BL-17A/2017G162, 2018RP-05

X-ray crystallographic analysis of *Stenotrophomonas maltophilia* DPP7

Yasumitsu Sakamoto[1,*], Saori Roppongi[2], Akihiro Nakamura[3], Nobutada Tanaka[4]
[1] School of Pharmacy, Iwate Medical University
[2] School of Medicine, Iwate Medical University
1-1-1 Idaidori, Yahaba, Iwate 028-3694, Japan
[3] Department of Science of Technology Innovation, Nagaoka University of Technology,
1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan
[4] School of Pharmacy, Kitasato University
5-9-1 Shirokane, Minato-Ku, Tokyo 108-8641, Japan

1 Introduction

The carbapenems, which are considered as the last trump card of antimicrobial agents. However, MDRB (MultiDrug-Resistant Bacteria) emerged due to microbial substitution caused by the inappropriate use of carbapenems with a broad antibacterial spectrum.

Nosocomial infections caused by MDRB are major problem for immunocompromised hospitalized patients. MRSA (Methicillin Resistant *Staphylococcus Aureus*), MDRP (MultiDrug-Resistant *Pseudomonas aeruginos*a), CRE (Carbapenem-Resistant *Enterobacteriaceae*), Sm (*Stenotrophomonas maltophilia*) are widely known as representative MDRB. Only new quinolone antibiotics are effective against these bacteria. In recent years, resistant strains of bacteria to new quinolones have emerged[1], and there is a need to develop new antibacterial agents. *Stenotrophomonas maltophilia* is known as a NFGNR (Non-Fermenting Gram-Negative Rod). Bacterial DPP7 is a peptidase, an enzyme important for the uptake of peptides, which are the nutritional source of certain NFGNRs.

2 Experiment

A synthetic gene coding for full-length SmDPP7 (residues 1–720), codon-optimized for Escherichia coli expression, was purchased from GenScript. An E. coli BL21 Gold (DE3) (Agilent Technologies) transformant harboring the mature SmDPP7 sequence (23-720) with signal peptide of DAP BII inserted into the pET-22b (Novagen) expression plasmid was used for the production of recombinant SmDPP7. The SmDPP7 were purified by precipitation with 35–70% ammonium sulfate and hydrophobic column chromatography using a HiPrep 16/10 Butyl column (Cytiva). The eluate was desalted using a HiPrep 26/10 desalting column (Cytiva) and finally subjected to anion-exchange column chromatography using a Mono Q 5/50 GL column (Cytiva).

The 5 mg/ml SmDPP7 solution was mixed with 100 mM Val-Tyr solution in a volume ratio of 19:1, resulting in a final protein concentration of 4.75 mg/ml and a final ligand concentration of 5 mM. 1 µl of the protein solution was mixed with an equal volume of the reservoir solution (20% (w/v) PEG8000 and 0.2 M ammonium acetate). The drop is suspended over 200 µl of the reservoir solution in a 48-well plate to crystallize at 293K[2]. The obtained crystal in a droplet was directly transferred to cryo protectant (16% (w/v) PEG8000 and 0.16 M ammonium acetate 20% Glycerol) prior to X-ray data collection performed under cryogenic condition. Crystal was mounted in nylon loops and flash-cooled in a cold nitrogen gas stream at 100 K immediately before data collection. Data collection was performed by the rotation method at 100 K using an Pilatus3 S 6M detector with synchrotron radiation ($\lambda = 0.98$ Å) on beamline BL17A of the Photon Factory. The Laue group and unit-cell parameters were determined using xia2/DIALS[3] with XDS[4].

3 Results and Discussion

The diffraction data from a SmDPP7-VY crystal were collected to 2.03 Å resolution. Data-collection statistics are summarized in Table 1.

Table 1: Data collection statistics

Beamline	BL17A
Wavelength	0.98
Temperature	100 K
Space group	$P\,2_1$
Cell dimensions (a, b, c, α, β, γ)	137.16, 75.26, 161.20, 90, 108.22, 90
Resolution	2.03 (2.07-2.03)
No. of Reflections	1378355 (59701)
Unique reflections	201373 (9639)
Multiplicity	6.8 (6.2)
Completeness	99.8 (96.6)
I/sigma	10.0 (2.0)
$CC_{1/2}$	0.997 (0.793)
R_{merge}	0.116 (0.921)
Wilson B	26.7

Values for the highest resolution shells are given in parentheses.

Acknowledgement

We thank Drs. Y. Yamada, M. Hikita and N. Matsugaki of the Photon Factory. This research was supported by a Grants-in-Aid for Scientific Research(C) 16K08322, 21K06071, the Takeda Science Foundation, Basis for Supporting Innovative Drug Discovery and Life Science Research(BINDS) 0026.

References

[1] E. Kim *et al*., *BMC Infect. Disea.* **19**, 754 (2019).
[2] A. Nakamura *et al*., *Sci Rep.* **11**, 7929 (2021).
[3] G. Winter *et al*., *Acta Crystallogr. D* **74,** 85-97 (2018)
[4] W. Kabsch *et al*., *Acta Crystallogr. D* **66,** 125-132 (2010)

* sakamoto@stbio.org