

Crystallization and preliminary crystallographic studies on the chromo shadow domain (CSD) of mouse heterochromatin protein M31

Yu Gao,^{a,b} Yi Ding,^a Prim B. Singh^c and Zihe Rao^{a*}

^aLaboratory of Structural Biology and MOE Laboratory of Protein Science School of Life Science and Engineering, Tsinghua University, Beijing 100084, People's Republic of China,

^bInstitute of Basic Medical Sciences, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100005, People's Republic of China, and ^cNuclear Reprogramming Laboratory, Division of Gene Expression and Development, Roslin Institute (Edinburgh), Midlothian EH25 9PS, Scotland

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

Members of the heterochromatin protein 1 (HP1) class of non-histone chromosomal proteins are components of heterochromatin and are involved in the epigenetic regulation of the genome. HP1 proteins are modular and consist of two sequence-related domains called the chromodomain (CD) and the chromo shadow domain (CSD). In order to investigate the role of the murine HP1-like protein M31 in heterochromatin formation and gene silencing, recombinant CSD was overexpressed in *Escherichia coli* and crystallized using the hanging-drop vapour-diffusion method with PEG 4000 as precipitant. Diffraction data to 2.9 Å were collected from a native crystal belonging to space group $C222_1$, with unit-cell parameters $a = 60.0$, $b = 95.6$, $c = 91.7$ Å, $\alpha = \beta = \gamma = 90^\circ$.

Received 21 December 2001

Accepted 2 April 2002

1. Introduction

The highly conserved heterochromatin protein (HP1) class of non-histone chromosomal proteins are constituents of chromosomal complexes involved in a variety of functions, including transcriptional repression and re-assembly of the nucleus at the end of mitosis (Jones *et al.*, 2000). There are three HP1 isotypes in mammals: HP1 α , HP1 β (also known as MoMOD1 or M31) and HP1 γ (also known as MoMOD2 or M32) (Singh *et al.*, 1991; Saunders *et al.*, 1993). HP1 proteins consist of two sequence-related domains: the N-terminal CD and C-terminal CSD (Aasland & Stewart, 1995). These two domains have probably arisen by gene duplication and are separated by a 'hinge' region, which is supposedly flexible and surface exposed (Ball *et al.*, 1997). The solution structures of the HP1 β CD and CSD have been recently determined by NMR spectroscopy (Ball *et al.*, 1997; Brasher *et al.*, 2000); the X-ray crystal structure of the CSD from *Schizosaccharomyces pombe* HP1-like protein, swi6p, has also been determined (Cowieson *et al.*, 2000). The structure of the whole HP1 molecule has not yet been solved. Thus, it remains unclear whether the CD and the CSD are constrained by long-range interactions and whether the 'hinge' is indeed as featureless and as flexible as is currently thought.

The CD and the CSD each consist of an antiparallel three-stranded β -sheet packed against one ($\alpha 2$) or two ($\alpha 1$, $\alpha 2$) α -helices, respectively (Ball *et al.*, 1997; Brasher *et al.*, 2000; Cowieson *et al.*, 2000). Conserved non-polar residues provide the backbone of this

characteristic fold and form a hydrophobic groove on one side of the β -sheet. Interestingly, this groove is occluded by neighbouring residues in the CSD, but is relatively open in the CD, providing sites for protein-protein interactions. Apart from their similarities, the CD and the CSD also have unique features. Helix $\alpha 1$ (which, together with helix $\alpha 2$, forms part of the CSD) is absent in the CD (Brasher *et al.*, 2000; Cowieson *et al.*, 2000). The CSD, at least in its recombinant non-modified form, dimerizes in solution, whereas the CD remains monomeric under the same conditions. The CSD dimer interface centres on helix $\alpha 2$, which interacts symmetrically and at an angle of 35° with helix $\alpha 2$ of a neighbouring subunit and forms a 'non-polar' pit that can accommodate pentapeptides with the consensus-sequence motif PxVxL (Smothers & Henikoff, 2000). The CD, but not the CSD, has also been reported to interact with the N-terminal tail of histone H3 when the latter is methylated at a specific lysine residue (Lys9) (Bannister *et al.*, 2001; Lachner *et al.*, 2001). Almost all other interactions between HP1 and nuclear proteins have been mapped in the CSD and result from the binding of pentapeptide motif-containing proteins to the 'non-polar' pit, a unique feature of the CSD as explained above (Jones *et al.*, 2000).

In this short report, we explore further the structure of the mammalian M31 (HP1 β) protein and have for the first time crystallized its CSD. Diffraction data have been collected to a resolution of 2.9 Å from a native crystal belonging to space group $C222_1$, with unit-cell parameters $a = 60.0$, $b = 95.6$, $c = 91.7$ Å, $\alpha = \beta = \gamma = 90^\circ$.

2. Materials and methods

2.1. Expression and purification

A construct corresponding to the M31 CSD (residues 104–175) was generated by PCR and subcloned into the PGEX-4T-1 expression vector. Expression of the GST-CSD fusion in *E. coli* strain BL21 (DE3) was induced with 1 mM IPTG. Expressing *E. coli* cells were harvested, resuspended in buffer A (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM K₂HPO₄ pH 7.3) and sonicated. After centrifugation at 15 000 rev min⁻¹ for 30 min at 277 K, the supernatant was subjected to chromatography on a glutathione Sepharose 4B disposable column (Amersham Pharmacia Biotech, USA) equilibrated with ten bed volumes of buffer A. The column was washed with 30 bed volumes of buffer A and thrombin protease was added to a final concentration of 30 U per milligram of fusion protein in order to remove GST from the fusion protein. After incubation at 289 K for 16 h, eluate containing the CSD was collected and concentrated. Final purification of the protein was performed by gel filtration on a Superdex75 HR 10/30 (Amersham Pharmacia, USA) previously equilibrated with buffer B (10 mM Tris-HCl pH 8.0 containing 50 mM NaCl). The purity of CSD was greater than 95% by SDS-PAGE analysis.

2.2. Crystallization

The M31 CSD was concentrated to a protein concentration of ~20 mg ml⁻¹ in 50 mM NaCl and 10 mM Tris-HCl pH 8.0.

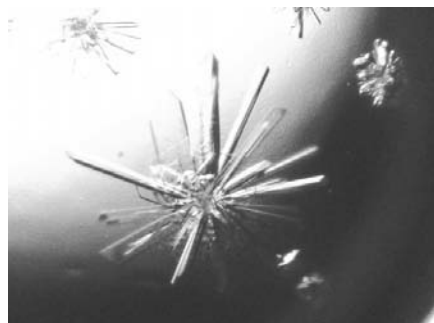


Figure 1
Crystal of M31 CSD. Crystal dimensions are 0.3 × 0.1 × 0.1 mm.

Initial screening was performed at 291 K by the hanging-drop vapour-diffusion method using sparse-matrix (Jancarik & Kim, 1991) screen kits from Hampton Research (Crystal Screens I and II). Each drop contained 1 µl of protein and 1 µl of reservoir solution.

Initial trials yielded needles which were often observed to be aggregated into large clusters. The crystallization trials were optimized with different pHs and buffers to yield rod-like crystals in 1–2 weeks at 291 K (Fig. 1). The optimized reservoir solution was 30% PEG 4000, 0.2 M sodium acetate, 0.1 M Tris-HCl pH 8.5.

2.3. Data collection and processing

Crystals were picked up using a fibre loop and flash-frozen in a stream of cold nitrogen gas. Data were collected at ~110 K on a MAR345 image-plate detector using Cu Kα radiation from an in-house Rigaku rotating-anode X-ray generator operating at 48 kV

Table 1

Data-collection and processing statistics.

Values in parentheses correspond to the highest resolution shell.	
Space group	C222 ₁
Unit-cell parameters (Å, °)	$a = 60.0, b = 95.6,$ $c = 91.7,$ $\alpha = \beta = \gamma = 90$
Completeness (%)	98.4 (97.2)
No. of unique reflections	5851
Mean redundancy	6.1
R_{merge} (%)	10.9 (36.6)
$I/\sigma(I)$ (highest shell)	12.5
No. of molecules per asymmetric unit	4
Matthews coefficient	2.07
Solvent content (%)	45

and 98 mA ($\lambda = 1.5418 \text{ \AA}$). The crystal-to-detector distance was 170 mm. The crystals diffracted to 2.9 Å and a data set was collected from a single crystal (Fig. 2). Each frame was exposed for 90 s and oscillated through 1.5°. All diffraction data were integrated and scaled using the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997) (Table 1).

We are grateful to Bin Geng and Qiang Zhao for technical assistance and to Feng Gao for assistance with data collection. We thank Zhilong Wang and Yanhui Xu for valuable discussion. The project was supported by the following grants: Wellcome Trust No. 062405/Z/00Z, NSFC Nos. 39870174 and 39970155, Project '863' No. 2001AA233011 and Project '973' Nos. G1999075602, G1999011902 and 1998051105.

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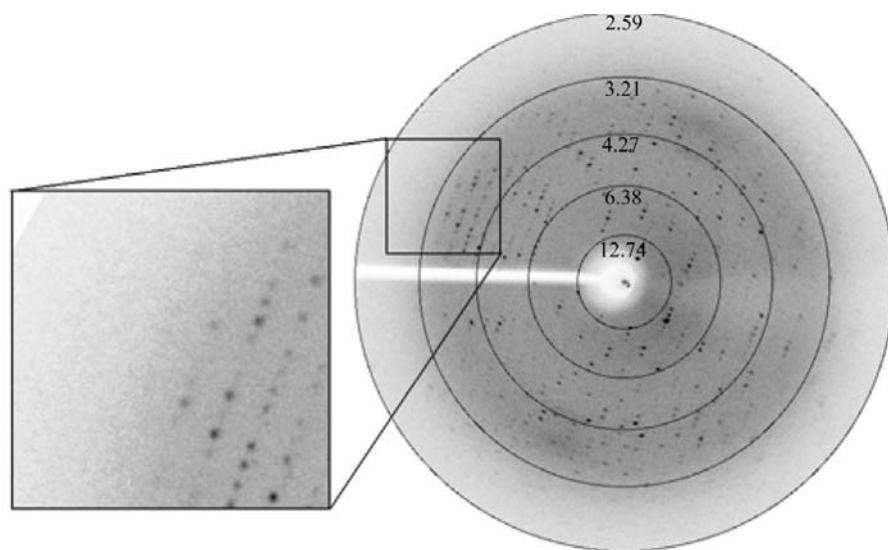


Figure 2
X-ray diffraction image from a M31 CSD crystal. A 1.5° oscillation image taken from a crystal of M31 CSD at 110 K. This image was collected in-house on a MAR345 detector. The resolution is 2.5 Å at the edge of the plates. The enlargement shows the spots at the highest resolution.

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