# NEW ENZYMES ACTING ON BIOACTIVE COMPOUNDS AND UNIQUE CATALYSIS

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We have studied various biocatalysts, and have clarified their reaction mechanism and expression mechanism (1-7). Screening is very important to give us a new materials and new phenomena. We have been interested in how bioactive compounds included in foods and plants are metabolized in nature, and therefore, we recently started screening of enzymes that act on these compounds. We have looked for microorganisms degrading each of them and have investigated enzymes involved in the degradation of the target compound. Here, I show you a few examples; particularly, natural compounds containing a methylenedioxyphenyl group, the structure of which includes a unique ring.

At first, we were interested in metabolism of sesamin, which is a type of lignin and is included in a sesame seed. Sesamin, which contains methylenedioxyphenyl groups, is a biologically active compound with antioxidative, cholesterol-lowering, and so on. While some microbial metabolites of sesamin have been identified, sesamin-metabolic pathways remain unclear at both the enzyme and gene levels. By using the enrichment culture technique, we isolated a bacterium, Sinomonas sp. growing on a medium containing sesamin as a sole-carbon source. The purified enzyme from the strain cleaved the methylenedioxy bridge of sesamin, and catalyzed the conversion of sesamin to sesamin mono-catechol, and conversion of the resultant sesamin mono-catechol to sesamin di-catechol. Interestingly, tetrahydrofolate was found to be required for higher enzyme activity. The enzyme surprisingly catalyzed methylene group transfer from sesamin or sesamin mono-catechol to tetrahydrofolate. E. coli transformant cells carrying the enzyme gene are a good tool for the production of useful sesamin catechols, which show higher bioactivity than sesamin. Based on site-directed mutagenesis and biochemical analysis, we propose a new and unique catalytic mechanism of the enzyme.

Second, an alkaloid, piperine containing a methylenedioxyphenyl group is included in a black pepper and shows a wide range of biological properties (e.g., antioxidant, antiinflammatory, antitumor, antimycobacterial and insecticidal activities). We obtained piperine-degrading No.14 strain (which was identified as Rhodococcus sp.) that grew on media containing piperine as a sole-carbon source. Piperine was found to be metabolized into piperic acid in the No.14 strain. The purified piperine-degrading enzyme was found to cleave the carbon-nitrogen bond in piperine. This is a new enzyme; there have been no reports concerning tertiary amide-degrading enzymes.

Third, we also have been involved in studies on metabolism of piperonal, which contains a methylenedioxyphenyl group. Piperonal is a component of the essential oil of the heliotrope flower, and is frequently used in perfumes and cosmetics. We have obtained a piperonal-degrading microorganism and identified piperonal-converting enzyme, which produces piperonylic acid and $H_2O_2$. Cofactor analysis of the purified enzyme indicates that the enzyme contain FAD, molybdopterin cytosine dinucleotide cofactor (MCD), and [2Fe-2S] cluster. We continue studying these enzyme unique catalysis.

References:

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