

Mystery of biological plastic synthesis machinery unveiled

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Plastics and other polymers are used every day. These polymers are mostly made from fossil resources through petrochemical refinery process. On the other hand, many microorganisms naturally synthesize polyesters known as polyhydroxyalkanoates (PHAs) as distinct granules inside the cell. PHAs are a family of microbial polyesters that have attracted much attention as biodegradable and biocompatible plastics and elastomers that can substitute petrochemical counterparts. There have been numerous papers and patents on gene cloning and metabolic engineering of PHA biosynthetic machineries, biochemical studies, and production of PHAs; simple Google search with "polyhydroxyalkanoates" returns with 223,000 document pages. PHAs have always been considered an amazing example of biological polymer synthesis. It is astounding to see PHAs of 500 kDa to sometimes as high as 10,000 kDa can be synthesized in vivo by PHA synthase, the key polymerizing enzyme in PHA biosynthesis. Thus, there has been great interest in determining the crystal structure of PHA synthase over the last 30 years, but unfortunately without success. Thus, the characteristics and molecular mechanisms of PHA synthase have so far been under a dark veil.

In two papers published back-to-back in *Biotechnology Journal* online on November 30, 2016, a Korean research team led by Professor Kyung-Jin Kim at Kyungpook National University and Distinguished Professor Sang Yup Lee at the Korea Advanced Institute of Science and Technology (KAIST) reported the crystal structure of PHA synthase from *Ralstonia eutropha*, the best studied bacterium for PHA

production. The research team also reported the structural basis for the detailed molecular mechanisms of PHA biosynthesis. The crystal structure has been deposited to Protein Data Bank in February 2016. After deciphering the crystal structure of the catalytic domain of PHA synthase in addition to other structural studies on whole enzyme and related proteins, the team performed experiments to elucidate mechanisms of the enzyme reaction, validating detailed structures, enzyme engineering, and N-terminal domain studies among others.

Through several biochemical studies based on crystal structure, authors show that PHA synthase exists as a dimer and is divided into two distinct domains, the N-terminal domain (RePhaC1ND) and the C-terminal domain (RePhaC1CD). The RePhaC1CD catalyzes the polymerization reaction via a non-processive ping-pong mechanism using a Cys-His-Asp catalytic triad. The two catalytic sites of the RePhaC1CD dimer are positioned 33.4 Å apart, suggesting that the polymerization reaction occurs independently at each site. This study also presents the structure-based mechanisms for substrate specificities of various PHA synthases from different classes.

Professor Lee, who has been working on this topic for more than 20 years, said, "The results and information presented in these two papers have been very much awaited not only in the PHA community, but also metabolic engineering, bacteriology/microbiology, and in general biological sciences communities. The structural information on PHA synthase together with reaction mechanisms deciphered will be valuable for understanding the detailed mechanisms of biosynthesizing this important energy/redox storage material, and also for the rational engineering of PHA synthases to produce designer bioplastics from various monomers more efficiently."

More information: Jieun Kim et al. Crystal structure of polyhydroxyalkanoate synthase C-terminal domain and reaction

mechanisms, *Biotechnology Journal* (2016). [DOI: 10.1002/biot.201600648](https://doi.org/10.1002/biot.201600648)

Yeo-Jin Kim et al. Structure and function of the N-terminal domain of polyhydroxyalkanoate synthase, and the proposed structure and mechanisms of the whole enzyme, *Biotechnology Journal* (2016). [DOI: 10.1002/biot.201600649](https://doi.org/10.1002/biot.201600649)

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