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Crystal Structures of Shark Immunoglobulin New Antigen Receptors (IgNARs)

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Introduction

Immunoglobulin new antigen receptors (IgNARs) are a unique antibody isotype found in the serum of sharks [1,2]. They are bivalent, but target antigen through a single immunoglobulin variable domain (~13kDa) displaying two complementarity determining region (CDR) loops. In contrast, conventional antibodies have a variable heavy (V_H) and light (V_L) domains format (~26kDa) and bind antigen through up to six CDRs. To compensate for their reduced size, IgNARs encode unusually long and structurally complex CDR-3s, which display a high degree of variability.

To date, three IgNAR isotypes have been identified, which vary in the number and configuration of their framework cysteine residues, and time of appearance in shark development. Type 3 IgNARs, the last discovered, display core sequence similarity to type 2 and limited diversity in both the size and composition of their CDR loop regions. They appear early in shark development prior to maturation of the full adaptive immune response. Both type 1 and -2 IgNAR levels increase as the shark immune system is exposed to exogenous antigen, and show significant diversity consistent with extensive antibody affinity maturation.

Recently, both our laboratory [3] and [4] have reported crystallographic structures for IgNAR variable domains (type 2 and 1, respectively), which provide significant insight into their evolutionary origin and antigen-binding strategy. Interestingly, the IgNAR immunoglobulin fold resembles I-set proteins (eg cell adhesion molecules) as much as it does conventional V-set immunoglobulins (eg V_H/V_L antibodies; T-cell receptors), suggesting an early divergence amongst the molecules of the shark immune system [5]. The structures also clearly delineate the type 1 and type 2 isotypes. For type 2, a disulphide bridge usually, though not in our first structures, links the CDR1 and CDR3 regions producing a loop structure extending high above the immunoglobulin framework. In contrast, for type 1 two conserved framework cysteine residues form disulphide bridges with matching residues within the extended CDR3, distending the loop laterally. These appear to be two related strategies to enhance stability, and concurrently position the extended loop allowing access to cleft-like epitopes, such as the lysozyme active site in one of the reported structures [4], in a manner similar to that observed in camelid V_HHs, the only other naturally occurring single domain antibodies.

Now, we have obtained X-ray data and solved the structure of a fully natural type 2 IgNAR variable domain.

Experimental

X-ray diffraction data for small crystal of type 2 IgNAR was collected at the multipole wiggler beamline BL5. BL5 is equipped with collimating mirror, double-crystal Si(111) monochromator and focusing mirror which focuses X-rays to the ADSC Quantum 315 CCD detector. Data were collected at -160ºC; the crystals required no added cryoprotectant. The data processing was carried out using the DENZO/SCALEPACK suite. Diffraction data statistics are summarized in below.

Space Group	$P2_12_12$
Unit cell (Å) (*a*,*b*,*c*)	38.27,68.32,39.51
Resolution (Å)	2.1
Wavelength (Å)	1.000
Unique reflections	6048
Multiplicity	7.2
Completeness (%)	98.6
I/σ(I)	15.2
χ^2	1.03
R_{merge} (%)	12.6

Results and Discussion

The structure of type 2 IgNAR was determined by molecular replacement using CCP4 MOLREP. The search model was the IgNAR structure [3] without the CDR3 loop. The final refinement included converged to R/R_{free} values of 0.217/0.280, respectively, for data up to 2.1 Å resolution. The coordinates have been deposited in the Protein Data Bank under the accession code 2COQ. The structure of a fully natural type 2 IgNAR variable domain possesses a disulphide bridge linking the CDR1 and -3 loops and the complete structure analysis will be published elsewhere [6].

References

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