RP105/MD-1

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1 Introduction

The Toll-like receptor 4 (TLR4)/MD-2 heterodimer senses lipopolysaccharide (LPS). Radioprotective 105 (RP105), a TLR-related molecule, is similar to TLR4 in that the extracellular leucine-rich repeats associate with MD-1, the MD-2-like molecule. MD-2 has a unique hydrophobic cavity that directly binds to lipid A, the active center of LPS. LPS-bound MD-2 opens the secondary interface with TLR4, leading to dimerization of TLR4/MD-2. MD-1 also has a hydrophobic cavity that accommodates lipid IVa, a precursor of lipid A, suggesting a role for the RP105/MD-1 heterodimer in sensing LPS or related microbial products. Little is known, however, about the structure of the RP105/MD-1 heterodimer or its oligomer. Here, we have determined the crystal structures of mouse and human RP105/MD-1 complexes at 1.9 and 2.8 Å resolutions, respectively [1].

2 Experiment

The extracellular domains of human RP105 (residues 21–626) and mouse RP105 (residues 21–626) were inserted into the expression vector pMT/BiP/V5-His of the Drosophila Expression System. Human MD-1 (residues 20–162) and mouse MD-1 (residues 20–162), fused to a C-terminal thrombin cleavage site located upstream of Protein A tags, were also inserted into the vector. Drosophila S2 cells were cotransfected with the RP105, MD-1, and pCoHygro vectors, and stably transfected cells were selected. Protein from culture supernatant was purified by IgG Sepharose affinity chromatography, Protein A tag cleavage by thrombin, saccharide trimming by endo Hf, Hitrap SP cation exchange, and finally, Superdex 200 gel filtration chromatography. Crystallization experiments were performed using the sitting-drop and hanging drop vapor-diffusion methods at 20°C. Crystals of mouse RP105/MD-1 were obtained with a reservoir solution containing 19% (w/v) PEG8000 and 100 mM Tris HCl (pH 9.0). Crystals of the human RP105/MD-1 were obtained with a reservoir solution containing 20% (w/v) PEG2000MME and 100 mM Tris HCl (pH 9.0). All diffraction datasets were collected on beamline NE3A at the Photon Factory (Tsukuba, Japan) under cryogenic conditions at 95 K. Phasing was performed using the autoSHARP (1) program and improved with the solvent flattening method using DM in the CCP4 suite..

3 Results and Discussion

RP105 is a curved solenoid that is characteristic of LRR-containing TLR family members. MD-1 resides on one side of the RP105 concave surface. Despite some differences in organization, the overall structure is very similar to that of the 1:1 TLR4/MD-2 complex. The crystallographic asymmetric unit contains two copies of the 1:1 RP105/MD-1 complex facing each other and forming a 2:2 RP105/MD-1 complex. Gel filtration and small-angle X-ray scattering analyses of RP105/MD-1 confirmed that both mouse and human RP105/MD-1 were dimeric in solution. The dimeric structure of RP105/MD-1 contrasts with those of reported 'm'-shaped TLR dimers such as the LPS-binding 2:2 TLR4/MD-2 complex [2], where the N-termini of the TLRs extend to opposite ends and their C-termini interact in the middle. As a result, two TLR4 molecules face each other in the C-terminal domain of the 2:2 TLR4/MD-2 complex. In contrast, the 'm'-shaped 2:2 RP105/MD-1 complex is structured such that the C-termini of RP105 extend to opposite ends and the N-termini interact in the middle. Because of this inverse arrangement, the C-terminal domains of RP105 in the 2:2 RP105/MD-1 complex are separated by approximately 100 Å.

Our structural study suggests that RP105/MD-1 is involved in lipid signaling as well as LPS signaling. Further studies of the relationship between RP105/MD-1 and lipid molecules would provide a new regulatory mechanism of activation for members of the TLR family.

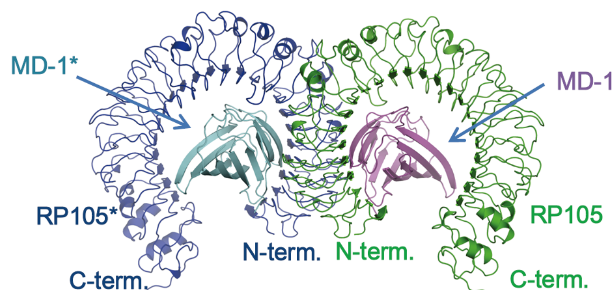

Fig. 1: Overall structure of RP105/MD-1 complex.

References

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