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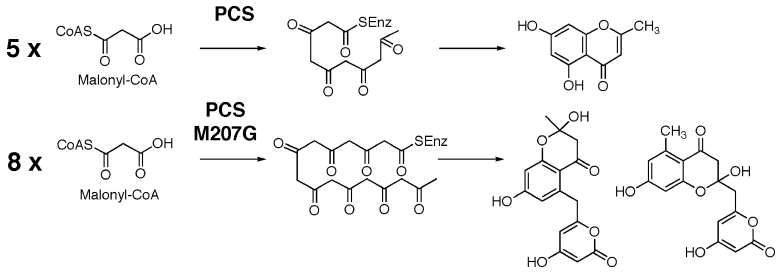

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A Plant Type III Polyketide Synthase that Produces Pentaketide Chromone

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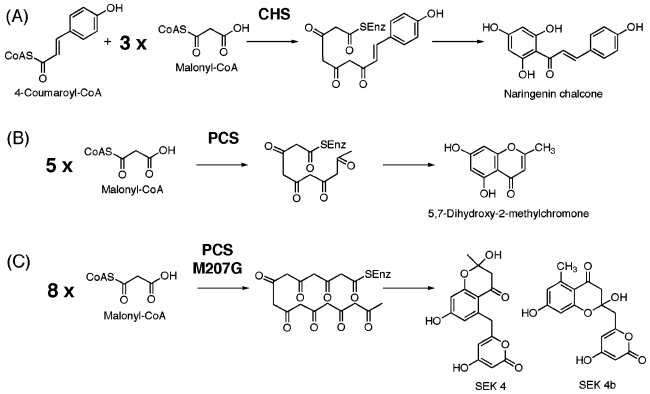
A growing number of functionally diverse type III polyketide synthases (PKSs), the chalcone synthase (CHS) (EC 2.3.1.74) superfamily enzymes, have been cloned and sequenced from various plants,[1] which include recently reported a diketide (benzalacetone)[2a,b] and a heptaketide (aloesone) synthase (ALS)[2c] from *Rheum palmatum*. In addition, bacterial type III PKSs, such as a pentaketide 1,3,6,8-tetrahydroxynaphthalene-producing enzyme, have been also reported.[3,4] The CHS-superfamily enzymes are structurally and mechanistically distinct from the type I (modular type) and type II (subunit type) PKSs, using free CoA thioesters as substrates without the involvement of acyl carrier protein, to carry out a complete series of decarboxylation, condensation, cyclization, and aromatization reactions with a single active site. The homodimer of 40−45 kDa proteins typically selects 4-coumaroyl-CoA as a starter and performs up to three condensations with malonyl-CoA to produce naringenin chalcone, (4,2′,4′,6′-tetrahydroxychalcone), which is the biosynthetic precursor of flavonoids (Scheme 1A). Recent crystallographic and site-directed mutagenesis studies have revealed structural and functional details of the plant and bacterial type III PKSs.[1−6]

Here we report a novel plant-specific type III PKS that catalyzes formation of a pentaketide chromone, 5,7-dihydroxy-2-methylchromone, from five molecules of malonyl-CoA (Scheme 1B). Remarkably, replacement of a single amino acid residue Met207 (corresponding to the *Medicago sativa* CHS active-site residue Thr197) yielded a mutant enzyme that efficiently produces aromatic octaketides, SEK4 and SEK4b, the products of the minimal PKS for the benzoisochromanequinone actinorhodin (*act* from *Streptomyces coelicolor*)[7] (Scheme 1C). A cDNA encoding the pentaketide chromone synthase (PCS) (the GenBank accession no. AY823626) was cloned and sequenced from young roots of aloe (*Aloe arborescens*), a medicinal plant rich in aromatic polyketides including chromones and anthraquinones, by RT-PCR using degenerate primers based on the conserved sequences of known CHSs as described before.[2] A 1212-bp open reading frame encoded a M_r 44,568 protein with 403 amino acids. The deduced amino acid sequence showed 50−60% identity to those of CHS-superfamily enzymes from other plants (58% identity (232/403) with *M. sativa* CHS,[5a] and 50% identity (206/403) with *R. palmatum* ALS[2c] that catalyzes formation of a heptaketide, aloesone (2-acetonyl-7-hydroxy-5-methylchromone), from acetyl-CoA and six molecules of malonyl-CoA). *A. arborescens* PCS maintains an almost identical CoA binding site, and the catalytic triad of Cys164, His303, and Asn336 (numbering in *M. sativa* CHS) is absolutely conserved in all type III PKSs. Furthermore, most of the active-site residues including Met137, Gly211, Gly216, Pro375, as well as Phe215, and Phe265,[1] are conserved in PCS (Figure 1). The CHS-based homology modeling predicted that *A. arborescens* PCS has the same three-dimensional overall fold as *M. sativa* CHS,[5a] with the total cavity volume (1124 Å^3) slightly larger than that of CHS (1019 Å^3) and almost as large as that of *R. palmatum* ALS (1173 Å^3).[2c]

Scheme 1. Formation of Polyketides by CHS-Superfamily Enzymes



Recombinant PCS was heterologously expressed in *Escherichia coli* BL21(DE3)pLysS as fusion protein with GST at the N-terminal (pET vector). After cleavage of the GST-tag, the purified enzyme gave a single band with molecular mass of 44 kDa on SDS-PAGE, while the native PCS appeared to be a homodimer since it had molecular mass of 88 kDa as determined by gel filtration. *A. arborescens* PCS efficiently accepted malonyl-CoA as a sole substrate to yield a single product with a parent ion peak $[M + H]^+$ at *m/z* 193 on LC-ESIMS. Spectroscopic data (^{1}H NMR, LC-MS, and UV) of the product obtained from a large-scale enzyme reaction (1.0 mg from 20 mg of malonyl-CoA) are completely identical with those of an authentic 5,7-dihydroxy-2-methylchromone. The aromatic pentaketide has been isolated from several plants and is known to be a biosynthetic precursor of khellin and visnagin, the anti-asthmatic furochromones found in *Ammi visnaga*.[8] Interestingly, acetyl-CoA, resulting from decarboxylation of malonyl-CoA, was also accepted as a starter substrate but not so efficiently as in the case of *R. palmatum* ALS.[2c] This was confirmed by the ^{14}C incorporation rate from [1-^{14}C]acetyl CoA in the presence of cold malonyl-CoA, while the yield of the pentaketide from [2-^{14}C]malonyl-CoA was almost at the same level in the presence or absence of cold acetyl-CoA in the reaction mixture. The recombinant PCS showed the K_M = 71.0 μM and k_{cat} = 445 × 10^{-3} min^{-1}, with a broad pH optimum within a range of 6.0−8.0. On the other hand, like other type III PKSs,[1,9] *A. arborescens* PCS showed the promiscuous substrate specificity; the enzyme also accepted aromatic (4-coumaroyl, cinnamoyl, and benzoyl) and aliphatic (*n*-hexanoyl, *n*-octanoyl, and *n*-decanoyl) CoA esters as a starter substrate; however, it yielded only triketide and teraketide α-pyrones.

One of the characteristic features of *A. arborescens* PCS is that the CHS active-site residues, Thr197, Gly256, and Ser338 (numbering in *M. sativa* CHS),[5a] are uniquely replaced with Met, Leu, and Val, respectively. Interestingly, the three residues are also missing in the heptaketide-forming *R. palmatum* ALS[2c] (T197A/G256L/S338T), and in *Gerbera hybrida* 2-pyrone synthase (2PS)[6] (T197L/

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Figure 1. Comparison of primary sequences of *A. arborescens* PCS and other CHS-superfamily enzymes. M.s CHS, *M. sativa* CHS; A.h STS, *Arachis hypogaea* stilbene synthase; G.h 2PS, *G. hybrida* 2PS; R.p ALS, *R. palmatum* ALS. The active-site residues conserved in the CHS-superfamily enzymes (Cys164, Phe215, His303, and Asn336, numbering in *M. sativa* CHS) are marked with #, and residues for the CoA binding, with +.

G256L/S338I) that also selects acetyl-CoA as a starter to produce a triketide pyrone. A CHS triple mutant (T197L/G256L/S338I) has been shown to yield an enzyme that was functionally identical to 2PS, suggesting the substitutions are responsible for the starter substrate specificity of the enzymes.[6b] To test the hypothesis, a mutant enzyme was constructed in which Met207 (corresponding to Thr197 in CHS) was replaced by Thr. However, the point mutation did not significantly affect the enzyme activity; PCS M207T mutant was functionally almost identical to the wild-type PCS. In contrast, when Met207 was substituted with Gly, there was a dramatic change in the enzyme activity; PCS M207G mutant efficiently afforded two new products with a parent ion peak $[M + H]^+$ at *m*/*z* 319 on LC-ESIMS, which were identified as aromatic octaketides SEK4 and SEK4b (ratio 1:4), the shunt products of the minimal type II PKS for actinorhodin,[7] by direct comparison with authentic compounds. Here formation of only a trace amount of 5,7-dihydroxy-2-methylchromone was detected by LC-MS. The pentaketide-forming PCS was thus transformed into an octaketide-producing enzyme by the single amino acid mutation. This is the first demonstration of a type III PKS catalyzing seven successive polyketide chain elongation reactions.

In conclusion, *A. arborescens* PCS is a novel plant-specific type III PKS that produces an aromatic pentaketide from five molecules of malonyl-CoA. Site-directed mutagenesis revealed that Met207 determines the polyketide chain length and the product specificity; PCS M207G mutant yielded SEK4 and SEK4b from eight molecules of malonyl-CoA.[10] This provided new insights into the catalytic functions and specificities of type III PKSs. Further characterization of the enzymes including their three-dimensional structure will be reported in due course.

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Supporting Information Available: Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews, see: (a) Schröder, J. In *Comprehensive Natural Products Chemistry*; Elsevier: Oxford, 1999; Vol. 2, pp 749−771. (b) Austin, M. B.; Noel, J. P. *Nat. Prod. Rep.* **2003**, *20*, 79−110. (c) Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 380−416.

(2) (a) Abe, I.; Takahashi, Y.; Morita, H.; Noguchi, H. *Eur. J. Biochem.* **2001**, *268*, 3354−3359. (b) Abe, I.; Sano, Y.; Takahashi, Y.; Noguchi, H. *J. Biol. Chem.* **2003**, *278*, 25218−25226. (c) Abe, I.; Utsumi, Y.; Oguro, S.; Noguchi, H. *FEBS Lett.* **2004**, *562*, 171−176.

(3) (a) Funa, N.; Ohnishi, Y.; Fujii, I.; Shibuya, M.; Ebizuka, Y.; Horinouchi, S. *Nature* **1999**, *400*, 897−899. (b) Funa, N.; Ohnishi, Y.; Ebizuka, Y.; Horinouchi, S. *J. Biol. Chem.* **2002**, *277*, 4628−4635. (c) Funa, N.; Ohnishi, Y.; Ebizuka, Y.; Horinouchi, S. *Biochem. J.* **2002**, *367*, 781−789. (d) Izumikawa, M.; Shipley, P. R.; Hopke, J. N.; O'Hare T.; Xiang, L.; Noel, J. P.; Moore, B. S. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 510−515. (e) Austin, M. B.; Izumikawa, M.; Bowman, M. E.; Udwary, D. W.; Ferrer, J.-L.; Moore, B. S.; Noel, J. P. *J. Biol. Chem.* **2004**, *279*, 45162−45174.

(4) (a) Saxena, P.; Yadav, G.; Mohanty, D.; Gokhale, R. S. *J. Biol. Chem.* **2004**, *278*, 44780−44790. (b) Sankaranarayanan, R.; Saxena, P.; Marathe, U.; Gokhale, R. S.; Shanmugam, V. M.; Rukmini, R. *Nat. Struct. Mol. Biol.* **2004**, *11*, 894−900. (c) Pfeifer, V.; Nicholson, G. J.; Ries, J.; Recktenwald, J.; Schefer, A. B.; Shawky, R. M.; Schröder, J.; Wohlleben, W.; Pelzer, S. *J. Biol. Chem.* **2001**, *276*, 38370−38377. (d) Tseng, C. C.; McLoughlin, S. M.; Kelleher, N. L.; Walsh, C. T. *Biochemistry* **2004**, *43*, 970−980.

(5) (a) Ferrer, J. L.; Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Nat. Struct. Biol.* **1999**, *6*, 775−784. (b) Jez, J. M.; Ferrer, J. L.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Biochemistry* **2000**, *39*, 890−902. (c) Jez, J. M.; Noel, J. P. *J. Biol. Chem.* **2000**, *275*, 39640−39646. (d) Jez, J. M.; Bowman, M. E.; Noel, J. P. *Biochemistry* **2001**, *40*, 14829−14838. (e) Tropf, S.; Kärcher, B.; Schröder, G.; Schröder, J. *J. Biol. Chem.* **1995**, *270*, 7922−7928. (f) Suh, D. Y.; Fukuma, K.; Kagami, J.; Yamazaki, Y.; Shibuya, M.; Ebizuka, Y.; Sankawa, U. *Biochem. J.* **2000**, *350*, 229−235. (g) Suh, D. Y.; Kagami, J.; Fukuma, K.; Sankawa, U. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 725−730. (h) Jez, J. M.; Bowman, M. E.; Noel, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5319−5324. (i) Austin, M. B.; Bowman, M. E.; Ferrer, J.-L.; Schröder, J.; Noel, J. P. *Chem. Biol.* **2004**, *11*, 1179−1194.

(6) (a) Eckermann, S.; Schröder, G.; Schmidt, J.; Strack, D.; Edrada, R. A.; Helariutta, Y.; Elomaa, P.; Kotilainen, M.; Kilpeläinen, I.; Proksch, P.; Teeri, T. H.; Schröder, J. *Nature* **1998**, *396*, 387−390. (b) Jez, J. M.; Austin, M. B.; Ferrer, J.; Bowman, M. E.; Schröder, J.; Noel, J. P. *Chem. Biol.* **2000**, *7*, 919−930.

(7) (a) Fu, H.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *J. Am. Chem. Soc.* **1994**, *116*, 4166−4170. (b) Fu, H.; Hopwood, D. A.; Khosla, C. *Chem. Biol.* **1994**, *1*, 205−210.

(8) Dewick, P. M. In *Medicinal Natural Products, A Biosynthetic Approach*, 2nd ed.; Wiley: West Sussex, 2002.

(9) (a) Abe, I.; Morita, H.; Nomura, A.; Noguchi, H. *J. Am. Chem. Soc.* **2000**, *122*, 11242−11243. (b) Morita, H.; Takahashi, Y.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 190−195. (c) Morita, H.; Noguchi, H.; Schröder, J.; Abe, I. *Eur. J. Biochem.* **2001**, *268*, 3759−3766. (d) Abe, I.; Takahashi, Y.; Noguchi, H. *Org. Lett.* **2002**, *4*, 3623−3626. (e) Abe, I.; Takahashi, Y.; Lou, W.; Noguchi, H. *Org. Lett.* **2003**, *5*, 1277−1280. (f) Abe, I.; Watanabe, T.; Noguchi, H. *Phytochemistry* **2004**, *65*, 2447−2453. (g) Oguro, S.; Akashi, T.; Ayabe, S.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 561−567.

(10) An analogous result in Type II PKS has been reported, see: Tang, Y.; Tsai, S.-C.; Khosla, C. *J. Am. Chem. Soc.* **2003**, *125*, 12708−12709.

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