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Crystallization and preliminary X-ray diffraction data of the rat histone H1^0 globular domain

Takuto Matsui, Shinsuke Nishimura, Kenichi Kijima, Yasushi Kawasaki, Fumio Tashiro and Shigetoshi Miura*

Department of Biological Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan

Correspondence e-mail:
smiura@rs.noda.tus.ac.jp

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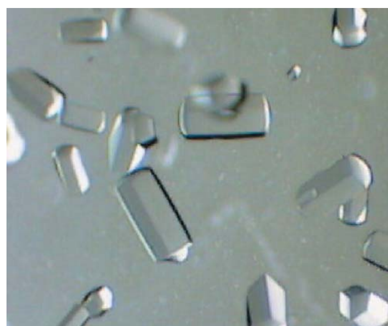
The linker histones H1 are a family of lysine-rich proteins that associate with the stretch of DNA that enters and exits the nucleosome. The linker histones facilitate the compaction and condensation of chromatin. The globular domain of histone H1^0, a specific subtype of histone H1, was crystallized at 288 K using the microbatch under silicone oil method with potassium phosphate as a precipitating agent. Diffraction data were collected to a resolution of 1.98 Å. The crystal belongs to the trigonal space group $P3_121$, with unit-cell parameters $a = 54.13$, $b = 54.13$, $c = 71.99$ Å, and contains one molecule per asymmetric unit. The V_M value and solvent content were calculated to be 3.04 Å^3 Da^{-1} and 59.6%, respectively.



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1. Introduction

In the eukaryotic cell, DNA is highly compacted and organized into chromatin. The proteins that are primarily responsible for the organized higher order structure of chromatin are the histones, which are basic small proteins named H2A, H2B, H3, H4 and H1 (Wolffe, 1998). Two copies of each of the histone proteins H2A, H2B, H3 and H4 are assembled into an octamer that has 146 base pairs (bp) of DNA wrapped around it to form a nucleosome core. The presence of a linker histone H1 in the nucleosome unit protects an additional ~20 bp of DNA against micrococcal nuclease digestion, which results in production of a particle, chromatosome, comprised of about 168 bp of DNA, a core histone octamer and one molecule of linker histone (Whitlock & Simpson, 1976; Noll & Kornberg, 1977; Allan *et al.*, 1980). The repeating nucleosome cores further assemble into higher order structures that are stabilized by the linker histone H1.

In higher organisms, the linker histone H1 forms a family of proteins with a conserved structure consisting of a central globular domain flanked by a long lysine-rich carboxyl-terminal domain and a much shorter amino-terminal domain (Allan *et al.*, 1980). Histones H1 have long been considered to be general repressors for transcription, since nucleosome-bound H1 stabilize the folded states of chromatin (Weintraub, 1984). A specific H1 subtype, H1^0, has been shown to accumulate in many terminally differentiated cells and is believed to contribute to cell differentiation (Zlatanova & Doenecke, 1994). Recently, other aspects of the role of linker histones in regulated transcription, DNA repair and apoptosis have been reported, suggesting a rather active and dynamic interaction between DNA and linker histones (Liang *et al.*, 2005; Ju *et al.*, 2006; Konishi *et al.*, 2003). Fluorescent recovery after photobleaching (FRAP) has been successfully employed to demonstrate the dynamic nature of the binding between linker histones and chromatin in live cells (Lever *et al.*, 2000; Misteli *et al.*, 2000). The basic residues contributing to nucleosomal binding are mapped onto the atomic model of the globular domain of H1^0 modelled from the crystal structure of the globular domain of an avian linker histone H5 that has 72% sequence identity to the rat linker histone H1^0 (Brown *et al.*, 2006; Ramakrishnan *et al.*, 1993). Interpretation of the mutagenesis data largely depends on the structural information on GH5.

In this paper, we report the purification, crystallization and preliminary crystallographic analysis of the globular domain of the rat linker histone $H1^0$ ($GH1^0$), which is composed of 92 amino-acid residues with a molecular weight of 9.9 kDa. Its crystal structure may further promote our understanding of the role of histone H1 in the compaction and organization of chromatin.

2. Protein expression and purification

The gene coding for the globular domain of histone $H1^0$ ($GH1^0$; Ala18–Phe107) was prepared by amplifying the corresponding region of the full-length rat cDNA (accession No. NM_012578) obtained using RT-PCR from the total RNA of K2 cells (Nakamura *et al.*, 1999). The primers used for PCR amplification of the DNA fragment corresponding to $GH1^0$ were 5′-GCGGGGATCCGCCGCCAA-GAAGTC-3′ and 5′-GCCGAATTCAGAAAGCCACCGACCTC-TTG-3′. These primers incorporated an additional *Bam*HI site at the 5′-end and an *Eco*RI site at the 3′-end of the resulting PCR product. After digestion with *Bam*HI and *Eco*RI, the PCR fragment was inserted into pGEX-2T (Amersham Biosciences). The nucleotide sequence of the insert was checked with the original sequence to ascertain whether there were any errors. $GH1^0$ was prepared from bacterial strain BL21 harbouring pGEX-$GH1^0$, which expresses the globular domain of histone $H1^0$ as a glutathione *S*-transferase (GST) fusion protein. The bacterial cells were cultured in M9 medium and expression of the fusion protein was induced with 0.5 m*M* IPTG at 298 K for 16 h. Harvested and pelleted cells were suspended in a sonication buffer consisting of 50 m*M* Tris–HCl pH 7.4 containing 1 m*M* EDTA and 1 m*M* PMSF. After sonication and centrifugation at 21 000*g* for 20 min at 277 K, NaCl was added to the recovered supernatant to 0.5 *M* with stirring in an ice bath for 20 min. The mixture was centrifuged at 80 000*g* for 90 min at 277 K. The supernatant was applied onto a glutathione Sepharose column (Amersham Biosciences) equilibrated with 50 m*M* Tris–HCl pH 7.4 containing 0.5 m*M* EDTA, 0.1 *M* NaCl and 1 m*M* PMSF. After the column had been washed with the same buffer, the fusion protein was eluted with buffer containing 10 m*M* glutathione (reduced form). Thrombin digestion was carried out in 50 m*M* Tris–HCl pH 8.0 containing 0.1 *M* NaCl and 2.5 m*M* $CaCl_2$ at 277 K for 4 h. The $GH1^0$ resulting from thrombin digestion retained an additional two amino acids, Gly-Ser, at the N-terminus of the protein. The digested products were charged onto a cation-exchange column (CMFF, Amersham Biosciences) equilibrated with 50 m*M* Tris–HCl pH 8.0 containing 0.1 *M* NaCl and eluted by applying a linear gradient of NaCl. Combined fractions containing $GH1^0$ were further applied onto a gel-filtration column (Superdex75 prep-grade, Amersham Biosciences) equilibrated with 50 m*M* Tris–HCl pH 7.8 containing 0.3 *M* KCl. Protein concentrations were estimated using a BCA protein-assay kit (Pierce) with BSA as a standard.

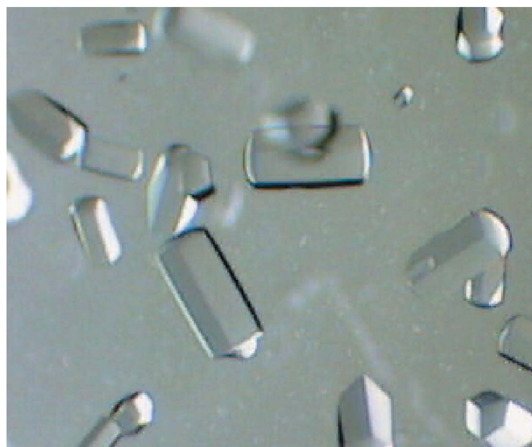

Figure 1
Crystal of rat histone $H1^0$ globular domain obtained in a droplet containing 2.0 *M* potassium phosphate pH 9.0. The crystals reach typical dimensions of 0.15 × 0.15 × 0.3 mm.

Table 1
Data-collection statistics for a $GH1^0$ crystal.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.54
Temperature (K)	100
Resolution (Å)	47.17–1.98 (2.11–1.98)
Space group	$P3_121$
Unit-cell parameters (Å)	$a = b = 54.13$, $c = 71.99$
No. of molecules per ASU	1
V_M (Å^3 Da^{-1})	3.04
Total observations	90029 (7142)
Independent reflections	8538 (1418)
Completeness (%)	95.42 (89.75)
Redundancy	10.54 (5.04)
Average $I/\sigma(I)$	6.79 (2.32)
R_{sym}† (%)	10.8 (22.5)

† $R_{sym} = \sum_{\mathbf{h}} \sum_l |I_{\mathbf{h}l} - \langle I_{\mathbf{h}} \rangle| / \sum_{\mathbf{h}} \sum_l \langle I_{\mathbf{h}} \rangle$, where I_l is the lth observation of reflection **h** and $\langle I_{\mathbf{h}} \rangle$ is the weighted average intensity for all observations I of reflection **h**.

3. Crystallization

For crystallization experiments, a solution of $GH1^0$ in 5 m*M* Tris–HCl pH 7.5 with a concentration in the range 16–19 mg ml^{-1} was used. Crystallization was performed using the microbatch under silicone oil method at 288 K (D'Arcy *et al.*, 1996). Drops containing 3 µl protein solution and 3 µl precipitant solution were placed under 100 µl silicon oil in 96-well plates. The vapour-diffusion rate was controlled by changing the mixing ratio of vapour-permeable and nonpermeable silicone oils. The mixing ratios were varied from 60:40 to 20:80 and the best results were obtained using a 50:50 ratio. The initial crystallization conditions were screened using Crystal Screen I (Hampton Research) and potassium phosphate buffers at a range of phosphate concentrations (1.0, 1.5, 2.0, 2.5 *M*) and pH values (6.5, 7.0, 7.5, 8.0). Several crystals were obtained using potassium phosphate buffers at pH 8.0 with the protein solution at a concentration of 18 mg ml^{-1}. To optimize the conditions in order to obtain crystals suitable for X-ray diffraction, conditions with potassium phosphate at 1.5, 2.0 and 2.5 *M* and pH 8.0–9.0 were surveyed. The crystals appeared within 10 d and grew to approximate dimensions of 0.15 × 0.15 × 0.3 mm at 2.0 *M* potassium phosphate pH 9.0 (Fig. 1).

4. X-ray analysis

Crystals of $GH1^0$ were picked up from a 96-well plate in a mounted cryo-loop (Hampton Research), dipped into a droplet of Paratone-N (Hampton Research) and immediately flash-cooled in a cold nitrogen-gas stream at 100 K. X-ray diffraction images of the crystal were collected at 100 K in the nitrogen-gas stream using Montel mirror optics and a Proteum-R CCD detector and Cu $K\alpha$ radiation generated by a MacScience M18XHF rotating-anode generator. Diffraction data for the crystals were collected to a resolution of 1.98 Å and were integrated using *SAINT-Plus* and scaled using *PROSCALE* software according to the manuals provided by the manufacturer (Bruker Co., Germany). Autoindexing of the diffrac-

tion data showed that the crystals belong to a hexagonal or trigonal space group, with unit-cell parameters a = 54.13, b = 54.13, c = 71.99 Å, α = 90, β = 90, γ = 120°. The systematic extinctions and symmetry of the diffraction data indicate that the space group of the crystals corresponds to $P3_121$ or $P3_221$ (trigonal). The preliminary X-ray crystallographic statistics of GH1^0 are summarized in Table 1.

Based on the molecular weight of 9.9 kDa, the V_M value is calculated to be (3.04/n) Å^3 Da^{-1}, where n is the number of molecules in the asymmetric unit. The statistics of the reported crystals suggest that the value of n is most likely to be 1 (Matthews, 1968; Kantardjieff & Rupp, 2003). Assuming one molecule of GH1^0 to be present per asymmetric unit, the solvent content was calculated to be 59.6%. The structure of GH1^0 was successfully solved by the molecular-replacement method using the program *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994) with the atomic coordinates of the globular domain of chicken histone H5 (PDB code 1hst; Ramakrishnan *et al.*, 1993) as a search model. The interpretable electron-density maps unequivocally indicate the correct space group of the crystal to be $P3_121$. A full description of the structure determination will be published elsewhere.

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