Journal of Structural Biology 191 (2015) 381–387

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Technical Note

Crystal structure of the first WW domain of human YAP2 isoform

Sergio Martinez-Rodriguez [b], Julio Bacarizo [a], Irene Luque [b], Ana Camara-Artigas [a,*]

[a] Department of Chemistry and Physics, University of Almería, Agrifood Campus of International Excellence (ceiA3), Research Centre for Agricultural and Food Biotechnology (BITAL), Carretera de Sacramento s/n, Almería 04120, Spain
[b] Department of Physical Chemistry, University of Granada, Avda. de Fuentenueva s/n, Granada 18071, Spain

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 6 August 2015
Accepted 6 August 2015
Available online 7 August 2015

Keywords:
WW domain
Crystal structure

ABSTRACT

The WW domains are the smallest modular domains known. The study of the structural basis of their stability is important to understand their physiological role. These domains are intrinsically flexible, which makes them difficult to crystallize. The first WW domain of the human Yes tyrosine kinase Associated Protein (YAP) has been crystallized and its structure has been solved by X-ray diffraction at 1.6 Å resolution. Crystals belong to the orthorhombic space group $P2_12_12$ with unit cell parameters $a = 42.67$, $b = 43.10$ and $c = 21.30$. The addition of proline and other small-molecule additives improves drastically the quality of the crystals. The interactions that stabilize this minimal modular domain have been analysed. This crystal structure reveals that, besides the stabilization of the hydrophobic core of the protein by the aromatic cluster formed by Trp177–Phe189–Pro202, some salt-bridges interactions might affect the stability of the domain.

The WW domain is a small modular domain (approximately 35–40 residues) that takes its name from the presence of two highly conserved tryptophan residues. It was first identified in the Yes tyrosine kinase Associated Protein (YAP) in the 90's (Sudol et al., 1995) and since then it has been found in a variety of proteins with very diverse functions: protein trafficking, receptor signalling, RNA processing, ubiquitin ligation and control of cytoskeleton (Ingham et al., 2005). In these proteins, WW domains appear in single or multi-copy and develop their role in the cell by interacting with proline rich motifs (PRMs) in their partners proteins.

WW domains were firstly classified in four different classes according to their ligands binding preferences: class I-PPxY; class II-PPLP; class III-RPPP(R); and class IV-po(S/T)P. Afterwards, the use of peptide library screens allowed the identification of new classes of WW domains, some of them showing unclassified ligand binding preferences, which do not belong to any of the classes already defined (Otte et al., 2003). Since their discovery WW domains have been the focus of attention as they are related to several important human diseases (McLoughlin and Miller, 2008; Passani et al., 2000; Staub et al., 1996; Sudol et al., 2012). Some of these domains have been found in proteins belonging to the ESCRT machinery, where they play a role in virus budding (Heidecker et al., 2004; Yasuda et al., 2003). Therefore, these domains emerge as potential targets to develop new therapeutic agents.

YAP plays a key role as an effector of the mammalian Hippo tumour suppressor pathway (Pan, 2010). Two major isoforms of the YAP have been described in human tissues: YAP1, containing one WW domain; and YAP2, containing two WW domains (Sudol et al., 1995). Each isoform shows different transcriptional activity: YAP2 is a more potent activator compared with YAP1 (Komuro et al., 2003). The first WW domain, which is present in both isoforms, comprises amino acids 171–204 and the second WW domain, which is present only in YAP2, is located between amino acids 233 and 263. Both are class I WW domains recognizing non phosphorylated PPxY containing sequences (Sudol et al., 2012).

Up to the date, most of the structures of WW domains have been obtained in solution by NMR techniques, but very few of them have been solved by X-ray diffraction. These domains show an intrinsic conformational flexibility impairing in great extent the formation of crystal contacts and, therefore, it complicates the crystallization process. Nowadays only six crystallographic structures of the isolated domain have been solved (Meiyappan et al., 2007; Mortenson et al., 2013; Qi et al., 2013). In the case of the WW domains of the human YAP2 (hYAP2), their structures

Abbreviations: YAP, Yes tyrosine kinase Associated Protein; IPTG, isopropyl-β-thio-D-galactopyranoside; PRM, proline rich motif; MR, molecular replacement; ASA, accessible surface area; NDSB, non-detergent sulfobetaine; MBCD, methyl β-cyclodextrin.

* Corresponding author.
E-mail address: acamara@ual.es (A. Camara-Artigas).

http://dx.doi.org/10.1016/j.jsb.2015.08.001
1047-8477/

are only known by solution NMR techniques (Aragon et al., 2011, 2012; Pires et al., 2001).

Here we report the first crystallographic structure of the *h*YAP2-WW1 at 1.6 Å resolution. Due to its small size and the absence of disulfide bonds and cofactors, these domains are an attractive model system for understanding the principles of protein folding on the simplest level. The reported structure shows long-range and water mediated interactions not previously described in the NMR structures of the YAP2 WW domains, which might be of relevance for molecular dynamics and folding studies of this domain. This structure might also help to shed some light on the controversy between the two-state and the downhill folding models (Jager et al., 2006; Liu et al., 2008).

1. Cloning, overexpression and purification of the first WW domain of the human Yes-Associated Protein (hYAP2-WW1)

An optimized DNA sequence for over-expression in *Escherichia coli* encoding for amino acids Phe165–Gln209 (corresponding to the first WW domain of human YAP2; GenBank acc. No. P46937.2) was purchased from Geneart AG. The gene fragment was subcloned into the pETM-30 vector. This construction allows the overexpression of the *h*YAP2-WW1 fused to an N-terminal His6-tagged Glutation-S-Transferase (N-His-GST-*h*YAP2-WW1), allowing removal of the N-His-GST fragment by proteolysis with TEV protease. Plasmid-encoding *h*YAP2-WW1 construct was expressed in a BL21 (DE3) strain of *E. coli* (Novagen). Cells were grown in LB medium with kanamycin at 37 °C until $OD_{600nm} \sim 0.7$. Expression was induced with 0.2 mM IPTG and the culture was continued at 32 °C for another 5 h. Cells were disrupted in ice by sonication and the supernatant was loaded onto a Ni–NTA column (Clontech) and purified as previously described (Iglesias-Bexiga et al., 2015). Finally, for protein crystallization, the protein was applied to a Superdex 75 16/60 gel filtration column (GE Healthcare) in an AKTA prime FPLC system (GE Healthcare), using 20 mM Tris–HCl pH 8.0 as running buffer. SEC-FPLC purified *h*YAP2-WW1 was concentrated to 15–20 mg mL^{-1} and dialyzed against 20 mM Tris–HCl pH 8.0. *h*YAP2-WW1 samples were then flash-frozen into liquid N_2, and stored at −80 °C until use. Protein purity was determined at different stages of the purification by SDS–PAGE electrophoresis. Protein concentrations were determined from the absorbance at 280 nm (ε_{280} = 12,490 M^{-1} cm^{-1}) (Gill and von Hippel, 1989).

2. Crystallization and data collection

Crystals were obtained by the vapour diffusion technique using a sitting or hanging drop setup at 20 °C, with drops made by mixing equal volumes (1 μL) of protein solution (20 mg mL^{-1}) and reservoir solution. The drops were equilibrated against 0.1 mL of reservoir solution. Crystals of the *h*YAP2-WW1 grew in presence of 1.5 M ammonium sulphate, 0.1 M sodium acetate (pH 5.0) as precipitant solution. Crystals were improved by using the same precipitant solution supplemented with 100 mM proline, 5 mM NDSB-201 and 5 mM MBCD. Before data collection crystals were transferred into a cryoprotectant solution (MitiGen oil) and flash-cooled in liquid nitrogen. X-ray diffraction was collected in a cold nitrogen stream maintained at 110 K at the beamline XALOC of the ALBA Synchrotron Radiation Facility (Spain) using a wavelength of 0.979 Å and a Dectris Pilatus 6 M detector (Juanhuix et al., 2014). Data were indexed and integrated with the package autoPROC (Vonrhein et al., 2011). Data scaling was performed using Aimless (Evans, 2011). The crystallographic parameters and statistics of data collection are listed in Table 1.

Table 1
Data collection and processing.

Diffraction source	ALBA BL13-XALOC
Wavelength (Å)	0.9793
Temperature (K)	100
Detector	Dectris Pilatus 6M
Crystal-detector distance (mm)	216.62
Rotation range per image (°)	0.500
Total rotation range (°)	215
Exposure time per image (s)	0.23
Space group	P21212
a, *b*, *c* (Å)	42.67, 43.10, 21.30
α, β, γ (°)	90, 90, 90
Mosaicity (°)	0.48
Resolution range (Å)	42.670–1.600 (1.630–1.600)
Total No. of reflections	38,300 (1901)
No. of unique reflections	5514 (278)
Completeness (%)	99.200 (96.900)
Redundancy	6.900 (6.800)
$\langle I/\sigma(I)\rangle$	12.000
Rr.i.m.[a]	0.112 (7.363)
Overall *B* factor from Wilson plot ($Å^2$)	12.780

Values for the outer shell are given in parentheses.
[a] Estimated Rr.i.m. = Rmerge[$N/(N-1)$]1/2, where N = data multiplicity.

3. Structure solution and refinement

The structure of the *h*YAP2-WW1 was solved by molecular replacement (MR) with PHASER (Bunkoczi et al., 2013). Several models of the NMR solution structure of the YAP65-WW1 domain in complex with PRMs are available at the PDB, however the MR searches using these models were unsuccessful. A new MR search with PHASER was performed by using the multi-search procedure with several WW ensembles; the models were obtained from the coordinates of the WW and WW-containing proteins structures solved by X-ray diffraction sharing at least 40% of sequence identity with the hYAP2-WW1: Pin1 peptidyl-prolyl cis–trans isomerase from homo sapiens, PDB codes 1PIN (Ranganathan et al., 1997), 3TSC (Graber et al., 2011), 2ITK (Zhang et al., 2007), 4GWT and 4GWV (Mortenson et al., 2013); and FE65-WW domain in complex with *h*Mena peptide, PDB codes 2HO2, 2OEI and 2IDH (Meiyappan et al., 2007). In spite of the low sequence identity of these WW domains, a MR solution was obtained with the coordinates of the human Fe65-WW domain (PDB code 2HO2) as search model. Model building and refinement were carried out with COOT (Emsley and Cowtan, 2004) and PHENIX (Adams et al., 2010). Structure solution and refinement statistics are listed in Table 2.

Table 2
Structure solution and refinement.

Resolution range (Å)	21.551–1.600 (1.832–1.600)
Completeness (%)	98.8
No. of reflections, working set	9439 (3151)
No. of reflections, test set	452 (148)
Final Rcryst	0.178 (0.199)
Final Rfree	0.194 (0.241)
No. of non-H atoms	
Protein	385
Solvent	47
Total	432
R.m.s. deviations	
Bonds (Å)	0.011
Angles (°)	1.356
Average *B* factor ($Å^2$)	16.70
Protein	15.70
Ramachandran plot	
Most favoured (%)	100
Allowed (%)	0

Values for the outer shell are given in parentheses.