Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of recombinant histone HPhA from the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3

Ti Li,[a] Xin Ji,[b] Fei Sun,[a] Renjun Gao,[b] Shugui Cao,[b] Yan Feng[b] and Zihe Rao[a]*

[a]Laboratory of Structural Biology, Department of Biological Science and Technology and MOE Laboratory of Protein Science, Tsinghua University, Beijing 100084, People's Republic of China, and [b]Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130023, People's Republic of China

Correspondence e-mail: raozh@xtal.tsinghua.edu.cn

Received 6 December 2001
Accepted 18 March 2002

Recombinant archaeal histone from the hyperthermophile *Pyrococcus horikoshii* OT3 (HPhA) was crystallized by the hanging-drop vapour-diffusion method. Crystals grew at 291 K in 200 m*M* $(NH_4)_2SO_4$, 100 m*M* sodium acetate buffer pH 4.6, 19% PEG 4000. Diffraction data were obtained to a resolution of 2.3 Å from a single frozen crystal, which belonged to space group $P2_1$ with unit-cell parameters $a = 34.99$, $b = 46.89$, $c = 35.02$ Å, $\alpha = \gamma = 90$, $\beta = 104°$. The asymmetric unit contained two molecules and had a solvent content of ∼35%.

1. Introduction

Archaeal histones are small basic DNA-binding proteins that wrap DNA into nucleosome-like structures, participating both in the organization of DNA and in the control of gene expression (Reeve *et al.*, 1997; Sandman *et al.*, 2001). Archetypal histones have primary sequences and structural similarity to the eukaryotic histones and therefore appear to have related functions and to have evolved from a common ancestor (Starich *et al.*, 1996; Pereira *et al.*, 1997). Over 30 archaeal histone sequences have been determined from mesophilic, thermophilic and hyperthermophilic archaea, ranging in length from 66 to 72 residues, and are 50–98% identical in sequence (Sullivan *et al.*, 2000).

The hyperthermophilic archeaon *P. horikoshii* has two histone genes, designated phso51 and phso46 (Kawarabayasi *et al.*, 1998), encoding proteins HPhA and HPhB, respectively, that are approximately 80% identical in sequence. HPh belongs to the HMf family of archaeal histones; it binds to double-stranded DNA and increases its resistance to thermal denaturation (Li *et al.*, 1998). *P. horikoshii* grows optimally at 368 K; hence, histones isolated from this archaeaon are stable at extreme temperature. Compared with the most extensively studied archaeal histones HMfA and HMfB (from *Methanothermus fervidus*, which grows optimally at 356 K; Starich *et al.*, 1996; Sandman *et al.*, 1990; Decanniere *et al.*, 2000), HPhA shows 62 and 58% sequence identity, respectively, and has higher temperature resistance (Soares *et al.*, 1998).

Here, we report the crystallization and preliminary X-ray characterization of the archaeal histone HPhA. We aim to reveal the similarity and differences between this archetypal histone from the hyperthermophile *P. horikoshii* OT3, HMfA and HMfB in *M. fervidus* (Decanniere *et al.*, 2000) and eukaryotic histones (Luger *et al.*, 1997). We also hope to understand the structural basis of the thermal stability of HPhA at extreme temperatures.

2. Materials and methods

Recombinant (r) HPhA was synthesized in *Escherichia coli* by expression of the HPhA gene cloned from the hyperthermophile *P. horikoshii* OT3. HPhA was purified using the following steps: (i) DNase I incubation for 1 h at 310 K, (ii) heat treatment at 368 K for 10 min with addition of 3 *M* NaCl and (iii) desalting followed by Hi-Trap heparin-affinity chromatography and gel filtration. The purified HPhA protein was concentrated to ∼20 mg ml^{-1} in 10 m*M* Tris–HCl buffer pH 7.5 containing 25 m*M* NaCl.

Crystallization trials were carried out in tissue-culture plates using the hanging-drop vapour-diffusion method. Preliminary crystallization conditions were established using Hampton Research Crystal Screen kits (Jancarik *et al.*, 1991) at 291 K, followed by refinement of the conditions through variation of the precipitant concentration.

Data were collected in-house at 115 K using a MAR Research image plate and a 4.8 kW Rigaku rotating-anode generator producing Cu $K\alpha$ radiation of wavelength 1.54 Å. Crystals were soaked in the crystallization buffer with 12% glycerol added as a cryoprotectant before data collection. Data processing was performed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

© 2002 International Union of Crystallography
Printed in Denmark – all rights reserved

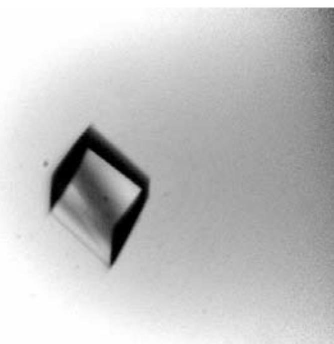

Figure 1
Photomicrograph of a prism-like crystal of HPhA; the dimensions of the crystal are approximately 0.1 × 0.08 × 0.04 mm.

3. Results and discussion

3.1. Crystallization

Several crystals were obtained from the 98 crystallization conditions tested, although most were very small needles that grew in clusters. Of these conditions, three in which prism-like crystals appeared were very promising. They all had the same pH of 4.6 with different precipitants: monomethyl ether 2K, PEG 4K and PEG 8K. It seems that pH 4.6 is optimum for the crystallization of this protein, since there were no good crystals observed with pH > 4.6. Crystals obtained using the three different precipitants had the same shape and were shown to have the same space group by X-ray diffraction. By further refinement through varying the concentrations of protein and precipitant, larger prism-like crystals which were reproducible and suitable for X-ray diffraction were obtained. The best crystals (Fig. 1) were obtained in 3 d in a hanging drop consisting of 1 µl of 15–20 mg ml^{-1} protein solution and 1 µl of reservoir solution comprising 100 m*M* NaAc buffer pH 4.6, 19% PEG 4000 and 200 m*M* $(NH_4)_2SO_4$. These crystals diffracted to 2.3 Å resolution and were stable on storage, as demonstrated by their X-ray diffraction to 2.3 Å resolution after storage for about 20 d at 291 K.

3.2. Data collection and preliminary X-ray crystallographic analysis

Diffraction data to 2.3 Å resolution were collected in-house from a HPhA crystal (Fig. 2). The crystal belonged to space group $P2_1$, with unit-cell parameters a = 34.99, b = 46.89, c = 35.02 Å, $\alpha = \gamma = 90$, $\beta = 104°$. Assuming two molecules in the asymmetric unit, the Matthews coefficient was 1.77 $Å^3$ Da^{-1} and the solvent content was about 35%. R_{merge} was 6.3% and the completeness was 98.1%. Diffraction data statistics are shown in Table 1.

Initial attempts to solve the crystal structure of HPhA were performed by molecular replacement. The archaeal histones HMfA and HMfB of *M. fervidus*, which show 62 and 58% sequence identity, respectively, served as the search models. Based on the single chain in the asymmetric unit, a dimer was generated by dyad symmetry. A molecular-replacement solution was obtained using the *CNS* package (Brünger *et al.*, 1998). After rigid-body refinement in the resolution range 30–3.5 Å, the *R* factor was 45%. Model building and refinement of the structure are in progress.

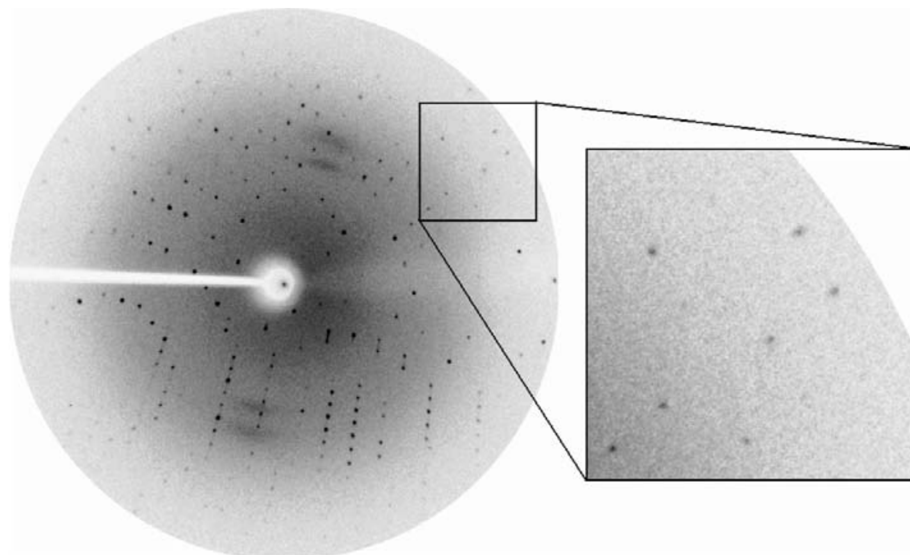

Figure 2
A representative image of the HPhA diffraction data; the maximum resolution at the edge of the image plate is 2.3 Å. The image on the right is an enlarged version of the selected square on the left.

Table 1
Diffraction data statistics..

Values in parentheses are for the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters	
a (Å)	34.99
b (Å)	46.89
c (Å)	35.02
$\alpha = \gamma$ (°)	90
β (°)	104
Wavelength (Å)	1.54
Resolution (Å)	99–2.3 (2.38–2.3)
Completeness (%)	98.1 (88.5)
Mean redundancy	7.2 (5.7)
No. of reflections	35632
Unique reflections	4932
$R_{merge}(I)$†	0.063 (0.185)
Mean $I/\sigma(I)$	16.1 (10.4)

† $R_{merge}(I) = \sum |I - \langle I \rangle| / \sum I$.

The authors are grateful to Feng Gao for help in data collection. This research was supported by the following grants: NSFC Nos. 39870174 and 39970155 and Project '973' Nos. G1999075602, G1999011902 and 1998051105.

References

Brünger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Decanniere, K., Babu, A. M., Sandman, K., Reeve, J. N. & Heinemann, U. (2000). *J. Mol. Biol.* **303**, 35–47.
Jancarik, J., Scott, W. G., Milligan, D. L., Koshland, D. E. Jr & Kim, S.-H. (1991). *J. Mol. Biol.* **221**, 31–34.
Kawarabayasi, Y. *et al.* (1998). *DNA Res.* **5**, 55–76.
Li, W. T., Grayling, R. A., Sandman, K., Edmondson, S., Shriver, J. W. & Reeve, J. N. (1998). *Biochemistry*, **37**, 10563–10572.
Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. (1997). *Nature (London)*, **389**, 251–260.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pereira, S. L., Grayling, R. A., Lurz, R. & Reeve, J. N. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 12633–12637.
Reeve, J. N., Sandman, K. & Daniels, C. J. (1997). *Cell*, **89**, 999–1002.
Sandman, K., Bailey, K. A., Pereira, S. L., Soares, D., Li, W. T. & Reeve, J. N. (2001). *Methods Enzymol.* **334**, 116–29.
Sandman, K., Krzycki, J. A., Dobrinski, B., Lurz, R. & Reeve, J. N. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 5788–5791.
Soares, D., Dahlke, I., Li, W. T., Sandman, K., Hethke, C., Thomm, M. & Reeve, J. N. (1998). *Extremophiles*, **2**, 75–81.
Starich, M. R., Sandman, K., Reeve, J. N. & Summers, M. F. (1996). *J. Mol. Biol.* **255**, 187–203.
Sullivan, S. A., Aravind, L., Makalowska, I., Baxevanis, A. D. & Landsman, D. (2000). *Nucleic Acids Res.* **28**, 320–322.