Crystallization and preliminary diffraction analysis of the phosphatase domain of PTPN14 in the human papillomavirus E7 binding-defective mutant form

Dahwan Lim^{1,2}, Chang Hoon Lee³, Ho-Chul Shin¹, Seung Jun Kim^{1,*} and Bonsu Ku^{1,*}

¹Disease Target Structure Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea

²Department of Biochemistry, Chungnam National University, Daejeon 34134, Republic of Korea

³Center for Information-Based Drug Research, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea

*Correspondence: ksj@kribb.re.kr, bku@kribb.re.kr

A tumor suppressor protein PTPN14 interacts with the oncoprotein E7 of human papillomaviruses, leading to its proteasomal degradation. Introduction of F1044S, G1055Q, and E1095A mutations into its phosphatase domain restored the antitumor activity by impairing the interaction with E7, showing the therapeutic potential of this mutant form against human papillomavirus-involved cancers. In this study, the phosphatase domain of PTPN14 containing the three mutations was produced in an *Escherichia coli* expression system, purified using Ni-NTA affinity and size exclusion chromatographies, and then crystallized. X-ray diffraction data with a maximum resolution of 1.50 Å were successfully collected, and a preliminary diffraction analysis was conducted. Our crystals belonged to the *P2*₁ space group with unit cell parameters of *a* = 43.9 Å, *b* = 74.3 Å, *c* = 47.8 Å, and β = 102.0°. The asymmetric unit contains one protein molecule with a 45% solvent content and a 2.2 Å³/Da Matthews coefficient.

INTRODUCTION

Classical protein tyrosine phosphatases (PTPs), which catalyze the removal of a phosphate group from phosphorylated tyrosine residues, can be divided into receptor-type PTPs localized to the plasma membrane and nonreceptor-type PTPs that reside in the cytoplasm (Tonks, 2006; Lee et al., 2015). PTPN14, also called PTP-Pez, PTP36, or PTPD2, is a nonreceptor-type PTP. PTPN14 functions as a key regulator of the Hippo signaling pathway (Mello et al., 2017), one of the central cascades controlling cellular proliferation and apoptosis, and is intimately associated with tumorigenesis (Saucedo and Edgar, 2007; Meng et al., 2016). Numerous studies have indicated that PTPN14 is an important tumor suppressor as it inhibits the oncogenic activity of yes-associated protein, the core downstream effector of the Hippo cascade (Wang et al., 2012; Huang et al., 2013; Michaloglou et al., 2013; Wilson et al., 2014). Furthermore, PTPN14 is targeted for proteasome-mediated degradation by the oncoprotein E7 of human papillomaviruses, the causative agents of cervical and other cancers (White et al., 2016; Szalmás et al., 2017). Human papillomavirus E7 recognizes the phosphatase domain of PTPN14 via direct binding; this was structurally determined by X-ray crystallographic and small-angle X-ray scattering analyses (Yun et al., 2019; Lee et al., 2021). The E7 binding-defective mutant PTPN14 was prepared such that Phe1044, Gly1055, and Glu1095 in the phosphatase domain were substituted with serine, glutamine, and alanine, respectively (Yun et al., 2019).

Subsequent biochemical and cellular analyses proved that this mutant is resistant to degradation in HeLa cervical cancer cells and effective in the suppression of cancer cell proliferation, migration, and invasion (Yun et al., 2019). In other words, PTPN14 with F1044S, G1055Q, and E1095A (SQA) mutations was verified as an effective tumor suppressor; therefore, it appears to have therapeutic potential against cervical cancer, one of the most prevalent cancers in women (Bray et al., 2018). In a first step to characterize this mutant protein at the atomic level, we attempted to express, purify, and crystallize the phosphatase domain of the SQA mutant form of PTPN14. We obtained crystals diffracted to 1.50 Å resolution and conducted a preliminary diffraction analysis.

RESULTS AND DISCUSSION

The phosphatase domain of human PTPN14 (residues 886–1187) containing F1044S, G1055Q, and E1095A mutations, referred to as PTPN14_{PTP}(SQA) in this manuscript, was cloned and expressed in an *Escherichia coli* expression system (Table 1). The recombinant PTPN14_{PTP}(SQA) protein was purified using Ni-NTA affinity and size-exclusion chromatographies. Using the final protein sample, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to visualize its purity by Coomassie blue staining (Figure 1). The protein band of PTPN14_{PTP}(SQA) appeared as a single band on the SDS gel, located between the 35 kDa and 43 kDa molecular

weight standards on the SDS gel (Figure 1), consistent with the calculated molecular weight (35.2 kDa) obtained from the ExPASy server (https://web.expasy.org/cgi-bin/compute_pi/pi_tool).

After purification, automated screening of protein crystallization conditions was performed using the sitting drop vapor diffusion

TABLE 1 | PTPN14_{PTP}(SQA) production information

Source organism	Human
DNA source	cDNA
Forward primer*	CGCGGCAGCCATATGTTGATGCCACCCGG GTTCCC
Reverse primer*	TGCGGCCGCAAGCTTTAAATGAGTCTGGAG TTTTGGAGGAA
Expression vector	pET28a
Expression host	<i>E. coli</i> BL21(DE3) RIL
Complete amino acid sequence of the construct produced**	SHMVDATRVPMDERFRTLKKKLEEGMVFEYE QIPKKKANGIFSTAALPENAEERSRIREVVPYEEN RVELIPTKENNTGYINASHIKVWVGGAEWYIAT QGPLPHTCHDFWQMVWEQGVNVIAVMTAEED GGRTKSHRYWPKLGSKHSSATYGKFKVTTKS RTDSVCYATTQLKVKHLLSGQERTVWHLQYTD WPDHGCPEDEVQGFLSYLEAIQSVRRHTNSML EGTKNRHPPIVHCSAGVGRTGVLLSELMIYC LEHNEKVEVPMMLRLLLREQRMFMIQTIAQYKFV YQVLIQFLQNSRLI

*Restriction enzyme sites in the primers (NdeI for forward and HindIII for reverse primers) are underlined.

**The F1044S, G1055Q, and E1095A mutations are marked in red and underlined.

FIGURE 1 | Purified PTPN14_{PTP}(SQA). Recombinant PTPN14_{PTP}(SQA) purified using HiLoad™ 25/600 Superdex™ 75pg column was visualized on an SDS gel by Coomassie blue staining. An arrowhead indicates PTPN14_{PTP}(SQA), the calculated molecular weight of which is 35.2 kDa. MW, molecular weight marker; kDa, kilodalton; A₂₈₀, absorbance values at 280 nm; mAU, milli absorbance units.

method. Initial crystals were obtained using a 40 mg/ml protein sample in C3 of a PEG/Ion Screen, containing 20% polyethylene glycol (PEG) 3,350 and 200 mM sodium acetate trihydrate. Crystals were optimized by altering the protein concentration to 30 mg/ml and the sodium acetate trihydrate concentration in the reservoir solution to 50 mM (Figure 2 and Table 2). X-ray diffraction data to 1.50 Å resolution were collected on beamline 11C at the Pohang Accelerator Laboratory (PAL; Figure 3) (Park et al., 2017). The space group of PTPN14_{PTP}(SQA) crystals was identified as *P*2₁, with unit cell parameters of *a* = 43.9 Å, *b* = 74.3 Å, *c* = 47.8 Å, and β = 102.0°. The total number of reflections was 2,838,848, merged to 46,882 unique reflections, with a merging *R* factor of 5.2% and a completeness value of 98.0%. The data collection and processing statistics are presented in Table 3. A single PTPN14_{PTP}(SQA) molecule was presumed to be present in an asymmetric unit, with the solvent content and Matthews coefficient calculated as 45% and 2.2 Å³/Da, respectively. Structure determination is currently underway; the molecular replacement method is employed using the phosphatase domain structure of wild-type PTPN14 (PDB code: 2BZL) as a search model.

METHODS

Preparation of recombinant protein

The fragment containing residues 886–1187 of human PTPN14 (GenBank accession number NP_005392.2) was amplified by

FIGURE 2 | Crystals of PTPN14_{PTP}(SQA). Initial (left) and optimized (right) crystals are shown with scale bars indicating 0.1 mm.

TABLE 2 | Crystallization

Method	Sitting drop vapor diffusion
Plate type	96-well sitting drop crystallography plate
Temperature (K)	291
Protein concentration (mg/ml)	30
Buffer composition of protein solution	50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 2 mM dithiothreitol
Composition of reservoir solution	20% (w/v) PEG 3,350 and 50 mM sodium acetate trihydrate
Cryoprotectant	20% glycerol
Volume of drop (μl)	0.4 (protein:reservoir = 1:1)
Volume of reservoir (μl)	70

FIGURE 3 | X-ray diffraction image. Representative X-ray diffraction photograph of the PTPN14_{PTP}(SQA) crystal with a 1.0° oscillation range. Circles represent resolution ranges as indicated. The maximum resolution at the edge of the film is 1.4 Å.

polymerase chain reaction and cloned into the pET28a plasmid (Novagen) using the forward and reverse primers described in Table 1. It was used as the template to prepare the mutant form of the PTPN14 PTP domain containing F1044S, G1055Q, and E1095A substitutions. The resulting recombinant pET28a plasmid was transformed into *E. coli* BL21(DE3) RIL (Novagen) competent cells. The cells were cultured in Luria-Bertani broth at 37°C until the optical density at 600 nm reached 0.6. Expression of PTPN14_{PTP}(SQA) was induced by treatment with 0.5 mM isopropyl-β-D-thiogalactopyranoside. After incubation at 25°C for 16 h, the cells were centrifuged at 1800 × *g* for 30 min at 4°C and resuspended in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 3 mM β-mercaptoethanol (β-ME) for sonication on ice. Cell debris was removed by centrifugation of the cell lysates at 18000 × *g* for 50 min at 4°C. The supernatant was loaded onto Ni-NTA agarose resin (QIAGEN), washed with a wash buffer containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 3 mM β-ME, and 30 mM imidazole. A buffer solution containing 50 mM Tris (pH 7.5), 200 mM NaCl, 3 mM β-ME, and 300 mM imidazole was used for protein elution. The N-terminal His₆-tag was removed by thrombin treatment at 4°C for 16 h. The sample was then subjected to a HiLoad™ 25/600 Superdex™ 75pg column (GE Healthcare) equilibrated with a buffer containing 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 2 mM dithiothreitol. The purified protein was concentrated to 40 mg/ml for crystallization.

Crystallization and X-ray data collection

Protein crystallization conditions were screened using commercial solutions. On day 3, initial crystals grew under

TABLE 3 | Data collection and processing statistics

Diffraction source	Beamline 11C, PAL
Wavelength (Å)	0.9794
Temperature (K)	100
Space group	<i>P</i> 2 ₁
a, b, c (Å)	43.9, 74.3, 47.8
α, β, γ (°)	90, 102.0, 90
Resolution (Å)	50.0–1.5 (1.53–1.50)*
Total No. of reflections	2,838,848
No. of unique reflections	46,882
Completeness (%)	98.0 (97.6)
R _{merge} (%)	5.2 (33.0)
Average I/σ(I)	27.6 (6.2)
Redundancy	6.1

*Values in parentheses are for the highest resolution shell.

the C3 condition of the PEG/Ion Screen (Hampton Research), containing 20% PEG 3,350 and 200 mM sodium acetate trihydrate. Improved crystals were obtained by altering the protein concentration from 40 to 30 mg/ml and the reservoir solution composition to 20% PEG 3,350 and 50 mM sodium acetate trihydrate, as described in Table 2. X-ray diffraction data were collected on beamline 11C at PAL (Park et al., 2017) and processed using *HKL2000* (Otwinowski and Minor, 1997), as summarized in Table 3.

ACKNOWLEDGEMENTS

We are grateful to PAL, Korea, for the use of beamline 11C. This work was supported by the National Research Foundation, funded by the Ministry of Science and ICT, Korea (2020R1C1C1008451 and 2019M3E5D6063955) and by the Korea Research Institute of Bioscience and Biotechnology Research Initiative Program (KGM9952112).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

Original Submission: Sep 28, 2021

Revised Version Received: Oct 19, 2021

Accepted: Oct 19, 2021

REFERENCES

- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**, 394–424.
- Huang, J.M., Nagatomo, I., Suzuki, E., Mizuno, T., Kumagai, T., Berezov, A., Zhang, H., Karlan, B., Greene, M.I., and Wang, Q. (2013). YAP modifies cancer cell sensitivity to EGFR and survivin inhibitors and is negatively regulated by the non-receptor type protein tyrosine phosphatase 14. *Oncogene* **32**, 2220–2229.

- Lee, H., Yi, J.S., Lawan, A., Min, K., and Bennett, A.M. (2015). Mining the function of protein tyrosine phosphatases in health and disease. *Semin Cell Dev Biol* **37**, 66-72.
- Lee, H.S., Kim, M.W., Jin, K.S., Shin, H.C., Kim, W.K., Lee, S.C., Kim, S.J., Lee, E.W., and Ku, B. (2021). Molecular analysis of the interaction between human PTPN21 and the oncoprotein E7 from human papillomavirus genotype 18. *Mol Cells* **44**, 26-37.
- Mello, S.S., Valente, L.J., Raj, N., Seoane, J.A., Flowers, B.M., McClendon, J., Biegging-Rolett, K.T., Lee, J., Ivanochko, D., Kozak, M.M., Chang, D.T., Longacre, T.A., Koong, A.C., Arrowsmith, C.H., Kim, S.K., et al. (2017). A p53 super-tumor suppressor reveals a tumor suppressive p53-Ptpn14-Yap axis in pancreatic cancer. *Cancer Cell* **32**, 460-473.e466.
- Meng, Z., Moroishi, T., and Guan, K.L. (2016). Mechanisms of Hippo pathway regulation. *Genes Dev* **30**, 1-17.
- Michaloglou, C., Lehmann, W., Martin, T., Delaunay, C., Hueber, A., Barys, L., Niu, H., Billy, E., Wartmann, M., Ito, M., Wilson, C.J., Digan, M.E., Bauer, A., Voshol, H., Christofori, G., et al. (2013). The tyrosine phosphatase PTPN14 is a negative regulator of YAP activity. *PLoS One* **8**, e61916.
- Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* **276**, 307-326.
- Park, S.Y., Ha, S.C., and Kim, Y.G. (2017). The protein crystallography beamlines at the Pohang Light Source II. *Biodesign* **5**, 30-34.
- Saucedo, L.J., and Edgar, B.A. (2007). Filling out the Hippo pathway. *Nat Rev Mol Cell Biol* **8**, 613-621.
- Szalmás, A., Tomaić, V., Basukala, O., Massimi, P., Mittal, S., Kónya, J., and Banks, L. (2017). The PTPN14 tumor suppressor is a degradation target of human Papillomavirus E7. *J Virol* **91**, e00057-17.
- Tonks, N.K. (2006). Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* **7**, 833-846.
- Wang, W., Huang, J., Wang, X., Yuan, J., Li, X., Feng, L., Park, J.I., and Chen, J. (2012). PTPN14 is required for the density-dependent control of YAP1. *Genes Dev* **26**, 1959-1971.
- White, E.A., Münger, K., and Howley, P.M. (2016). High-risk human papillomavirus E7 proteins target PTPN14 for degradation. *mBio* **7**, e01530-16.
- Wilson, K.E., Li, Y.W., Yang, N., Shen, H., Orillion, A.R., and Zhang, J. (2014). PTPN14 forms a complex with Kibra and LATS1 proteins and negatively regulates the YAP oncogenic function. *J Biol Chem* **289**, 23693-23700.
- Yun, H.Y., Kim, M.W., Lee, H.S., Kim, W., Shin, J.H., Kim, H., Shin, H.C., Park, H., Oh, B.H., Kim, W.K., Bae, K.H., Lee, S.C., Lee, E.W., Ku, B., and Kim, S.J. (2019). Structural basis for recognition of the tumor suppressor protein PTPN14 by the oncoprotein E7 of human papillomavirus. *PLoS Biol* **17**, e3000367.