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Conformational Plasticity Revealed by the Cocrystal structure of NKG2D and its Class I MHC-like Ligand ULBP3
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Beamline(s): X9B

Introduction: NKG2D, a member of CTLR superfamily and distantly related to NKG2A, B, C, and E, is found on NK cells to trigger cytotoxicity against certain tumor cells, on CD8* αβ T and γδ T cells to provide a co-stimulatory signal against virally infected cell. Efforts in searching for NKG2D ligands have led to the identification of MICa, MICB, and ULBP in human, Rae-1 and H60 in mice. The sequence identities among MICA and ULBP are about 25%, making NKG2D an unique activating receptor with the ability to bind diverse MHC class I-like ligands. The structural basis of such broad specificity of NKG2D is yet unknown.

Methods and Materials: The complex of NKG2D and ULBP3 was prepared by mixing both components in a 1:1 molar ratio and concentrating to 8-15 mg/ml for crystallization. Single crystals were obtained by vapor diffusion in hanging drops at room temperature from 10% PEG 3350-8000 and 50 mM MES at pH 6.0. After brief soaking in precipitant solutions containing 25% glycerol, crystals were flash frozen at 100 K. A SeMet MAD dataset was collected from the crystals containing SeMet-ULBP3 at the X9B beamline (NSLS) and processed with HKL2000. Six selenomethionines were found in the difference electron density map with phases from NKG2D using program CNS, version 1.0. After density modification including solvent flipping, the electron density of ULBP3 was traced unambiguously. Model adjustments and rebuilding were done using program O. The positional and individual B-factor refinement was carried out using a maximum likelihood target function of CNS v1.0.

Results: The structure of a human NKG2D receptor in complex with its ligand ULBP3 has been determined by a combination of molecular replacement and multiwavelength anomalous dispersion (MAD) methods and refined to 2.6 Å resolution [1]. The refined (2Fo-Fc) electron density is continuous throughout the complex except for one surface loop of ULBP3, which is from residue 90 to 96 at the C-terminal end of the α1-helix and is away from the receptor interface. The refined R-factors are 23.0% and 27.2% for Rcryst and Rfree, respectively. Each crystallographic asymmetric unit contains one NKG2D dimer and one ULBP3 molecule.

Conclusions: In the NKG2D/ULBP3 complex, the structure of ULBP3 resembles that of the α1 and α2 domains of classical MHC molecules without a bound peptide. The lack of α3 and β2m domains is compensated by replacing two hydrophobic patches at the underside of class I MHC β-sheet floor with a group of hydrophilic and charged residues in ULBP3. NKG2D binds diagonally across the ULBP3 α-helices, creating a complementary interface, an asymmetrical subunit orientation and local conformational adjustments in the receptor. The interface is stabilized primarily by hydrogen bonds and hydrophobic interactions. Unlike the KIR receptors that recognize a conserved HLA region by a lock-and-key mechanism, NKG2D recognizes diverse ligands by an induced-fit mechanism.

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References: