Crystal Structures of Innate Immune RNA Receptor TLR8

Toll-like receptor 8 (TLR8) recognizes single-stranded RNA and activates innate immunity. TLR8 is also activated by synthetic imidazoquinoline compounds, which have the potential to be used for antiviral drugs. We report the first crystal structures of the unliganded and ligand-induced activated TLR8 dimers. These structures are m-shaped dimers in which two C-termini converge in the middle. Ligands were recognized at two equivalent sites located within the dimerization interface. The C-termini of the two TLR8 protomers were brought into closer proximity in the liganded form, suggesting that ligand binding enables the subsequent dimerization of the intercellular TIR domains and downstream signaling.

Innate immunity is the first line of defense against microbial invasions into the human body. Microbial components such as lipopeptide, peptidoglycan, and nucleic acids stimulate the innate immunity through a repertoire of pathogen sensor proteins. Toll-like receptors (TLRs) are one of the most important families among the pathogen sensors that recognize a wide variety of pathogens. The TLR molecule consists of an extracellular leucine-rich repeat (LRR) domain, transmembrane domain, and intracellular TIR domain. The LRR and TIR domains are responsible for ligand recognition and signaling, respectively. The typical TLR is believed to exist as a monomer and form an activated dimer upon ligand binding. TLR8 consists of 26 LRR units, and senses guanosine/uridine rich single-stranded RNA whose sequence was derived from virus RNA [1, 2]. Moreover, TLR8 is activated by imidazoquinoline synthetic ligands, some of which are used for medical treatment [3, 4]. Interestingly, TLR8 and TLR9 form preformed dimers, suggesting that their activation mechanisms are different from those of other TLRs [5, 6]. However, the structural basis of the ligand recognition and signaling by these TLRs has not been elucidated.

We determined the crystal structures of unliganded and liganded TLR8 ectodomain [7]. Although recombinant TLR8 was already cleaved at the long inserted loop (Z-loop) between LRR14 and LRR15, the resultant N- and C-terminal fragments were associated with each other in the purification steps. Both unliganded and liganded TLR8 were eluted as dimers in gel-filtration chromatography, suggesting that TLR8 ectodomain creates preformed dimers. Crystallization was conducted to determine the structures of an unliganded form of TLR8 and liganded forms of TLR8 with imidazoquinoline synthetic ligands, namely, CL097, CL075, and R848. The crystal structure of the unliganded form was determined at 2.3 Å resolution, and the liganded forms at 2.0–2.7 Å resolutions (TLR8/CL097, TLR8/CL075, TLR8/R848 form 1-3).

One protomer from TLR8 dimers composed a ring-shaped structure in which N- and C-terminal fragments interacted directly (Fig. 1) though Z-loop was cleaved. The consensus β strands of LRR14 and LRR15 interacted to form a β-sheet structure as well as other LRRs, which was positioned within the concave face of TLR8 monomer.

Consistent with the result of gel filtration chromatography, TLR8 formed m-shaped dimers in which two C-termini converged in the middle in both the unliganded and liganded forms (Fig. 1).

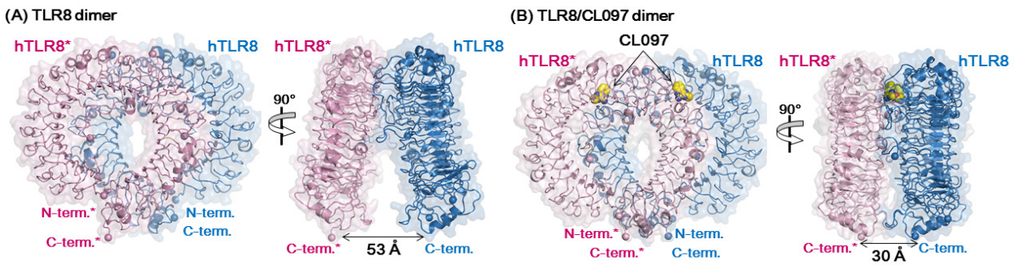

Figure 1: Dimeric structures of TLR8 unliganded form (A) and liganded form (B) (TLR8/CL097 complex) are shown. Two TLR8 protomers are drawn with different colors

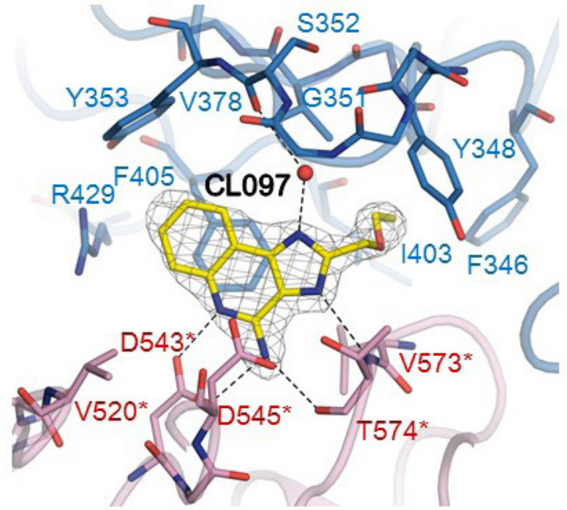

Figure 2: Ligand binding site of TLR8/CL097 complex. The electron density around CL097 was contoured at the 3.0-sigma level in the F_O-F_C map. The water molecule is shown by a red sphere and hydrogen bonds by dashed lines.

The second TLR8 and its residues in the dimeric TLR8 are indicated with asterisks. The C-termini of the two TLR8 protomers were separated by 53 Å in the unliganded form, whereas the C-termini were brought into close proximity (~30 Å) in the liganded forms.

Ligands were located in the two equivalent sites on the dimerization interface, which consisted mainly of LRR14-15 and LRR17*-18*. The electron densities corresponding to the ligands were clearly observed (Fig. 2). Several characteristic interactions between ligand and TLR8 were observed. The benzene ring of chemical ligands formed a stacking interaction with F405. The amidine group formed hydrogen bonds with D543* and T574*. The hydrophobic group of ligands protruded into a hydrophobic pocket formed by F346, Y348, V573* and so on. The functional importance of these residues interacting with ligands at the cellular level was confirmed by NF-κB reporter assay of their alanine mutants.

Upon ligand binding, the dimerization interface was rearranged to increase the protein-protein and ligand-mediated interactions from 1,290 Å^2 to 2,150 Å^2. The C-termini of the two TLR8 protomers were brought into closer proximity in the liganded form than in the unliganded form by hinge motions and ring rotations of the two protomers, thus enabling the subsequent dimerization of the intercellular TIR domains and downstream signaling. These results will contribute to drug design targeting TLR8.

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BEAMLINES

AR-NE3A and AR-NW12A

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