Crystal Structure of the Ly49I Natural Killer Cell Receptor Reveals Variability in Dimerization Mode within the Ly49 Family

N. Dimasi, M.W. Sawicki, L.A. Reineck and R.A. Mariuzza

Center for Advanced Research in Biotechnology, W.M. Keck Laboratory of Structural Biology, University of Maryland Biotechnology Institute, Rockville, MD

Natural killer (NK) cells are a fundamental component of the innate immune system that plays a key role in immune surveillance against tumor cells [1,2]. Thus, cancer patients with low NK cell activity tend to develop metastases more frequently than those with normal activity levels. In addition, patients with impaired NK function due to congenital or acquired immunodeficiencies, including X-linked severe combined immunodeficiency, Chediak-Higashi syndrome and AIDS, have a relatively high frequency of certain types of malignancies, particularly leukemias, lymphomas and Kaposi’s sarcomas.

The cytolytic activity of NK cells is regulated through a balance of activating and inhibitory signals mediated through distinct classes of receptors found on their surface. The dominant signal received by an NK cell through its interaction with normal levels of MHC class I on target cells is inhibitory. When the level of MHC class I is reduced through tumorigenic processes, this inhibitory signal is released, and the NK cell is activated. In this way, cells with abnormal MHC class I expression become the targets of NK lytic activity that results from loss of inhibition of NK cell activation.

Two distinct structural families of receptors are responsible for the regulation of NK cells: the C-type lectin-like NK receptors (Ly49A through W, NKG2D, CD94/NKG2) and the immunoglobulin-like NK receptors (KIRs, LIRs) [3,4]. These receptors recognize either classical MHC class I molecules (Ly49, CD94/NKG2, KIRs, LIRs), or MHC class I homologs such as MICA (NKG2D).

Crystal structures of several C-type lectin-like NK receptors, both free and bound to their MHC class I ligands, have been reported [3,4]. However, the structure of a member of the Ly49 family in uncomplexed form has so far not been determined. Here we report the crystal structure of the extracellular C-type lectin-like domain (CTLD) of the mouse inhibitory NK receptor Ly49I, a type II transmembrane glycoprotein. Ly49I recognizes the MHC class I molecule H-2Kb in peptide-dependent manner. To produce soluble Ly49I protein for crystallization, a cDNA construct encoding the CTLD, but lacking the stem and transmembrane regions, was assembled in vitro using a recursive PCR technique. The Ly49I CTLD was expressed as inclusion bodies in E. coli, folded in vitro, and purified by size exclusion and cation exchange chromatography. X-ray diffraction data extending to 3.0 Å were measured from one flash-frozen Ly49I crystal on beamline X9B of the National Synchrotron Light Source at Brookhaven National Laboratory using a Quantum-4 CCD detector. Data were processed and scaled using HKL2000/SCALEPACK [5]. The protein crystallized in space group R3 (a = b = 91.7 Å, c = 89.6 Å, α = β = 90.0°, γ = 120°). The structure was solved by molecular replacement using the Ly49A monomer [6] (PDB entry 1QO3) as a search model, and contains two molecules in the asymmetric unit. The structure was refined to an Rcryst of 27.9% and Rfree of 28.3% at 3.0 Å resolution.

As shown in Figure 1, Ly49I adopts a fold similar to those of the CTLDs of Ly49A [6] and NKG2D [7,8], and to that of the carbohydrate recognition domain of rat mannose-binding protein (MBP-A) [9], a true C-type lectin. Like Ly49A and NKG2D, Ly49I lacks the conserved Ca2+-binding sites found in MBP-A. The Ly49I monomer consists of two α-helices (α1 and α2) and two anti-parallel β-sheets. The two β-sheets are formed by β-strands β0, β1 and β5, and β-strands β2, β2’, β3 and β4, respectively. In addition, strand β2 forms a short β-hairpin with strand β2’. There are four intrachain disulfide bonds in the Ly49I CTLD, all of which appear to be conserved among Ly49 family members [3,4,6]. The Cys167-Cys253 and Cys232-Cys245 disulfides are invariant in all C-type animal lectins. The third disulfide, Cys145-Cys150, is characteristic of all long-form C-type lectins. The fourth disulfide, Cys163-Cys251, is unique to the Ly49 family and links the N-terminus of strand β5 to helix α1. The loops that connect the secondary structure elements constitute the regions of Ly49I most different when compared to Ly49A, or other members of the CTLD superfamily of protein modules.

At the cell surface, Ly49I exists as a disulfide-linked homodimer, stabilized by an interchain disulfide bond between paired cysteines within the stem region [3,4]. The Ly49I CTLD behaves as a monomer in size exclusion chromatography, but in the crystal two copies of Ly49I form a non-crystallographic dimer of approximate dimensions 39 Å x 35 Å x 74 Å, with a root-mean-square
deviation in α-carbon positions of 0.93 Å between the two monomers.

The structure of Ly49I reveals significant variability in dimerization mode within the Ly49 family of NK receptors. Whereas the portion of the dimer interface formed by anti-parallel β0 strands is similar to those of the Ly49A [6] and NKG2D [7,8] CTLDs, the Ly49I monomers are further linked by a β-hairpin between the C-terminal half of strand β0 and the N-terminal end of β1 (Figure 2). On the other hand, the α2 helix is not involved in the interface, opening up the Ly49I dimer compared to Ly49A and NKG2D. As a result, the putative MHC class I-binding surfaces of the Ly49I dimer are somewhat more separated spatially than the corresponding surfaces of Ly49A, and much more so than those of the NKG2D dimer (Figure 2). These structural differences probably reflect the fundamentally different ways in which Ly49 and NKG2D receptors recognize their respective ligands: whereas the single MICA binding site of NKG2D is formed by the precise juxtaposition of two monomers [8], each Ly49 monomer appears to contain an independent binding site for MHC class I [4,10,11]. Hence, the structural constraints on dimerization geometry may be relatively relaxed within the Ly49 family.

Acknowledgments
This work was supported by NIH grants AI47900 and AI36900. Coordinates have been deposited in the Protein Data Bank under accession code 1JA3. Research carried out at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Division of Materials Sciences and Division of Chemical Sciences, under Contract No. DE-AC02-98CH10886.
Figure 2. Structures of the Ly49I, Ly49A and NKG2D dimers and regions of interaction with MHC class I or MICA. Side views of the Ly49I (A), Ly49A (D) and NKG2D (G) homodimers with N-termini at the bottom. In these ribbon models, the β-strands are green, the α-helices are red and yellow, and the loops are gold. Top views of the Ly49I (B), Ly49A (E) and NKG2D (H) dimers. In these surface representations, the regions corresponding to the binding sites for MHC class I on Ly49I and Ly49A, or to the MICA binding site on NKG2D [8], are outlined in magenta. For Ly49A, the surface on each monomer at the Site 2 interface with H-2Dd [6,10,11] is shown. The corresponding regions of Ly49I are hypothetical and were obtained by structure-based sequence homology based on the Site 2 interaction in the Ly49A/H-2Dd complex [6]. Side views of the Ly49I (C), Ly49A (F) and NKG2D (I) dimers in which the distances between corresponding features defining the binding surfaces of these NK receptors [8] are indicated.

References