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Expression, purification and preliminary crystallographic analysis of recombinant human small glutamine-rich tetratricopeptide-repeat protein

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Human small glutamine-rich tetratricopeptide-repeat protein (hSGT) is a 35 kDa protein implicated in a number of biological processes that include apoptosis, cell division and intracellular cell transport. The tetratricopeptide-repeat (TPR) domain of hSGT has been cloned and expressed in *Escherichia coli* and purified. Here, the crystallization and preliminary diffraction analysis of the TPR domain of hSGT is reported. X-ray diffraction data were processed to a resolution of 2.4 Å. Crystals belong to space group $P2_12_12$, with unit-cell parameters a = 67.82, b = 81.93, c = 55.92 Å, $\alpha = \beta = \gamma = 90^\circ$.

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1. Introduction

Having been identified as a binding partner of the nonstructural protein of parvovirus H-1 (Cziepluch *et al.*, 1998), cellular small glutamine-rich tetratricopeptide-repeat protein (SGT), also referred to as viral U-binding protein (UBP), has been implicated in a number of biological processes that include apoptosis (Wang *et al.*, 2005; Winnefeld *et al.*, 2006; Yin *et al.*, 2006), cell division (Winnefeld *et al.*, 2004) and intracellular cell transport (Buchanan *et al.*, 2007). Human SGT (hSGT) has also been shown to interact with the human immunodeficiency virus type 1 (HIV-1) viral-encoded protein U (Vpu) and the major structural component of the viral capsid Gag (Callahan *et al.*, 1998; Handley *et al.*, 2001). Vpu is a 16 kDa type I integral membrane phosphoprotein that forms oligomeric structures *in vivo* and *in vitro* (Maldarelli *et al.*, 1993; Strebel *et al.*, 1989). Vpu facilitates the degradation of CD4 receptor in the endoplasmic reticulum of infected cells (Willey *et al.*, 1992) and enhances the release of virus particles from the plasma membrane (Strebel *et al.*, 1988, 1989). Gag is the principal structural component of the viral core and is synthesized in the cytoplasm. In myristylated form, Gag is largely associated with cellular membranes. Intermolecular interaction of Gag results in the formation of viral capsids (Vogt & Simon, 1999).

It has been demonstrated that the overexpression of hSGT in HIV-1-infected cells reduces the efficiency of viral particle release (Callahan *et al.*, 1998). As this experiment was performed using the Hela cell line which lacks the CD4 receptor, the effect of hSGT is unlikely to be related to Vpu-mediated degradation of CD4. Although hSGT can bind both Vpu and Gag, the hSGT–Gag complex dissociates in the presence of Vpu. This suggests that the role of Vpu is to dissociate hSGT–Gag complexes and enhance HIV-1 virus release (Callahan *et al.*, 1998). As it has been observed that Vpu can affect the cellular localization of both hSGT and Gag, it is probable that the interaction between Vpu and hSGT prevents hSGT from binding to Gag and inhibiting the proper transport of the protein (Handley *et al.*, 2001). Thus, the overexpression of hSGT inhibits Vpu-mediated viral release.

SGT consists of three structural units: an N-terminal self-association domain, a tetratricopeptide-repeat (TPR) domain and a C-terminal glutamine-rich domain (Callahan *et al.*, 1998; Cziepluch *et al.*, 1998; Liou & Wang, 2005). TPR domains have been identified in a wide range of proteins in various organisms ranging from bacteria to humans. They mediate protein–protein interactions and the assembly of multi-protein complexes and are involved in a variety of processes such as cell-cycle regulation and Rac-mediated activation of NADPH

oxidase (Sikorski *et al.*, 1990; King *et al.*, 1995; Ponting, 1996). Each TPR domain consists of several 34-amino-acid motifs in tandem and generally forms a conserved antiparallel pair of helices (Blatch & Lassle, 1999; D'Andrea & Regan, 2003; Lamb *et al.*, 1995). The TPR domain of hSGT is composed of three TPR motifs in tandem, with the highest homology (39%) observed with the TPR domain of protein phosphatase 5 (Yang *et al.*, 2005). Here, we report the cloning, expression, purification, crystallization and preliminary analysis of the TPR domain of hSGT.

2. Experimental procedures

2.1. Cloning, expression and purification

The open reading frame encoding hSGT was amplified from a B-lymphocyte cDNA expression library (Fielding *et al.*, 2006). The 5′ primer 5′-CGGGATCCGAGGAGGACTCAGCAGAGGCAGAG-CGC-3′ and 3′ primer 5′-CCGCTCGAGTTAGGGGGCCTCCCG-CAGCTTCAGCT-3′ were used to clone the TPR domain (residues 85–210) of hSGT in an expression plasmid with an amino-terminal glutathione *S*-transferease (GST) fusion partner (pGEX6p1; GE Healthcare). The sequence identity was confirmed by DNA sequencing (DNA core facility, IMCB, Singapore).

The fusion protein was expressed in *Escherichia coli* BL21 (DE3) (Novagen). Cultures were grown at 310 K in Luria–Bertani (LB) medium supplemented with 100 μg ml^{-1} ampicillin. On reaching an OD_{600} of 0.8, the cultures were cooled to 301 K and induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.2 m*M*. After an incubation period of 4 h, cells were harvested at 4000*g* for 15 min. Bacterial pellets were resuspended in lysis buffer (50 m*M* Tris–HCl pH 7.4, 300 m*M* NaCl and 2 m*M* DTT) supplemented with Complete Protease Inhibitor (Roche). For purification, cells were subjected to sonication. The lysate was cleared by centrifugation (1 h, 20 000*g*) and the supernatant was loaded onto a 5 ml glutathione Sepharose column (GE Healthcare) pre-equilibrated with lysis buffer. The column was washed to remove unbound material. Removal of the GST tag from the N-terminus of hSGT-TPR was achieved by proteolytic cleavage using recombinant 3C protease (GE Healthcare). Typically, glutathione Sepharose beads containing the fusion protein were unpacked and resuspended in 30 ml lysis buffer containing 3C protease. Cleavage took place overnight at 277 K with constant rotation. The supernatant containing cleaved hSGT-TPR was separated by pouring the resin into an empty Econo-Pac column (BioRad). The elutant was concentrated using an Amicon Ultra (5 kDa cutoff, Millipore, catalogue No. UFC800596) and further purified by size-exclusion chromatography using a Superdex S75 column (GE Healthcare) pre-equilibrated in 10 m*M* Tris–HCl pH 7.4, 50 m*M* NaCl and 2 m*M* DTT. Peak fractions were analyzed by SDS–PAGE to assess purity (Fig. 1). Protein concentration was determined by spectrometry using the molar extinction coefficient of hSGT-TPR (13 535 M^{-1} cm^{-1}) at 280 nm. The molar extinction coefficient was determined from the protein sequence of hSGT-TPR using the *ProtParam* tool (Gasteiger *et al.*, 2005). Pure hSGT-TPR was concentrated to 35 mg ml^{-1}. As a consequence of cleavage, five amino acids (GPLGS) remained fused to the N-terminus of hSGT-TPR.

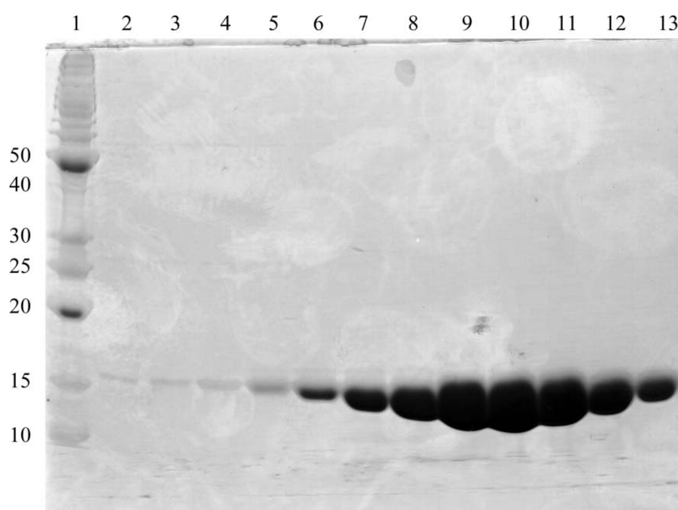

Figure 1
SDS–PAGE analysis of the purification stages of hSGT-TPR. Lane 1, protein molecular-weight standards (kDa); lanes 2–13, fractions collected from the gel-filtration column.

2.2. Crystallization

hSGT-TPR was crystallized by the sitting-drop vapour-diffusion method at 288 K using 24-well plates. 1 μl protein solution was mixed with 1 μl reservoir solution (Crystal Screens I and II; Hampton Research). The volume of reagent in the reservoir was 0.5 ml. X-ray diffraction-quality crystals were obtained within two months from drops containing 1 μl protein solution (hSGT-TPR at 35 mg ml^{-1}) and 1 μl 4 *M* sodium formate. No other conditions tested produced crystals. Crystals grew to maximum dimensions of 0.3 × 0.3 × 0.15 mm (Fig. 2).

2.3. X-ray diffraction analysis

For cryoprotection, the crystal was soaked in cryosolution (6 *M* sodium formate) and flash-cooled in liquid nitrogen. Native data sets were collected on beamline ID 14-1 at the European Synchrotron Radiation Facility. 180 images were collected with 1° oscillation on a Quantum CCD detector with 2 s exposure. The crystal-to-detector distance was set to 366 mm. Raw data were integrated and scaled using the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

The TPR domain of hSGT was cloned and expressed in *E. coli* BL21 (DE3). Crystals first appeared within two months of incubation. Native data were collected from a single crystal of hSGT-TPR grown

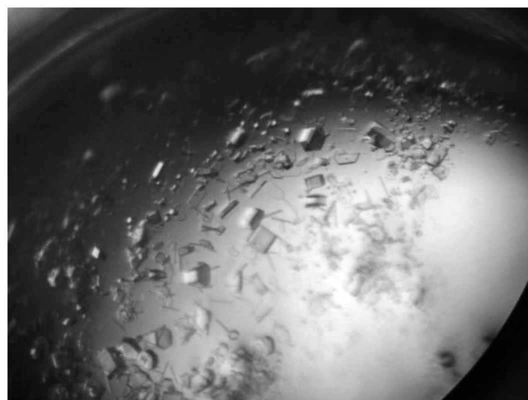

Figure 2
Crystals of hSGT-TPR. Crystals grew to typical maximum dimensions of 0.3 × 0.3 × 0.15 mm.

Table 1
Statistics of preliminary data analysis.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12$
Unit-cell parameters (Å, °)	a = 67.82, b = 81.93, c = 55.92, $\alpha = \beta = \gamma = 90$
No. of molecules per ASU	2
Resolution (Å)	30–2.4 (2.49–2.4)
Wavelength (Å)	0.95
Observed reflections	61085 (5856)
Unique reflections	12726 (1246)
Redundancy	4.8 (4.7)
Completeness (%)	100 (99.0)
$R_{\rm merge}$ (%)	6.7 (21.5)
$I/\sigma(I)$	19.2 (6.8)

in 4 *M* sodium formate (Fig. 2). The space group was determined to be $P2_12_12$, with unit-cell parameters a = 67.82, b = 81.93, c = 55.92 Å, $\alpha = \beta = \gamma = 90°$. The asymmetric unit contains two copies of hSGT-TPR; the crystal volume per unit molecular weight, $V_{\rm M}$, was calculated to be 2.7 Å^3 Da^{-1}, corresponding to a solvent content of 54.4% (Matthews, 1968). Crystallographic statistics of the native data are summarized in Table 1. Crystallization of full-length hSGT is actively being pursued, as is a molecular-replacement solution for hSGT-TPR using the structure of the TPR domain of protein phosphatase 5 (PDB code 1wao; Yang *et al.*, 2005) as a search model.

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