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Crystallization and preliminary X-ray analysis of wild-type and V103L mutant Myb R2 DNA-binding domain

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The R2 subdomain of the mouse c-Myb DNA-binding domain and its V103L mutant have been crystallized by the vapour-diffusion method using highly concentrated sodium citrate at pH 6.8 as a precipitant. Using ammonium sulfate as precipitant in MES buffer only produced crystals for the mutant R2. All crystals are isomorphous and belong to space group $P2_12_12_1$. The unit-cell dimensions for wild-type R2 crystals grown from sodium citrate precipitant are $a = 28.83$, $b = 40.18$, $c = 49.23$ Å. Crystals contain one R2 molecule per asymmetric unit. They are stable during 3 d exposure to X-rays and diffract to 1.37–1.45 Å resolution.

1. Introduction

c-Myb, a protooncogene product, is a transcriptional regulatory protein (Graf, 1992; Lüscher & Eisenman, 1990). It contains three functional domains responsible for DNA binding, transcriptional activation and negative regulation of DNA binding (Sakura *et al.*, 1989). The DNA-binding domain consists of three homologous tandem subdomains (R1, R2 and R3) of 51–52 amino-acid residues, each of which has a very similar architecture of folding and core region, and each of which has a motif related to the helix–turn–helix structures in the prokaryotic repressors or in the eukaryotic homeodomains (Ogata *et al.*, 1992, 1994, 1995). However, R2 is much less stable (melting temperature $T_m = 316$ K) than R1 and R3 (T_m of 334 and 330 K, respectively) in the DNA-free state (Sarai *et al.*, 1993). Comparison of the solution structures of R1, R2 and R3 has revealed the presence of a cavity inside the hydrophobic core of R2 which is adjacent to a valine residue at position 103, and it has been suggested that this cavity is responsible for the lower thermal stability of R2 (Ogata *et al.*, 1996). Substitution of the valine residue at position 103 by leucine (with a bulkier side chain) resulted in the reduction of the cavity volume and a significant increase in stability (T_m = 339 K). However, the DNA-binding activity and the *trans*-activating capacity are reduced by the V103L mutation, indicating the importance of the cavity in the hydrophobic core and the conformational flexibility of R2 in the binding of DNA (Ogata *et al.*, 1996). Thus, R2 may play an important role in the subtle regulation of specific recognition of target sequences. A detailed comparative analysis of high-resolution structures of the wild type and in the V103L mutant R2 not only may aid in understanding the relation between the cavity in the hydrophobic core and the thermal stability of the protein (Eriksson *et al.*, 1992), but may also suggest a structural basis for the reduction of the DNA-binding activity of R2 by the V103L mutation. Here, we report the crystallization of the wild-type and the V103L mutant Myb R2.

2. Experimental

2.1. Peptide syntheses

The R2 subdomain (residues 90–141) of mouse c-Myb protein and its V103L mutant were synthesized by the usual solid-phase method using an FMOC strategy with a Shimadzu-PSSM8 peptide synthesizer. The linker-functionalized resin TGS-CHA, purchased from Shimadzu, was used for the synthesis in order to provide the peptides with amide-type C-termini. The elongated peptides were cleaved from the resin with a trifluoroacetic acid solution containing several cation scavengers and then purified by reversed-phase HPLC. Analysis by electrospray ionization mass spectroscopy showed the correct products.

2.2. Crystallization

Crystallization trials were conducted at 297 K in 24-well plates using the sitting-drop vapour-diffusion method. Sets of screening conditions [Crystal Screen I and Crystal Screen II, as described by Jancarik & Kim (1991) and by Cudney *et al.* (1994)] were supplied by Hampton Research and used for initial screening. Drops consisting of 1 µl protein solution (10 mg ml^{-1} Myb R2 and 10 m*M* dithiothreitol, pH adjusted to 6.8 with KOH) and 1 µl reservoir solution were equilibrated against 0.5 ml of reservoir solution. No crystals

Table 1
Crystal parameters and data-collection statistics.

Crystal type	Myb $R2_{V103\text{-}SC}$	Myb $R2_{L103\text{-}SC}$	Myb $R2_{L103\text{-}AS}$
a (Å)	28.83	28.91	28.95
b (Å)	40.18	40.16	40.11
c (Å)	49.23	49.35	48.81
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Solvent content (%)	45.9	45.9	45.3
Number of frames/crystals	180/1	221/1	177/1
Oscillation range (°)	2	2	1.5
Scan speed (° min^{-1})	0.5	0.5	0.5
Crystal-to-detector distance (mm)	70	70	60
Observations ($I \geq \sigma_I$)	91470	114461	90490
Unique reflections ($I \geq \sigma_I$)	9268	9394	11133
Mosaicity (°)	0.18	0.19	0.21

Myb $R2_{V103\text{-}SC}$.

Resolution (Å)	$\langle I\rangle/\langle\sigma_I\rangle$	R_{merge}† (%)	Completeness (%)
15.00–3.24	68.6	0.039	99.8
3.24–2.58	54.5	0.056	99.8
2.58–2.25	42.7	0.069	99.9
2.25–2.05	35.2	0.084	99.7
2.05–1.90	25.0	0.106	99.8
1.90–1.79	14.6	0.152	99.9
1.79–1.70	10.7	0.191	99.9
1.70–1.63	7.0	0.257	99.8
1.63–1.56	4.7	0.318	97.2
1.56–1.51	3.1	0.372	93.3
15.00–1.51	38.9	0.059	98.7

Myb $R2_{L103\text{-}SC}$.

Resolution (Å)	$\langle I\rangle/\langle\sigma_I\rangle$	R_{merge}† (%)	Completeness (%)
15.00–3.24	91.0	0.028	99.6
3.24–2.58	80.6	0.039	99.9
2.58–2.25	69.2	0.047	100.0
2.25–2.05	58.7	0.054	99.9
2.05–1.90	44.5	0.068	99.9
1.90–1.79	30.0	0.089	99.8
1.79–1.70	21.9	0.109	99.8
1.70–1.63	14.5	0.142	99.0
1.63–1.56	10.3	0.187	98.5
1.56–1.51	7.6	0.240	97.3
15.00–1.51	64.0	0.040	99.4

Myb $R2_{L103\text{-}AS}$.

Resolution (Å)	$\langle I\rangle/\langle\sigma_I\rangle$	R_{merge}† (%)	Completeness (%)
15.00–3.03	47.8	0.048	98.2
3.03–2.41	46.6	0.065	99.2
2.41–2.10	37.1	0.075	98.7
2.10–1.91	33.7	0.081	98.7
1.91–1.78	25.3	0.094	98.6
1.78–1.67	21.0	0.105	98.4
1.67–1.59	15.7	0.121	97.4
1.59–1.52	12.1	0.144	95.4
1.52–1.46	8.8	0.179	94.6
1.46–1.41	6.0	0.249	92.5
15.0–1.41	37.6	0.064	97.2

† $R_{merge} = \sum|I_j - \langle I_j\rangle|/\sum\langle I_j\rangle$, where I_j is the intensity of reflection j and $\langle I_j\rangle$ is the average intensity of reflection j.

appeared in two months. Several grid-screen solutions with increased salt or PEG concentrations at pH values of 6, 6.5, 7 and 7.5 were prepared and tested in a similar manner as for Crystal Screen I and Crystal Screen II. Perfectly shaped single crystals appeared within one week using grid screens containing sodium citrate or ammonium sulfate. To produce larger crystals, the successful conditions were adjusted and the concentration of protein was increased from 10 to 25 mg ml^{-1}. All the crystals grew in the form of square pyramids. The wild-type Myb R2 crystals (Myb $R2_{V103\text{-}SC}$) only grew from 1.65 *M* sodium citrate at pH 6.8, to a maximum size of 0.3 mm, while the V103L mutant crystals of Myb R2 grew to a maximum size of 0.6 mm from two conditions, the first containing 3.15 *M* ammonium sulfate in 0.1 *M* MES buffer at pH 6.8 (Myb $R2_{L103\text{-}AS}$) and the second containing 1.85 *M* sodium citrate at pH 6.8 (Myb $R2_{L103\text{-}SC}$).

2.3. Data collection

The diffraction data sets were collected on a MacScience machine with a DIP2030 imaging plate, using Cu $K\alpha$ radiation filtered using a 0.15 mm nickel filter and focused using a double mirror. X-rays were generated using a rotating-anode generator with a focus size of 0.2 × 2 mm (collimator 0.3 mm), which was powered by an M06XHF[22]-Fine generator operated at 50 kV and 50 mA. In order to reduce the air absorption, flowing helium gas was used in the mirrors. Crystals were mounted in a glass capillary with an arbitrary orientation, and data were collected at two settings for each crystal: the first oscillation range was 180°, while the second oscillation range was limited by the lifetime of the crystals. All intensity data were indexed, integrated and scaled with *DENZO* and *SCALEPACK* (Otwinowski, 1986). Further details of the data-collection procedure, crystal parameters and data-processing statistics are summarized in Table 1.

3. Results and discussion

All the crystals appear to be well diffracting and stable to X-rays for 3–4 d. Myb $R2_{V103\text{-}SC}$ and Myb $R2_{L103\text{-}SC}$ crystals diffract to 1.45 Å, while the recorded resolution of Myb $R2_{L103\text{-}AS}$ crystals is at least 1.37 Å and is limited by the geometry of diffractometer, *i.e.* the flat imaging plate and the crystal-to-detector distance. All three crystals are orthorhombic with space group $P2_12_12_1$. The unit-cell parameters are very similar except for the c axis in Myb $R2_{L103\text{-}AS}$, which is 0.54 Å shorter than in Myb $R2_{L103\text{-}SC}$ and may be the result of crosslinking of adjacent protein molecules by sulfate ions in Myb $R2_{L103\text{-}AS}$. The solvent-content calculations (Matthews, 1968; Table 1) show that only one molecule of Myb R2 can be located in the asymmetric unit of each crystal. The high-resolution diffraction data obtained, with its high completeness and redundancy, may reveal in detail the differences caused by the V103L mutation and explain the difference in thermostability between the wild-type and the V103L mutant Myb R2. Crystal structure determination by the

molecular-replacement method using a solution structure of Myb R2 (Ogata *et al.*, 1995) as a search model is in progress.

Note added in proof: High-resolution crystals of three further R2 mutants, V103I, V103Abu and V103Nva, were obtained under conditions similar to those described above.

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