【Grant-in-Aid for Scientific Research (S)】

Studies on innovative technology for modification of structural skeleton of natural products and production of artificial novel natural compounds

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Project Information	Project Number : 23H05474 Project Period (FY) : 2023-2027 Keywords : Natural product chemictry, middle-molecular weight molecules, genetic engineering, biosynthesis

Purpose and Background of the Research

●Outline of the Research

It is often challenging to synthesize mid-molecular natural compounds by organic synthesis; thus, developing biosynthesis-based structural modification technology is strongly desired. In our previous studies, we have developed an innovative biosynthetic gene modification technology, which we named "module editing technology," and have made it possible to create mid-molecular natural compounds with the intended skeletal modification. In this study, we aim to establish a technology that covers a broader range of natural compounds and rapidly and efficiently modifies their structural units, thereby contributing to the needs of society in both basic and applied research.

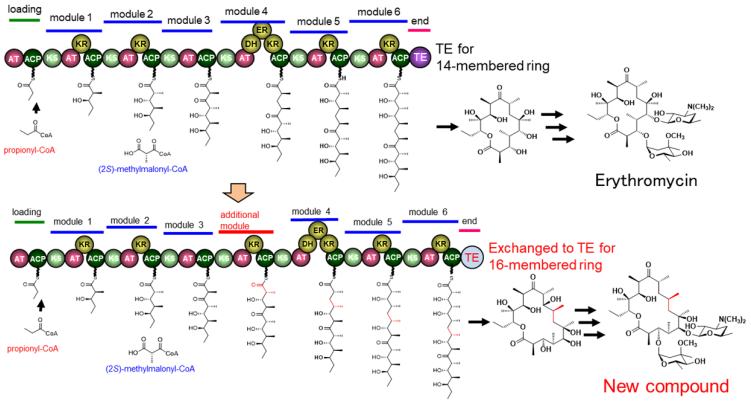

Figure 1. The core concept of this research. Creation of artificial new natural compounds with the intended skeleton by modifying "modular" biosynthetic enzymes at will.

●Purpose and Background of the Research

Middle-molecular weight compounds (middle molecules) with molecular weights ranging from 400 to 2,000 are attracting attention as new drug resources, filling the gap between small molecules such as aspirin and H2 blockers and large molecules such as antibody drugs (Herceptin and Opdivo) and biologics (insulin, erythropoietin, etc.). Natural compounds exhibit diverse structures and potent activity, making them an ideal resource for building a library of middle molecules. However, developing derivatives of natural-derived middle molecules by organic synthesis is still challenging. Therefore, developing synthetic biological structural modification techniques is considered the most effective strategy. Utilizing "modular" enzymes such as polyketide synthases and non-ribosomal peptide synthetases should solve these problems, which many natural product chemists have dreamed of. Still, most studies have been individualistic, and no practical methodology exists. Therefore, to make breakthroughs, it is necessary to comprehensively study the technologies to control the functions of these valuable "modular" enzymes and the structural biology for highly efficient molecular design. Based on the above, this study aims to develop practical combinatorial biosynthetic technologies using modular enzymes.

	Small molecules	Natural middle molecules (macrolides, peptides etc.)	Large molecules (antibodies, biologics etc.)
Structure	ex. Aspirin Mw 180	ex. Rapamycin Mw 900	Ex. Herceptin Mw 148,000
Molecular weight	< 500	500～2000	about 150,000
Molecular formula	$C_9H_8O_4$	$C_{51}H_{81}NO_{12}$	LC: $C_{1032}H_{1603}N_{277}O_{335}S_6$ (214 AA) HC: $C_{2192}H_{3387}N_{583}O_{671}S_{16}$ (449 AA)
Way of produce	Organic synthesis	Organic synthesis or biosynthesis (plants and microbes)	Biosynthesis (animal)
Gene size	–	107 kbp	642 bp (LC) + 1,347 bp (HC)
Reaction steps (excluding protein syntheses)	3	Organic synthesis: 34 steps, yield 2% (～5 years) Biosynthesis: 60 steps in cells / 1 week Number of genes (enzymes): 75	Biosynthesis: 0 step (excluding sugar attachment) Number of genes: 2
Structural design	Relatively easy	Difficult (beyond the human knowledge)	Easy (combinations of 20 amino acids)

Insulin: Mw 5807、$C_{257}H_{383}N_{65}O_{77}S_6$ (21 AA+30 AA)

Figure 2. Comparison of natural middle molecules with other modalities

Expected Research Achievements

●Realizing combinatorial biosynthesis using proprietary technology

Modular enzymes are giant enzymes consisting of many modules, a set of enzyme domains, that form the backbone of polyketides and peptides by passing biosynthetic intermediates one after another, like a car assembly line. Therefore, it has been thought that mother skeleton modification is possible by replacing, deleting, or adding gene regions corresponding to the modules. However, the huge size of the genes and the high similarity of each module made precise genetic modification impossible with conventional gene manipulation techniques. The module editing technology we have developed overcomes this problem and is versatile for modifying the mother skeletons of natural middle molecules with a one-to-one correspondence between compound structural design and genetic modification. Based on this original technology, we aim to realize combinatorial biosynthesis utilizing any modular enzyme by constructing a theory for efficient intermediate delivery between modules and developing practical parts based on the theory. We believe that this research will enhance "synthetic biology", which is expected to be used for industrial applications, into a technology that can be applied in various fields. We aim to contribute to "biomanufacturing" in multiple areas, including drug discovery, by using Japan's strengths in microorganism libraries and knowledge and expertise in natural product chemistry. We hope this will help strengthen Japan's bio-industry competitiveness, promote the creation of new industries, and secure our country's international superiority.

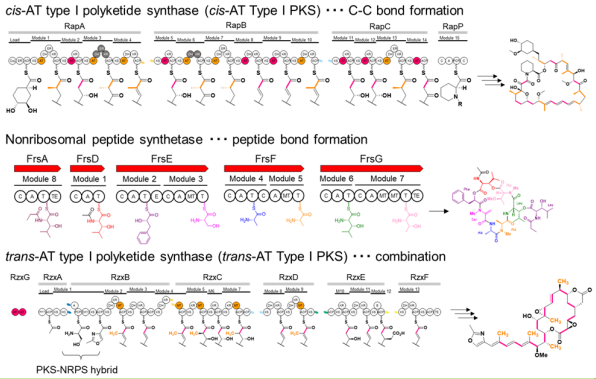

Figure 3. "Modular" enzymes responsible for natural middle molecules biosynthesis

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