Acta Cryst. (1998). D**54**, 433–435

Crystallization and preliminary diffraction studies of the extracellular region of human p58 killer cell inhibitory receptor (KIR2)

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(*Received 20 July 1997; accepted 15 September 1997*)

Abstract

Molecules of the human killer cell inhibitory receptor (KIR) family, which belong to the immunoglobulin superfamily (IgSF), are expressed on the surface of natural killer (NK) cells and some subsets of T cells. These receptors function to mediate the inhibition or activation of cytotoxic activity by recognizing HLA class I molecules on the target cell. The extracellular region of a p58 KIR's specific for HLA-Cw1,3,7 (KIR2) has been overproduced in *Escherichia coli* and purified. The recombinant KIR2 has been crystallized in 9–10% poly(ethylene glycol) methyl ether (average M_r = 8000), 50m*M* HEPES, 8% ethylene glycol, 0.5% octyl-β-glucoside, pH 7.5, at 294 K using the sitting-drop vapour-diffusion method. Preliminary X-ray diffraction studies reveal the space group to be hexagonal ($P6_122$ or $P6_522$) with lattice constants $a = b = 95.3$, $c = 130.8$ Å. A native data set (3 Å resolution) has been collected at the Photon Factory ($\lambda = 1.0$ Å).

1. Introduction

Natural killer (NK) cells lyse tumour and virally infected cells (Malnati *et al.*, 1993; Brutkiewicz & Welsh, 1995). The presence of major histocompatibility complex (MHC) class I molecules expressed on normal target cells generally inhibits NK cell cytotoxicity. NK cells recognize MHC class I alleles *via* clonally distributed surface receptors (Yokoyama, 1995; Leibson, 1995; Raulet & Held, 1995; Gumpertz & Parham, 1995). Of such receptors, human killer cell inhibitory receptors (KIR's) are transmembrane glycoproteins expressed on NK cells and certain subsets of T cells (Colonna, 1996; Lanier & Phillips, 1996).

Sequence analysis indicates that KIR's consist of N-terminal extracellular regions of two or three immunoglobulin superfamily (IgSF) domains with or without an intracellular signal transduction tail that includes two immune receptor tyrosine inhibitory motifs (ITIM's) (Colonna & Samaridis, 1995; Wagtman *et al.*, 1995; D'Andrea *et al.*, 1995; Döhring *et al.*, 1996). KIR's are encoded by a small family of genes localized on human chromosome 19 at 19q13.4 (Baker *et al.*, 1995; Suto *et al.*, 1996). The diversity of KIR cDNA's, including alternative splicing variants (Döhring *et al.*, 1996), results in receptors which recognize distinct polymorphic determinants of HLA class I molecules. Two KIR's, KIR1 and KIR2, each containing two IgSF domains, recognize distinct subgroups of HLA-C alleles; KIR1 is specific for HLA-Cw2,4,5,6 and KIR2 is specific for HLA-Cw1,3,7, whereas KIR's with three IgSF domains recognize either HLA-B alleles of the HLA-Bw4 serotype or HLA-A alleles.

Recently, the binding site of KIR's on HLA-C molecules has been mapped by analyzing the cytotoxicity of NK cells towards mutant HLA-C transfected cells (Mandelboim *et al.*, 1997). It has also been shown that a soluble recombinant version of KIR1 consisting of the extracellular region, produced in *E. coli* and refolded, can specifically bind recombinant HLA-Cw4 (Fan *et al.*, 1996). In this paper, we report the production and crystallization of the soluble extracellular region of KIR2 (sKIR2).

2. Expression and purification

Plasmid pGEM2 (Promega) was used as an expression vector for the production of the extracellular domain of KIR2 (Maenaka *et al.*, 1998). The gene encoding the Shine–Dalgano and signal peptide sequence of pelB, the extracellular domain of KIR2 (residues 1–224), a c-*myc* linker and $(\text{histidine})_6$-tag were inserted in tandem between the *Hin*dIII and *Eco*RI restriction sites of pGEM2 under the control of a T7 promoter. The resulting expression plasmid was designated pKMATHNK2. *E. coli* strain BL21(DE3)pLysS cells (Novagen) harbouring pKMATHNK2 produced and secreted the recombinant sKIR2 to the periplasmic space and media (yield approximately 0.2 mg l^{-1}). The recombinant protein was purified by chelate affinity chromatography (Ni-NTA superflow, Quiagen) followed by ion-exchange chromatography (MonoQ, Pharmacia).

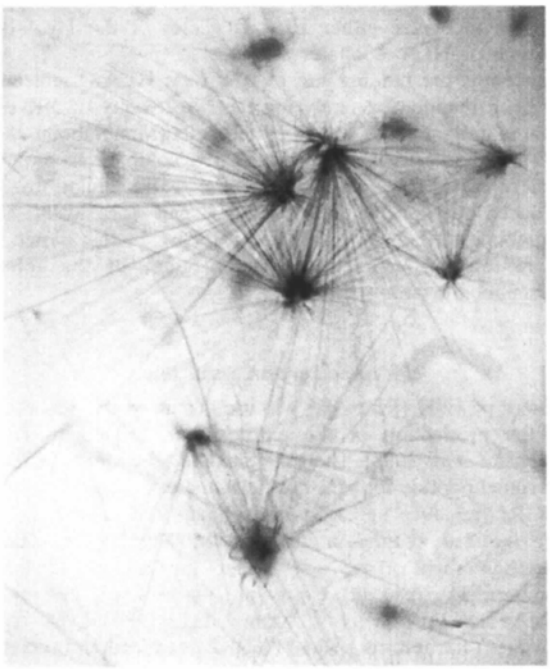
3. Crystallization

Crystals were obtained by the sitting-drop vapour-diffusion method using microbridges (Harlos, 1992) in Linbro tissue-culture plates. Preliminary crystallization trials were conducted at 294 K using Crystal Screen I and II (Hampton Research). Typically, 1 µl of a 5 mg ml^{-1} protein solution in 20 m*M* Tris, pH 8.0 was mixed in a 1:1 ratio with the crystallization reservoir solution and the 2 µl drops incubated at 294 K. Crystal screen II condition 37 [10% poly(ethylene glycol) methyl ether (average M_r = 8000) (PEG 8000), 0.1 *M* HEPES, 8% ethylene glycol, pH 7.5)] produced very fine needles (Fig. 1*a*). The addition of 0.5% octyl-β-glucoside in subsequent crystallization trials at this condition yielded highly ordered rod-shaped crystals of hexagonal cross section. The protocol was scaled up using larger sitting drops (8 µl) prepared by the above method

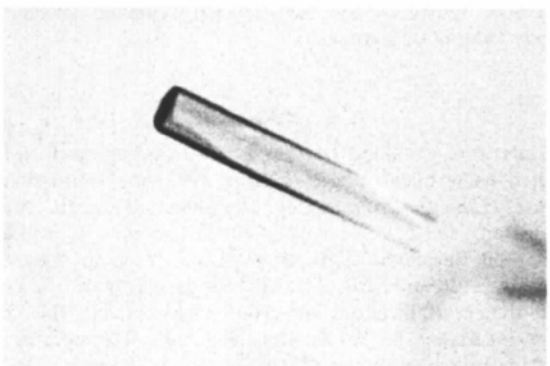
(4 µl of protein solution plus an equal volume of crystallization solution 9–10% PEG 8000, 50 m*M* HEPES, 8% ethylene glycol, 0.5% octyl-β-glucoside, pH 7.5). Crystals appeared within 7 d and reached maximum size (0.05 × 0.05 × 0.25 mm) after 2 weeks (Fig. 1*b*).

4. X-ray analysis

Preliminary characterization of the crystals at BM14 of the ESRF (Grenoble, France) indicated a hexagonal or trigonal space group with unit-cell dimensions of approximately $a = b = 95.0$, $c = 131$ Å. Data to a Bragg spacing of 3 Å were collected from harvested crystals using 1.0 Å wavelength X-ray radiation at station BL-6A of the Photon Factory synchrotron radiation source (Tsukuba, Japan) utilizing a Weissenberg Camera (Sakabe, 1991) and a Fuji imaging-plate system (BA100). All diffraction data were autoindexed, integrated and corrected for Lorentz and polarization effects with the program *DENZO* (Otwinowski, 1993). Scaling and merging of the data in *SCALEPACK* (Otwinowski, 1993) indicated a 622 point group ($R_{\rm merge}$ = 16.5% on data to 3.5 Å resolution, data completeness = 83.8%) and allowed the cell dimensions to be refined to $a = b = 95.3$, $c = 130.8$ Å with $\alpha = \beta = 90$, $\gamma = 120°$. Examination of the distribution of intensities for the 00l reflections indicated the space group to be $P6_122$ or $P6_522$. For these space groups the cell dimensions are consistent with an asymmetric unit containing one sKIR2 molecule with 60% solvent. A full structure determination is in progress.



(*a*)



(*b*)

Fig. 1. Crystals of sKIR2. (*a*) fine needle crystals grown from 10% PEG8000, 8% ethylene glycol in 50 m*M* HEPES at pH7.5; (*b*) rod-shaped crystal grown from the same solution with the addition of 0.5% octyl-β-glucoside.

We thank Drs N. Sakabe and N. Watanabe for their advice on use of the Weissenberg camera at the Photon Factory, Tsukuba, Japan, and Dr V. Stojanof for her assistance on BM14 at the ESRF. We also thank Drs I. Kumagai, M. Matsushima, K. Tokunaga, S. Sugiyama and K. Tsumoto for their advice and helpful discussion. We are grateful to Dr T. Yabe, Mr T. Nakayama and colleagues in the Japanese Red Cross Blood Center for their encouragement. We thank Drs S. Ikemizu, J. Tormo, S. Reid, J. Bravo-Sicilia, J.-S. Ren, D. K. Stammers, Mr M. Deller and A. Hopkins for their help in data collection and also Ms Jessica R. Wyer for DNA sequencing. KM is supported by a JSPS Research Fellowship for Young Scientists. This work is supported in part by a grant-in-aid for scientific research on priority areas (No. 2782) from the Ministry of Education, Science, and Culture of Japan. The OCMS is supported by the BBSRC, MRC and EPSRC. DIS and EYJ are supported by the MRC and Royal Society, respectively.

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