Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

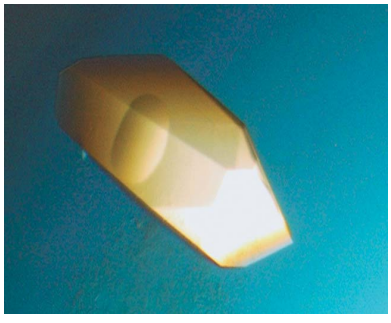
Crystallization and X-ray diffraction analysis of a novel immune-type receptor from *Ictalurus punctatus* and phasing by selenium anomalous dispersion methods

David A. Ostrov,[a]* José A. Hernández Prada,[a] Robert N. Haire,[b] John P. Cannon,[b,c] Andrew T. Magis,[a] Kate Bailey[a] and Gary W. Litman[b,c,d]

[a]Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, 1600 SW Archer Road, Gainesville, FL 32610, USA, [b]Department of Pediatrics, University of South Florida College of Medicine, USF/ACH Children's Research Institute, 830 First Street South, St Petersburg, FL 33701, USA, [c]Immunology Program, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Avenue, Tampa, FL 33612, USA, and [d]Department of Molecular Genetics, All Children's Hospital, 801 Sixth Street South, St Petersburg, FL 33701, USA

Correspondence e-mail: ostroda@pathology.ufl.edu

Received 30 August 2007
Accepted 29 October 2007



X-ray diffraction data from crystals of a novel immune-type receptor (NITR10 from the catfish *Ictalurus punctatus*) were collected to 1.65 Å resolution and reduced to the primitive hexagonal lattice. Native and selenomethionine derivatives of NITR10 crystallized under different conditions yielded $P3_121$ crystals. SeMet NITR10 was phased to a correlation coefficient of 0.77 by SAD methods and experimental electron-density maps were calculated to 1.65 Å. Five NITR10 molecules are predicted to be present in the asymmetric unit based on the Matthews coefficient.

1. Introduction

Novel immune-type receptors (NITRs), which consist of an N-terminal immunoglobulin-type variable (V) region, a transmembrane region and a cytoplasmic tail, are encoded in large diversified multigene families (Rast *et al.*, 1995; Strong *et al.*, 1999). The vast majority of NITRs contain either activating or inhibitor signaling motifs (Yoder *et al.*, 2001, 2004). As with immunoglobin and T-cell antigen receptors (TCRs), the V regions of NITRs can be classified into major families. NITRs are apparently restricted to various lineages of bony fish, including pufferfish (*Spheroides nephelus*; Strong *et al.*, 1999), zebrafish (*Danio rerio*; Yoder *et al.*, 2001, 2004) and channel catfish (*Ictalurus punctatus*; Hawke *et al.*, 2001; Strong *et al.*, 1999; Yoder *et al.*, 2001, 2004). Based on their predicted structures, we have suggested that NITRs may represent the bony fish equivalent of certain natural killer (NK) cell receptors that have been described in higher vertebrates (Eason *et al.*, 2004). Expression of NITRs in cell lineages that exhibit morphological and functional similarities to mammalian NK receptors has been demonstrated in channel catfish (Hawke *et al.*, 2001). Two types of NITR genes have been described: one with an extracellular domain consisting of a single V domain and one with a V and a V/C2 domain (Piyaviriyakul *et al.*, 2007). Here, we describe the crystallization conditions and X-ray diffraction analysis of NITR10, a single V-region-containing NITR.

2. Materials and methods

2.1. Purification of NITR10

cDNA sequences encoding the variable domain of catfish NITR10 (Hawke *et al.*, 2001) were amplified from plasmids by PCR and cloned into the bacterial expression vector pET-Blue1 for expression and refolding. The plasmid used was an excised phagemid containing the NITR10 wild-type cDNA that was isolated by cDNA library screening (Hawke *et al.*, 2001). PCR oligonucleotide primers were designed based on published catfish NITR10 cDNA sequences (GenBank accession No. AF397463). The sequences of the primers are ipNITR10-XC-F1, 5′-ATGGACATCAAGGAGCTTCATGTG (ATG start codon plus AF397463 nucleotides 64–84), and ipNITR10-XC-R1, 5′-CTATCAAAATTGCAGACGTGTTCCAG (antisense stop codons CTA TCA plus AF397463 antisense nucleotides 374–

Table 1
Data-collection and reduction statistics.

Values in parentheses are for the highest resolution shell.

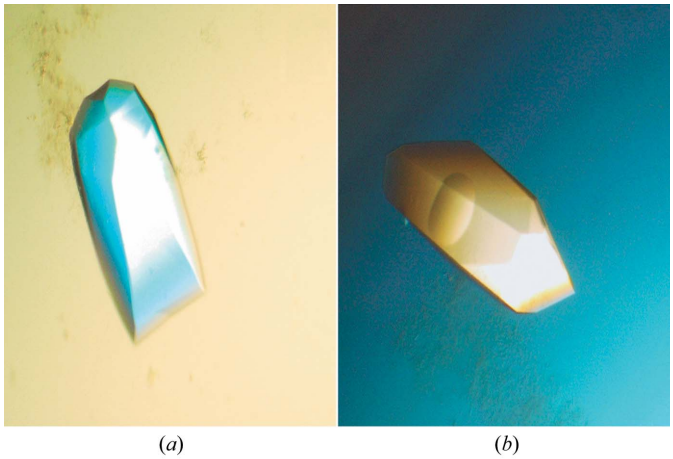
Data set	NITR10	SeMet NITR10 (remote)
No. of frames	480	180
Crystal-to-detector distance (mm)	110	150
Exposure (s)	8	10
Wavelength (Å)	0.9791	0.9393
Unique reflections	91403	151358
Redundancy	7.0 (3.6)	2.9 (2.8)
Resolution range (Å)	30–1.56 (1.57–1.56)	30–1.65 (1.66–1.65)
Space group	$P3_121$	$P3_121$
Unit-cell parameters (Å, °)	a = 90.246, c = 136.352, α = 90.0, γ = 120.0	a = 90.566, c = 137.235, α = 90.0, γ = 120.0
Oscillation step (°)	0.25	0.5
Mosaicity (°)	0.300	0.278
$\langle I/\sigma(I)\rangle$	40.7 (2.1)	24.3 (2.6)
Reflections >3σ (%)	89.0 (31.2)	76.1 (34.5)
Completeness (%)	99.6 (95.6)	99.6 (100)
R_{merge} (%)	4.6 (49.2)	4.3 (36.8)
Unit-cell volume (Å^3)	961718.8	974932.3
V_M (Å^3 Da^{-1})	2.49	2.52
Solvent (%)	50.58	51.25
No. of molecules	5	5

393). Individual clones were sequenced for confirmation of the coding sequences. The peptide encoded by the amplicon represents the NITR V domain, beginning at the first residue after the predicted signal peptide and extending through the end of the J-like sequence of the domain (amino acids 22–131 of GenBank peptide AAL35551).

The construct was transformed into *Escherichia coli* Tuner strain (Novagen) for IPTG-induced expression. A 2 l culture was grown to an OD_{600} of 0.5–0.9 at 310 K and 100 m*M* IPTG was added to a final concentration of 1 m*M*. The culture was grown for an additional 5 h at 310 K. The induced bacterial cultures were centrifuged and stored overnight in 20% sucrose, 10 m*M* EDTA. Refolding and purification of NITR10 was performed in the same manner as previously described (Hernández Prada *et al.*, 2004).

2.2. Preparation of native and derivative crystals

NITR crystals were grown in hanging drops using the vapor-diffusion method (McPherson, 1999). In order to obtain maximally sized crystals of native NITR10 and its selenomethionine derivative, the drop-composition ratio was varied. Crystals of NITR10 formed in hanging drops consisting of 3 µl protein solution (10 mg ml^{-1} in 10 m*M* Tris pH 8.0, 50 m*M* NaCl) and 2 µl reservoir solution (1.5 *M* sodium citrate pH 8.0; Fig. 1*a*). X-ray analysis was performed on crystals grown in 5 µl drops. Samples were mixed on siliconized slides and allowed to equilibrate against 1 ml reservoir solution. Sparse-matrix crystallization (Jancarik & Kim, 1991) was used for initial screening. All crystallization experiments were performed at room temperature.



(*a*) (*b*)

Figure 1
Crystals of NITR10 ranged in size from 0.1 to 0.3 mm in their longest dimension. (*a*) NITR10; (*b*) selenomethionine derivative of NITR10. Crystallization conditions are described in the main text.

2.3. X-ray data collection

Data were collected on beamline X6A at the National Synchroton Light Source (Brookhaven National Laboratory, Upton, New York, USA). Briefly, X6A was equipped with a CrystalLogic single-axis diffractometer, a Kappa goniometer head and an ADSC 210 CCD detector. The optical system consisted of an Si(111) channel-cut monochromator and an Oxford Danfysik toroidal focusing mirror. Crystals were flash-frozen in a nitrogen-gas stream delivered from an Oxford cryosystem.

HKL-2000 (Otwinowski & Minor, 1997) was used to process the diffraction images. After scaling and merging of the integrated intensities, *XPREP* (Sheldrick, 1991) was used to aid in space-group determination. The molecular weight of crystallized proteins was estimated from mass-spectrometry analysis and unit-cell contents were predicted using the Matthews Probability Calculator (Kantardjieff & Rupp, 2003). Data-collection and processing statistics are listed in Table 1.

2.4. Phasing

SHELX (Uson & Sheldrick, 1999) identified Se atoms in the asymmetric unit and phases were calculated from a single anomalous dispersion data set. The program *DM* applied density modification and *ARP*/*wARP* (Lamzin & Wilson, 1997; Morris *et al.*, 2002; Perrakis *et al.*, 1999) automatically traced the chain. Experimental phases for NITR10 selenomethionyl data were calculated to a resolution of 1.65 Å.

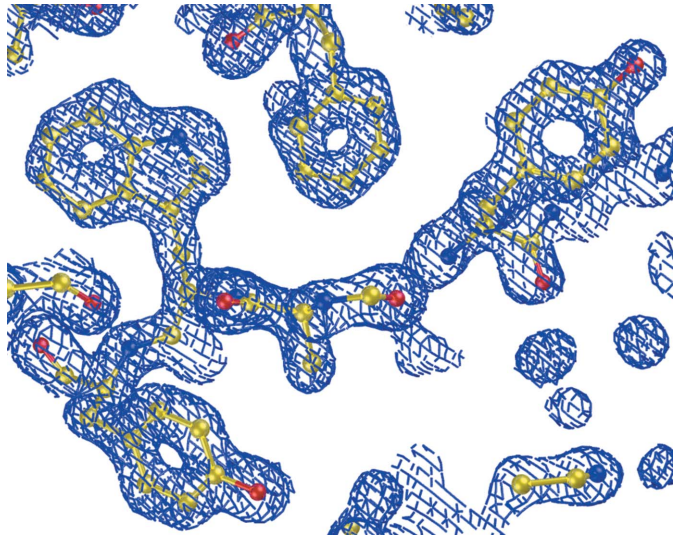


Figure 2
$2F_o - F_c$ σ_A-weighted electron-density maps of NITR10 phased from the anomalous dispersion of selenomethionine-derivative crystals. The map of NITR10 is centered on a cluster of aromatic residues. Experimental phases were obtained to 1.65 Å from a SAD experiment (remote). The map contour is 1σ. This figure was rendered with *Ray-tracer* in *MIFit* (McRee, 1999).

3. Results and discussion

Native NITR10 crystals were grown in 1.5 *M* sodium citrate pH 8.0. Data were collected from single crystals two weeks after setting up crystallization trials. Reflections were measured to 1.56 Å and were reduced to a primitive hexagonal lattice with unit-cell parameters $a = b = 90.246$, $c = 136.352$ Å (Table 1). The unit cell belonged to space group $P3_121$ or $P3_221$. A Matthews coefficient of $V_M = 2.49$ Å^3 Da^{-1} for this crystal predicted the presence of five molecules in the asymmetric unit and a solvent content of 50.58% using a unit-cell volume of 961 718.8 Å^3 and a molecular weight of 12 887.5 Da.

Currently, there are no solved structures of NITRs. In order to generate unbiased phase information from NITR crystals, selenomethionyl NITR10 crystals were utilized for X-ray analysis. A single cryocooled crystal provided data that were used in phasing and structural refinement. X-ray absorption spectroscopy of the selenomethionyl protein crystal was used to select wavelengths for data collection of anomalous reflections. In this study, we report X-ray diffraction data collected at a wavelength of 0.9393 Å at NSLS beamline X6A from a selenomethionyl protein crystal and at 0.9791 Å from a native crystal of NITR10. The X-ray data from the native and selenomethionyl protein crystals were isomorphous to each other.

Single anomalous dispersion phasing was carried out by *SHELX* to a correlation coefficient of 0.77 using data from a selenomethionyl protein crystal. NITR10 molecules were traced with *ARP*/*wARP*, resulting in a structure solution with the predicted five molecules in the asymmetric unit (Fig. 2). The model for NITR10 is currently being refined against the solved structure of SeMet NITR10. Based on these preliminary results, this novel class of immune receptor appears to have important structural similarities to the antigen receptors utilized in adaptive immunity.

We would like to thank Jean Jakoncic for support, discussion and interpretation of X-ray diffraction data.

References

Eason, D. D., Cannon, J. P., Haire, R. N., Rast, J. P., Ostrov, D. A. & Litman, G. W. (2004). *Semin. Immunol.* **16**, 215–226.
Hawke, N. A., Yoder, J. A., Haire, R. N., Mueller, M. G., Litman, R. T., Miracle, A. L., Stuge, T., Shen, L. L., Miller, N. & Litman, G. W. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 13832–13837.
Hernández Prada, J. A., Haire, R. N., Cannon, J. P., Litman, G. W. & Ostrov, D. A. (2004). *Acta Cryst.* D**60**, 2022–2024.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kantardjieff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
Lamzin, V. S. & Wilson, K. S. (1997). *Methods Enzymol.* **277**, 269–305.
McPherson, A. (1999). *Crystallization of Biological Macromolecules.* New York: Cold Spring Harbor Laboratory Press.
McRee, D. E. (1999). *J. Struct. Biol.* **125**, 156–165.
Morris, R. J., Perrakis, A. & Lamzin, V. S. (2002). *Acta Cryst.* D**58**, 968–975.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Perrakis, A., Morris, R. & Lamzin, V. S. (1999). *Nature Struct. Biol.* **6**, 458–463.
Piyaviriyakul, P., Kondo, H., Hirono, I. & Aoki, T. (2007). *Fish Shellfish Immunol.* **22**, 467–476.
Rast, J. P., Haire, R. N., Litman, R. T., Pross, S. & Litman, G. W. (1995). *Immunogenetics*, **42**, 204–212.
Sheldrick, G. (1991). *XPREP. Space Group Determination and Reciprocal Space Plots.* Siemens Analytical X-ray Instruments, Madison, Wisconsin, USA.
Strong, S. J., Mueller, M. G., Litman, R. T., Hawke, N. A., Haire, R. N., Miracle, A. L., Rast, J. P., Amemiya, C. T. & Litman, G. W. (1999). *Proc. Natl Acad. Sci. USA*, **96**, 15080–15085.
Uson, I. & Sheldrick, G. M. (1999). *Curr. Opin. Struct. Biol.* **9**, 643–648.
Yoder, J. A. *et al.* (2004). *Proc. Natl Acad. Sci. USA*, **101**, 15706–15711.
Yoder, J. A., Mueller, M. G., Wei, S., Corliss, B. C., Prather, D. M., Willis, T., Litman, R. T., Djeu, J. Y. & Litman, G. W. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 6771–6776.