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

# Protein preparation, crystallization and preliminary X-ray analysis of the C-terminal domain of human RSK1 serine/threonine protein kinase

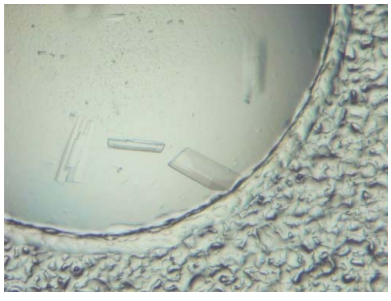
**Tian-Min Fu,[a]‡ Dan Li,[a]‡ Jie Nan,[a] Lanfen Li,[a] Yafeng Xue[b] and Xiao-Dong Su[a,c]***

[a]College of Life Sciences, Peking University, Beijing 100871, People's Republic of China, [b]AstraZeneca Global Structural Chemistry, AstraZeneca R&D Molndal, SE-431 83, Molndal, Sweden, and [c]Peking University Shenzhen Graduate School, Shenzhen 518055, People's Republic of China

‡ These authors contributed equally to this work.

Correspondence e-mail: xdsu@pku.edu.cn

Received 29 August 2007
Accepted 17 October 2007





As a substrate of extracellular signal-related kinase (ERK), the p90 ribosome S6 kinase 1 (RSK1) is at the terminus of the Ras/ERK pathway. Residues 411–735 of human RSK1, covering the C-terminal serine/threonine kinase catalytic domain and the functionally important tail, were cloned into an *Escherichia coli* expression vector. The protein was expressed, purified and crystallized. The crystals diffracted to 2.7 Å and belonged to space group $P2_1$, with unit-cell parameters $a$ = 39.8, $b$ = 143.8, $c$ = 59.9 Å, $\beta$ = 95.7°.

## 1. Introduction

The Ras/extracellular signal-regulated kinase (ERK) pathway, a ubiquitous pathway in eukaryotic organisms, can regulate many important cellular programs including cell proliferation, growth, motility and apoptosis (Pearson *et al.*, 2001). As a substrate of ERK, the p90 ribosome S6 kinase 1 (RSK1) is at the terminus of the Ras/ERK pathway. RSK1 belongs to the RSK family of serine/threonine kinases. In humans, the RSK family contains four members (RSK1–4) and two related homologues, mitogen- and stress-activated kinase 1 (MSK1) and mitogen- and stress-activated kinase 2 (MSK2) (Roux *et al.*, 2003). Members of the RSK family show high amino-acid identity and have similar overall structures. All of them are composed of two non-identical protein kinase catalytic domains, a linker region and short N-terminal and C-terminal tails. The N-terminal kinase domain acts to phosphorylate all known exogenous substrates of RSK, while the C-terminal kinase domain and linker region are responsible for the regulation of N-terminal kinase activity (Bjorbaek *et al.*, 1995; Richards *et al.*, 1999). Activated RSK has been shown to phosphorylate many substrates located both in the cytoplasm and the nucleus. The substrates of RSK include cAMP response element-binding protein (CREB), the oestrogen receptor-$\alpha$ (ER-$\alpha$), filamin A, Bad, Myt1, c-Fos, histone H3 and I$\kappa$B (Xing *et al.*, 1996; Joel *et al.*, 1998; Woo *et al.*, 2004; Bonni *et al.*, 1999; Palmer *et al.*, 1998; Sassone-Corsi *et al.*, 1999; Schouten *et al.*, 1997). Although RSK plays an important role in cellular processes, the precise mechanism of RSK activation remains unclear. To date, a large amount of biochemical data related to the activation of RSK has been obtained; however, no structure has been reported for any RSK-family members. Here, we report the preparation, crystallization and preliminary X-ray analysis of the RSK1 C-terminal kinase domain. The structural information will contribute to the understanding of the RSK1 activation mechanism.

## 2. Materials and methods

### 2.1. Gene cloning and expression

The coding sequence of RSK1(411–735) containing the C-terminal serine/threonine protein kinase domain was amplified by the polymerase chain reaction (PCR) with forward primer 5′-GGATTCCATATGAACCTGGTTTTTAGTGAC-3′ and reverse primer 5′-CAGCTCGAGTCACAGGGTGGTGGATGG-3′ using commercial RSK1 cDNA (Proteintech Group Inc., People's Republic of China) as a template. The PCR product was cloned into the *Escherichia coli*

**Table 1**
Data-collection statistics for RSK1(411–735).

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Wavelength (Å) | 0.98 |
| Resolution (Å) | 50–2.7 (2.80–2.70) |
| Completeness (%) | 97.9 (97.6) |
| $R_{sym}$† (%) | 7.8 (34.8) |
| Mean $I/\sigma(I)$ | 9.8 (3.5) |
| Space group | $P2_1$ |
| Unit-cell parameters (Å, °) | $a$ = 39.8, $b$ = 143.8, $c$ = 59.9, $\beta$ = 95.7 |
| No. of observed reflections | 48516 |
| No. of unique reflections | 17784 |
| Molecules per ASU | 2 |
| $V_M$ (Å$^3$ Da$^{-1}$) | 2.2 |
| Solvent content (%) | 44.8 |

† $R_{sym} = |I_{obs} - I_{avg}|/\sum I_{obs}$, where the summation is over all reflections.

expression vector pET-15b (Novagen) at the *Nde*I and *Xho*I cloning sites introduced into the PCR primers. The *E. coli* expression strain Rosetta was transformed with the pET-15b-RSK1(411–735) plasmid. Cells were cultured at 310 K in Luria–Bertani (LB) medium containing 50 µg ml$^{-1}$ ampicillin until the mid-exponential growth phase was reached with an $OD_{600}$ of 0.5–0.7. At this point, RSK1(411–735) expression was induced by addition of isopropyl $\beta$-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 m*M* and the culture was then grown at 291 K for 20 h. Cells were harvested by centrifugation at 6700*g* for 10 min at 277 K.

### 2.2. Protein purification

The cells were suspended in buffer containing 20 m*M* Tris–HCl, 0.5 *M* NaCl, 1 m*M* phenylmethanesulfonyl fluoride (PMSF) pH 7.5 and lysed by sonication. The cell lysate was centrifuged at 34 700*g* for 50 min at 277 K. The supernatant was applied onto a 5 ml Hitrap Ni column (GE Healthcare, USA) equilibrated with buffer *A* (20 m*M* Tris–HCl, 0.5 *M* NaCl pH 7.5). Unbound proteins were eluted using buffer *A* and loosely bound proteins were eluted using 20% buffer *B* (20 m*M* Tris–HCl, 0.5 *M* NaCl, 0.5 *M* imidazole pH 7.5) in buffer *A*. Tightly bound proteins, mainly RSK1(411–735), were then eluted with 60% buffer *B* in buffer *A*. RSK1 (411–735) was further purified using a HiLoad 16/60 Superdex 75 column (GE Healthcare, USA); 20 m*M* sodium citrate pH 6.0 was used as eluant. The purity of the protein was examined by 15% SDS–PAGE at each purification step. All purifications were performed using an ÄKTA Explorer (GE Healthcare, USA).

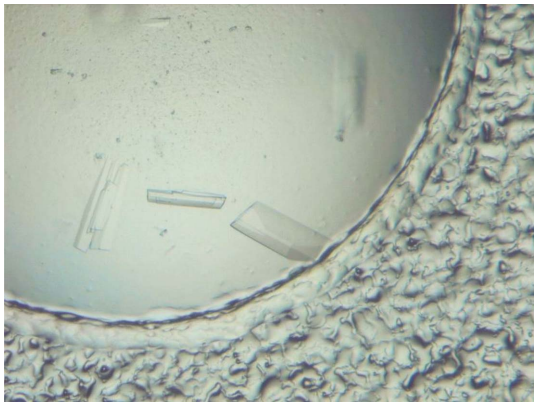


**Figure 1**
Crystals of RSK1(411–735) grown from a sitting drop on a 48-well SBS microplate. The dimensions of the largest crystal are approximately 0.3 × 0.1 × 0.04 mm.

### 2.3. Crystallization

The purified RSK1(411–735) with a His tag fused at the N-terminus was concentrated to 5 mg ml$^{-1}$ by ultrafiltration (Millipore Amicon, USA) and subjected to Crystal Screen I, Crystal Screen II, Index Screen (Hampton Research, USA) and JBScreen Kinase (Jena Bioscience, Germany) as initial screening kits. Additive Screen (Hampton Research, USA) was used for optimization. Crystallization trials were performed at 290 K using the sitting-drop method on newly designed 48-well SBS (ANSI/SBS 1-2004; American National Standards Institute/Society for Biomolecular Sciences, USA) microplates (XtalQuest Inc., Beijing, People's Republic of China). A mixture of 1 µl protein solution and 1 µl reservoir solution was equilibrated against 100 µl reservoir solution.

### 2.4. Data collection

X-ray diffraction data were collected on a MAR 165 CCD detector at beamline 3W1A at Beijing Synchrotron Radiation Facility (BSRF), Beijing, People's Republic of China. The crystal was flash-frozen in liquid nitrogen and maintained at 100 K using nitrogen gas (Oxford Instruments Inc., UK) during data collection. The data were processed using *HKL*-2000 (Otwinowski & Minor, 1997).

## 3. Results and discussions

The correct sequence of the construct was confirmed by DNA sequencing (Augct Biotechnology, Beijing, People's Republic of China). RSK1(411–375) produced in the *E. coli* strain Rosetta harbouring the recombinant plasmid was soluble and was purified to homogeneity in two steps. From 0.5 l culture, about 5 mg RSK1(411–735) with a purity of >95% could normally be produced. SDS–PAGE showed that the overexpressed target protein had an apparent molecular weight of 38.0 kDa. This molecular weight matches the theoretical molecular weight of the recombinant RSK1(411–735): 36.5 kDa with a 2.3 kDa fusion peptide including the hexahistidine tag.

Microcrystals were obtained under several conditions. After optimizing the initial crystallization conditions, single crystals with dimensions of 0.3 × 0.1 × 0.04 mm were obtained in 0.1 *M* HEPES pH 7.5, 17.1%(*w*/*v*) PEG 3350, 4%(*v*/*v*) acetonitrile or 18%(*w*/*v*) PEG 3350, 100 m*M* ammonium formate, 4%(*v*/*v*) acetonitrile after 2 d. Sitting drops on the 48-well SBS microplates were necessary to obtain the best crystals (Fig. 1). The crystals diffracted to a resolution of 2.7 Å and belonged to space group $P2_1$, with unit-cell parameters $a$ = 39.8, $b$ = 143.8, $c$ = 59.9 Å, $\beta$ = 95.7°. Assuming the presence of two molecules per asymmetric unit, the Matthews coefficient is 2.2 Å$^3$ Da$^{-1}$ and the solvent content is 44.8% (Matthews, 1968). Data statistics are listed in Table 1. Phase-determination trials using molecular replacement are in progress.

The authors would like to thank Mats Örmo, Margareta Ek, Lisa Wissler, Linda Öster, Cristian Johansson, AstraZeneca Global Structural Chemistry, AstraZeneca R&D Molndal for help in setting up formulation screening for thermostability and further crystallization screening during the visit of DL and JN to AstraZeneca Molndal. X-DS is supported by the National Natural Science Foundation of China (NSFC) grants 30325012 and 30530190.

## References

Bjorbaek, C., Zhao, Y. & Moller, D. E. (1995). *J. Biol. Chem.* **270**, 18848–18852.

Bonni, A., Brunet, A., West, A. E., Datta, S. R., Takasa, M. A. & Greenberg, M. E. (1999). *Science*, **286**, 1358–1362.
Joel, P. B., Smith, J., Sturgill, T. W., Fisher, T. L., Blenis, J. & Lannigan, D. A. (1998). *Mol. Cell. Biol.* **18**, 1978–1984.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, Z. (1997). *Methods Enzymol.* **276**, 307–326.
Palmer, A., Gavin, A. C. & Nebreda, A. R. (1998). *EMBO J.* **17**, 5037–5047.
Pearson, G., Robinson, F., Gibson, T. B., Xu, B. E., Karandikar, M., Berman, K. & Cobb, M. H. (2001). *Endocr. Rev.* **22**, 153–183.
Richards, S. A., Fu, J., Romanelli, A., Shimamura, A. & Blenis, J. (1999). *Curr. Biol.* **9**, 810–820.
Roux, P. P., Richard, S. A. & Blenis, J. (2003). *Mol. Cell. Biol.* **23**, 4796–4804.
Sassone-Corsi, P., Mizzem, C. A., Cheung, P., Crosio, C., Monaco, L., Jacquot, S., Hanauer, A. & Allis, C. D. (1999). *Science*, **285**, 886–891.
Schouten, G. J., Vertegaal, A. C. O., Whiteside, S. T., Israel, A., Toebes, M., Dorsman, J. C., Van der Eb, A. J. & Zantema, A. (1997). *EMBO J.* **16**, 3133–3144.
Woo, M. S., Ohta, Y., Rabinovitz, I., Stossel, T. P. & Blenis, J. (2004). *Mol. Cell. Biol.* **24**, 3025–3035.
Xing, J., Ginty, D. D. & Greenberg, M. E. (1996). *Science*, **273**, 959–963.