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Crystallization and preliminary X-ray diffraction analysis of a biologically active fragment of CD55

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Crystals have been grown of two of the domains of CD55. This is the first report of crystallization of a short consensus repeat (SCR) domain containing protein. CD55 is a widely expressed polymorphic glycoprotein, which functions as a complement regulator by inhibiting assembly and promoting destruction of C3 and C5 convertases. As a key regulator of complement, CD55 is implicated in the hyperacute rejection of xenografts from pigs into primates. It is also commonly hijacked as a receptor by viruses (*e.g.* medically important echoviruses and coxsackieviruses) and bacterial pathogens (*e.g.* certain pathogenic strains of *Escherichia coli*). Here, crystallization of a virus-binding fragment expressed in yeast, consisting of two of the four extracellular SCR domains of CD55, is reported. The recombinant domains have been crystallized in 30% polyethylene glycol (PEG), 0.2 *M* sodium acetate, 0.1 *M* sodium acetate trihydrate pH 4.6 using the sitting-drop vapour-diffusion method. Two crystal forms are observed (orthorhombic and monoclinic) and a native data set to 1.65 Å resolution has been collected from the monoclinic form at the Synchrotron Radiation Source, Daresbury, UK.

1. Introduction

CD55 is an intrinsic membrane glycoprotein widely distributed throughout the body. It functions as a complement-regulatory protein by inhibiting the assembly and accelerating the decay of the C3 and C5 convertases of both the classical and alternative pathways. Sequencing of cDNA indicated that CD55 consists of four homologous short consensus repeats (SCRs) linked to a glycosylphosphatidylinositol membrane anchor by an *O*-glycosylated serine- and threonine-rich region (Lublin & Atkinson, 1989). The most membrane-distal SCR domain is not involved in the control of complement (Brodbeck *et al.*, 1996; Coyne *et al.*, 1992). To date, the only structures of SCR domains are from NMR studies (Barlow *et al.*, 1992; Norman *et al.*, 1991; Wiles *et al.*, 1997) and these have shown the fold to consist of five β-strands linked by a pair of disulfide bridges in the centre of the molecule.

CD55 is also commonly used as a cell-surface receptor by various pathogenic organisms. Mapping of the various sites for recognition of CD55 by viruses and bacteria has shown that for many viruses [*e.g.* echovirus 11 (Lea *et al.*, 1998), echovirus 7 (Bergelson *et al.*, 1994; Caras *et al.*, 1987, Clarkson *et al.*, 1995) and coxsackievirus B3 (Bergelson *et al.*, 1995)] and the pathogenic *Escherichia coli* (Nowicki *et al.*, 1993) the membrane-proximal SCR domains are the most important. Indeed, surface plasmon resonance has revealed that for echovirus 11, 80% of the binding affinity may be accounted for by the interaction between the virus and SCR 3 (Lea *et al.*, 1998).

CD55 is also important in mediating the complement-dependent hyperacute rejection of xenotransplants from pigs to primates (reviewed in Weiss, 1998). This currently provides one of the major challenges to be overcome if xenotransplantation is to become a reality; pig herds expressing human CD55 (either alone or in combination with other complement regulators such as CD46 and CD59) have already been engineered. In view of its common role as a pathogen receptor, a fuller understanding of the mode in which CD55 interacts with other molecules (to perform both its physiological and pathological functions) is required, so that the risk of adaptation of pig viruses to humans created by transplantation from such herds may be more fully understood. Such an understanding requires detailed structural information.

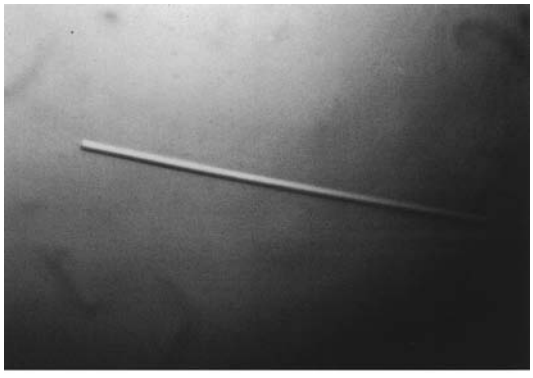
CD55 is therefore seen to be a medically interesting molecule both for its physiological function as a complement regulator, its involvement in xenograft rejection and because of its common hijacking by pathogenic organisms as a facilitator of cell entry. We have therefore decided to initiate X-ray crystallographic studies on various expressed constructs in the hope of providing a structural context for this biological information.

Table 1
Data-collection and processing statistics.

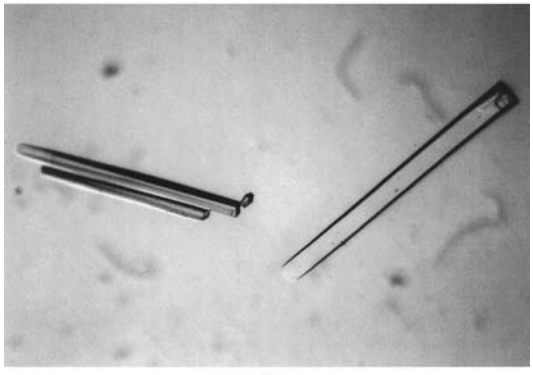
	Orthorhombic form	*C*2, type *A*	*C*2, type *B*
Unit-cell parameters (Å, °)	a = 31.4, b = 36.9, c = 106.8	a = 125.7, b = 20.4, c = 52.7 , β = 111.5	a = 124.9, b = 20.6, c = 41.4 , β = 99.9
Crystal-to-detector distance (mm)	300	152	275
Maximum resolution (Å)	3.0	1.65	2.8
Rotation range for each exposure (°)	3.0	3.0	3.0
Number of images	46	107	69
Number of observations	18223	161246	17616
Number of unique reflections	2650	14289	2751
$R_{\rm merge}$ (outer shell) (%)	12 (24, 3.25–3.0 Å)	8 (27, 1.72–1.65 Å)	10 (23, 2.95–2.80 Å)
Completeness (outer shell) (%)	96 (84, 3.25–3.0 Å)	92 (79, 1.72–1.65 Å)	100 (99, 2.95–2.80 Å)

2. Results and discussion

Several different domain deletion constructs of CD55 were expressed and purified as previously described (Powell *et al.*, 1997) for crystallization trials. With the exception of a construct consisting of domains 1 and 2 alone (which was soluble at concentrations in excess of 15 mg ml^{-1} in Tris pH 7.5), protein solubility often prevented concentration above ∼3 mg ml^{-1}. Initial crystallization screens were performed using the Crystal Screens 1 and 2 (Hampton Research, California, USA) with constructs consisting of domains 2, 3 and 4 (D234), domains 1 and 2 (D12), domains 2 and 3 (D23) and domains 3 and 4 (D34). Although several apparently crystalline fragments were seen, further optimization of the conditions did not yield crystals suitable for X-ray diffraction analysis. A search for buffer conditions which increased the solubility was made and it was found that the D34 construct ($M_r \simeq 14$ kDa) could be concentrated to ∼10 mg ml^{-1} in a buffer consisting of 0.01 *M* HEPES buffer at pH 7.4 with 0.15 *M* NaCl, 3 m*M* EDTA and 0.005% surfactant P20. Screening of this more concentrated material resulted in small crystals in Crystal Screen I (Hampton Research, California, USA) in condition number 10 (30% PEG 4000, 0.2 *M* ammonium acetate, 0.1 *M* sodium acetate trihydrate pH 4.6). Optimization of crystal-growth conditions by serial dilution of this condition and growth of crystals at 285 K has yielded two crystal forms suitable for X-ray diffraction analysis. Crystals grow over a range of dilutions varying from 100 to 80% of the stock Crystal Screen condition.



(*a*)



(*b*)

Figure 1
Crystals of CD55 domains 3 and 4. (*a*) Orthorhombic crystal form; crystal is ∼0.5 mm in the longest dimension. (*b*) Monoclinic crystal form; crystal on the right is ∼0.3 mm in the longest dimension.

X-ray diffraction data have been collected from both crystal forms at station 9.6 at the Synchrotron Radiation Source, Daresbury. Data were collected from crystals cooled to 100 K and mounted in fibre loops. Crystals of form 1 (Fig. 1*a*) have a needle-like morphology commonly being 0.5–1.0 mm in length but between 0.02 and 0.05 mm in the other two dimensions. These crystals are orthorhombic, point group 222 (space group $P2_12_12$ or $P2_12_12_1$) and diffract to 3.0 Å resolution. The unit-cell dimensions are a = 31.4, b = 36.9, c = 106.8 Å. This is the most common crystal morphology. Crystals of the other morphology grow infrequently under the same conditions in the same drops as the orthorhombic form and are the much thicker needles shown in Fig. 1(*b*) (usually 0.4 × 0.1 × 0.05 mm). These crystals belong to space group *C*2 and diffract beyond 1.65 Å. The unit-cell parameters are either a = 125.7, b = 20.4, c = 52.7 Å, β = 111.5° or a = 124.9, b = 20.6, c = 41.4 Å, β = 99.8°. Crystals of all forms grow in the same drops, with the orthorhombic form predominating. All data were collected from crystals flash-frozen to liquid-nitrogen temperatures using a cryoprotectant solution made by addition of 20% glycerol to 80% Crystal Screen solution 10.

Data-collection and processing statistics are presented for both crystal forms in Table 1. The difference between the resolution limits for the two *C*2 forms related simply to the exposure time and crystal volume. Data-ccollection time at the synchrotron was limited and exposures were set so as to allow collection of a relatively complete data set in the time available. The 1.65 Å data were not overlapped at the resolution limit but were very weak, hence the lack of completeness in the outer shell. The calculated packing parameter, V_m (Matthews, 1968), is 2.4 Å^3 Da^{-1} for the orthorhombic form and 2.3 and 1.8 Å^3 Da^{-1} for the monoclinic forms, assuming one molecule in the asymmetric unit for all forms. These values relate to solvent contents varying between 31 and 48% for the different forms. The low solvent content seems to explain why such relatively small crystals show ordered diffraction to high resolution. Molecular replacement using a variety of approaches and starting models has not yielded a definitive solution; we are therefore in the process of collecting data from heavy-atom soaks.

This first report of crystals grown from a protein predicted to contain SCR domains shows that once solubility problems can be overcome it is possible to produce crystals which diffract to high resolution. The SCR fold is present in several medically important proteins, and the prospect of detailed structural knowledge is therefore exciting.

3. Materials and methods

3.1. Protein purification and concentration

Soluble CD55 fragments were expressed in the yeast *Pichia pastoris* (Powell *et al.*, 1997) with a C-terminal oligohistidine tag. Purification was by a single-step Ni-NTA column (Qiagen, Dorking, England) and

yielded material of a purity greater than 95%. Protein size was checked by reducing and non-reducing PAGE analysis. Buffer exchange performed in microconcentrators (microcon-3, 3000 kDa cutoff; Millipore, Ashby Road, Bedford, MA, USA) spun at 10000 rev min^{-1} in a bench-top microfuge.

3.2. Crystallization

Crystallization trials were set up as sitting drops on polypropylene micro-bridges (Harlos, 1992). Drops consisted of 1 µl protein solution plus 1 µl well solution. Plates were set up at 277, 285, 293 and 303 K. Once initial conditions for the domain 3 and 4 crystals were identified, optimization proceeded by dilution with water of a stock mother liquor consisting of 30% PEG 4000, 0.2 *M* sodium acetate, 0.1 *M* sodium acetate trihydrate pH 4.6. Protein concentration varied between 8 and 12 mg ml^{-1} as estimated by absorbance at 280 nm.

3.3. X-ray diffraction analysis

Crystals were cryo-protected by short soaks in a solution consisting of 80% stock mother liquor solution and 20% glycerol. Crystals were mounted in the beam in a 200 µm diameter fibre loop in a stream of nitrogen at 100 K (Oxford Cryosystems Cryostream, Eynsham, England). X-ray diffraction data were collected on a 300 mm MAR Research image-plate detector at a wavelength of 0.87 Å. The data were auto-indexed and integrated using the program *DENZO* and scaled and merged using the program *SCALEPACK* (Otwinowski & Minor, 1997).

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