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X-ray crystal structure analysis of lasso-peptide epimerase, MslH

Yu Nakashima[1] and Hiroyuki MORITA[1,*]
[1]Institute of Natural Medicine, University of Toyama, Toyama 930-0194, Japan

1 Introduction

Lasso peptide MS-271, as an inhibitor of calmodulin-activated myosin light chain kinase from *Streptomyces* sp. M-271 [1-2], is classified as ribosomally synthesized and post-translationally modified peptides (RiPPs) (Fig. 1). MS-271 form consists of 21 amino acids and has a D-tryptophan at the *C*-terminal. This lasso peptide is initially produced by the ribosome as the precursor peptide MslA and matured by several post-translational modifications. MslH, which is identified in the MS-271 biosynthetic gene cluster (*msl*), catalys the epimerization on the *C*-terminal MslA Trp21 at the Cα center, leading to the formation of *epi* MslA (Fig. 1) [3-4]. The intricate catalytic process, encompassing the catalytic site and cofactors, has remained an enigma. The current investigation employed X-ray crystallography and interaction with the MslA core peptide analogue to determine that MslH is a peptide epimerase that relies on metal ions. Crystallographic studies of MslH have further indicated that this enzyme employs acid/base chemistry to enable the reversible epimerization of the *C*-terminal Trp21 of MslA [5].

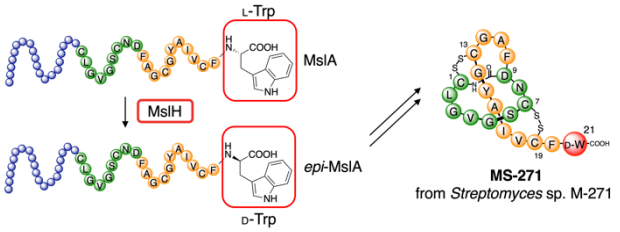

Fig. 1: Reaction scheme of MslH and structure of MS-271.

2 Experiment

Crystallization – Diffraction-quality crystals of the *N*-His_6-tagged 5 mg/mL MslH with/without 1.89 mg/mL MslAW21G-MslB1 complex, in which MslA W21 was substituted G21 as an inactivated form, were obtained at 20 °C, in 200 mM $MgCl_2$, 100 mM Tris-HCl (pH 8.0-9.0), and 28% (w/v) PEG400 by using sitting-drop vapor-diffusion method.

Data collection – The crystals were transferred into the soaking solution with 20% (v/v) glycerol for 10 sec for cryoprotection and then flash cooled at -196 °C in a nitrogen-gas stream. The X-ray diffractions of crystals were collected at BL-1A, processed and scaled with *XDS* and *AIMLESS* in the *CCP4* program package. The MslH:*apo* structure was solved by S-SAD, using the AutoSol and the MslH:MslAW21G complex structure was solved by the molecular replacement method with *Phaser-MR* using the MslH:*apo* structure (PDB code: 8GQ9) as a template. The structures were modified manually with *Coot* and refined with *PHENIX*.

3 Results and Discussion

The MslH:*apo* and MslH:MslAW21G complex structures were solved by X-ray crystallography at 2.30 Å and 2.12 Å resolution, with the final *R*-value of 17.7% (R_{free} = 20.5%) and 18.2% (R_{free} = 20.7%), respectively, as the dimeric form. The *C*-terminal carboxyl group of MslAW21G was coordinated with the Ca(II) metal in the active-site cavity within the dimer form of MslH, and the amide moiety of MslA F20-G21 has polar interactions with the main-chain carbonyl oxygen of MslH G60 (2.6 Å) and the side-chain of His295 (2.5 Å) (Fig. 2). The Cα center of MslA G21 located in the middle of MslH H88 and H295, interacting with MslH D91 and D11, respectively. This crystallographic observation indicates that MslH employs an “acid-base” chemistry in the epimerization reaction to generate *epi*-MslA from MslA (Fig. 1).

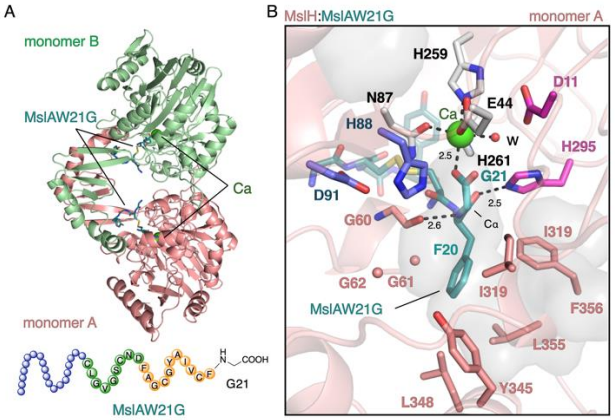

Fig. 2: Crystal structure of the MslH:MslAW21G (PDB code: 8ITG) complex structure. (A) Overall structure of MslH:MslAW21G as the dimeric form and the structure of MslAW21G. (B) Close-up view of the active site. The dashed lines represent the distances in Å.

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References

[1] K. Yano *et al.*, *Bioorg. Med. Chem.* **4**, 115-120 (1996).

[2] R. Katahira *et al*., *Bioorg. Med. Chem.* **4**, 121-129 (1996).
[3] Z. Feng *et al*., *Chembiochem* **19**, 2045-2048 (2018).
[4] Z. Feng *et al*., *Chem. Sci.* **12**, 2067-2074 (2020).
[5] Y. Nakashima *et al*., *Nat. Commun.* **14**, 4752 (2023).

* hmorita@inm.u-toyama.ac.jp