Acta Crystallographica Section F
Structural Biology Communications
ISSN 2053-230X

Crystallization and preliminary X-ray analysis of the C-type lectin domain of the spicule matrix protein SM50 from *Strongylocentrotus purpuratus*

Puneet Juneja,[a] Ashit Rao,[b] Helmut Cölfen,[b] Kay Diederichs[a] and Wolfram Welte[a]*

[a]Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany, and [b]Department of Chemistry, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany

Correspondence e-mail: wolfram.welte@uni-konstanz.de

Received 12 December 2013
Accepted 14 January 2014

Sea urchin spicules have a calcitic mesocrystalline architecture that is closely associated with a matrix of proteins and amorphous minerals. The mechanism underlying spicule formation involves complex processes encompassing spatio-temporally regulated organic–inorganic interactions. C-type lectin domains are present in several spicule matrix proteins in *Strongylocentrotus purpuratus*, implying their role in spiculogenesis. In this study, the C-type lectin domain of SM50 was overexpressed, purified and crystallized using a vapour-diffusion method. The crystal diffracted to a resolution of 2.85 Å and belonged to space group $P2_12_12_1$, with unit-cell parameters a = 100.6, b = 115.4, c = 130.6 Å, $\alpha = \beta = \gamma = 90^\circ$. Assuming 50% solvent content, six chains are expected to be present in the asymmetric unit.

1. Introduction

The phenomenon of biomineralization encompasses diverse and widespread processes that involve organic–inorganic interactions by which organisms form composite hierarchical materials (Lowenstam & Weiner, 1989; Mann, 2001; Fratzl & Weinkamer, 2007). The sea urchin spicule is a model system for investigating the mechanisms of calcium carbonate biomineralization (Wilt, 2002). The spicule has a mesocrystalline architecture composed of crystallographically aligned calcite particles (50–200 nm) organized in a matrix of proteins and amorphous calcium carbonate (Seto *et al.*, 2012). On account of this structure, the spicule has unique properties such as single-crystal-like diffraction and a conchoidal fracture surface typical of amorphous materials. Hence, the mechanisms underlying spicule formation have attracted interest from multiple disciplines (Knapp *et al.*, 2012; Rao *et al.*, 2013; Tester *et al.*, 2013).

Among the proteins regulating spicule formation, SM50 is a 48.5 kDa nonglycosylated, secreted protein with an alkaline pI (Benson *et al.*, 1987; Killian & Wilt, 1996). The N-terminal region of SM50 harbours a C-type lectin (CTL) domain (13.6 kDa), which can affect calcium carbonate mineralization (Killian & Wilt, 2008; Rao *et al.*, 2013). Although their functions are as yet unknown, CTL domains are also present in other proteins associated with the sea urchin spicule, such as the SM30 family (Livingston *et al.*, 2006). Thus, CTL domains appear to play an important role in calcium carbonate biomineralization. In nature, proteins with the CTL fold, such as the type II antifreeze proteins, phospholipase receptors and coagulation-factor-binding proteins, have diverse functions (Drickamer, 1999). These proteins can also bind to carbohydrate ligands, Ca^{2+} ions and form oligomers (Drickamer, 1993). To elucidate their role in biomineralization, structural and functional understanding of the CTL domain with respect to calcium carbonate mineralization is important. The highest sequence identity of the CTL domain of the SM50 protein from *Strongylocentrotus purpuratus* to a protein of known structure is 32% to snake-type CTL (PDB entry 3ubu; Gao *et al.*, 2012). The snake CTL binds specifically to blood platelet glycoproteins and inhibits adhesion and aggregation (Gao *et al.*, 2012). Here, we report the purification, crystallization and preliminary X-ray analysis of the CTL domain of SM50 spicule matrix protein in fusion with SUMO protein.

```
                10        20        30        40        50        60
GHHHHHHGSD SEVNQEAKPE VKPEVKPETH INLKVSDGSS EIFFKIKKTT PLRRLMEAFA

                70        80        90       100       110       120
KRQGKEMDSL RFLYDGIRIQ ADQTPEDLDM EDNDIIEAHR EQIGGTGQDC PAYYVRSQSG

               130       140       150       160       170       180
QSCYRYFNMR VPYRMASEFC EMVTPCGNGP AKMGALASVS SPQENMEIYQ LVAGFSQDNQ

               190       200       210       220       230
MENEVWLGWN SQSPFFWEDG TPAYPNGFAA FSSSPASPPR PGMPPTRSWP
```

Figure 1
Sequence of the SUMO-CTL fusion protein used for crystallization. The SUMO domain with an N-terminal His_6 tag is shown in green and the CLT domain of the SM50 protein (NCBI Reference Sequence NP_999775.1) is shown in magenta.

2. Experimental procedures

2.1. Overexpression and purification

Protein expression and purification were carried out as described previously (Rao *et al.*, 2013). Briefly, the CTL domain (13.6 kDa) of the larval spicule matrix protein SM50 from *S. purpuratus* N-terminally fused with a cleavable (Ulp1 protease) His_6-SUMO tag was cloned in pET-24a vector (Fig. 1). *Escherichia coli* BL21 CodonPlus-RIL cells were transformed with the pET-24a vector and cultured in LB medium at 30°C. On reaching an OD_{600} of 0.6, expression was induced with 0.5 m*M* IPTG overnight at 20°C. Cells were harvested and suspended in lysis buffer consisting of 20 m*M* HEPES, 50 m*M* NaCl, 10 m*M* β-mercaptoethanol (β-ME) pH 7.0, 10 pg ml^{-1} pepstatin, 0.5 m*M* PMSF, 0.5 mg ml^{-1} lysozyme (Roth, Germany) and were incubated for 30 min on ice. The resulting cell lysate was centrifuged at 16 000 rev min^{-1} for 30 min and the resulting supernatant was passed over an Ni–NTA column (Qiagen). After initial washing with five bed volumes of 20 m*M* HEPES, 50 m*M* NaCl, 0.5 m*M* imidazole, 10 m*M* β-ME pH 7.0, the protein was eluted with 20 m*M* HEPES, 50 m*M* NaCl, 200 m*M* imidazole, 10 m*M* β-ME pH 7.0. The eluted protein was concentrated with Vivaspin concentrators (5 kDa cutoff) and loaded onto a Superdex 75 column (GE Healthcare) for final purification in 20 m*M* HEPES, 50 m*M* NaCl, 10 m*M* β-ME pH 7.0. A single peak was observed and the peak fractions were analyzed by SDS–PAGE (Fig. 2). When the SUMO tag was cleaved, the CTL domain was unstable and aggregated; therefore, crystallization was performed using the SUMO-CTL fusion protein.

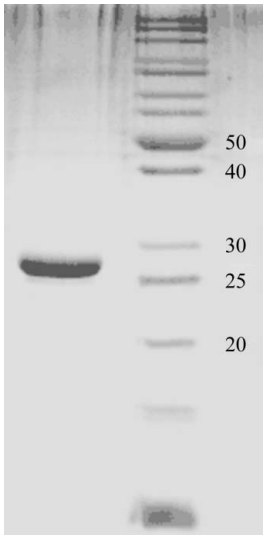

Figure 2
SDS–PAGE showing purified SUMO-CTL after size-exclusion chromatography purification (Superdex 75). The individual molecular weights of SUMO and the CTL domain are 12.1 and 13.6 kDa, respectively. SUMO-CTL runs at an apparent molecular weight near 25 kDa. The right lane contains molecular-weight marker (labelled in kDa).

2.2. Crystallization

Prior to crystallization, the protein was concentrated to 5 mg ml^{-1} (Bradford assay) and filtered through a 0.1 µm filter (Millipore). Crystallization screening was performed with the sitting-drop method in a 96-well format using a Gryphon Robot (Art Robbins Instruments). Crystals were obtained in a condition with reservoir solution consisting of 0.1 m*M* HEPES pH 7.5, 70% MPD using a protein:reservoir ratio of 1:1(*v*:*v*) (0.2 µl protein solution and 0.2 µl reservoir solution) at 18°C. Further optimization was performed in both hanging drops and sitting drops.

2.3. Data collection and processing

The harvested crystals were directly flash-cooled in liquid nitrogen. Data were collected on the X06DA beamline at the Swiss Light Source (SLS), Villigen, Switzerland. The best crystal diffracted isotropically to a resolution of 2.85 Å. The diffraction data set was processed with the *X-ray Detector Software* (*XDS* program package; Kabsch, 2010*a*,*b*). The space-group assignment was performed with *POINTLESS* (Evans, 2006) and further analysis of data quality was carried out with *phenix.xtriage* (Zwart *et al.*, 2005).

3. Results and discussion

The SUMO-CTL crystals appeared after three weeks. The crystals were very fragile. When the wells were opened, the crystals tended to

Figure 3
Crystals of SUMO-CTL fusion protein grown by the sitting-drop vapour-diffusion method with a protein:reservoir ratio of 1:1 in 0.1 m*M* HEPES pH 7.5, 70% MPD. The crystals grew to a length of 150 µm.

Table 1
Diffraction data statistics.

Values in parentheses are for the outer shell.

Diffraction source	X06DA, SLS
Wavelength (Å)	1.000
Rotation range per image (°)	0.1
Total rotation range (°)	180
Exposure time per image (s)	0.1
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 100.6, b = 115.4, c = 130.6
Mosaicity (°)	0.228
Resolution (Å)	2.85 (3.02–2.85)
Total No. of reflections	242290 (35688)
No. of unique reflections	36606 (5688)
Completeness (%)	99.5 (97.5)
Multiplicity	6.6 (6.2)
Mean $I/\sigma(I)$	6.38 (1.07)
$CC_{1/2}$† (%)	98.4 (42.7)
R_{merge} (%)	29.0 (166.1)

† Karplus & Diederichs (2012).

lose their crystalline shape and changed to spherical droplets resembling the droplets observed during phase separation of organic solvents such as PEG and MPD. Addition of glycerol and ethylene glycol did not improve the crystal quality. Crystals were optimized in hanging-drop and sitting-drop conditions, and the best diffracting crystals were obtained using the sitting-drop method (Fig. 3). Crystals were quickly harvested using a CryoLoop (Hampton Research) directly for flash-cooling in liquid nitrogen. The best crystal diffracted to a resolution of 2.85 Å and belonged to the orthorhombic space group $P2_12_12_1$ (Table 1). The SUMO-CTL fusion protein with a total molecular weight of 25.9 kDa is monomeric in solution as shown by analytical ultracentrifugation (Rao *et al.*, 2013). Further analysis with *phenix.xtriage* based on sequence composition gave a solvent content of 50.2% and a Matthews coefficient of 2.47 $Å^3\ Da^{-1}$ (Matthews, 1968), with six chains in the asymmetric unit. Attempts to solve the structure using the structures of SUMO protein (PDB entries 3qht, 3pge and 3tix; Gilbreth *et al.*, 2011; Freudenthal *et al.*, 2011; Schalch *et al.*, 2011), snake CTL (PDB entry 3ubu; Gao *et al.*, 2012) and a combination of both as molecular-replacement models were unsuccessful. Therefore, we are presently working on the crystallization of selenomethionine-labelled SUMO-CTL protein.

AR acknowledges Professor Dr Martin Scheffner for his support and encouragement. We would like to acknowledge Konstanz Research School Chemical Biology for financial assistance and the staff at the Swiss Light Source, Villigen, Switzerland for their support during data collection.

References

Benson, S., Sucov, H., Stephens, L., Davidson, E. & Wilt, F. (1987). *Dev. Biol.* **120**, 499–506.
Drickamer, K. (1993). *Biochem. Soc. Trans.* **21**, 456–459.
Drickamer, K. (1999). *Curr. Opin. Struct. Biol.* **9**, 585–590.
Evans, P. (2006). *Acta Cryst.* D**62**, 72–82.
Fratzl, P. & Weinkamer, R. (2007). *Prog. Mater. Sci.* **52**, 1263–1334.
Freudenthal, B. D., Brogie, J. E., Gakhar, L., Kondratick, C. M. & Washington, M. T. (2011). *J. Mol. Biol.* **406**, 9–17.
Gao, Y., Ge, H., Chen, H., Li, H., Liu, Y., Chen, L., Li, X., Liu, J., Niu, L. & Teng, M. (2012). *Proteins*, **80**, 1707–1711.
Gilbreth, R. N., Truong, K., Madu, I., Koide, A., Wojcik, J. B., Li, N.-S., Piccirilli, J. A., Chen, Y. & Koide, S. (2011). *Proc. Natl Acad. Sci. USA*, **108**, 7751–7756.
Kabsch, W. (2010*a*). *Acta Cryst.* D**66**, 133–144.
Kabsch, W. (2010*b*). *Acta Cryst.* D**66**, 125–132.
Karplus, P. A. & Diederichs, K. (2012). *Science*, **336**, 1030–1033.
Killian, C. E. & Wilt, F. H. (1996). *J. Biol. Chem.* **271**, 9150–9159.
Killian, C. E. & Wilt, F. H. (2008). *Chem. Rev.* **108**, 4463–4474.
Knapp, R. T., Wu, C.-H., Mobilia, K. C. & Joester, D. (2012). *J. Am. Chem. Soc.* **134**, 17908–17911.
Lowenstam, H. A. & Weiner, S. (1989). *On Biomineralization.* Oxford University Press.
Livingston, B. T., Killian, C. E., Wilt, F., Cameron, A., Landrum, M. J., Ermolaeva, O., Sapojnikov, V., Maglott, D. R., Buchanan, A. M. & Ettensohn, C. A. (2006). *Dev. Biol.* **300**, 335–348.
Mann, S. (2001). *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry.* Oxford University Press.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Rao, A., Seto, J., Berg, J. K., Kreft, S. G., Scheffner, M. & Cölfen, H. (2013). *J. Struct. Biol.* **183**, 205–215.
Schalch, T., Job, G., Shanker, S., Partridge, J. F. & Joshua-Tor, L. (2011). *Nature Struct. Mol. Biol.* **18**, 1351–1357.
Seto, J., Ma, Y., Davis, S. A., Meldrum, F., Gourrier, A., Kim, Y.-Y., Schilde, U., Sztucki, M., Burghammer, M., Maltsev, S., Jäger, C. & Cölfen, H. (2012). *Proc. Natl Acad. Sci. USA*, **109**, 3699–3704.
Tester, C. C., Wu, C.-H., Krejci, M. R., Mueller, L., Park, A., Lai, B., Chen, S., Sun, C., Balasubramanian, M. & Joester, D. (2013). *Adv. Funct. Mater.* **23**, 4185–4194.
Wilt, F. H. (2002). *Zool. Sci.* **19**, 253–261.
Zwart, P. H., Grosse-Kunstleve, R. W. & Adams, P. D. (2005). *CCP4 Newsl. Protein Crystallogr.* **43**, contribution 7.