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Crystallization and preliminary X-ray crystallographic analysis of full-length yeast tropomyosin 2 from *Saccharomyces cerevisiae*

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Tropomyosin is a highly conserved actin-binding protein that is found in most eukaryotic cells. It is critical for actin-filament stabilization and for cooperative regulation of many actin functions. Detailed structural information on tropomyosin is very important in order to understand the mechanisms of its action. Whereas structures of isolated tropomyosin fragments have been obtained at high resolution, the atomic structure of the entire tropomyosin molecule is still unknown. Here, the crystallization and preliminary crystallographic analysis of full-length yeast tropomyosin 2 (yTm2) from *Saccharomyces cerevisiae* are reported. Recombinant yTm2 expressed in *Escherichia coli* was crystallized using the hanging-drop vapour-diffusion method. The crystals belonged to space group $C2$, with unit-cell parameters $a = 154.8$, $b = 49.9$, $c = 104.0$ Å, $\alpha = \gamma = 90.0$, $\beta = 124.0^\circ$ and two molecules in the asymmetric unit. A complete native X-ray diffraction data set was collected to 3.5 Å resolution using synchrotron radiation.

1. Introduction

Tropomyosin is a highly conserved actin-binding protein that is present in most eukaryotic cells. It is an α-helical coiled-coil dimer that forms a head-to-tail polymer along the length of the actin filament.

The most studied role of tropomyosin is in vertebrate skeletal and cardiac muscles, where it is involved in the regulation of muscle contraction through interactions with troponin and actin (reviewed by Brown & Cohen, 2005).

Saccharomyces cerevisiae has two tropomyosin isoforms, yTm1 and yTm2, which are encoded by two separate genes, *TPM1* and *TPM2*, with yTm1 being about six times more abundant than yTm2 (Liu & Bretscher, 1989; Drees *et al.*, 1995). The yeast tropomyosins are the shortest to be identified to date, comprising 199 and 161 amino acids, respectively, equivalent to five and four actin-binding sites. All other tropomyosin isoforms currently identified have either 248 or 284 residues, spanning six or seven actin molecules, respectively.

Nonmuscle tropomyosin is believed to play an important role in stabilizing the actin cytoskeleton (Gunning *et al.*, 2005). Previous studies have shown that the simultaneous deletion of both tropomyosin genes in *S. cerevisiae* results in lethality, whereas disruption of the *TPM1* gene alone results in a temperature-sensitive phenotype and the disappearance of actin cables. This phenotype can be partially rescued by overexpression of the *TPM2* gene. Loss of yTm2 has no detectable phenotype (Drees *et al.*, 1995). Thus, the two proteins participate in the performance of some essential function in yeast. However, they seem to be functionally distinct.

It is widely believed that tropomyosin adopts a flexible rod-like structure and that this flexibility plays a crucial role in its functions. This high flexibility makes crystallization of full-length tropomyosin an extremely difficult task. Full-length tropomyosin formed crystals that diffracted X-rays poorly (Phillips *et al.*, 1986; Whitby *et al.*, 1992) and the structure has only been obtained at 7 Å resolution at best (Whitby & Phillips, 2000). High-resolution structures have only been obtained for isolated fragments of muscle tropomyosin stabilized either by a GCN4 leucine-zipper extension or by disulfide bonds between cysteine residues introduced at the end of the coiled coil

(Greenfield *et al.*, 1998, 2003, 2006; Brown *et al.*, 2001, 2005; Li *et al.*, 2002; Nitanai *et al.*, 2007).

In the atomic structure of an N-terminal fragment comprising 81 residues, specific regions have been identified in which consecutive alanine or serine residues are clustered at the hydrophobic core of the coiled coil. At the boundary of the alanine cluster, two paired chains are staggered with respect to each other, giving rise to a bend in the tropomyosin coiled coil (Brown *et al.*, 2001). These regions could provide the flexibility in the tropomyosin strand that is required for winding around the actin filament as well as for the regulation of muscle contraction. Neither yTm1 nor yTm2 contain such alanine clusters. Unlike muscle tropomyosins, the coiled coils of the tropomyosin from *S. cerevisiae* are probably not continuous. They seem to be interrupted at one position in yTm2 and two positions in yTm1; the phase of the heptad repeats shifts owing to a four-residue deletion (Strand *et al.*, 2001). It was suggested that these shifts might induce the bends in the yeast tropomyosin molecules. Thus, judging from the amino-acid sequence, the structure of yeast tropomyosin may differ from the structure of muscle tropomyosin.

To date, only the structure of muscle tropomyosin has been investigated intensively. Structural information on nonmuscle tropomyosin still remains completely unknown. In this paper, we report the crystallization and preliminary X-ray crystallographic analysis of full-length nonmuscle yeast tropomyosin 2 from *S. cerevisiae*.

2. Experimental procedures and results

2.1. Expression and purification

The yTm2 construct in pJC20 vector (Maytum *et al.*, 2001) was transformed into competent *Escherichia coli* cells [strain BL21 (DE3) pLys S] and expressed and purified as described previously (Maytum *et al.*, 2000) with modifications. Briefly, a 2 l culture was grown at 310 K to late exponential phase in Luria–Bertani medium, induced with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside and grown at 310 K for a further 4 h. The cell pellet was suspended in lysis buffer consisting of 20 m*M* Tris–HCl pH 7.4, 100 m*M* NaCl, 2 m*M* EDTA, lysed by sonication and heated at 353 K for 15 min. Precipitated proteins and cell debris were removed by centrifugation. Tropomyosin was then isoelectrically precipitated from the supernatant at pH 4.5. The precipitate was pelleted, resuspended in 40–50 ml running buffer (20 m*M* potassium phosphate pH 7.0, 100 m*M* NaCl) and applied onto a MonoQ HR column (GE Healthcare). The column was washed with a linear gradient of NaCl from 0.1 to 0.4 *M* in 20 m*M* potassium phosphate pH 7.0. The primary product containing the target protein was then applied onto CHT Ceramic Hydroxyapatite Type I (Bio-Rad) equilibrated with 10 m*M* potassium phosphate pH 7.0, 300 m*M* NaCl. The protein was eluted with a linear gradient of 10–500 m*M* potassium phosphate pH 7.0. Finally, tropomyosin was further purified on a HiTrap Phenyl HP column (GE Healthcare) with a linear gradient of 1.7–0.8 *M* ammonium sulfate in 10 m*M* Tris–HCl pH 7.0. The purity of the protein was examined by SDS–PAGE with Coomassie Brilliant Blue staining. Purified protein was dialyzed extensively against 10 m*M* Tris–HCl pH 7.0 and concentrated to 5–10 mg ml^{-1} using Centricon YM-10 (Millipore Corporation).

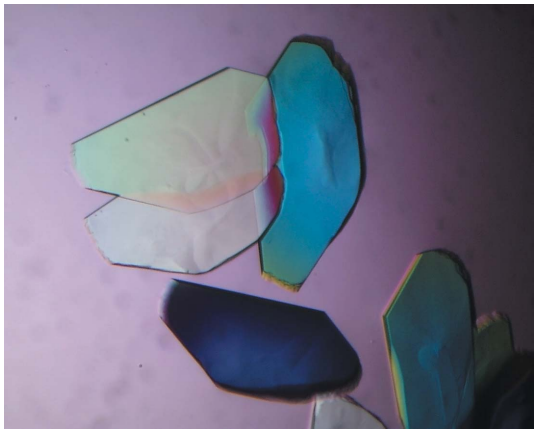

Figure 1
Crystals of yeast tropomyosin 2 from *S. cerevisiae* with dimensions of 0.6 × 0.2 × 0.02 mm.

Table 1
Diffraction data statistics for the native crystal of yTm2.

Values in parentheses are for the highest resolution shell.

Space group	*C*2
Unit-cell parameters (Å, °)	a = 154.8, b = 49.9, c = 104.0, $\alpha = \gamma$ = 90.0, β = 124.0
Wavelength (Å)	1.0000
Resolution (Å)	50–3.5 (3.63–3.5)
Completeness (%)	100 (89.64)
Total reflections	50357
Unique reflections	13885
Redundancy	3.7 (3.4)
$R_{\rm merge}(I)$† (%)	6.2 (24.5)
Mean $I/\sigma(I)$	14.1 (4.2)

† $R_{\rm merge}(I) = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl.

2.2. Crystallization

Initial crystallization experiments were performed using the hanging-drop vapour-diffusion method at three different temperatures (278, 286 and 293 K) in VDX plates (Hampton Research). The crystallization screening kits Crystal Screen, Crystal Screen 2 and Crystal Screen Cryo (Hampton Research) were used as initial screening conditions. 2 µl protein solution was mixed with an equal volume of precipitant solution and equilibrated against a 1 ml reservoir. Rod-like crystals of yeast tropomyosin 2 were obtained in Crystal Screen 2 condition No. 4 [35%(*v*/*v*) dioxane] at 286 K. The crystals diffracted to a maximum resolution of only 10 Å. To improve the crystal quality, the initial crystallization condition was further optimized by varying the additives and the concentrations of precipitant and protein. We found that in the presence of taurine yTm2 gave crystals of a completely different shape (Fig. 1). These crystals diffracted to 3.2 Å resolution. The crystals were obtained at 286 K in a drop containing 10 m*M* Tris–HCl pH 7.0, 15 m*M* taurine, 0.1%(*w*/*v*) *n*-octyl β-D-glucoside and 2 mg ml^{-1} protein that was equilibrated against 1 ml 25%(*v*/*v*) dioxane. The crystals appeared after 5–6 d and reached maximum dimensions of 0.6 × 0.2 × 0.02 mm in about 10 d.

2.3. X-ray diffraction data collection and processing

X-ray diffraction data were collected on RIKEN Structural Biology Beamline II (BL44B2) at SPring-8 using an ADSC Quantum 210 CCD detector (Area Detector Systems Corporation; Adachi *et al.*, 2001). Before collecting diffraction data, crystals were soaked for 20 min in cryoprotectant solution containing 80 m*M* Tris–HCl pH 7.0, 100 m*M* taurine, 15%(*v*/*v*) dioxane and 30%(*w*/*v*) ethylene glycol. Subsequently, crystals were mounted in a nylon loop (Hampton Research) and flash-cooled in a nitrogen stream at 100 K. Diffraction spots were indexed, integrated and scaled using the *HKL*-2000

package (Otwinowski & Minor, 1997). The crystals diffracted to beyond 3.2 Å resolution and belonged to space group $C2$. The unit-cell parameters were a = 154.8, b = 49.9, c = 104.0 Å, $\alpha = \gamma = 90.0$, $\beta = 124.0^\circ$. Assuming the presence of two molecules per asymmetric unit, the calculated Matthews coefficient (Matthews, 1968) was 2.18 $Å^3\ Da^{-1}$, which corresponds to a solvent content of 43.6%. A complete native X-ray diffraction data set was successfully collected at 3.5 Å resolution using synchrotron radiation. Data-collection statistics are summarized in Table 1.

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