NISR RESEARCH GRANT
2003 Young Investigator Research Grant

Characterization of the Biosynthetic Gene Cluster of Goadsporin which Promote Secondary Metabolism and Sporulation in Actinomycetes

Hiroyasu ONAKA

Biotechnology Research Center, Toyama Prefectural University

Research aims

Goadsporin which was isolated from *Streptomyces* sp. TP-A0584 is a 19-amino-acid polypeptide containing four oxazole and two thiazole rings within its backbone (Fig. 1). Goadsporin promotes secondary metabolism and morphogenesis in actinomycetes. There are no related reports on the substance which induces the secondary metabolism and morphogenesis in a wide variety of different *Streptomyces* species. The research aims of this study are to identify and characterize the goadsporin biosynthetic gene cluster, and propose the mode of action of it.

Methods and results

Cloning of the goadsporin biosynthetic gene cluster

Goadsporin is a peptide compound, therefore we proposed that goadsporin was biosynthesized ribosomally. It is predicted that unusual amino acids contained in goadsporin, thiazole, oxazole, and methyloxazole are derived from cysteine, serine, and threonine. We synthesized oligonucleotide probe corresponding to the precursor sequence of goadsporin and did colony hybridization with the oligoprobe. Several clones were obtained, and one of positive clone, pGSBC1 was selected.

Heterologous expression of goadsporin in Streptomyces lividans

pGSBC1 was transformed into *S. lividans* TK23, that is a surrogate host for heterologous expression. pGSBC1 was constructed on the basis of pTOYAMAcos cosmid vector which is integrated into the specific chromosome *attC* site in actinomycetes. pGSBC1 integrated strain produced goadsporin. Nucleotide sequencing of the pGSBC1 fragment revealed that *godA* is located on the end of the fragment and 14 ORFs are located on the downstream of *godA* spanning 20-kb. Subcloning experiments revealed that essential region for goadsporin biosynthesis is between *godA* and *orf5* spanning 20-kb as shown in Fig. 2. In conclusion, the ten genes, *godA*, *godB*, *godC*, *godD*, *godE*, *godF*, *godG*, *godH*, *godI*, and *godR* are responsible for goadsporin biosynthesis.

Proposed overall biosynthetic pathway of goadsporin

A proposed biosynthetic pathway for the goadsporin is shown in Fig. 3. The 49-residue *godA* polypeptide is a substrate, and it processed by putative goadsporin synthetase, *godD*, *E*, *F*, and *G*. They introduce six heterocycles and converts GodA to proGoadsporin. Proteolysis of proGoadsporin is performed by GodB which contains a conserved peptidase domain. Finally GodH, which encodes the putative acetyltransferase, catalyzes the N-acetylation of N-terminal to end goadsporin biosynthesis. GodI may be responsible for the resistance of goadsporin and/or translocation of goadsporin and its biosynthesis apparatus.

Conclusion

Goadsporin which was isolated from *Streptomyces* sp. TP-A0584 is a 19-amino-acid polypeptide containing four oxazole and two thiazole rings within its backbone. Except for goadsporin, linear polypeptides containing thiazole and oxazole rings have not been isolated from actinomycetes. Goadsporin promotes secondary metabolism and morpho-

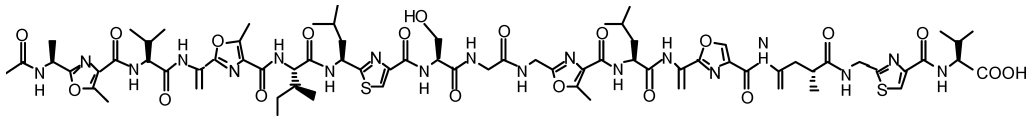

Fig. 1. The chemical structure of goadsporin.

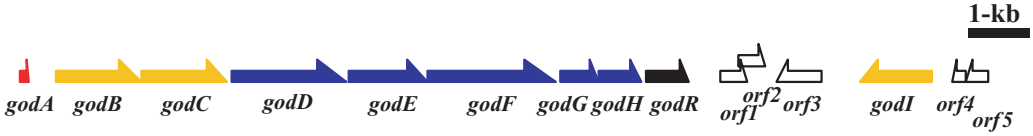

Fig. 2. Genetic organization of the goadsporin biosynthetic gene cluster.

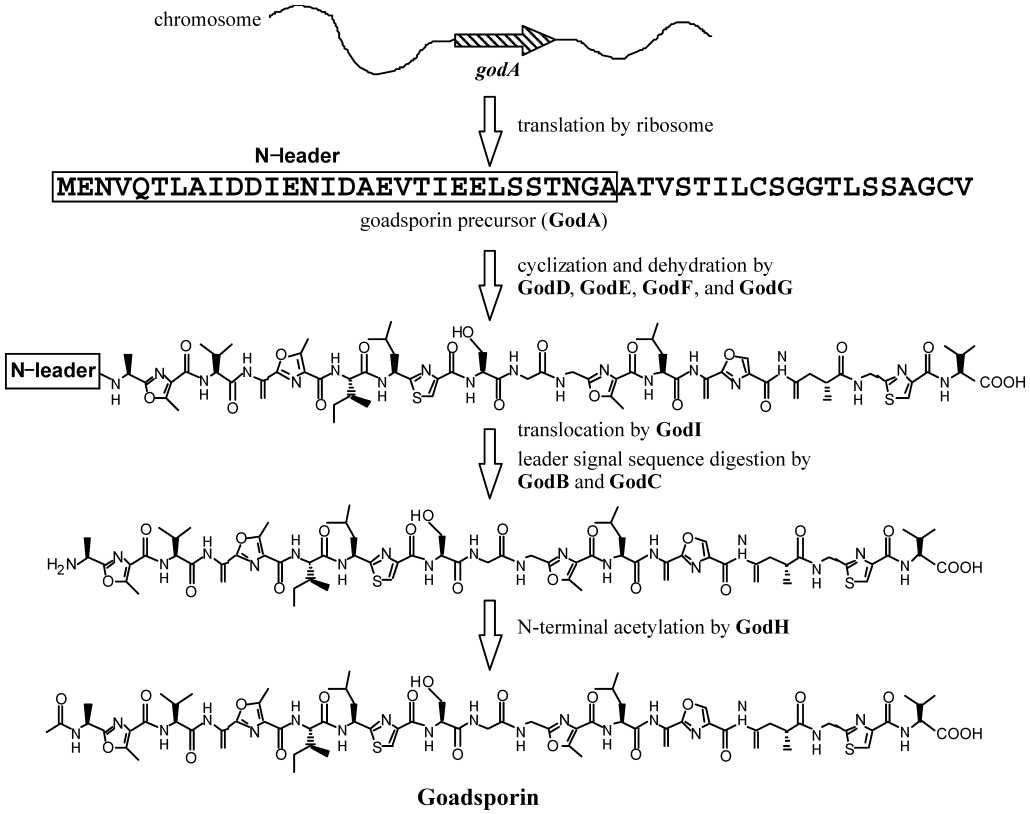

Fig. 3. Proposed overall biosynthetic pathway of goadsporin.

genesis in actinomycetes. For example, it promotes the formation of red pigment and sporulation at a concentration of 1 μM and growth inhibition occurred at more than 1 μM in *Streptomyces lividans*. Such an activity was observed against to a wide variety of actinomycetes, however no activity to the other organisms. The biosynthesis of goadsporin would be started by the translation of *godA* structural gene, and then the serine residue is modified to oxazole or dehydroalanine, threonine is to methyloxazole, and cystein is to thiazole. *godD*, *godE*, *godF*, and *godG* products would involve in such a post translational modification. Finally, the peptide sequence of N-terminal region would be digested and acetylated to afford mature goadsporin by *godC* and *godH* products. The genetic information of goadsporin biosynthesis was applied to the goadsporin derivative biosynthesis. The site directed mutagenesis of *godA* has generated five molecules of the derivative.

References

H. Onaka, H. Tabata, Y. Igarashi, Y. Sato and T. Furumai, Goadsporin, a chemical substance which promotes secondary metabolism and morphogenesis in streptomycetes. I. Purification and Characterization, *The Journal of Antibiotics*, **54**, 1036–1044, (2001)

Y. Igarashi, Y. Kan, K. Fujii, T. Fujita, K. Harada, H. Naoki, H. Tabata, H. Onaka and T. Furumai, Goadsporin, a chemical substance which promotes secondary metabolism and morphogenesis in streoptomycetes. II. Structure determination, *The Journal of Antibiotics*, **54**, 1045–1053 (2001)