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# Crystallization and preliminary X-ray crystallographic studies of the axin DIX domain

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Axin is a negative regulator of the canonical Wnt signalling pathway that mediates the phosphorylation of $\beta$-catenin by glycogen synthase kinase 3$\beta$. The DIX domain of rat axin, which is important for its homooligomerization and interactions with other regulators in the Wnt pathway, was purified and crystallized by the sitting-drop vapour-diffusion technique using polyethylene glycol 6000 and lithium sulfate as crystallization agents. Crystals belong to space group $P6_1$ or $P6_5$, with unit-cell parameters $a = b = 91.49$, $c = 84.92$ Å. An X-ray diffraction data set has been collected to a nominal resolution of 2.9 Å.

## 1. Introduction

Axin, which has been identified as a negative regulator of the canonical Wnt signalling pathway, is a scaffold protein that mediates the phosphorylation of $\beta$-catenin by glycogen synthase kinase 3$\beta$ (GSK-3$\beta$) in cooperation with the adenomatous polyposis (APC) protein. Axin, GSK-3$\beta$ and APC form a large protein assembly (the $\beta$-catenin destruction complex) that leads to the subsequent degradation of $\beta$-catenin by the ubiquitin–proteasome system (Aberle *et al.*, 1997; Orford *et al.*, 1997). In the presence of a Wnt signal, dishevelled (Dvl) acts to inhibit the phosphorylation of $\beta$-catenin by GSK-3$\beta$, resulting in the accumulation and translocation of $\beta$-catenin to the nucleus (Li, Yuan, Weaver *et al.*, 1999; Salic *et al.*, 2000). Axin, GSK-3$\beta$, APC and Dvl seem to play central roles in the canonical Wnt signalling pathway, although a number of proteins that bind to axin have been identified (Kikuchi, 1999*a*,*b*; Luo & Lin, 2004). Both axin and APC function as tumour suppressors. Mutation of these proteins results in the development of numerous human cancers by inappropriate activation of downstream target genes.

The structures of the APC, GSK-3$\beta$ and $\beta$-catenin binding regions of axin have been determined by X-ray crystallography of the complexes of axin with small peptide fragments of the respective binding partners. The C-terminus of axin, which is homologous to the N-terminal region of Dvl, is known as the DIX domain (Axin-DIX) and may be important for the homooligomerization of axin (Kishida *et al.*, 1999; Sakanaka & Williams, 1999; Hsu *et al.*, 1999). Using NMR spectroscopy, Capelluto and coworkers (Capelluto *et al.*, 2002; Capelluto & Overduin, 2005) suggested that the DIX domain of Dvl2 (Dvl2-DIX) has three helical regions in the presence of dodecylphosphocholine (DPC) micelles, although its overall structure is still unknown. They also demonstrated that the Dvl2 DIX domain associates with G-actin and DPC micelles and found a putative phospholipid-binding site within the DIX domain. Mutation of the site resulted in the loss of not only the ability to associate with cytoplasmic vesicles, but also the ability to stabilize $\beta$-catenin, which may relate to the fact that truncation of the DIX domain from Dvl abolishes the $\beta$-catenin accumulation activity (Yanagawa *et al.*, 1995; Kishida *et al.*, 1999; Li, Yuan, Xie *et al.*, 1999). Moreover, it has been reported that the DIX domain is required for Dvl1 to bind to axin (Kishida *et al.*, 1999; Julius *et al.*, 2000). Thus, structural and biochemical studies have shed light on the biological role of the Dvl DIX domain. However, the biological role of the axin DIX domain is still unclear apart from the formation of homomeric and heteromeric

**Table 1**
Data-collection statistics.

| | |
|---|---|
| Beamline | BL-5A, Photon Factory |
| Space group | $P6_1$ |
| Unit-cell parameters (Å) | $a = b = 91.49$, $c = 84.92$ |
| Wavelength (Å) | 1.0000 |
| Resolution range (Å) | 50–2.90 (3.00–2.90) |
| Measured reflections | 67284 |
| Unique reflections | 9081 |
| Completeness (%) | 99.9 (100.0) |
| $R_{\rm merge}$ | 8.8 (46.5) |
| Multiplicity | 7.4 (7.6) |
| $\langle I/\sigma(I)\rangle$ | 15.4 (3.7) |

complexes with the DIX domain-carrying proteins axin, Dvl and Ccd1 (Kishida *et al.*, 1999; Sakanaka & Williams, 1999; Hsu *et al.*, 1999; Julius *et al.*, 2000; Zhang *et al.*, 2000; Shiomi *et al.*, 2003). The structure of the axin DIX domain may provide insight into its function. Here, we report the preparation and crystallization of the axin DIX domain from rat (rAxin-DIX).

## 2. Experimental procedures and results

### 2.1. Protein expression and purification

The rat axin DIX domain cDNA encoding residues 713–832 was cloned by standard PCR methods into expression vector pMAL-c2x (New England Biolabs). The recombinant plasmid was transformed into *Escherichia coli* DH5$\alpha$ strain and grown in Luria–Bertani broth with 100 mg ml$^{-1}$ ampicillin at 310 K until the $OD_{600}$ reached 0.6. Expression of the recombinant fusion protein was then induced with 0.3 m*M* isopropyl $\beta$-D-thiogalactopyranoside for 3 h at 303 K. Cells were pelleted by centrifugation and frozen at 243 K. The pellet was resuspended in buffer containing 20 m*M* Tris pH 7.5, 1 m*M* dithiothreitol (DTT), 0.2 *M* NaCl, 0.1%(*v*/*v*) Triton X-100 and 1 m*M* phenylmethanesulfonyl fluoride and then disrupted by sonication on ice, after which cell debris was removed by centrifugation. Unless described otherwise, all the following steps were performed at 277 K. 2 ml amylose resin (New England Biolabs) was added to the supernatant, which was gently agitated for 3 h. The mixture was loaded onto a column and washed with 60 ml wash buffer (20 m*M* Tris pH 7.5 and 1 m*M* DTT). The bound protein was then eluted using elution buffer (20 m*M* Tris pH 7.5, 1 m*M* DTT and 10 m*M* maltose). Fractions were collected and checked by SDS–PAGE for size, purity and relative concentration. Selected fractions were combined, treated with trypsin (final concentration of 1 µg ml$^{-1}$) and gently agitated for 1 h to remove the N-terminal MBP tag. The protein solution was loaded onto a HiTrapQ column pre-equilibrated with 20 m*M* Tris pH 7.5, 100 m*M* NaCl, 1 m*M* DTT and a gradient of 0.1–0.4 *M* NaCl in the same buffer was run to separate rAxin-DIX from MBP. Selected fractions eluted from the HiTrapQ column were combined and then dialyzed against 20 m*M* Tris–HCl pH 8.5 and 1 m*M* DTT. To prevent intermolecular disulfide-bond formation, 3 m*M* *p*-chloromercuribenzoate (PCMB) was added to the pooled fractions from the HiTrapQ column so that the final ratio of Hg to SH groups was 3:1. Subsequently, the PCMB-treated rAxin-DIX solution was concentrated to 20 mg ml$^{-1}$. MALDI–TOF mass spectra and N-terminal sequence analysis indicated that the trypsin-cleaved protein is a mixture of the peptides Lys741–Asp832 and Ala742–Asp832, both of which contain the entire rAxin-DIX domain (Ser752–Asp832).

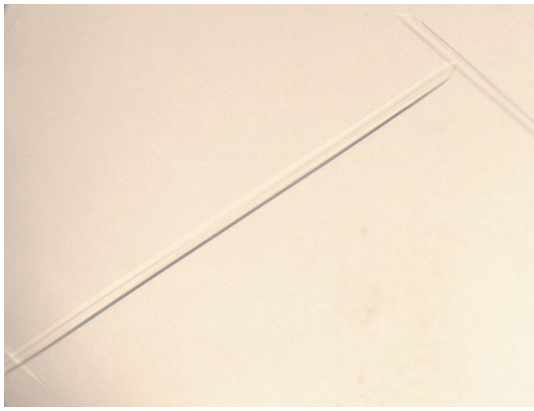

**Figure 1**
Crystals of rAxin-DIX.

### 2.2. Crystallization

Purified rAxin-DIX was concentrated to 20 mg ml$^{-1}$ and screened for crystallization conditions using the sitting-drop vapour-diffusion method. Initial screens were performed using the commercially available sparse-matrix kits Crystal Screens I and II and Crystal Screen Cryo (Hampton Research) at 283 K. Initial crystallization hits from Crystal Screen I condition No. 17 and Crystal Screen Cryo condition No. 17 were then optimized using Additive Screen and Detergent Screen kits (Hampton Research). In the final crystallization experiments, 5 µl purified protein solution (20 mg ml$^{-1}$ in 20 m*M* Tris–HCl pH 8.5) was mixed with the same volume of reservoir solution consisting of 14%(*w*/*v*) PEG 6000, 0.2 *M* $Li_2SO_4$, 0.1 *M* Tris–HCl pH 8.5, 5%(*v*/*v*) glycerol and 0.33 m*M* deoxy-BIGCHAP.

### 2.3. Data collection and crystallographic analysis

The crystals used for data collection (Fig. 1) were soaked in reservoir solution augmented with 30%(*v*/*v*) glycerol before being flash-cooled to 95 K in preparation for data collection. A data set was collected at the BL-5A beamline at the Photon Factory, Japan equipped with an ADSC Quantum 315 detector. 180 frames of 3 s exposure and 1.0° oscillation were collected. The crystal-to-detector distance was 220 mm. All data were integrated and merged using the *HKL*-2000 program package (Otwinowski & Minor, 1997). The data indicate that the space group belongs to the 6/*m* Laue symmetry class. Analysis of the 00*l* axial reflections shows a clear $l = 6n$ pattern, indicating that the space group is $P6_1$ or its enantiomorph $P6_5$, with unit-cell parameters $a = b = 91.49$, $c = 84.92$ Å. The data-collection statistics are given in Table 1. Initial SAD phasing using a mercury anomalous signal in combination with real-space averaging displayed a $\beta$-rich structure in space group $P6_1$. Model building using the $P6_1$ map is currently under way.

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