Purification, crystallization and preliminary X-ray diffraction studies of the β -catenin homolog HMP-2 from *Caenorhabditis elegans*

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β -Catenin is a multifunctional protein involved in both cell adhesion and Wnt signaling in metazoans. The nematode *Caenorhabditis elegans* is unusual in that it expresses four β -catenin paralogs with separate functions. *C. elegans* HMP-2 participates in cell adhesion but not in Wnt signaling, so structural and biochemical studies of this protein will help in understanding its unusual specialization and the evolution of β -catenin. HMP-2 was expressed, purified and crystallized in two different salt conditions. Crystals grown from a sodium formate condition diffracted to a resolution of 2 Å and belonged to space group C2, with unit-cell parameters $a = 165.2$, $b = 39.0$, $c = 101.1$ Å, $\beta = 116.7^\circ$. Crystals obtained from a lithium sulfate condition diffracted to 3 Å resolution and belonged to space group $P4_3$, with unit-cell parameters $a = b = 85.3$, $c = 138.7$ Å. Diffraction data were collected and processed from both crystal forms and the structure was solved by molecular replacement. Model refinement is in progress.

1. Introduction

In mammalian systems, β -catenin is an essential component of adherens junctions and also functions as a transcriptional coactivator in response to a Wnt signal, a key pathway regulating cell-fate determination. In the absence of a Wnt signal, a cytosolic pool of β -catenin is recruited to a 'destruction complex' by interactions with the adenomatous polyposis coli (APC) protein and axin, and is eventually degraded by the ubiquitin-dependent proteasome system. In the presence of a Wnt signal, β -catenin translocates to the nucleus and interacts with TCF/LEF transcription factor to turn on the transcription of Wnt response genes. The majority of cellular β -catenin is associated with adherens junctions through its interactions with E-cadherin and α -catenin. Structural studies showed that a positively charged groove formed by the superhelical structure of the central armadillo (arm) domain of β -catenin interacts with many β -catenin partners, including TCF/LEF transcription factors, APC, axin and E-cadherin (Huber *et al.*, 1997; Graham *et al.*, 2000; Huber & Weis, 2001; Graham *et al.*, 2001; Poy *et al.*, 2001; Eklof Spink *et al.*, 2001; Xing *et al.*, 2003, 2004; Ha *et al.*, 2004; Sun & Weis, 2011). The N-terminal and the C-terminal domains of β -catenin are mostly unstructured but are important for ubiquitin-dependent degradation and transcriptional activation, respectively. Cellular β -catenin binding partners display a range of affinities for β -catenin, with dissociation constants ranging from micromolar to sub-nanomolar, but they can be modulated by phosphorylation, as shown in biochemical and structural studies of E-cadherin and

Table 1
Data collection and processing.

Values in parentheses are for the outer shell.

Crystal	I	II
Wavelength (Å)	1.0332	1.0332
Detector	MAR 325 CCD	MAR 325 CCD
Crystal-to-detector distance (mm)	280	420
Total rotation range (°)	180	90
Space group	<i>C</i> 2	<i>P</i> 4 ₃
<i>a</i> , <i>b</i> , <i>c</i> (Å)	165.2, 39.0, 101.1	85.3, 85.3, 138.7
α , β , γ (°)	90, 116.7, 90	90, 90, 90
Resolution range (Å)	50–2.0 (2.07–2.00)	50–3.0 (3.11–3.00)
No. of unique reflections	36385 (3154)	19910 (1970)
Completeness (%)	93.0 (82.7)	99.9 (99.9)
Multiplicity	4.9 (4.4)	3.9 (3.8)
Solvent content (%)	42	66
$\langle I/\sigma(I) \rangle$	19.7 (5.6)	13 (2.2)
$R_{\text{merge}}^{\dagger}$	0.038 (0.22)	0.10 (0.48)
$CC_{1/2}^{\ddagger}$	0.999 (0.978)	0.997 (0.811)
Overall <i>B</i> factor from Wilson plot (Å ²)	29.6	77.8

$^{\dagger} R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the weighted mean of all measurements of reflection *hkl*. $^{\ddagger} CC_{1/2}$ is the Pearson correlation coefficient between random half data sets (Diederichs & Karplus, 2013).

APC (Huber & Weis, 2001; Ha *et al.*, 2004; Choi *et al.*, 2006; Liu *et al.*, 2006; Sun & Weis, 2011). The aberrant activation of β -catenin is related to various diseases, including cancers, and there have been efforts to design therapeutic agents targeting β -catenin using structural information from β -catenin complexes. However, the development of a drug targeting β -catenin with high specificity and low side effects is hindered by the facts that β -catenin is involved in both cell adhesion and Wnt signaling and that the core binding site on the arm domain of β -catenin is shared with several binding partners.

Although most metazoans have a single gene encoding β -catenin, the nematode *Caenorhabditis elegans* expresses four different β -catenin paralogs, HMP-2, WRM-1, BAR-1 and SYS-1, and only HMP-2 functions in cell–cell adhesion (Korswagen *et al.*, 2000). It has been shown that HMP-2 localizes at the cell membrane and interacts with the E-cadherin homolog HMR-1 (Costa *et al.*, 1998; Korswagen *et al.*, 2000). WRM-1, BAR-1 and SYS-1 do not bind to HMR-1 but interact with the TCF/LEF homolog POP-1. The functional separation of β -catenin paralogs in *C. elegans* could be advantageous to study cell adhesion and Wnt signaling pathways separately. Thus, structural and biochemical studies of HMP-2 will help in understanding its unusual specialization and the evolution of β -catenin.

2. Materials and methods

2.1. Macromolecule production

The clone for the gene encoding full-length HMP-2 was kindly provided by Dr Jeff Hardin (University of Wisconsin). Initial attempts to express and purify full-length HMP-2 failed owing to the insolubility of HMP-2. An N-terminal 53-amino-acid deletion construct (HMP-2 Δ N) was obtained by

subcloning the PCR product amplified using 5'-CACGAATTCAAATGCCAACTCAACAGCTGAAGC-3' forward and 5'-CACCTCGAGTCACAAATCGGTATC-GTACCAATTGTGATTAGG-3' reverse primers into the pGEX-TEV expression vector (restriction sites are shown in bold), which contains an N-terminal glutathione-S-transferase (GST) affinity tag followed by a *Tobacco etch virus* (TEV) protease cleavage site prior to the start of the HMP-2 54–678 sequence. The resulting expression construct was verified by DNA sequencing.

The recombinant plasmid was transformed into chemically competent *Escherichia coli* strain BL21 (DE3) CodonPlus cells and a single colony was picked and grown in Luria–Bertani (LB) medium containing ampicillin and chloramphenicol at 310 K overnight. For protein expression, 20 ml of the overnight culture was inoculated into 2 l LB medium containing antibiotics, and 0.5 mM IPTG was added when the OD₆₀₀ reached about 0.7. After 4 h of additional incubation at 303 K, the cells were harvested and lysed using an EmulsiFlex-C3 homogenizer (Avestin Inc., Ottawa, Canada) in phosphate-buffered saline (PBS) containing protease-inhibitor cocktail (Roche). The lysate was cleared by centrifugation at 26 500g for 30 min at 277 K, and the supernatant was loaded onto glutathione agarose (G-agarose) beads pre-equilibrated with PBS. The column was washed with ten column volumes of PBSTR buffer (PBS, 1 M NaCl, 5 mM DTT, 0.05% Tween 20) followed by two column volumes of T buffer (50 mM Tris–HCl pH 8.0, 100 mM NaCl, 3 mM DTT). The GST tag was removed by incubating the beads with TEV protease [20:1(w:w) substrate:TEV] overnight at 277 K. The HMP-2 released by TEV protease digestion was pooled and loaded onto a Mono Q column equilibrated with buffer QA (20 mM Tris–HCl pH 8.0, 20 mM NaCl, 0.5 mM EDTA, 2 mM DTT)

Figure 1
Gel-filtration (Superdex 200) profile of HMP-2 Δ N in 20 mM HEPES pH 8.0, 150 mM NaCl, 1 mM DTT. The column was calibrated using proteins of standard molecular mass (Sigma; catalog No. MWGF200-1KT). The observed molecular mass of HMP-2 Δ N is 70 kDa. A 4–20% SDS-PAGE of purified HMP-2 Δ N is shown in the inset; lane 1 contains purified HMP-2 Δ N and lane 2 contains standard molecular-mass markers (labelled in kDa).

and eluted using a linear gradient of 100–350 mM sodium chloride. The pooled fractions were further purified using a Superdex 200 HiLoad 26/600 column equilibrated with a buffer consisting of 20 mM HEPES pH 8.0, 150 mM NaCl, 1 mM DTT. The protein was homogeneously eluted as a monomeric form and the fractions were pooled and concentrated to 20 mg ml⁻¹ for crystallization.

2.2. Crystallization

Initial crystallization experiments were performed using commercially available crystallization screens from Qiagen. About 800 crystallization conditions were screened using a Phoenix crystallization robot (Art Robbins Instruments). Initial hits were observed in two different conditions: 1.75 M sodium formate pH 7.5 at 295 K (condition I) and 0.8 M lithium sulfate, 50 mM Tris–HCl pH 8.0 at 295 K (condition II). Optimization was carried out for each crystal form by varying the concentration of salt precipitant, the buffer, the pH and the incubation temperature. To reduce nucleation, the protein concentration was also decreased to 12 mg ml⁻¹. Larger diffraction-quality crystals were obtained using the hanging-drop vapour-diffusion method by equilibrating a mixture of 1 µl protein solution (12 mg ml⁻¹) and 1 µl reser-

voir solution against 500 µl reservoir solution consisting of 1.3 M sodium formate, 50 mM HEPES pH 7.5 (condition I) or 0.55 M lithium sulfate, 50 mM imidazole pH 8.0 (condition II). The crystals from conditions I and II appeared after 2 d incubation at 298 K and 24 h incubation at 293 K, respectively.

2.3. Data collection and processing

Crystals from the sodium formate condition (form I) were flash-cooled in liquid nitrogen using perfluoropolyether oil (PFO) as a cryoprotectant. Single crystals from the lithium sulfate condition (form II), which were obtained by the manipulation of a cluster of multilayered crystals, were cryoprotected using 20% glycerol in reservoir solution and were flash-cooled in liquid nitrogen. Crystal forms I and II diffracted to 2.0 and 3.0 Å resolution, respectively, and diffraction data sets were collected on beamline 11-1 at the Stanford Synchrotron Radiation Lightsource (SSRL). The diffraction images, each of which was obtained by 2 s exposure for 1° oscillation at a wavelength of 1.0332 Å on a MAR 325 CCD detector, were indexed and integrated using *HKL-2000* (Otwinowski & Minor, 1997) and each data set was scaled using *SCALEPACK* (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

Figure 2
Crystals of HMP-2ΔN. (a) Form I crystals grown from the initial hit (left; 1.75 M sodium formate pH 7.5 at 295 K) and the optimized condition (right; 1.3 M sodium formate, 50 mM HEPES pH 7.5 at 298 K). (b) Form II crystals obtained from lithium sulfate conditions: the initial hit (left; 0.8 M lithium sulfate, 50 mM Tris–HCl pH 8.0 at 295 K) and the optimized condition (right; 0.55 M lithium sulfate, 50 mM imidazole pH 8.0 at 293 K).

3. Results and discussion

Full-length HMP-2 was initially expressed and purified for structural and biochemical studies. However, only small amounts of soluble protein were obtained upon cell lysis, and the soluble lysates formed aggregates during purification as assessed by gel-filtration chromatography. To obtain soluble protein, the N-terminal 53 amino acids were truncated based on sequence alignment with the arm domain of mouse β -catenin (Supplementary Fig. S1). A new HMP-2 construct, HMP-2 Δ N, produced soluble protein in *E. coli*, and HMP-2 Δ N eluted as a monomer in gel-filtration chromatography

(Fig. 1). The final yield of purified HMP-2 Δ N was 2.5 mg per litre of cell culture. Initial hits from the crystallization screening experiment were found in 1.75 M sodium formate pH 7.5 at 295 K (form I) and 0.8 M lithium sulfate, 50 mM Tris-HCl pH 8.0 at 295 K (form II), and these conditions were optimized to produce larger diffraction-quality crystals (Fig. 2). In both cases, optimal crystals were obtained by reducing the concentrations of the salt precipitants to 1.3 M sodium formate and 0.55 M lithium sulfate, respectively.

Diffraction data were collected to 2.0 Å (form I) and 3.0 Å (form II) resolution at 100 K (Fig. 3). The space groups were determined to be C2 (form I) and $P4_1$ or $P4_3$ (form II) (Table 1). The expected molecular mass of monomeric HMP-2 Δ N deduced from its amino-acid sequence is 68.6 kDa. The Matthews coefficients (Matthews, 1968) calculated from the unit-cell parameters suggest that the C2 and $P4$ crystals contained one monomer in the asymmetric unit with 42% solvent content ($V_M = 2.12 \text{ Å}^3 \text{ Da}^{-1}$) and 66% solvent content ($V_M = 3.67 \text{ Å}^3 \text{ Da}^{-1}$), respectively. The data statistics are presented in Table 1.

The space-group ambiguity of the $P4$ crystal was resolved by a molecular-replacement (MR) search using *Phaser* (McCoy *et al.*, 2007), which only gave an MR solution for space group $P4_3$ using the mouse β -catenin arm domain structure (PDB entry 1i7w; Huber & Weis, 2001) as a search model. Initial refinement of the structure solution using *PHENIX* (Adams *et al.*, 2010) resulted in an R_{work} and an R_{free} of 39 and 44%, respectively. Unexpectedly, initial attempts to solve the C2 crystal form I by MR with the mouse β -catenin arm domain structure were unsuccessful, but a solution was obtained by using a partially refined structure from the 3 Å resolution data set as a search model. It is possible that an MR solution for the 2 Å resolution data set was discarded by packing analysis, perhaps owing to the lower solvent content and tight packing in the C2 crystal. Further structure refinement and model building are in progress. Structural analysis of the arm domain of HMP-2 will reveal the mechanism of binding specificity toward HMR-1 and will provide information about the evolutionally conserved characteristics of β -catenin in the cell-adhesion system.

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Figure 3
Diffraction images of HMP-2 Δ N. (a) Diffraction image collected from the crystal grown in condition I. The resolution at the edge of the detector is 1.9 Å. (b) Diffraction image from crystals grown in condition II. The resolution at the edge of the detector is 2.6 Å.

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