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研究課題名（和文）Structure-guided engineering of a biodegradable plastic synthase for tailor-made polymer production

研究課題名（英文）Structure-guided engineering of a biodegradable plastic synthase for tailor-made polymer production

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研究成果の概要（和文）：PHA合成酵素（PhaC）は、ポリヒドロキシアルカノエート（PHA）の生合成における重要な酵素であり、石油化学プラスチックの有望な代替品となる生分解性プラスチックです。本研究では、PhaCの結晶構造を決定し、触媒作用に重要な領域を特定しました。PhaCの出口トンネル内の特定の位置を変異させ、変異した酵素を精製し、その酵素活性を測定しました。さらに、PhaCの正確な触媒機構を生化学的および酵素学的分析を通じて解明しました。本研究の成果は、PhaCの基質特異性を広げるための将来のエンジニアリングにとって重要な指針を提供します。

研究成果の学術的意義や社会的意義
This study reveals the structure-function relationship of the biodegradable plastic-synthesizing enzyme PHA synthase (PhaC). It is utmost urgent to improve the efficiency and substrate range of PHA production, catalysed by PhaC, in order to reduce the production costs of the biodegradable plastics.

研究成果の概要（英文）：PHA synthase (PhaC) is the key enzyme in the biosynthesis of polyhydroxyalkanoate (PHA), a biodegradable plastic that offers a promising alternative to petrochemical plastics. In this study, the crystal structure of PhaC was determined, and important regions for catalysis were identified. Specific positions in the egress tunnel of PhaC were mutated, and the mutated enzymes were purified and their enzymatic activities measured. Additionally, the exact catalytic mechanism of PhaC was elucidated through biochemical and enzymatic analyses. The outcomes of this study provide an information for future engineering of PhaC to broaden its substrate specificity.

研究分野：Structural biology, Enzymology

キーワード：Biodegradable plastic　Polyhydroxyalkanoate　Enzyme engineering

１．研究開始当初の背景

Replacing the petrochemical plastic with biodegradable polymer, polyhydroxyalkanoate (PHA), is the utmost important step in reducing plastic pollutions. PHA synthase (PhaC) is the key enzyme in synthesizing PHA, with a higher preference to short-chain length (scl) substrates, but a lower preference to medium-chain length (mcl) substrates. Despite decades of studies, the key factor in determining the substrate specificity of PhaC remained elusive. This study aims to understand the role of tunnel in PhaC on its substrate specificity through mutational studies. In addition, this study also examines the proposed model of the catalytic mechanism of PhaC *via* biochemical analyses. This study will provide information for future engineering of PhaC for tailor-made PHA production with broader substrate specificity.

２．研究の目的

The purpose of this study is to understand the catalytic mechanism of PhaC through mutational analyses. In addition, this study also explores the importance of the residues located in the newly discovered tunnel egress in the substrate specificity of PhaC. In the second part of the study, this study aims to examine the processive catalytic mechanism of PhaC, whereas one active molecule adopt an open conformation and supported by an inactive molecule, forming a heterodimer for the polymerization of PHA.

３．研究の方法

This proposed study is planned to understand the substrate specificities of PhaC through mutagenesis studies around the tunnel. The mutational residues will be selected based on the tunnel discovered in the crystal structure **(*ia. Structural analysis*)**. The target residues will be mutated to amino acid with smaller side-chain to increase the volume of the tunnel. In addition, the mutational strategy also includes replacing the corresponding residues in $PhaC_{Ac}$ by residues found in PhaC with higher mcl-substrates preferences, such as PhaC from *Pseudomonas* sp. 6-13 **(*ib. Sequence alignment*)**. Once the mutational targets were determined, the amino acids will be mutated through site-directed mutagenesis using a pair of mutagenic primers in PCR reactions based on pGEX-6P3-expression vector harboring $phaC_{Ac}$ ***(ii. Site-directed mutagenesis)***. The mutated proteins will be expressed in *Escherichia coli Rosetta 2* (DE3) expression host and purified based on previous established methods using affinity chromatography, ion-exchange chromatography, and size-exclusion chromatography (*Chek et. al.,* ***Sci. Rep.*** *7, 5312, 2017)* ***(iii. Potein expressions and purifications)***. The substrate specificities of the mutants will be determined by comparing the rate of enzyme reactions towards scl-substrates (3HBCoA) and mcl-substrates (3HxCoA) *(Chek et. al.,* ***iScience*** *23(5):101084, 2020*) ***(iv. in vitro enzymatic activity assay)***. In addition, the relationship between the activities and the oligomerization of the mutants will be examined using analytical gel-filtration ***(v. Biochemical characterization)***. After the

identification of the importance residues, the second part of the project was focused on understanding the processive mechanism of PhaC. The catalytic mechanism of PhaC was examined through *in vitro* enzymatic assay.

４．研究成果

1) Based on the mutational analyses, the important residues affecting the substrate specificity and turnover rate of the PhaC were identified near to the exit of the tunnel egress. In the *in vitro* enzymatic assay, the reaction of the mutant was significantly enhanced for both 3HBCoA and 3HHxCoA substrates.
2) Based on the crystal structure of PhaC from *Aeromonas caviae*, the enzymes form a homodimer, which represent the functional unit of PhaC.Our enzyme assay system indicated that PhaC adopts processive mechanism with a single active site in the dimeric formation. The inactive PhaC molecule in the dimer is functioning to support the open conformation of the activated PhaC molecule.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　2件）

１．著者名 Tan Hua Tiang、Chek Min Fey、Miyahara Yuki、Kim Sun-Yong、Tsuge Takeharu、Hakoshima Toshio、Sudesh Kumar	４．巻 134
２．論文標題 Characterization of an (R)-specific enoyl-CoA hydratase from Streptomyces sp. strain CFMR 7: A metabolic tool for enhancing the production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)	５．発行年 2022年
３．雑誌名 Journal of Bioscience and Bioengineering	６．最初と最後の頁 288～294
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jbiosc.2022.07.005	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Tan Hua Tiang、Chek Min Fey、Neoh Soon Zher、Ang Shaik Ling、Yoshida Shosuke、Hakoshima Toshio、Sudesh Kumar	４．巻 206
２．論文標題 Characterization of the polyhydroxyalkanoate (PHA) synthase from Ideonella sakaiensis, a bacterium that is capable of degrading and assimilating poly(ethylene terephthalate)	５．発行年 2022年
３．雑誌名 Polymer Degradation and Stability	６．最初と最後の頁 110160～110160
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.polymdegradstab.2022.110160	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Soon Zher Neoh, Min Fey Chek, Hua Tiang Tan, Javier A. Linares-Pasten, Ardra Nandakumar, Toshio Hakoshima, Kumar Sudesh	４．巻 4
２．論文標題 Polyhydroxyalkanoate synthase (PhaC): The key enzyme for biopolyester synthesis	５．発行年 2022年
３．雑誌名 Current Research in Biotechnology	６．最初と最後の頁 87～101
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.crbiot.2022.01.002	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Neoh Soon Zher、Tan Hua Tiang、Trakunjae Chanaporn、Chek Min Fey、Vaithanomsat Pilanee、Hakoshima Toshio、Sudesh Kumar	４．巻 23
２．論文標題 N-terminal truncation of PhaCBP-M-CPF4 and its effect on PHA production	５．発行年 2024年
３．雑誌名 Microbial Cell Factories	６．最初と最後の頁 52
掲載論文のDOI（デジタルオブジェクト識別子） 10.1186/s12934-024-02329-w	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　3件／うち国際学会　3件）

1．発表者名 CHEK Min Fey
2．発表標題 Insights into the catalytic mechanism of PHA synthase, a biodegradable plastic synthesizing enzyme
3．学会等名 The 48th International Congress on Science, Technology and Technology-based Innovation (STT48). Walailak University, Nakhon Si Thammarat, Thailand (Nov 29-Dec 1, 2022).（招待講演）（国際学会）
4．発表年 2022年

1．発表者名 Min Fey CHEK
2．発表標題 Crystal structure of PHA synthase and the unexpected open-closed heterodimer
3．学会等名 The International Symposium on Biopolymers (ISBP 2021) - 3rd Webinar. Switzerland (May 20, 2021).（招待講演）（国際学会）
4．発表年 2021年

1．発表者名 Min Fey CHEK
2．発表標題 Crystal structure of bioplastic-synthesizing PHA synthase (PhaC) revealing an unexpected open-closed heterodimer
3．学会等名 SYMPOSIUM D: CRYSTALLOGRAPHY” of the 47th International Congress on Science, Technology and Technology-based Innovation (STT47). Nakhon Pathom, Thailand (October 5-7, 2021)（招待講演）（国際学会）
4．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関