

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

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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-2K^d 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 *R*3 ($a = b = 91.7$ Å, $c = 89.6$ Å, $\alpha = \beta = 90.0^\circ$, $\gamma = 120^\circ$). 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 R_{cryst} of 27.9% and R_{free} 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 ($\alpha 1$ and $\alpha 2$) and two anti-parallel β -sheets. The two β -sheets are formed by β -strands $\beta 0$, $\beta 1$ and $\beta 5$, and β -strands $\beta 2$, $\beta 2'$, $\beta 3$ and $\beta 4$, respectively. In addition, strand $\beta 2$ forms a short β -hairpin with strand $\beta 2'$. 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 $\beta 5$ to helix $\alpha 1$. 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

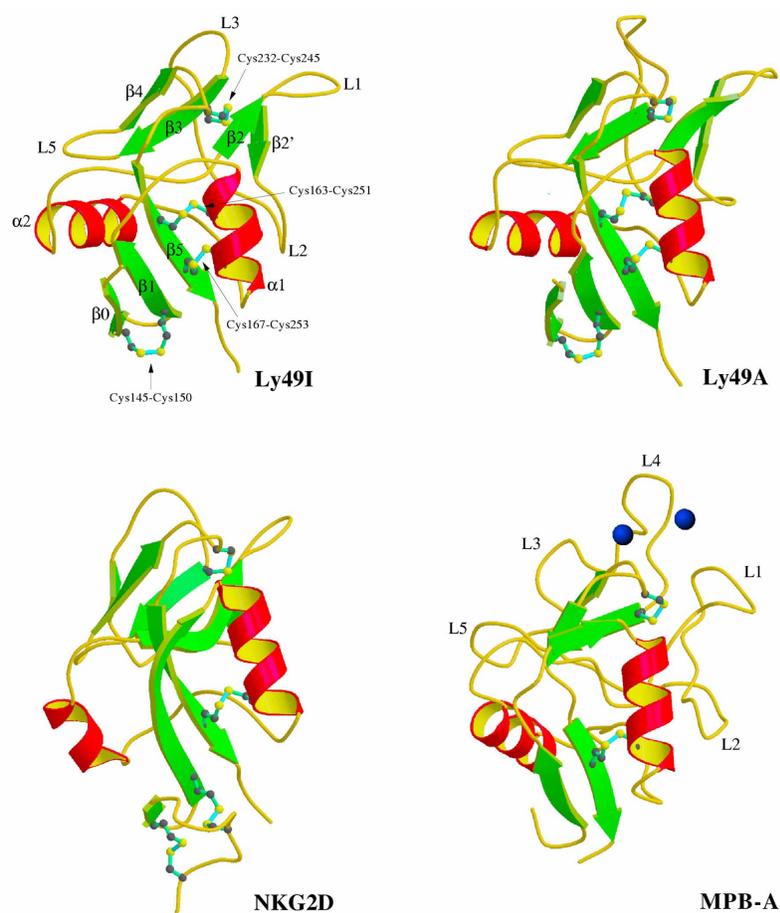


Figure 1. Structure of Ly49I and comparison with Ly49A, NKG2D and MBP-A. Ly49I displays a C-type lectin-like fold, in common with Ly49A (PDB entry code 1Q03), NKG2D (1HQ8) and MBP-A (1YTT). Secondary structure elements of Ly49I are labeled following the numbering for MBP-A [9], the family prototype, and are colored as follows: α -helices red and yellow, β -strands green, and loop regions gold. The disulphide bonds are shown in cyan as ball-and-stick representation. The Ca^{2+} ions bound to MBP-A are drawn as blue spheres.

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 β 0 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 β 0 and the N-terminal end of β 1 (Figure 2). On the other hand, the α 2 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

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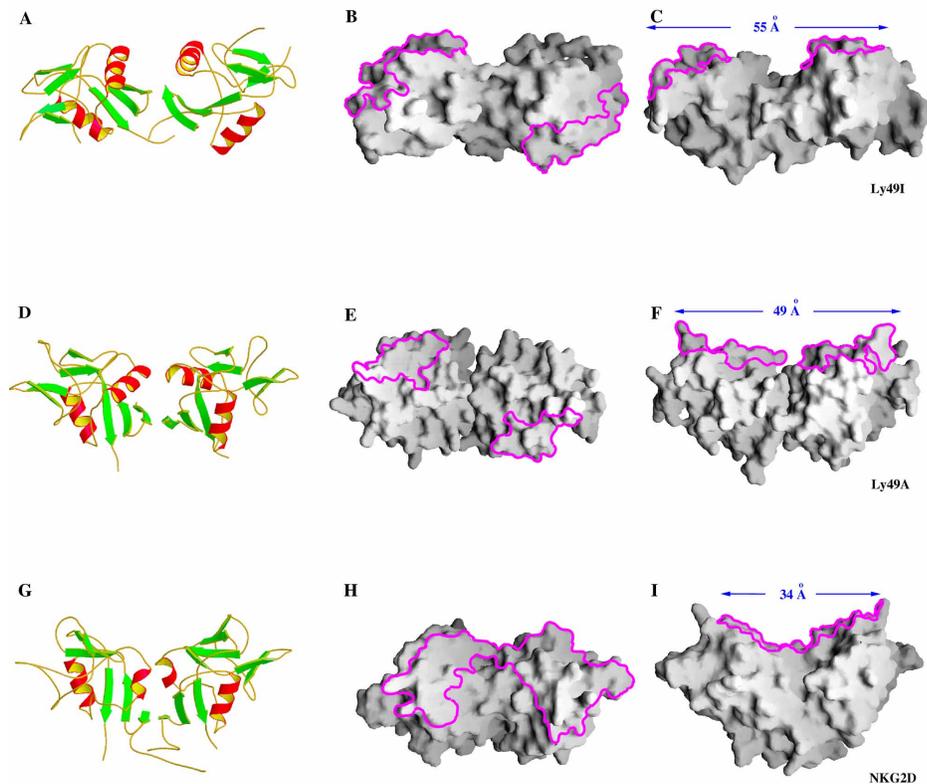


Figure 2. Structures of the Ly49I, Ly49A and NKG2D dimers and regions of interaction with MHC class I or MICA. Side views of the Ly49I (A), Ly49A (D) and NKG2D (G) homodimers with N-termini at the bottom. In these ribbon models, the β -strands are green, the α -helices are red and yellow, and the loops 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-2D^d [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-2D^d 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

- [1] L.L. Lanier, NK cell receptors. *Annu. Rev. Immunol.*, **16**, 359, 1998.
- [2] L.L. Lanier, On guard-activating NK cell receptors. *Nature Immunol.*, **2**, 23, 2001.
- [3] M.W. Sawicki, N. Dimasi, K. Natarajan, J. Wang, D.H. Margulies and R.A. Mariuzza, Structural basis of MHC class I recognition by natural killer cell receptors. *Immunol. Rev.*, **181**, 52, 2001.
- [4] Natarajan, N. Dimasi, J. Wang, R.A. Mariuzza and D.H. Margulies, Structure and function of natural killer (NK) cell receptors: multiple molecular solutions to self, non-self discrimination. *Annu. Rev. Immunol.*, **20**, 853, 2002.
- [5] Z. Otwinowski and W. Minor, Processing X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307, 1997.
- [6] J. Tormo, K. Natarajan, D.H. Margulies and R.A. Mariuzza, Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. *Nature*, **402**, 623, 1999.
- [7] D.W. Wolan, L. Teyton, M.G. Rudolph, B. Villmow, S. Bauer, D.H. Busch and I.A. Wilson, Crystal structure of the murine NK cell-activating receptor NKG2D at 1.95 Å. *Nature Immunol.* **2**, 248, 2001.
- [8] P. Li, D.L. Morris, B.E. Willcox, A. Steinle, T. Spies and R.K. Strong, Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nature Immunol.* **2**, 443, 2001.
- [9] W.I. Weis, R. Kahn, R. Fourme, K. Drickamer and W. Hendrickson, Structure of the calcium-dependent lectin domain from a rat mannose-binding protein determined by MAD phasing. *Science* **254**, 1608, 1991.
- [10] N. Matsumoto, M. Mitsuki, K. Tajima, W.M. Yokoyama and K. Yamamoto, The functional binding site for the C-type lectin-like natural killer cell receptor Ly49A spans three domains of its major histocompatibility complex class I ligand. *J. Exp. Med.* **193**, 147, 2001.
- [11] J. Wang, M.C. Whitman, K. Natarajan, J. Tormo, R.A. Mariuzza and D.H. Margulies, Binding of the NK inhibitory receptor Ly49A to its MHC-I ligand: crucial contacts include both H-2D^d and β 2m. *J. Biol. Chem.*, **277**, 1433, 2002.