The Engineered Type III Polyketide Synthase Produce Diverse Plant Polyphenols

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The chalcone synthase (CHS) superfamily of type III polyketide synthases (PKSs) are structurally simple homodimeric proteins that produce basic skeletons of flavonoids as well as a variety of plant polyphenols with remarkable biological activities.

The four unprecedented type III PKSs were cloned from medicinal plants; Rhubarb and Aloe, which catalyze formation of an aromatic di-, penta-, hepta- and octa-ketides selecting various acyl-CoA as a starter. Among them, the function of these three type III PKSs was shown to be swapped each other by steric modulation of the chemically inert single to triple residues lining the active-site cavity accompanied by conservation of the Cys-His-Asn catalytic triad. For example, the site-directed mutagenesis of octaketide synthase (OKS) from Aloe revealed that small-to-large substitutions of a single residue Gly207 (G207A, G207T, G207M, G207L, G207F, and G207W) resulted in loss of the octaketide-forming activity and concomitant formation of shorter chain length polyketides including a pentaketide chromone, and a hexaketide pyrone, depending on the size of side chain residues. In contrast, the three active site residues conserved in CHSs (T197, G256, S338) were replaced in the heptaketide synthase from Rhubarb. The octaketides-forming activity was dramatically increased in the triple mutant (T197G/G256L/S338T). In this striking example, changing chemically inert amino acid residues of CHS resulted in the enzyme producing SEK4/SEK4B that was not previously produced ever by prototype CHS, effectively turned the enzyme into a related type III PKS.

The other type III PKS, benzalacetone synthase (BAS) from Rhubarb, which normally produces a diketide benzalacetone by one-step decarboxylative condensation of *p*-coumaroyl-CoA with malonyl-CoA, efficiently catalyzes condensation of *N*-methylanthraniloyl-CoA (or anthraniloyl-CoA) with one molecule of malonyl-CoA (or methylmalonyl-CoA) to produce 4-hydroxy-2 (1*H*)-quinolones in the yield of 86%.

All the experiments have been performed with recombinant wild-type enzymes that are harvested from *E. coli*, though. The above results with an each single enzyme lead to the possibility to produce a reasonable source by introducing these genes into suitable plants to express them artificially. With the knowledge of the detailed structures and functions of those enzymes gleaned from the rational engineering approaches described above, we can judiciously engineer "artificial" versions of the biosynthetic pathways now linked to an artificial gene or a set of genes.

The several strains of Arabidopsis, a typical model plant, which were engineered by these genes, are now ready for investigation of their constituents. This provided novel strategies for the engineered biosynthesis of pharmaceutically important plant polyphenols.