博士論文（要約）

Studies on the biosynthesis of dialkylbenzene-containing natural products produced by *Streptomyces*

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Two natural products **1** and **2** have been isolated and identified from a rifampicin resistant mutant TW-R50-13 derived from *Streptomyces* sp. SANK 60404 in our laboratory. Both compounds contain a very rare *o*-tolyl group among natural products, and in addition, **1** has a relatively long polyene moiety. Feeding experiments using ^{13}C-labeled acetate have suggested that the backbone including the intact tolyl group of **1** is synthesized by polyketide synthase (PKS). Many natural products containing an *o*-dialkylbenzene moiety have been isolated, which exhibit a variety of biological activities such as antibacterial, antifungal, antitumor and antiangiogenic activities. Moreover, bioinformatics analysis suggests that bacteria producing these natural products encode highly reducing (HR) type II PKS genes in their genomes. It has been speculated that the *o*-dialkylated benzene moiety is formed from polyene precursor via an intramolecular 6π-electrocyclic reaction, followed by dehydrogenation. However, the biosynthetic mechanism of *o*-dialkylated benzene has remained elusive, and such an enzyme, if it actually exists, has never been documented.

Chapter 1. Gene inactivation experiments and bioinformatics analysis of the *mbg* cluster

The compounds **1** and **2** biosynthetic gene cluster (BGC) containing 31 discrete open reading frames (ORFs), which also encodes HR type II PKS, have been identified in our laboratory. This gene cluster was named *mbg.* Since the *mbg* cluster features eight KSs, three ACPs, and three DHs, it is difficult to elucidate how *o*-tolyl polyene moieties of **1** and **2** were synthesized, respectively. Therefore, genes associated with the biosynthesis of **1** and **2** in the *mbg* cluster were identified by *in vivo* gene inactivation experiments and bioinformatics analysis. As a result, the backbone polyenes of **1** and **2** would share part of the biosynthetic pathway by using a common set of HR type II PKS including *mbg8* (ACP), *mbg7* (KS), *mbg6* (KS) or *mbg25* (KR). On the other hand, the biosynthesis of intact backbone polyene of **1** also required extra HR type II PKS genes *mbg21* (KS) and *mbg9* (DH). These results indicated that after the *o*-tolyl moiety linked to an ACP was synthesized by a set of HR type II PKS, this intermediate would be catalyzed by other set of HR type II PKSs for the further chain elongation.

Chapter 2. Reconstitution of the biosynthesis of compounds 1 and 2 *in vitro*.

To reconstitute the biosynthesis of *o*-tolyl polyene moiety of **1** and **2** *in vitro*, 19 recombinant proteins were prepared and incubated with appropriate substrates at 30°C for 1 h (Reaction mixture 1). As a result, compounds **3**–**6**, where **5** and **6** are C_5N unit-less precursors of compounds **1** and **2**, were accumulated. Further incubation of **5** and **6** with the enzymes and substrates required for C_5N unit formation at 30 °C for 2 h (Reaction mixture 2), compound **1** and a tiny amount of **7**, which is the oxidized precursor of compound **2,** were detected. On the other hand, compounds **3**–**6** were undetectable when the hydrolysis step was skipped, suggesting that **3**–**6** remain to be tethered to ACPs during the biosynthesis and that formation of the *o*-tolyl group is carried out in an ACP-bound state.

Chapter 3. Elucidation of the biosynthetic pathway of compounds 5 and 6.

Since ACP Mbg10 and Mbg14 show 99% identical in Reaction mixture 1, only Mbg8 and Mbg10 were used for further *in vitro* experiments described in the present study (Reaction mixture 3). A series of *in vitro* assays indicated that Mbg8, not Mgb10 is responsible for the *o*-tolyl group formation and the resulting intermediate could be transferred to Mbg10 for further chain elongation. Subsequently, the four types of KS-CLF responsible for the biosynthesis of compounds **5** and **6** in Reaction mixture 3 were investigated. It was shown that compound **4**, as a starting unit attached to Mbg10, was elongated by Mbg21-Mbg22 (KS-CLF) to produce compound **6**, while elongated by Mbg16-Mbg15 (KS-CLF) or Mbg21-Mbg22 (KS-CLF) to produce compound **5**, which agrees with *in vivo* knockout data mentioned above. On the other hand, KS-CLFs Mbg7-Mbg3, Mbg6-Mbg5 are responsible the biosynthesis of *o*-tolyl group.

Chapter 4. Formation of the *o*-tolyl group in compound 4.

A minimal reaction mixture, which is sufficient for the biosynthesis of *o*-tolyl moiety, was defined and employed to study the *o*-tolyl group biosynthesis. It was identified that Mbg7-Mbg3 is works on the biosynthesis of polyene intermediate **3** and Mbg6-Mbg5 catalyzes the final C_2 extension. In addition, the final chain elongation requires the help of enzymes Mbg2 and Mbg17. Subsequently, only the elongated intermediate could undergo an intramolecular 6π-electrocyclic reaction followed by FAD/FMN-dependent dehydrogenation to form the *o*-tolyl structure. We speculate this proposed biosynthetic route as a common strategy for the formation of *o*-dialkylbenzene in nature because further bioinformatic analysis shows that all key proteins are conserved in the BGCs of natural products containing *o*-dialkylbenzene groups.

Conclusion

The present work provided detailed insights into the biosynthesis of compounds **1** and **2** including the formation of *o*-tolyl group, which not only expands a catalytic repertoire of pericyclic reactions found in biological processes but also unravels the biosynthetic schemes of other natural products containing an *o*-dialkylbenzene motif. Mbg2 represents the first example of a carbocyclase that acts on a polyene structure and catalyzes an intramolecular 6π-electrocyclic reaction.