Functional analysis of post-PKS enzymes in hitachimycin biosynthesis

Akimasa MIYANAGA

Department of Chemistry, Tokyo Institute of Technology

Research aims

Hitachimycin, a β-amino acid-containing macrolactam polyketide antibiotic isolated from *Streptomyces scabrisporus*, has both antiprotozoal and antitumor activities[1]. The main structural features of hitachimycin are the β-phenylalanine starter unit of its polyketide skeleton and a bicyclic structure with an internal five-membered carbocycle (Fig. 1). The bicyclic structure likely results from post-polyketide synthase (post-PKS) modification reactions. However, the mechanism underlying the formation of such a bicyclic structure remains unclear. In this study, we used gene-disruption and biochemical analyses to investigate putative enzymes that have been proposed to function in the post-PKS modification reactions of hitachimycin biosynthesis.

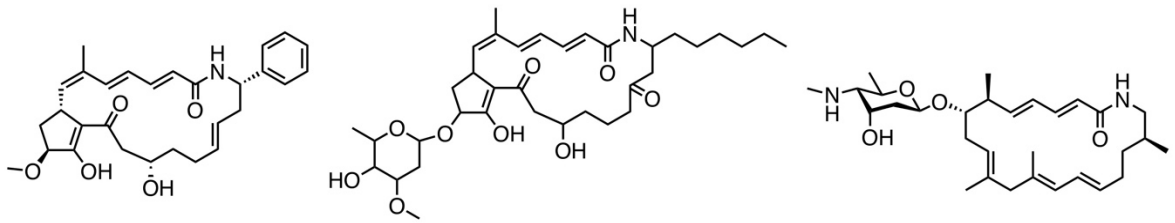

Figure 1. β-amino acid-containing macrolactam polyketide antibiotics

Methods

We previously reported the identification of the hitachimycin biosynthetic gene cluster of *S. scabrisporus*[2]. Within this cluster, six genes (*hitM1*, *hitM2*, *hitM3*, *hitM4*, *hitM5*, and *hitM6*) have been proposed to play roles in the post-PKS modification reactions of hitachimycin biosynthesis. While the *hitM1* and *hitM4* genes encode putative oxidoreductases, *hitM2* and *hitM5* encode putative isomerases, *hitM3* encodes a putative cytochrome P450 monooxygenase, and *hitM6* encodes a putative methyltransferase. Here, we constructed *S. scabrisporus* disruption mutants (disruptants) for these six genes (referred to as Δ*hitM1* to Δ*hitM6 strains*) and analyzed the compounds they were producing. Furthermore, we performed *in vitro* biochemical analyses of the reaction catalyzed by the

HitM4 protein using recombinant HitM4 and the hitachimycin biosynthetic intermediate produced by the Δ*hitM4* strain.

Results

To confirm that the *hitM1-6* genes are involved in hitachimycin biosynthesis, we first examined whether the Δ*hitM1* to Δ*hitM6* strains were producing hitachimycin. As expected, none of the disruptants were producing hitachimycin, showing that these genes are indeed involved in hitachimycin biosynthesis. Moreover, we found that some of these strains were accumulating hitachimycin derivatives. Among them, the Δ*hitM1* and Δ*hitM6* strains produced hitachimycin derivatives that contained a bicyclic structure. This observation strongly suggests that HitM1 and HitM6 catalyze reactions that are downstream of the formation of the bicyclic structure.

Conversely, the Δ*hitM4* strain produced a hitachimycin biosynthetic intermediate (referred to as compound 1) that does not contain a bicyclic structure. Therefore, it is likely that HitM4 enzymatic activity is involved in the formation of the bicyclic structure. To further investigate the function of HitM4, we expressed recombinant HitM4 in *E. coli* and purified the protein to homogeneity. We then optimized the reaction conditions and successfully detected the conversion of compound 1 by HitM4 *in vitro*.

Conclusions

In this study, we examined post-PKS modification reactions in the hitachimycin biosynthetic pathway. Our gene-disruption and biochemical analyses clearly showed that six enzymes (HitM1 to HitM6) are involved in the post-PKS modification reactions of hitachimycin biosynthesis. Our data suggest that, among these six enzymes, HitM4 is responsible for the first step of the post-PKS modification reactions. Although this study represents significant progress, further analyses are necessary to elucidate the mechanism underlying the formation of the bicyclic structure of hitachimycin.

References

1) Ōmura, S., Nakagawa, A., Shibata, K, and Sano, H. (1982) The structure of hitachimycin, a novel macrocyclic lactam involving β-phenylalanine. *Tetrahedron Lett.* **23:** 4713-4716.
2) Kudo, F. *et al.* (2015) Genome mining of the hitachimycin biosynthetic gene cluster: involvement of a phenylalanine-2,3-aminomutase in biosynthesis. *Chembiochem* **16:** 909-914.