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Crystallization and preliminary X-ray analysis of the V domain of human nectin-2

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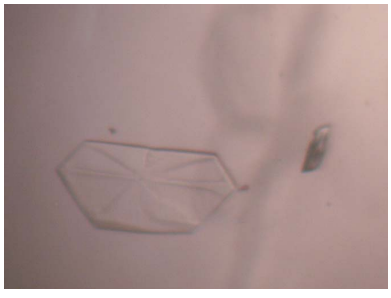

Nectin-2 belongs to a family of immunoglobulin-like cell adhesion molecules that are characterized by the presence of three immunoglobulin-like domains (V, C2 and C2) in the extracellular region. The V domain plays important roles in cell adhesion, NK cell activation and the entry of some herpesvirus. In this study, the V domain of human nectin-2 was expressed in *Escherichia coli* in the form of inclusion bodies, which were subsequently denatured and refolded. The soluble protein was crystallized using the hanging-drop vapour-diffusion method. The crystals diffracted to 1.85 Å resolution and belonged to space group $P2_1$, with unit-cell parameters a = 52.3, b = 43.9, c = 56.1 Å, β = 118.2°.

1. Introduction

Nectins are immunoglobulin-like cell adhesion molecules (CAMs) that participate in Ca^{2+}-independent intracellular interactions. The nectin family has four members: nectin-1 to nectin-4 (Takai & Nakanishi, 2003). Five nectin-like (Necl) molecules have also been discovered. The major difference between nectins and Necls is that nectins can associate with afadin, an F-actin-binding protein, through their conserved C-terminal motif, while Necls cannot form any such association. The nectin and Necl molecules share common domains, including an extracellular region consisting of three successive immunoglobulin-like (Ig-like) domains, *i.e.* V, C2 and C2, as well as a single transmembrane region and a cytoplasmic tail (Fuchs & Colonna, 2006).

Similar to other nectins, the major function of nectin-2 is the formation of adherens junctions between neighbouring epithelial cells. Two nectin-2 molecules that are present on the surface of the same cell initially form *cis*-dimers through the second Ig-like domain, which is followed by *trans*-dimerization between homodimers located on apposing cells *via* the first Ig-like V domain (Yasumi *et al.*, 2003; Takai & Nakanishi, 2003). In addition to its role in adhesion, nectin-2 also serves as a weak coreceptor for HSV-2 strains, PRV and certain viable mutants of HSV-1 and its V domain appears to be a critical site for viral entry (Ogita & Takai, 2006; Martinez & Spear, 2001). Nectin-2 is ubiquitously expressed on all cell types, but is specifically overexpressed on tumour cells and virus-infected cells (Pende *et al.*, 2005; Castriconi *et al.*, 2004). It is possible that this protein helps in fighting against abnormal cells by cooperating with CD155 and its receptor leukocyte adhesion molecule DNAM-1 (CD226) to activate natural killer (NK) cells (Bottino *et al.*, 2003; Pende *et al.*, 2005). To date, there is little structural information on the exact sites at which the interaction between nectin-2 and CD226 occurs. Participation of the V domain of nectin-2 is possible, taking into consideration the critical role played by this domain in HSV entry and the crystal structures of two nectin-like (Necl) molecules that have previously been determined (Zhang *et al.*, 2008; Dong *et al.*, 2006). Thus, a high-resolution crystal structure of the V domain of nectin-2 would supply valuable information on the interaction between nectin-2 and other proteins. Additionally, it would provide a structural basis for mutagenesis and modelling studies.

In this study, we expressed and purified the V domain of human nectin-2. Purified nectin-2 was crystallized using the hanging-drop vapour-diffusion method.

2. Materials and methods

2.1. Cloning, expression, refolding and purification

The DNA encoding the V domain of human nectin-2 (amino acids 24–125; NM_002856) was generated by PCR from a human kidney lymphocyte cDNA library (Stratagene) using the following specific primer pair: 5′-CCAACATATGCAGGATGTGCGAGTTCAAGTG-3′ (forward) and 5′-CCGCTCGAGTTAGACTCTGAGCCAGGTCATCCCTCG-3′ (reverse). The product thus obtained was inserted into the pET-21a (Novagen) expression vector between the *Nde*I and *Xho*I restriction-enzyme sites. The recombinant plasmid was identified by automated sequencing (Invitrogen). Positive recombinant plasmids were chemically transformed into *Escherichia coli* BL21 (DE3) and a single colony was picked and incubated at 310 K in Luria–Bertani (LB) medium containing 100 mg ml^{-1} ampicillin. When the culture density (OD_{600}) reached approximately 0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG; Sigma, USA) was added to a final concentration of 1 m*M* and the cells were induced for 4 h at 310 K. Proteins were expressed in the form of inclusion bodies without any tag.

The inclusion bodies were washed three times with wash buffer [0.5% Triton X-100, 50 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 10 m*M* EDTA, 10 m*M* β-mercaptoethanol (β-ME) and 0.1% NaN_3] and then with resuspension buffer (50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 10 m*M* EDTA, 10 m*M* β-ME and 0.1% NaN_3). They were finally dissolved in a guanidine-containing buffer [6 *M* guanidine hydrochloride, 50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 10 m*M* EDTA, 10%(*v*/*v*) glycerin and 10 m*M* DTT]. The inclusion bodies were renatured by dilution refolding in refolding buffer (100 m*M* Tris–HCl, 2 m*M* EDTA, 400 m*M* L-arginine–HCl, 0.5 m*M* oxidized glutathione, 5 m*M* reduced glutathione, 0.1 m*M* PMSF and 0.1 m*M* NaN_3 pH 8.0). Details of the procedures used have been described previously (Chen *et al.*, 2007). Soluble proteins were initally purified by gel-filtration chromatography on a HiLoad 16/60 Superdex 75pg column using an ÄKTA FPLC system (GE Healthcare). This was followed by ion exchange on a Resource Q negative ion-exchange column (GE Healthcare). The final purification step was gel filtration using an elution buffer containing 20 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl. Prior to crystallization, the target protein was concentrated to 5 and 10 mg ml^{-1} in the elution buffer.

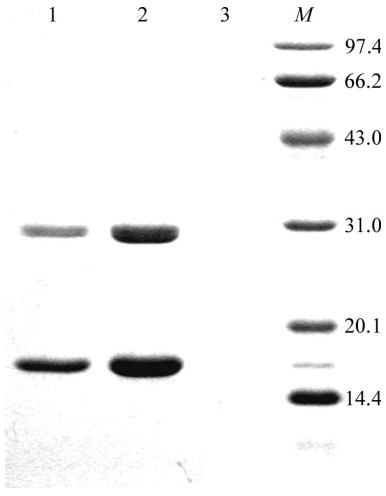

Figure 1
SDS–PAGE of the V domain of human nectin-2. Lanes 1, 2 and 3 represent different fractions after negative ion-exchange chromatography. Lane *M*, protein molecular-weight markers (kDa).

Table 1
Summary of data-collection statistics.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 52.3, b = 43.9, c = 56.1, β = 118.2
Molecules in ASU	2
Resolution range (Å)	50–1.85 (1.92–1.85)
Total No. of reflections	69512
Completeness (%)	99.8 (98.3)
R_{merge}†	0.036 (0.137)
Redundancy	3.6
Average $I/\sigma(I)$	41.4 (11.3)

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

2.2. Crystallization and X-ray diffraction analysis

1 µl protein solution was mixed with 1 µl reservoir solution and equilibrated against 200 µl reservoir solution. All crystallization attempts were performed at 291 K using the hanging-drop vapour-diffusion method. The conditions for crystal growth were initially screened using the Index kit (Hampton Research).

For X-ray diffraction measurements, the crystals were directly mounted using nylon loops and flash-cooled in a stream of gaseous nitrogen (Parkin & Hope, 1998) at 100 K without any cryopreservation agent. Diffraction data were collected using an in-house X-ray source (Rigaku MicroMax007 desktop rotating-anode X-ray generator with a Cu target operated at 40 kV and 30 mA) and an R-AXIS IV^{++} imaging-plate detector at a wavelength of 1.5418 Å. The collected intensities were indexed, integrated, corrected for absorption, scaled and merged using the *HKL*-2000 suite (Otwinowski & Minor, 1997).

3. Results and discussion

The two bands observed on the SDS–PAGE gel (Fig. 1) correspond to a monomer of approximate molecular weight 14 kDa and a dimer of approximate molecular weight 28 kDa. As reported previously, the hydrophobic interaction between two monomers prevents them from

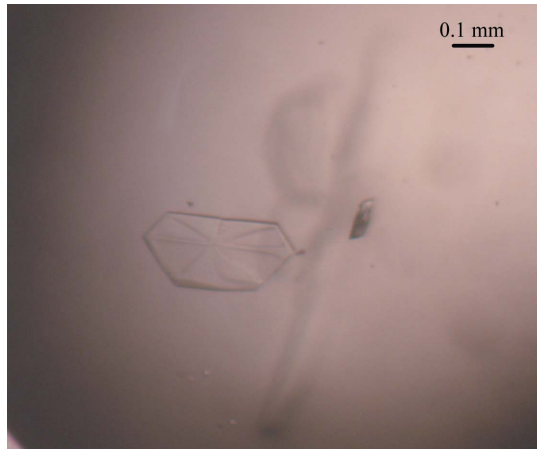

Figure 2
Crystals of the V domain of human nectin-2.

separating even under denaturing conditions (Dong *et al.*, 2006). Overall, the character of nectin-2 suits its role in connecting cells.

Crystals (Fig. 2) were formed at 291 K after 4 d with a reservoir solution composed of 0.2 *M* ammonium acetate, 0.1 *M* bis-tris pH 5.5 and 25% polyethylene glycol 3350. They diffracted to a resolution of 1.85 Å without further optimization. The data-processing statistics are summarized in Table 1. The crystals belonged to space group $P2_1$, with unit-cell parameters $a = 52.3$, $b = 43.9$, $c = 56.1$ Å, $\beta = 118.2^\circ$ and two molecules per asymmetric unit. The Matthews number (Matthews, 1968) was approximately 1.98 $Å^3$ Da^{-1}, corresponding to a solvent content of 38%.

The molecular-replacement method was employed using the structures of other homologous proteins (the maximum amino-acid sequence identity was 43% for CD155; PDB code 3eow) as search models. However, this approach did not provide sufficient phases for structure determination. We are currently preparing selenomethionine-substituted crystals for phase determination using the MAD technique.

To date, there have been no reports of crystal structures of members of the nectin family. This is the first report on the crystallization and preliminary X-ray analysis of the V domain of human nectin-2. We hope that these results will help in identifying the exact sites on nectin-2 that are involved in virus entry and binding to CD226.

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