Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of ribosomal protein L11 methyltransferase from *Thermus thermophilus* HB8

Tatsuya Kaminishi,[a,b] Hiroaki Sakai,[a] Chie Takemoto-Hori,[a] Takaho Terada,[a,c] Noriko Nakagawa,[c] Nobuko Maoka,[c] Seiki Kuramitsu,[a,c,d] Mikako Shirouzu[a,c] and Shigeyuki Yokoyama[a,b,c]*

[a]RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, [b]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, [c]RIKEN Harima Institute at SPring-8, 1-1-1 Kohto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan, and [d]Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Correspondence e-mail: yokoyama@biochem.s.u-tokyo.ac.jp

Received 28 November 2002
Accepted 26 February 2003

Ribosomal proteins are subjected to a variety of post-translational modifications, of which methylation is the most frequently found in all three kingdoms of life. PrmA is the only bacterial enzyme identified to date that catalyzes the methylation of a ribosomal protein. It is responsible for the introduction of nine methyl groups into the N-terminal domain of ribosomal protein L11. The PrmA protein from *Thermus thermophilus* HB8 was crystallized and a preliminary X-ray diffraction analysis was performed. A cryocooled crystal diffracted X-rays beyond 1.9 Å using synchrotron radiation.

1. Introduction

The ribosome is a large ribonucleoprotein consisting of three RNA molecules (rRNAs) and more than 50 ribosomal proteins. Many of these ribosomal components are subjected to various modifications. In all three kingdoms of life, the most frequently found modifications are methylated forms of nucleotides and amino-acid residues (Alix, 1988; Decatur & Fournier, 2002). While methyltransferases (MTases, EC 2.1.1) for rRNA have been investigated in great detail (Grosjean & Benne, 1998), there is little information available on the ribosomal protein MTases. Two genes in *Escherichia coli* have been implicated in the methylation of ribosomal proteins: *prmA* for L11 and *prmB* for L3 (Colson *et al.*, 1979). The *prmA* gene is dispensable in *E. coli* (Vanet *et al.*, 1994), but is widely conserved in bacteria and also in plastids. To date, the PrmA protein is the only ribosomal protein MTase in bacteria that has been purified and examined for enzymatic activity (Vanet *et al.*, 1994). It contains a set of highly conserved amino-acid residues specific to the *S*-adenosyl-L-methionine (AdoMet) dependent methyltransferase family (Vanet *et al.*, 1993; Bujnicki, 2000). The PrmA protein is involved in the introduction of nine methyl groups into the N-terminal domain of L11 (Colson, 1977; Alix *et al.*, 1979; Colson *et al.*, 1979). Trimethylations take place at the three independent sites: the N-terminal amino group of the Ala1 residue (the initiating fMet residue is removed) and the side-chain amino groups of the Lys3 and Lys39 residues (Lederer *et al.*, 1977; Dognin & Wittmann-Liebold, 1980).

L11 has been shown to participate in a number of biologically intriguing processes. It binds to a highly conserved stretch of rRNA to constitute the GTPase-associated region of the ribosome (Thompson *et al.*, 1979; Schmidt *et al.*, 1981). This region is involved in regulating the GTPase activity of the elongation factors (Cundliffe, 1986). Thiostrepton and micrococcin, which are antibiotics that contain thiazole rings, inhibit this interplay by binding to the interface between L11 and the RNA fragment (Wimberly *et al.*, 1999). L11 also plays a critical role in the stringent response *in vivo* (Parker *et al.*, 1976; Ambulos *et al.*, 1988; Ochi, 1990*a*,*b*), particularly through its N-terminal 36 residues (Yang & Ishiguro, 2001). It has been suggested that the N-terminal domain of L11 alternates between different conformational states in order to function as a molecular switch (Wimberly *et al.*, 1999). Although the residues trimethylated by PrmA may be involved in this molecular-switch mechanism, two of them (Ala1 and Lys3) are missing in the crystal structure of the L11–RNA complex (Wimberly *et al.*, 1999).

In the present study, we report the purification, crystallization and preliminary X-ray diffraction analysis of PrmA, the ribosomal protein L11 methyltransferase from *Thermus thermophilus* HB8. The protein contains 254 amino-acid residues and has a molecular weight of 27.6 kDa.

2. Experimental and results

2.1. Purification and crystallization

The protein solution for crystallization was prepared by the RIKEN Structural Genomics Initiative (Yokoyama *et al.*, 2000). PrmA was overexpressed in *E. coli* strain BL21(DE3). Cells were harvested and sonicated in 20 m*M* Tris–HCl buffer pH 8.0 containing 50 m*M* NaCl and 5 m*M* β-mercaptoethanol. The cell lysate was then subjected to heat treatment (10 min, 343 K) and denatured proteins origi-

nating from *E. coli* were spun down by centrifugation (1 h, 277 K, 185 000*g*). Soluble proteins in the supernatant were precipitated with 1.5 *M* ammonium sulfate and dialyzed against 20 m*M* Tris–HCl pH 8.0. Resource Q (Amersham Biosciences), Bio-Scale CHT-10 (Bio-Rad), BioAssist Q (Tosoh) and HiLoad 16/60 Superdex 75 pg (Amersham Biosciences) columns were subsequently employed at ambient temperature to purify PrmA to homogeneity. The protein was finally concentrated to 12 mg ml^{-1} in 20 m*M* Tris–HCl buffer pH 8.0 containing 150 m*M* NaCl and 1 m*M* dithiothreitol.

We set up initial crystallization trials at 293 K using the hanging-drop vapour-diffusion method. The commercially available kits Crystal Screen and Crystal Screen 2 (Hampton Research) were used for reservoir solutions. The volume of the reservoir solutions was 200 µl and the drops contained 1 µl each of the protein and reservoir solutions. Spherulites appeared overnight in a drop containing 50 m*M* MES pH 6.5, 5%(*v*/*v*) dioxane and 800 m*M* ammonium sulfate (Fig. 1*a*). We then varied the pH, the concentrations of ammonium sulfate and dioxane and the volume of the protein and reservoir solutions mixed in the drops, but no single crystals were obtained. Therefore, reagents from Additive Screens 1, 2 and 3 (Hampton Research) were subsequently tested. Several diols successfully gave single crystals and crystals with sharp edges grew in the presence of 1,6-hexanediol. We again optimized the crystallization conditions by changing the temperature and the volume of the solutions mixed in the drops. Finally, single crystals of about 60 × 80 × 300 µm in size were obtained within 2 d in drops containing 20 m*M* MES pH 6.8, 700 m*M* ammonium sulfate, 4%(*v*/*v*) dioxane and 160 m*M* 1,6-hexanediol (Fig. 1*b*).

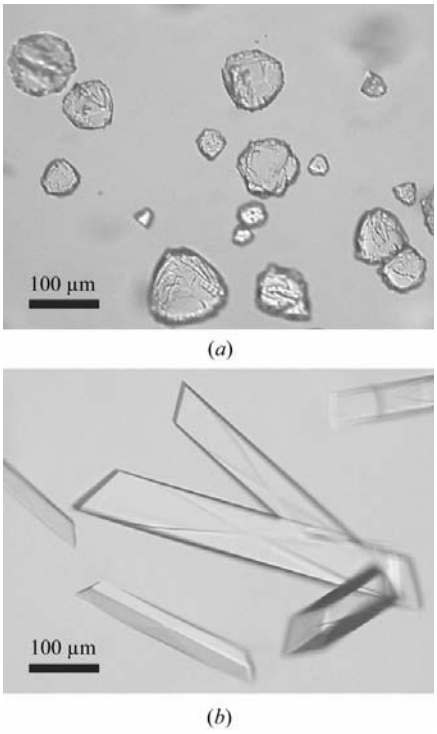

Figure 1
Improvement of PrmA crystallization, starting with spherulites (*a*) and ending with single crystals with sharp edges (*b*).

2.2. X-ray diffraction experiments

The reservoir solution was supplemented with 320 m*M* 1,6-hexanediol and used to harvest the crystals. However, the crystals immediately cracked when transferred to this solution. We thus refined the concentrations of the components and found that the crystals could be stabilized in a harvesting solution containing 50 m*M* MES pH 6.8, 1.44 *M* ammonium sulfate, 5%(*v*/*v*) dioxane and 320 m*M* 1,6-hexanediol. One of the crystals was mounted in a quartz capillary tube and an X-ray diffraction experiment was carried out in the laboratory using Cu $K\alpha$ radiation. An FR-D rotating-anode X-ray generator (Rigaku) operating at 50 kV and 60 mA was used. Diffraction was observed to only 3.5 Å at 293 K on an R-AXIS IV++ image-plate area detector (Rigaku). The resulting data were processed with the *HKL*2000 suite (Otwinowski & Minor, 1997). The crystal belongs to the monoclinic space group *C*2, with unit-cell parameters $a = 83.30$, $b = 77.77$, $c = 62.73$ Å, $\beta = 130.77^\circ$. Assuming one PrmA molecule in the asymmetric unit, the solvent content was calculated to be 55.5% ($V_M = 2.8$ Å^3 Da^{-1}; Matthews, 1968).

Typical antifreezing reagents were then tested for the collection of data at cryogenic temperatures. Although the PrmA crystals were extremely unstable in the presence of most of the cryoprotectants, 10%(*v*/*v*) propylene glycol in the harvest solution prevented ice formation without damaging the crystals. Crystals were dipped in this solution and then flash-frozen in liquid nitrogen. One of the cryocooled crystals diffracted X-rays to better than 1.9 Å resolution at 100 K using synchrotron radiation at beamline BL44B2 of SPring-8 (Adachi *et al.*, 2001). The crystal belongs to space group *C*2, like the crystal measured at 293 K, but the unit-cell had contracted in every dimension to $a = 81.75$, $b = 75.69$, $c = 61.93$ Å, $\beta = 130.45^\circ$. The data-collection statistics are given in Table 1.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.97–1.90 Å).

Wavelength (Å)	1.0000
Resolution (Å)	50.00–1.90
Total reflections	87044
Unique reflections	22573
Redundancy	3.8 (3.8)
Completeness (%)	98.5 (98.0)
$I/\sigma(I)$	28.5 (4.8)
$R_{\rm merge}$† (%)	4.0 (29.4)

† $R_{\rm merge} = \sum_h \sum_i |I_{h,i} - \langle I_h \rangle| / \sum_h \sum_i |I_{h,i}|$, where h and i indicate unique reflection indices and symmetry-equivalent indices, respectively.

2.3. Search for heavy-atom derivatives for phasing

Although the crystal or solution structures of many AdoMet-dependent MTases are available (Fauman *et al.*, 1999), no PrmA protein has yet been structurally characterized. Therefore, we first aimed towards phasing by the multiple isomorphous replacement (MIR) method. Crystals were soaked in the harvest solution containing 0.4–10 m*M* of heavy-atom compounds such as platinum potassium thiocyanate, mercury (II) cyanide and samarium (III) acetate hydrate. X-ray diffraction experiments were carried out at the same SPring-8 beamline. The X-ray wavelength was tuned from crystal to crystal, so that anomalous scattering from the respective heavy atoms could be utilized for phase determination. We managed to find Patterson peaks for some of the crystals and used the program *SOLVE* to locate the heavy-atom sites and calculate initial phases (Terwilliger & Berendzen, 1999). However, no interpretable electron-density maps were obtained by the MIR method because of the non-isomorphism among the crystals and/or the low occupancies of the heavy atoms. In the course of further screening, crystals soaked in 2 m*M* potassium tetrachloroaurate(III) gave strong and consistent peaks in both the isomorphous and anomalous Patterson maps. We are now attempting to solve the crystal structure by the multiwavelength anomalous dispersion method with these promising Au derivatives.

This work was in part supported by a grant for the Organized Research Combination System (ORCS) from the Ministry of Education, Culture, Sports, Science and Technology.

References

Adachi, S., Oguchi, T., Tanida, H., Park, S.-Y., Shimizu, H., Miyatake, H., Kamiya, N., Shiro,

Y., Inoue, Y., Ueki, T. & Iizuka, T. (2001). *Nucl. Instrum. Methods Phys. Res. A*, **467–468**, 711–714.
Alix, J. H. (1988). *Advances in Post-translational Modifications of Proteins and Aging*, edited by V. Zappia, pp. 371–385. New York: Plenum Press.
Alix, J. H., Hayes, D., Lontie, J. F., Colson, C., Glatigny, A. & Lederer, F. (1979). *Biochimie*, **61**, 671–679.
Ambulos, N. P. Jr, Rogers, E. J., Alexieva, Z. & Lovett, P. S. (1988). *J. Bacteriol.* **170**, 5642–5646.
Bujnicki, J. M. (2000). *Acta Microbiol. Pol.* **49**, 19–29.
Colson, C. (1977). *Mol. Gen. Genet.* **154**, 167–173.
Colson, C., Lhoest, J. & Urlings, C. (1979). *Mol. Gen. Genet.* **169**, 245–250.
Cundliffe, E. (1986). *Structure, Function and Genetics of Ribosomes*, edited by B. Hardesty & G. Kramer, pp. 586–604. New York: Springer–Verlag.
Decatur, W. A. & Fournier, M. J. (2002). *Trends Biochem. Sci.* **27**, 344–351.
Dognin, M. J. & Wittmann-Liebold, B. (1980). *Eur. J. Biochem.* **112**, 131–151.
Fauman, E. B., Blumenthal, R. M. & Cheng, X. (1999). *S-Adenosylmethionine-Dependent Methyltransferases: Structures and Functions*, edited by X. Cheng & R. M. Blumenthal, pp. 1–38. Singapore: World Scientific.
Grosjean, H. & Benne, R. (1998). *Modification and Editing of RNA*. Washington, DC: ASM Press.
Lederer, F., Alix, J. H. & Hayes, D. (1977). *Biochem. Biophys. Res. Commun.* **77**, 470–480.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Ochi, K. (1990*a*). *J. Gen. Microbiol.* **136**, 2405–2412.
Ochi, K. (1990*b*). *J. Bacteriol.* **172**, 4008–4016.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Parker, J., Watson, R. J. & Friesen, J. D. (1976). *Mol. Gen. Genet.* **144**, 111–114.
Schmidt, F. J., Thompson, J., Lee, K., Dijk, J. & Cundliffe, E. (1981). *J. Biol. Chem.* **256**, 12301–12305.
Terwilliger, T. C. & Berendzen, J. (1999). *Acta Cryst.* D**55**, 849–861.
Thompson, J., Cundliffe, E. & Stark, M. (1979). *Eur. J. Biochem.* **98**, 261–265.
Vanet, A., Plumbridge, J. A. & Alix, J. H. (1993). *J. Bacteriol.* **175**, 7178–7188.
Vanet, A., Plumbridge, J. A., Guerin, M. F. & Alix, J. H. (1994). *Mol. Microbiol.* **14**, 947–958.
Wimberly, B. T., Guymon, R., McCutcheon, J. P., White, S. W. & Ramakrishnan, V. (1999). *Cell*, **97**, 491–502.
Yang, X. & Ishiguro, E. E. (2001). *J. Bacteriol.* **183**, 6532–6537.
Yokoyama, S., Hirota, H., Kigawa, T., Yabuki, T., Shirouzu, M., Terada, T., Ito, Y., Matsuo, Y., Kuroda, Y., Nishimura, Y., Kyogoku, Y., Miki, K., Masui, R. & Kuramitsu, S. (2000). *Nature Struct. Biol.* **7**, *Suppl.*, 943–945.