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Expression, crystallization and preliminary X-ray studies of the PDZ domain of Dishevelled protein

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Dishevelled (Dsh) protein is an important component of the Wnt signal-transduction pathway. It has three relatively conserved domains: DIX, PDZ and DEP. The PDZ domain of the *Xenopus laevis* homolog of Dsh, which consists of residues 254–348, was overexpressed as a soluble protein in *Escherichia coli*, purified and crystallized. The crystals were obtained by the vapor-diffusion method, using 1.4 *M* sodium formate as a precipitant. The crystals diffracted to 2.3 Å resolution. The space group was determined to be $P6_122$ or $P6_522$, with unit-cell dimensions $a = b = 95.9$, $c = 93.9$ Å.

1. Introduction

PDZ domains are molecular-recognition modules that mediate protein–protein interactions. They are often associated with cell junctions and have been implicated in the clustering of membrane proteins as well as in the assembly of multiprotein complexes (Ponting *et al.*, 1997). Originally, PDZ domains were named GLGF domains (Gly-Leu-Gly-Phe is a relatively conserved element of their sequence). The structure of the PDZ domain from the post-synaptic density protein PSD-95 demonstrated that GLGF loop was involved in the binding of the COOH-terminal group of the target peptide (Doyle *et al.*, 1996). PDZ domains are present in a diverse set of proteins, including tyrosine phosphatases, membrane-associated guanylate kinases, neuronal nitric oxide synthase, syntrophin, Dishevelled proteins and many others (Sato *et al.*, 1995; Ponting *et al.*, 1997; Hall, 1994; Adams *et al.*, 1993).

Dsh plays an important role in the Wnt signal-transduction pathway (Tseisen *et al.*, 1994; Klingensmith *et al.*, 1994; reviewed by Cadigan & Nusse, 1997). Wnts are a family of secreted glycocoproteins that function as signaling molecules during a number of different developmental decisions. They are implicated in such processes as cell proliferation and cell-fate determination during embryonic development in a variety of organisms, ranging from *Drosophila melanogaster* to mammals (reviewed by Dale, 1998; Cadigan & Nusse, 1997). Dsh was shown to be activated in response to the binding of Wnts to their receptors and to transmit the signal downstream by inhibiting the activity of the GSK3/APC/Axin/β-catenin complex. In the absence of the Wnt signal, β-catenin is phosphorylated by the complex and rapidly degraded. The inhibition of this complex activity results in the increase of the level of cytoplasmic β-catenin. As a result, β-catenin is able to accumulate in cytoplasm and nucleus and form a complex with transcription factors of the LEF/TCF family, thus leading to the activation of the transcription of Wnt-responsive genes.

Dsh is a cytoplasmic protein containing three domains that are conserved between different Dsh homologs: DIX, PDZ and DEP (Klingensmith *et al.*, 1994; Tseisen *et al.*, 1994). To date, the exact biochemical mechanism of action of Dsh is not known. However, there is strong evidence that the PDZ domain of Dsh is critical for the activation of the Wnt pathway. In a cell-culture assay, overexpression of wild-type Dsh resulted in the accumulation of β-catenin, while overexpression of Dsh lacking the PDZ domain failed to do so (Yanagawa *et al.*, 1995). Additionally, Dsh without a PDZ domain acted as dominant-negative in a secondary axis induction assay in *Xenopus* embryos (Sokol, 1996). However, in other systems PDZ domains were shown to be dispensable (Axelrod *et al.*, 1998).

Here, we report the overexpression, purification and crystallization of recombinant PDZ domain as well as preliminary X-ray characterization. The structural information should help in understanding the mechanism of Dsh at the molecular level.

2. Materials and methods

2.1. Expression and purification

Recombinant PDZ domain from Dishevelled protein was expressed in *E. coli* BL-21 (DE3) as a fusion protein with six histidines on a COOH-terminal (pET-21b, Novagen). Since the PDZ domain was insoluble after induction at 310 K and was only partially soluble at room

Table 1
Data-collection statistics.

Values in parentheses refer to the outer resolution shell.

Data set	Resolution (Å)	R_{merge} (%)	Completeness (%)
Native	20–2.7	4.7 (19)	99.1 (99.7)
Se-Met crystals	20–2.4	4.2 (30)	98.9 (99.9)

temperature, it was induced at 285 K for 36 h with 1 m*M* IPTG. The cells were pelleted in a JA-10.5 rotor at 6500*g* for 5 min and then either used immediately or stored frozen at 249 K. The bacteria were resuspended in 50 m*M* sodium phosphate pH 8, 200 m*M* NaCl, 5 m*M* β-mercaptoethanol, 10 µg ml^{-1} Pefablock (20 ml of buffer per litre of culture) and lysed by sonication. The lysate was centrifuged in a Ja-25.5 rotor at 40 000*g* for 20 min and the supernatant was added to Talon resin (Clontech) pre-equilibrated with a lysis buffer (1 ml of bed volume per litre of culture). The suspension was incubated at 285 K for 20 min to facilitate binding and the Talon beads were then washed twice with lysis buffer, loaded onto the gravity-flow column (Biorad) and washed once with 10 m*M* Tris pH 8, 100 m*M* NaCl, 5 m*M* β-mercaptoethanol, 1 m*M* imidazole. The protein was eluted by addition of 10 m*M* Tris pH 8, 100 m*M* NaCl, 5 m*M* β-mercaptoethanol, 50 m*M* imidazole and further purified by anion-exchange chromatography using a HQ anion-exchange column (Perseptive) run with a linear gradient from buffer *A* (10 m*M* Tris pH 8.5, 1 m*M* DTT) to buffer *B* (10 m*M* Tris pH 8.5, 2 *M* NaCl, 1 m*M* DTT). The buffer was then exchanged to 10 m*M* Tris pH 8, 25 m*M* NaCl using a desalting column and the protein was concentrated to 6–8 mg ml^{-1} in a Centricon concentrator (Amicon). The protein used for crystallization consisted of amino acids 254–348 from Dsh protein with one additional vector-derived Met on the N-terminus and eight amino acids (GEHHHHHH) on the C-terminus. The homogeneity of the protein was confirmed by electrospray mass spectrometry.

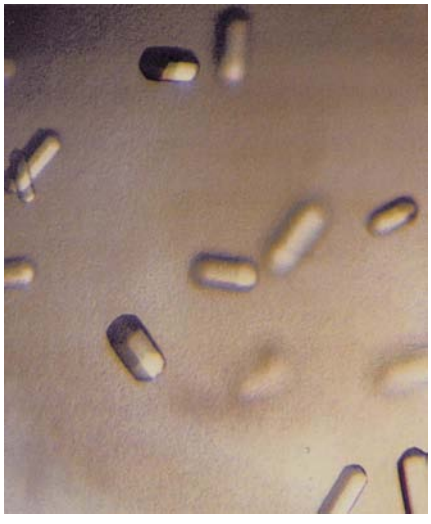

Figure 1
Photograph of selenomethionine-derivatized Dsh PDZ domain crystals. Typical crystal dimensions are 50 × 50 × 150 µm.

The protein in which selenomethionine was substituted for methionine was expressed in methionine auxotroph cell line B834 (DE3) (Novagen) as in Doublié (1997) and purified according to an identical protocol.

2.2. Crystallization

Crystallization conditions were surveyed using Crystallization Screen 1 (Hampton Research) using the hanging-drop vapor-diffusion method with 4 µl drops (2 µl protein solution plus 2 µl mother liquor) equilibrated against 1 ml reservoir at room temperature. Most of the drops initially gave strong precipitation. After approximately 10 d, small hexagonally shaped crystals appeared in 100 m*M* sodium acetate pH 4.6, 2 *M* sodium formate conditions.

Selenomethionine-derivative crystals grew in similar conditions but at lower salt concentration. Optimized conditions were 100 m*M* sodium acetate pH 4.7, 1.4 *M* sodium formate. Crystals formed from the precipitate after 7–8 d and grew to 150 µm in the largest dimension.

2.3. Crystallographic characterization

A complete data set was collected on beamline 5.0.2 at the Macromolecular Crystallography Facility at the Advanced Light Source, LBNL, using an ADSC QUANTUM 4 CCD-based detector. Data were collected at 100 K using an Oxford Cryosystems Cryostream low-temperature system.

3. Results and discussion

The borders of the Dsh PDZ domain were chosen by comparison with other PDZ domains of known structure. The soluble PDZ domain with a His-tag on the COOH-terminus was obtained by overexpression in *E. coli* strain BL-21 (DE3), purified by metal affinity and anion-exchange chromatography and crystallized by the vapor-diffusion method. Single crystals (see Fig. 1) were soaked in a cryoprotection solution (sodium acetate pH 4.6, 2 *M* sodium formate, 15% glycerol) for 2 min, mounted on cryoloops and flash-frozen in liquid nitrogen. These crystals diffracted to 2.5 Å resolution. A complete native data set was collected to 2.7 Å (see Table 1 for data-collection statistics). Space group and unit-cell parameter determination was performed using the *DENZO* and *SCALEPACK* data-processing software (Otwinowski & Minor, 1997). Possible space groups were determined to be $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 95.9$, $c = 93.9$ Å. Assuming two molecules per asymmetric unit, the Matthews coefficient (V_m) was calculated to be 2.61 Å^3 Da^{-1}, corresponding to a solvent content of 50% (Matthews, 1968). Attempts to determine the crystal structure of the PDZ domain of Dsh by molecular replacement with other PDZ domains (Doyle *et al.*, 1996; Daniels *et al.*, 1998) as models using the programs *AMoRe* and *EPMR* were unsuccessful. Since there are four naturally occurring methionine residues in this domain, we expressed selenomethionine-derivatized protein for MAD structure determination.

The PDZ domain was expressed in methionine auxotroph *E. coli* strain B834 (DE3). The expression conditions were similar to those for the unsubstituted protein. All four methionines plus one extra N-terminal methionine derived from the plasmid sequence were substituted with selenomethionines. The substitution was verified by electrospray mass spectrometry. The selenomethionine-derivatized crystals grew under conditions similar to those for wild-type crystals but with lower salt concentration. They were cryoprotected with sodium acetate pH 4.7, 1.4 *M* sodium formate and 15% glycerol and then flash-frozen. The crystals diffracted to 2.4 Å resolution (see Table 1 for data-collection statistics). Selenomethionine-derivatized crystals were obtained in the same space group as the wild-type crystals, but had slightly different unit-cell parameters: $a = b = 96.5$, $c = 93.8$ Å.

Structure determination using MAD is presently under way.

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References

Adams, M. F., Butler, M. H., Dwyer, T. M., Peters, M. F., Murname, A. A. & Froehner, S. C. (1993). *Neuron*, **11**, 531–540.

Axelrod, J. D., Miller, J. R., Shulman, J. M., Moon, R. T. & Perrimon, N. (1998). *Genes Dev.* **12**(16), 2610–2622.

Cadigan, M. M. & Nusse, R. (1997). *Genes Dev.* **11**, 3286–3305.

Dale, T. C. (1998). *Biochem. J.* **329**(2), 209–223.

Daniels, D. L., Cohen, A. R., Anderson, J. M. & Brünger, A. T. (1998). *Nature Struct. Biol.* **5**(4), 317–325.

Doublié, S. (1997). *Methods Enzymol.* **276**, 523–530.

Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M. & MacKinnon, R. (1996). *Cell*, **85**(7), 1067–1076.

Hall, A. V. (1994). *J. Biol. Chem.* **269**, 33082–33090.

Klingensmith, J., Nusse, R. & Perrimon, N. (1994). *Genes Dev.* **8**, 118–130.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Ponting, C. P., Phillips, C., Davies, K. E. & Blake, D. J. (1997). *Bioessays*, **19**(6), 469–479.

Sato, T., Irie, S., Kitada, S. & Reed, J. C. (1995). *Science*, **268**(5209), 411–415.

Sokol, S. Y. (1996). *Curr. Biol.* **6**(11), 1456–1467.

Tseisen, H., Purcell, J., Bennet, M., Kansagawa, D., Syed, A. & Marsh, J. L. (1994). *Development*, **120**, 347–360.

Yanagawa, S., van Leeuwen, F., Wodarz, A., Klingensmith, J. & Nusse, R. (1995). *Genes Dev.* **9**(9), 1087–1097.