Acta Crystallographica Section F
**Structural Biology and Crystallization Communications**
ISSN 1744-3091

# Crystallization and preliminary X-ray analysis of the yeast tRNA-thiouridine modification protein 1 (Tum1p)

**Rui Qiu,‡ Fengbin Wang,‡ Meiruo Liu, Zhenxing Yang, Tong Wu and Chaoneng Ji***

State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, People's Republic of China

‡ These authors contributed equally to this work.

Correspondence e-mail: chnji@fudan.edu.cn

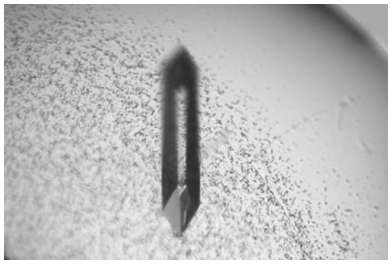
Received 19 May 2011
Accepted 24 June 2011

Yeast tRNA-thiouridine modification protein 1 (Tum1p), a crucial component of the Urm1 system, is believed to play important roles in protein urmylation and tRNA-thiouridine modification. Previous studies have demonstrated that the conserved residue Cys259 in the C-terminal rhodanese-like domain of Tum1p is essential for these sulfur-transfer activities. Here, recombinant Tum1p protein has been cloned and overexpressed in *Escherichia coli* strain BL21 (DE3). After purification, crystals of Tum1p were obtained by the hanging-drop vapour-diffusion method and diffracted to 1.9 Å resolution. The preliminary X-ray data showed that the tetragonal Tum1p crystal belonged to space group $I4_1$, with unit-cell parameters $a = b = 120.94$, $c = 48.35$ Å. The asymmetric unit of the crystal was assumed to contain one protein molecule, giving a Matthews coefficient of 2.41 Å$^3$ Da$^{-1}$ and a solvent content of 49.0%.

## 1. Introduction

Ubiquitin and ubiquitin-like proteins (UBLs) are small modifiers that regulate many important cellular processes *via* covalent conjugation to target substrates (Pickart & Fushman, 2004; Sun & Chen, 2004; Ulrich, 2002). Although limited sequence identity has been found among UBLs, they all seem to share a similar enzyme-reaction cascade: the C-terminal glycine–glycine motif possessed by most UBLs is activated by adenylation and thus forms a thioester bond to activating enzyme E1; activated UBLs are then transferred to conjugating enzyme E2 *via* transthioesterification and finally ligated to their specific protein substrates with the help of ligase E3 (Hershko *et al.*, 2000; Hochstrasser, 2000; Pedrioli *et al.*, 2008; Welchman *et al.*, 2005).

Eukaryotic ubiquitin-related modifier (Urm1p), the most ancient UBL (Xu *et al.*, 2006), has been shown to play many important roles in yeast (Yu & Zhou, 2008), such as budding (Goehring *et al.*, 2003*b*), nutrient sensing (Rubio-Texeira, 2007), high-temperature sensitivity (Furukawa *et al.*, 2000), antioxidant stress response (Goehring *et al.*, 2003*a*) and post-translational modification of the elongator subunit (Fichtner *et al.*, 2003). Like many UBLs, Urm1p contains a C-terminal glycine–glycine motif and a $\beta$-grasp-fold structural motif. Intriguingly, it can also covalently modify Ahp1p, the yeast thioredoxin peroxidase protein, mediated by the E1-like protein Uba4p (Furukawa *et al.*, 2000; Goehring *et al.*, 2003*a*). Recently, Van der Veen and coworkers have significantly extended this observation by detecting 21 further substrates that are uniquely modified by Urm1p in response to oxidative stress in human cells (Van der Veen *et al.*, 2011). However, compared with other UBLs Urm1p shows a significantly higher sequence identity to bacterial sulfur carriers such as MoaD (molybdopeterin; Pedrioli *et al.*, 2008; Singh *et al.*, 2005). Recently, several independent groups have described a new function of Urm1p as a sulfur carrier in the 2-thiolation of 5-methoxycarbonylmethyl-2-thiouridine (mcm$^5$s$^2$U; Bjork *et al.*, 2007; Dewez *et al.*, 2008; Huang *et al.*, 2008; Leidel *et al.*, 2009; Nakai *et al.*, 2008; Noma *et al.*, 2009). Thus, Urm1p is regarded as a molecular fossil in the evolutionary link



between prokaryotic sulfur carriers and eukaryotic ubiquitin-like proteins (Pedrioli *et al.*, 2008).

Several genome-wide screens have indentified five components that are responsible for the protein-urmylation and tRNA-modification branches of the Urm1 pathway: Urm1p, Tum1p, Uba4p, Ncs2p and Ncs6p (Huang *et al.*, 2008; Leidel *et al.*, 2009; Nakai *et al.*, 2008; Noma *et al.*, 2009). In addition, the cysteine desulfurase Nfs1p is believed to be an upstream component of the system (Marelja *et al.*, 2008; Nakai *et al.*, 2004); it cannot be detected by the screen since it is required for cell survival (Nakai *et al.*, 2001). Briefly, *in vitro* assays revealed that using pyridoxal 5-phosphate (PLP) as a cofactor, Nfs1p accepts sulfur from cysteine to form a persulfide (Ikeuchi *et al.*, 2006; Nakai *et al.*, 2004) and this bond can subsequently be transferred to the rhodanese-like domain (RLD) of Tum1p or Uba4p. Tum1p is believed to be an important Nfs1p activator as well as an optional persulfide mediator; it is not essential as Nfs1p can transfer persulfide sulfur to Uba4p directly, but it can very significantly enhance the activity of Nfs1p. The E1-like enzyme Uba4p then activates Urm1p by forming an acyl adenylate intermediate at the C-terminus of Urm1p and forms an acyl disulfide bond between Cys397 of Uba4p and the C-terminal glycine of Urm1p (Noma *et al.*, 2009; Schmitz *et al.*, 2008). Thiocarboxylated Urm1p is then released, which is the substrate catalyzed by the Ncs6p–Ncs2p complex in the 2-thiolation modification of $mcm^5s^2U$ and is also the activated Urm1p used in urmylation (Dewez *et al.*, 2008).

Sequence alignment has detected two rhodanese-like domains in the N-terminal and C-terminal regions of Tum1p. Rhodaneses are widespread enzymes that detoxify cyanide by converting it to thiocyanate, and conserved cysteine residues play a crucial role in their catalytic activity and substrate recognization (Bordo & Bork, 2002). Interestingly, site-directed mutagenesis demonstrates that Cys259 in the Tum1p C-terminal RLD is the only site used for persulfide formation, since the N-terminal RLD seems to be an inactive RLD, with no conserved cysteine residue being found on alignment. High-throughput screening of the subcellular locations of yeast proteins indicates that Tum1p is localized in the mitochondria and cytoplasm, while Nfs1p is mainly located in the mitochondria and Uba4p in the cytoplasm (Huh *et al.*, 2003; Kumar *et al.*, 2002; Sickmann *et al.*, 2003). Based on these results, Tum1p might be a shuttling protein between the mitochondria and cytoplasm and thus transfer persulfide sulfur from Nfs1p in the mitochondria to Uba4p in the cytoplasm (Noma *et al.*, 2009).

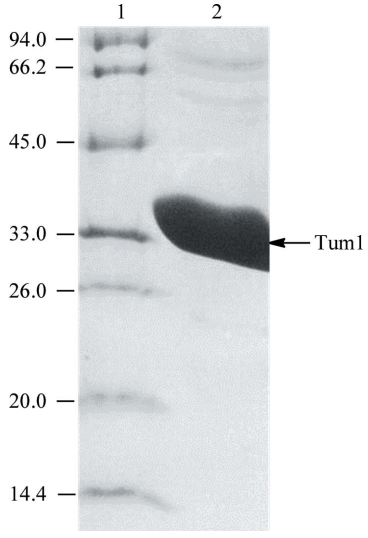



**Figure 1**
Coomassie Blue-stained 12% reducing SDS–PAGE gel showing the purity of the Tum1p used for crystallization. Lane 1, molecular-weight marker (labelled in kDa); lane 2, Tum1p sample used in crystallization trials at a 1/10 concentration.

## 2. Materials and methods

### 2.1. Cloning, expression and purification

We cloned the *Saccharomyces cerevisiae* Tum1p gene (GeneBank ID NM_001183670.1) into a pET28b (Novagen, USA) plasmid with an N-terminal $His_6$ tag using the following synthesized primers: forward, 5′-GGACTAGTATGCCATTATTTGATCTTATTTCTC-3′; reverse, 5′-CACCTCGAGTTAATCTCTGTTTTCAGCAATCC-3′. After confirmation by sequencing, pET28b-Tum1p was transformed into *Escherichia coli* strain BL21 (DE3). Bacteria were grown in LB medium (containing 50 µg $l^{-1}$ kanamycin and 34 µg $l^{-1}$ chloramphenicol) at 310 K to an $OD_{600}$ around 0.8 and protein expression was induced by the addition of 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) at 298 K for 8 h. Cells were harvested by centrifugation at 5000*g* for 10 min.

The cell pellet was resuspended in a standard buffer (40 m*M* $Na_2HPO_4$, 10 m*M* $NaH_2PO_4$, 300 m*M* NaCl, 10 m*M* imidazole pH 8.0) and disrupted by pressure (JN-3000 PLUS, JNBIO, People's Republic of China) at 277 K. After centrifugation at 27 000*g* at 277 K for 30 min, the supernatant was passed through a pre-equilibrated Ni–NTA Superflow column (Qiagen). Briefly, after washing the column with standard buffer containing 10 and 35 m*M* imidazole to remove other proteins, the $His_6$-tagged Tum1p was washed out with elution buffer (40 m*M* $Na_2HPO_4$, 10 m*M* $NaH_2PO_4$, 300 m*M* NaCl, 250 m*M* imidazole pH 8.0) and dialyzed into 5 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl. The purity and concentration of the protein samples were subsequently analyzed by SDS–PAGE (Fig. 1) and Bradford assay (Pierce). Fractions containing Tum1p were at 20 mg $ml^{-1}$ after dialysis and were frozen at 203 K until use in crystallization.

### 2.2. Crystallization and X-ray data collection

Preliminary crystallization conditions were screened using Crystal Screen Lite and Crystal Screen 2 (Hampton Research, USA) with the hanging-drop vapour-diffusion method at 293 K. Tetragonal crystals were obtained by mixing 1 µl 15 mg $ml^{-1}$ protein with 1 µl reservoir solution and equilibrating the drop over 500 µl reservoir solution

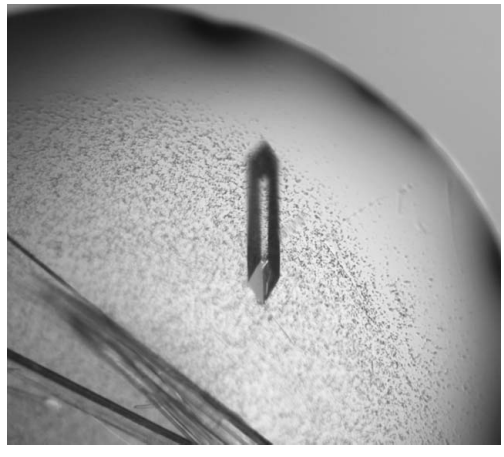


**Figure 2**
A crystal of Tum1p grown using a reservoir solution consisting of 0.1 *M* sodium cacodylate trihydrate pH 6.5, 0.2 *M* ammonium sulfate and 15%(*w*/*v*) polyethylene glycol 8000. The dimensions of the crystal are about 0.2 × 0.2 × 1.0 mm.

**Table 1**
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Source | Beamline BL17U, SSRF |
| Wavelength (Å) | 0.93219 |
| Resolution range (Å) | 50–1.9 (1.93–1.90) |
| Space group | $I4_1$ |
| Unit-cell parameters (Å) | $a = b = 120.94$, $c = 48.35$ |
| Observed reflections | |
| Total | 1822023 |
| Unique | 27813 |
| Completeness (%) | 99.7 (97.4) |
| Molecules per asymmetric unit | 1 |
| $V_M$ (Å$^3$ Da$^{-1}$) | 2.41 |
| $R_{merge}$† (%) | 7.9 (29.2) |
| Average $I/\sigma(I)$ | 30.7 (6.8) |
| Mosaicity (°) | 0.694 |
| Solvent content (%) | 49.0 |

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection $hkl$, $\sum_{hkl}$ is the sum over all reflections, $\sum_i$ is the sum over $i$ measurements of reflection $hkl$ and $\langle I(hkl)\rangle$ is the weighted average intensity of all observations $i$ of reflection $hkl$.

consisting of 0.1 *M* sodium cacodylate trihydrate pH 6.5, 0.2 *M* ammonium sulfate and 15%(*w*/*v*) polyethylene glycol 8000. The crystals obtained grew to maximal dimensions of about 0.2 × 0.2 × 1.0 mm within 3 d (Fig. 2). Prior to data collection, a single crystal was soaked in mother liquor containing 30%(*v*/*v*) dimethyl sulfoxide as a cryoprotectant and flash-cooled directly in a liquid-nitrogen stream at 100 K.

X-ray diffraction data were collected using an exposure of 1.2 s per image on the BL17U-MX beamline at Shanghai Synchrotron Radiation Facility (SSRF: λ = 0.93219 Å at 100 K) using a MAR DTB detector system. One complete data set was obtained by collecting 360 images with 1° oscillation. The data were processed using the *HKL*-2000 package (Otwinowski & Minor, 1997).

## 3. Results and discussion

In this study, recombinant Tum1p protein was successfully cloned and overexpressed in *E. coli*. After purification, Tum1p was crystallized by the hanging-drop vapour-diffusion technique. From the screens, tetragonal crystals of Tum1p suitable for X-ray diffraction analysis could be obtained under two conditions: (i) 0.1 *M* sodium cacodylate trihydrate pH 6.5, 0.2 *M* ammonium sulfate, 15%(*w*/*v*) polyethylene glycol 8000 and (ii) 0.1 *M* sodium citrate tribasic dihydrate pH 5.6, 10%(*v*/*v*) 2-propanol, 10%(*w*/*v*) polyethylene glycol 4000. Our results revealed that condition (i) was better. The statistics of the preliminary crystallographic data for the Tum1p crystal are given in Table 1. The crystals of Tum1p belonged to space group $I4_1$, with unit-cell parameters $a = b = 120.94$, $c = 48.35$ Å. Calculation of the Matthews coefficient indicated that there was one molecule in the asymmetric unit with a Matthews coefficient of 2.41 Å$^3$ Da$^{-1}$, corresponding to a solvent content of 49.0%.

We are attempting to determine the structure of Tum1p by the molecular-replacement method. The X-ray crystal structure of Tum1p may provide information on how it interacts with Uba4p and Nfs1p as a persulfide mediator and an important Nfs1p activator. Also, it may provide us with further information regarding its presumptive shuttling activity. Therefore, determination of the Tum1p structure would provide us with a novel illustration of the entire Urm1 system.

We thank the staff of Shanghai Synchrotron Radiation Facility beamline BL17U for assistance in data collection. This work was supported by the National Basic Research Program of China (973 Program; 2007CB914304 and 2009CB825505), the National Natural Science Foundation of China (30770427), the New Century Excellent Talents in University (NCET-06-0356), the Shanghai Leading Academic Discipline Project (B111) and the National Talent Training Fund in Basic Research of China (No. J0630643).

## References

Björk, G. R., Huang, B., Persson, O. P. & Byström, A. S. (2007). *RNA*, **13**, 1245–1255.
Bordo, D. & Bork, P. (2002). *EMBO Rep.* **3**, 741–746.
Dewez, M., Bauer, F., Dieu, M., Raes, M., Vandenhaute, J. & Hermand, D. (2008). *Proc. Natl Acad. Sci. USA*, **105**, 5459–5464.
Fichtner, L., Jablonowski, D., Schierhorn, A., Kitamoto, H. K., Stark, M. J. & Schaffrath, R. (2003). *Mol. Microbiol.* **49**, 1297–1307.
Furukawa, K., Mizushima, N., Noda, T. & Ohsumi, Y. (2000). *J. Biol. Chem.* **275**, 7462–7465.
Goehring, A. S., Rivers, D. M. & Sprague, G. F. Jr (2003*a*). *Eukaryot. Cell*, **2**, 930–936.
Goehring, A. S., Rivers, D. M. & Sprague, G. F. Jr (2003*b*). *Mol. Biol. Cell*, **14**, 4329–4341.
Hershko, A., Ciechanover, A. & Varshavsky, A. (2000). *Nature Med.* **6**, 1073–1081.
Hochstrasser, M. (2000). *Nature Cell Biol.* **2**, E153–E157.
Huang, B., Lu, J. & Byström, A. S. (2008). *RNA*, **14**, 2183–2194.
Huh, W.-K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S. & O'Shea, E. K. (2003). *Nature (London)*, **425**, 686–691.
Ikeuchi, Y., Shigi, N., Kato, J., Nishimura, A. & Suzuki, T. (2006). *Mol. Cell*, **21**, 97–108.
Kumar, A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K.-H., Miller, P., Gerstein, M., Roeder, G. S. & Snyder, M. (2002). *Gene Dev.* **16**, 707–719.
Leidel, S., Pedrioli, P. G., Bucher, T., Brost, R., Costanzo, M., Schmidt, A., Aebersold, R., Boone, C., Hofmann, K. & Peter, M. (2009). *Nature (London)*, **458**, 228–232.
Marelja, Z., Stöcklein, W., Nimtz, M. & Leimkühler, S. (2008). *J. Biol. Chem.* **283**, 25178–25185.
Nakai, Y., Nakai, M. & Hayashi, H. (2008). *J. Biol. Chem.* **283**, 27469–27476.
Nakai, Y., Nakai, M., Hayashi, H. & Kagamiyama, H. (2001). *J. Biol. Chem.* **276**, 8314–8320.
Nakai, Y., Umeda, N., Suzuki, T., Nakai, M., Hayashi, H., Watanabe, K. & Kagamiyama, H. (2004). *J. Biol. Chem.* **279**, 12363–12368.
Noma, A., Sakaguchi, Y. & Suzuki, T. (2009). *Nucleic Acids Res.* **37**, 1335–1352.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pedrioli, P. G., Leidel, S. & Hofmann, K. (2008). *EMBO Rep.* **9**, 1196–1202.
Pickart, C. M. & Fushman, D. (2004). *Curr. Opin. Chem. Biol.* **8**, 610–616.
Rubio-Texeira, M. (2007). *FEBS Lett.* **581**, 541–550.
Schmitz, J., Chowdhury, M. M., Hänzelmann, P., Nimtz, M., Lee, E.-Y., Schindelin, H. & Leimkühler, S. (2008). *Biochemistry*, **47**, 6479–6489.
Sickmann, A., Reinders, J., Wagner, Y., Joppich, C., Zahedi, R., Meyer, H. E., Schonfisch, B., Perschil, I., Chacinska, A., Guiard, B., Rehling, P., Pfanner, N. & Meisinger, C. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 13207–13212.
Singh, S., Tonelli, M., Tyler, R. C., Bahrami, A., Lee, M. S. & Markley, J. L. (2005). *Protein Sci.* **14**, 2095–2102.
Sun, L. & Chen, Z. J. (2004). *Curr. Opin. Cell Biol.* **16**, 119–126.
Ulrich, H. D. (2002). *Eukaryot. Cell*, **1**, 1–10.
Van der Veen, A. G., Schorpp, K., Schlieker, C., Buti, L., Damon, J. R., Spooner, E., Ploegh, H. L. & Jentsch, S. (2011). *Proc. Natl Acad. Sci. USA*, **108**, 1763–1770.
Welchman, R. L., Gordon, C. & Mayer, R. J. (2005). *Nature Rev. Mol. Cell Biol.*, **6**, 599–609.
Xu, J., Zhang, J., Wang, L., Zhou, J., Huang, H., Wu, J., Zhong, Y. & Shi, Y. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 11625–11630.
Yu, J. & Zhou, C.-Z. (2008). *Proteins*, **71**, 1050–1055.