Biodesign

pISSN 2288-6982 | eISSN 2288-7105

CRYSTALLIZATION P 56-59

Crystallization and preliminary diffraction analysis of human TEAD1, a transcriptional enhancer factor that controls the Hippo signaling pathway

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Transcriptional enhancer activation domain (TEAD) proteins are transcription factors that promote the expression of genes involved in organogenesis, embryonic development, and tumorigenesis. TEAD functions downstream of the Hippo signaling pathway as one of the pivotal regulators that controls cell viability and proliferation, tissue growth, and organ size, by interacting with yes-associated protein (YAP) via its C-terminal YAP-binding domain (YBD). As YAP is a well-known oncoprotein and its interaction with TEAD has been shown to be critical for the function of YAP, TEAD proteins are emerging as a potential therapeutic target for cancer. In this study, the YBD of the TEAD1 protein was produced from an *Escherichia coli* expression system, purified using a Ni-NTA affinity chromatography, HiTrap Q anion exchange chromatography, and size exclusion chromatography, and then successfully crystallized. X-ray diffraction data were collected to the resolution of 1.70 Å. Preliminary diffraction analysis revealed that the TEAD1 YBD crystals belong to the space group $P2_12_12_1$ with unit cell parameters of a = 36.5 Å, b = 89.4 Å, c = 135.6 Å, and that two TEAD1 YBD molecules are contained in the asymmetric unit with a solvent content of 45.2%.

INTRODUCTION

The Hippo signaling pathway is one of the key regulators that controls cell proliferation and apoptosis, tissue growth and regeneration, and the size of organs (Saucedo and Edgar, 2007; Zhao et al., 2011). Plenty of evidence has indicated that its dysregulation can lead to carcinogenesis in various organs including the breast, liver, lung, ovary, and prostate (Harvey et al., 2013; Zhao et al., 2008a). Hippo signaling is coordinated by a number of factors that constitute and/or regulate the central kinase signaling cascade in which Mst1/2, Lats1/2, and yes-associated protein (YAP) kinases are involved (Meng et al., 2016). YAP is a key downstream effector of this pathway that transfers signals to the nucleus, and it is also well-known as a potent oncoprotein (Huang et al., 2005; Overholtzer et al., 2006). When Hippo signaling is "on", phosphorylated YAP remains in the cytosol by binding to the 14-3-3 protein. Once the pathway is turned "off", YAP is dephosphorylated and enters the nucleus where it interacts with transcription factors, such as the transcriptional enhancer activation domain (TEAD) family members, to act as a transcription coactivator (Vassilev et al., 2001). Consequently, the YAP–TEAD complex activates downstream gene expression that is associated with cell proliferation and tissue growth. Previous studies have indicated that the intermolecular interaction with TEAD is inevitable for the function of YAP in controlling cell proliferation (Lamar et al., 2012; Vassilev et al., 2001; Zhao et al., 2008b). Moreover, in *Drosophila*, it was revealed that while the YAP homologue is indispensable for normal tissue formation and homeostasis, the TEAD homologue is largely not (Huang et al., 2005; Wu et al., 2008), collectively indicating that the TEAD proteins could be a putative therapeutic target for the treatment of cancer, as a mediator of the Hippo signaling pathway (Holden and Cunningham, 2018; Pobbati et al., 2015; Santucci et al., 2015; Zhou et al., 2015).

The human TEAD protein family is composed of four members, TEAD1–4, which commonly contain an N-terminal DNA-binding TEA domain and a C-terminal YAP-binding domain (YBD). The three dimensional structure of the YBD of TEADs in the apo form (Kaan et al., 2017; Noland et al., 2016; Tian et al., 2010) or in a complex with YAP (Chen et al., 2010; Li et al., 2010; Zhou et al., 2015) or with other proteins (Jiao et al., 2014; Kaan et al., 2017; Pobbati et al., 2012) have been under intensive investigation and have mostly been determined by X-ray crystallography,

TABLE 1 | TEAD1 production information

Source organism	Human
DNA source	cDNA
Forward primer	CCGCGCGGCAGCCATATGCGCAGTATTGGTACCACC
Reverse primer	TGGTGGTGGTGCTCGAGTTCAGTCCTTTACAAGCCT
Expression vector	pET28a
Expression host	*E. coli* BL21 (DE3) RIL
Complete amino acid sequence of the construct produced	MRSIGTTKLRLVEFSAFLEQQRDPDSYNKHLFVHIGH ANHSYSDPLLESVDIRQIYDKFPEKKGGLKELFGKGP QNAFFLVKFWADLNCNIQDDAGAFYGVTSQYESSEN MTVTCSTKVCSFGKQVVEKVETEYARFENGRFVYRIN RSPMCEYMINFIHKLKHLPEKYMMNSVLENFTILLVVT NRDTQETLLCMACVFEVSNSEHGAQHHIYRLVKD

TABLE 2 | Crystallization

Method	Sitting drop vapor diffusion
Plate type	96-well sitting drop crystallography plate
Temperature (K)	291
Protein concentration (mg/mL)	10
Buffer composition of protein solution	50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 1 mM dithiothreitol
Composition of reservoir solution	4% (v/v) 2-propanol, 100 mM sodium phosphate dibasic/citric acid (pH 4.5), and 200 mM lithium sulfate
Volume of drop (μL)	2 (protein: reservoir = 1:1)
Volume of reservoir (μL)	80

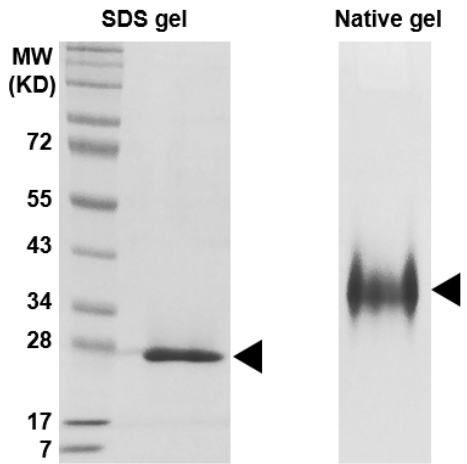

FIGURE 1 | **Purified TEAD1 YBD.** The purified TEAD1 YBD protein was visualized on an SDS (left) or native gel (right) by Coomassie blue staining. The molecular weight markers are shown on the SDS gel. The TEAD1 YBD is indicated by arrowheads.

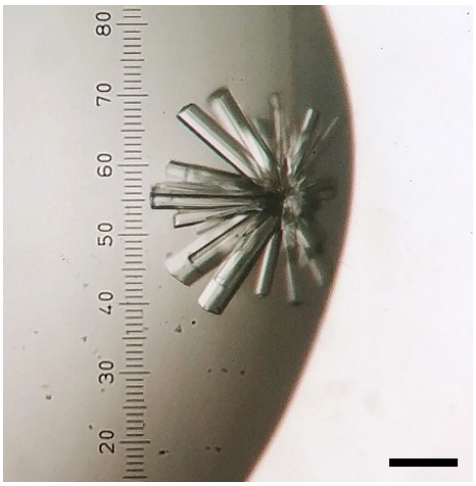

FIGURE 2 | **Crystals of human TEAD1 YBD.** Crystals were obtained with a reservoir solution containing 4% (v/v) 2-propanol, 100 mM sodium phosphate dibasic/citric acid (pH 4.5), and 200 mM lithium sulfate at 18°C. The scale bar indicates 0.1 mm.

except for the structure of apo TEAD1 YBD, which has not been elucidated. In this study, in an effort to obtain structural information for the TEAD1 YBD in the apo form, we attempted to express, purify, and crystallize this protein. We finally obtained crystals for the TEAD1 YBD that were diffracted to 1.70 Å resolution, and we carried out a preliminary diffraction analysis of the crystals.

RESULTS AND DISCUSSION

The YBD of human TEAD1 (residues 209–426) was cloned and expressed in an *Escherichia coli* expression system (Table 1). The TEAD1 YBD protein was purified using Ni-NTA affinity chromatography, HiTrap Q anion exchange chromatography, and size exclusion chromatography. The purity of the final protein sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by Coomassie blue staining, together with the molecular weight markers (Figure 1, left). On the SDS gel, the protein band appeared slightly below the 28 kDa protein standard, which was consistent with its calculated molecular weight (25.4 kDa). The homogeneity of the final sample was further confirmed by native gel electrophoresis (Figure 1, right). The TEAD1 YBD protein was visualized as a single band on both gels with approximately >98% purity (Figure 1).

The purified protein was subjected to automated protein crystallization screening trials using the sitting drop vapor diffusion method. Finally, optimized TEAD1 YBD crystals were obtained by use of a precipitant solution containing 4% (v/v) 2-propanol, 100 mM sodium phosphate dibasic/citric acid (pH 4.5), and 200 mM lithium sulfate (Figure 2 and Table 2). The cryoprotectant solution was prepared by adding 15% ethylene glycol to the precipitant solution. X-ray diffraction data were collected on beamline 7A at the Pohang Accelerator Laboratory (PAL) to 1.70 Å resolution (Figure 3) (Park et al., 2017). The space group of our TEAD1 YBD crystals was revealed to be $P2_12_12_1$ with unit cell parameters of a = 36.5 Å, b = 89.4 Å, c = 135.6 Å,

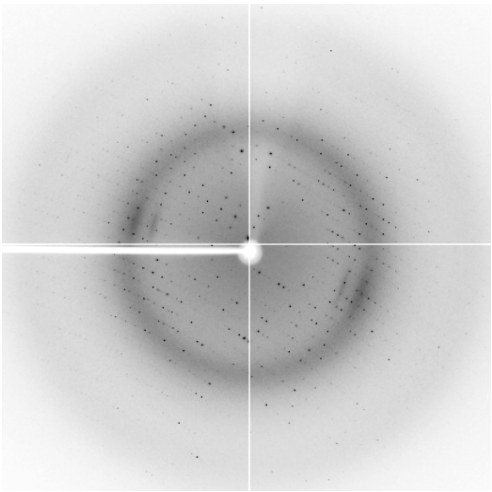

FIGURE 3 | Representative X-ray diffraction image. X-ray diffraction photograph of the TEAD1 YBD crystal with 1.0° oscillation range and maximum resolution at the edge of the film of 1.7 Å.

TABLE 3 | Data collection and processing statistics

Diffraction source	Beamline 7A, Pohang Accelerator Laboratory
Wavelength (Å)	0.9794
Temperature (K)	100
Space group	$P2_12_12_1$
a, b, c (Å)	36.5, 89,4, 135.6
α, β, γ (°)	90, 90, 90
Resolution (Å)	50.0–1.7 (1.73–1.70)*
Total No. of reflections	620,451
No. of unique reflections	49,632
Completeness (%)	99.5 (99.2)
R_{merge} (%)	6.6 (29.0)
Average I/σ(I)	35.4 (5.1)
Redundancy	6.0

*Values in parentheses are for the highest resolution shell.

and α = β = γ = 90°. The total number of reflections was 620,451, and these were merged to produce 49,632 unique reflections with a merging R factor of 6.6% and a completeness value of 99.5%. The data collection statistics are shown in Table 3. The solvent content and Matthews coefficient were calculated to be 45.2% and 2.24 $Å^3$/Da, respectively, when the asymmetric unit of the crystals contained two molecules of TEAD1 YBD. Structure determination by the molecular replacement method using the structure of TEAD1 YBD in a complex with YAP (PDB code: 3KYS) as a search model is currently underway.

METHODS

Cloning and purification of the recombinant protein

The YBD of human TEAD1 (GenBank accession number NP_068780.2; residues 209–426) was amplified by the polymerase chain reaction and cloned into the pET28a plasmid (Novagen) by means of the forward and reverse primers described in Table 1. The recombinant pET28a plasmid containing the TEAD1 YBD DNA was transformed into *E. coli* BL21(DE3) RIL competent cells (Novagen). Cells were incubated at 37°C until the OD_{600} reached 0.6 and were then treated with 0.5 mM isopropyl-β-D-thiogalactopyranoside to induce protein expression, which was followed by a subsequent incubation at 18°C for 16 h. After centrifugation at 1800x*g* for 30 min at 4°C, the cells were resuspended in a lysis buffer containing 50 mM Tris-HCl (pH 7.5) and 200 mM NaCl and then sonicated on ice. After centrifugation of the cell lysate at 18000x*g* for 50 min at 4°C to remove cell debris, the supernatant was loaded onto Ni-NTA agarose resin (QIAGEN). The resin was washed first with wash buffer 1 containing 50 mM Tris-HCl (pH 7.5), 1 M NaCl, and 3 mM β-mercaptoethanol (βME), and subsequently, with wash buffer 2 containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 3 mM βME, and 50 mM imidazole. Protein was eluted with 40 mL of a buffer containing 50 mM Tris (pH 7.5), 50 mM NaCl, 3 mM βME, and 300 mM imidazole. The N-terminal His_6-tag was removed by thrombin treatment at 4°C for 16 h. The sample was next subjected to a HiTrap™ Q HP 5 mL anion exchange column (GE Healthcare) that was equilibrated with 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 1 mM dithiothreitol. The TEAD1 YBD protein eluted in the unbound fraction. Finally, the protein was loaded onto a HiLoad™ 25/600 Superdex™ 75pg column (GE healthcare) that was equilibrated with a buffer containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 1 mM dithiothreitol. Finally, the fractions containing the purified protein were collected and concentrated to 10 mg/mL for crystallization.

Crystallization and X-ray data collection

Initial crystallizations were conducted using commercial screening solutions. Initial crystals were obtained on day 1 or 2 with the conditions from E6 of Wizard Classic 2 HT96 (Molecular Dimensions), which contained 10% (v/v) 2-propanol, 100 mM sodium phosphate dibasic/citric acid (pH 4.2), and 200 mM lithium sulfate. Improved crystals were obtained by altering the pH condition from pH 4.2 to pH 4.5 and the concentration of 2-propanol from 10% to 4%, as described in Table 2. X-ray diffraction data collected on beamline 7A at the PAL (Park et al., 2017) were processed using the program *HKL*2000 (Otwinowski and Minor, 1997), as summarized in Table 3.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

We are grateful for the use of beamline 7A at the Pohang Accelerator Laboratory in Korea. This work was supported by the National Research Foundation funded by the Ministry of Science and ICT of Korea (Grant number 2015M3A9B5030308). This work was also supported by the Korea Research Institute of Chemical Technology Research Initiative Program (KK1803) and by the Korea Research Institute of Bioscience and Biotechnology Research Initiative Program for Creative Research.

Original Submission: Aug 8, 2018
Revised Version Received: Sep 3, 2018
Accepted: Sep 4, 2018

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