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Crystallization and preliminary X-ray diffraction studies of the carbohydrate-recognition domain of SIGN-R1, a receptor for microbial polysaccharides and sialylated antibody on splenic marginal zone macrophages

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SIGN-R1, or CD209b, is a mouse C-type lectin receptor that is expressed at high levels on macrophages in lymphoid tissues, especially within the marginal zone of the spleen. SIGN-R1 can bind and mediate the uptake of various microbial polysaccharides, including dextrans, lipopolysaccharides and pneumococcal capsular polysaccharides. It has been shown that SIGN-R1 mediates the clearance of encapsulated pneumococcus, complement fixation *via* binding C1q independent of antibody and innate resistance to pneumococcal infection. Recently, SIGN-R1 has also been demonstrated to bind sialylated antibody and mediate its activity to suppress autoimmunity. The carbohydrate-recognition domain (CRD) of SIGN-R1 has been cloned and overexpressed in a soluble secretory form in mammalian Chinese hamster ovary (CHO) cells. The CRD protein of SIGN-R1 was purified from CHO cell-culture supernatant and concentrated for crystallization using the hanging-drop vapour-diffusion method at 291 K. Crystals grew from a mixture of 2 *M* ammonium sulfate in 0.1 *M* bis-tris pH 5.5. Single crystals, which belonged to the monoclinic space group *C*2 with unit-cell parameters $a = 146.72$, $b = 92.77$, $c = 77.06$ Å, $\beta = 121.66^\circ$, allowed the collection of a full X-ray data set to a maximum resolution of 1.87 Å.

1. Introduction

Streptococcus pneumoniae is one of the most common and important human pathogens and causes serious life-threatening diseases such as acute otitis media, pneumonia, sepsis and meningitis. Pneumococcal infections are associated with high morbidity and mortality, especially amongst children, the elderly and immune-depressed patients. The widespread emergence of antibiotic resistance and the lack of highly effective pneumococcal vaccines against all serotypes of this organism give urgency to the elucidation of the molecular processes that are involved in its pathogenicity (Kristinsson, 1997; Pelton, 2000). Recognition of pathogens by the immune system is crucial for the initiation and maintenance of protective immunity. Pattern-recognition receptors, including C-type lectins and Toll-like receptors, discriminate the molecular patterns expressed by pathogens and facilitate differential recognition of pathogens and microbial products (Gordon, 2002).

The innate immune responses provide a critical rapid defence mechanism that acts before the maturation of acquired immunity. Investigations have revealed that SIGN-R1, or CD209b, is a C-type lectin receptor that is primarily found on subsets of macrophages in splenic marginal zone and lymph-node medulla in mouse and mediates the uptake of dextrans (Geijtenbeek *et al.*, 2002; Kang *et al.*, 2003) and lipopolysaccharides (Nagaoka *et al.*, 2005). Furthermore, SIGN-R1 plays an essential role in host defence against *S. pneumoniae* by mediating the recognition of capsular polysaccharide and the clearance of these bacteria (Kang *et al.*, 2004; Park *et al.*, 2009; Lanoue *et al.*, 2004). Moreover, SIGN-R1 contributes to innate resistance by an unusual complement-activation pathway that is independent of immunoglobulin (fulfilling the role of IgM) *via* direct binding with C1q, an essential subcomponent of the classical com-

plement pathway (Kang *et al.*, 2006). Recently, SIGN-R1 has been identified as a lectin receptor that is required for the anti-inflammatory activity of intravenous sialylated immunoglobulin, suggesting that it is a key player in the new therapeutic approach of using sialylated immunoglobulin to suppress autoimmune diseases (Anthony *et al.*, 2008).

SIGN-R1, a C-type lectin receptor, has a structure consisting of an N-terminal cytosolic domain, a single transmembrane domain, a neck domain and a carbohydrate-recognition domain (CRD) at the C-terminus. We generated and expressed in mammalian cells the soluble form of the CRD part of SIGN-R1 (CRD_SIGN-R1), which contains 134 amino-acid residues. Here, we describe the initial results obtained for the crystallization of CRD_SIGN-R1 and its X-ray diffraction data to 1.87 Å resolution.

2. Experimental procedures

2.1. Expression and purification of CRD_SIGN-R1

To produce a soluble secretory form of CRD_SIGN-R1 protein tagged with a FLAG epitope, the sequences for a signal peptide and a FLAG epitope were fused to the sequence of the CRD portion of SIGN-R1. The construct of soluble FLAG-tagged CRD_SIGN-R1, (SF-CRD_SIGN-R1; GenBank accession No. EU697459) was stably transfected into Chinese hamster ovary (CHO) cells. SF-CRD_SIGN-R1 protein was purified from the culture supernatant of CHO/SF-CRD_SIGN-R1 cells using Anti-FLAG M1 Affinity Gel (Sigma–Aldrich, St Louis, Missouri, USA) following the manufacturer's instructions. 1 mg of protein with a molecular weight of 16.5 kDa was obtained at the end of the purification, which was further concentrated to 3.5 mg ml^{-1} by centrifugation using an Amicon Ultra-10 membrane in 0.01 *M* Tris–HCl pH 8 and 0.1 *M* NaCl buffer.

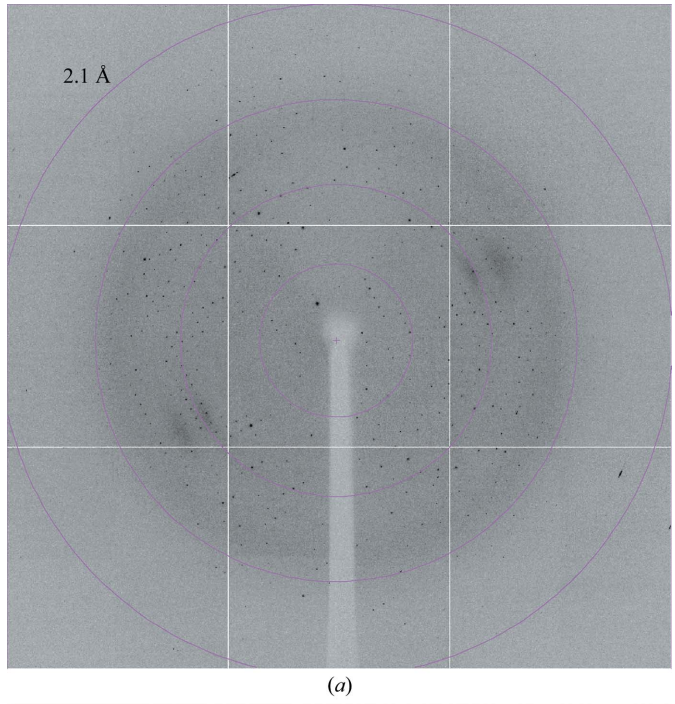
2.2. Crystallization

Initial assays were carried out by the sitting-drop vapor-diffusion method at 291 K on Innovaplate SD-2 microplates (Innovadyne Technologies Inc.), mixing 250 nl protein solution with 250 nl precipitant solution and equilibrating against 70 µl well solution. High-throughput techniques with a NanoDrop robot (Innovadyne Technologies Inc.) were used to assay crystallization conditions using CRD_SIGN-R1 at 3.5 mg ml^{-1} in 0.01 *M* Tris–HCl pH 8 and 0.1 *M* NaCl with the PACT Suite and JCSG+ Suite from Qiagen and JBScreen Classics 1, 4, 5 and 7 from Jena Bioscience. Successful initial conditions were optimized by hand using hanging-drop methods, mixing 1 µl protein solution with 1 µl precipitant solution and equilibrating against 500 µl well solution.

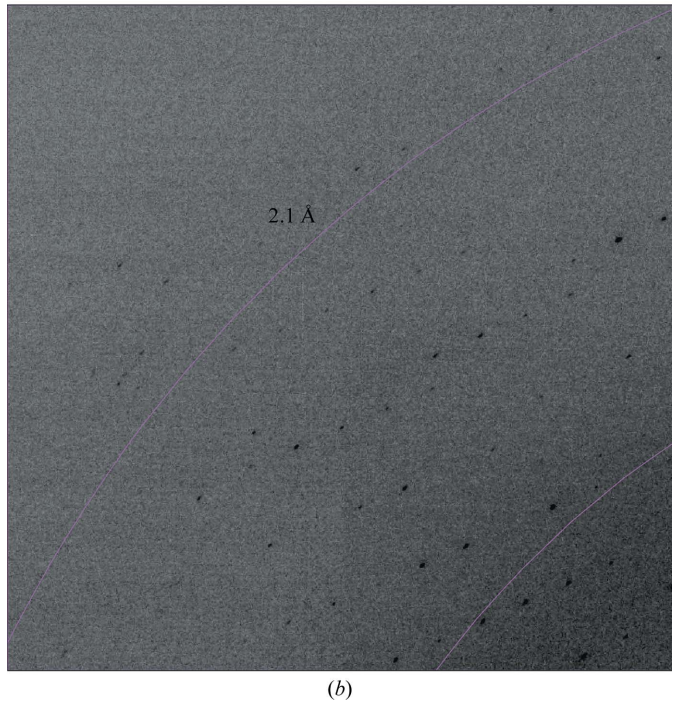
Figure 1
CRD_SIGN-R1 crystals obtained using 1.7 *M* ammonium sulfate and 0.1 *M* bis-tris pH 5.5. The approximate dimensions of the crystals were 0.06 × 0.06 × 0.01 mm.

2.3. X-ray data collection and processing

All crystals were soaked for 5 s in a cryosalt protective solution consisting of 50%(*v*/*v*) saturated lithium sulfate in the crystallization solution prior to flash-cooling to 100 K using a cryogenic system. A native data set was collected using synchrotron radiation on beamline ID23-1 at ESRF (Grenoble) using an ADSC Quantum Q315r



(*a*)



(*b*)

Figure 2
(*a*) X-ray diffraction pattern of CRD_SIGN-R1 crystals (oscillation range 1.0°); (*b*) enlargement showing the highest resolution area.

Table 1
Data-collection statistics for CRD_SIGN-R1 crystals.

Values in parentheses are for the highest resolution shell.

Crystal data	
Space group	*C*2
Unit-cell parameters	
a (Å)	146.72
b (Å)	92.77
c (Å)	77.06
β (°)	121.66
Data collection	
Temperature (K)	100
Wavelength (Å)	1.07225
Resolution (Å)	74.58–1.87 (1.97–1.87)
Unique data	52474 (2478)
Multiplicity	3.9 (2.8)
Data completeness (%)	93.30 (72.54)
Average $I/\sigma(I)$	11.80 (1.70)
Molecules per ASU	4
Matthews coefficient (Å^3 Da^{-1})	3.49
Solvent content (%)	64.73
$R_{\rm merge}$†	0.06 (0.57)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith measurement of reflection hkl and $\langle I(hkl)\rangle$ is the weighted mean of all measurements.

detector and a wavelength of 1.072250 Å. The collected images were processed and scaled using *MOSFLM* (Leslie, 2006) and *SCALA* (Collaborative Computational Project, Number 4, 1994), respectively.

3. Results

The CRD_SIGN-R1 microcrystals grew under six different conditions. Four of these used ammonium sulfate as the main precipitant agent: (i) 2 *M* ammonium sulfate in 0.1 *M* sodium acetate pH 4.6, (ii) 2 *M* ammonium sulfate with 0.2 *M* sodium chloride in 0.1 *M* sodium cacodylate pH 6.5, (iii) 2 *M* ammonium sulfate in 0.1 *M* Tris–HCl pH 8.5 and (iv) 2 *M* ammonium sulfate in 0.1 *M* bis-tris pH 5.5. The other two conditions used different agents: (v) 2.1 *M* DL-malonic acid pH 7 and (vi) 1.6 *M* trisodium citrate. Good-quality crystals with a rhombohedral shape were obtained using 2 *M* ammonium sulfate in 0.1 *M* bis-tris pH 5.5. The crystals reached maximum dimensions of 0.06 × 0.06 × 0.01 mm in two weeks (Fig. 1).

An X-ray data set was collected to 1.87 Å resolution from a single CRD_SIGNR-1 crystal and displayed apparently weak but well defined diffraction patterns (Fig. 2). X-ray data processing showed good processing statistics (Table 1). The crystal belonged to the monoclinic space group *C*2, with unit-cell parameters a = 146.72, b = 92.77, c = 77.06 Å, β = 121.66°. Specific volume calculations based on the molecular weight of CRD_SIGNR-1 and the unit-cell parameters indicated the presence of four molecules in the asymmetric unit with 64% solvent content and a Matthews coefficient $V_{\rm M}$ of 3.49 Å^3 Da^{-1} (Matthews, 1968). Structure determination was initiated using the human DC-SIGN structure (PDB code 1k9j; Feinberg *et al.*, 2001), which shows 68% sequence identity, as the initial structural model. Molecular replacement was performed with the *MOLREP* program (Vagin & Teplyakov, 1997) using reflections to 3.5 Å resolution. Four single and unambiguous solutions for the rotation and translation functions were obtained, which yielded a final correlation coefficient of 0.60 and an *R* factor of 0.49. The space group was confirmed to be *C*2, with four monomers in the asymmetric unit. Structural refinement of the CRD_SIGNR-1 model is currently in progress.

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