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Digging for answers, smelling a hint of success and tasting triumph

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Abstract

The C₁₂ 'earthy' odorant geosmin is derived from the C₁₅ metabolite farnesyl diphosphate. Metabolic transformation now seems to be catalyzed by a bifunctional protein having two operatively independent sesquiterpene synthase domains. The domains are catalytically linked through the passive diffusion of a C₁₅ alcohol product of the N-terminal catalytic domain to the C-terminal catalytic domain for the final steps of geosmin formation.

Nothing stirs the passions of farmers and gardeners more than the sweet smell of a newly tilled field or a recently prepared planting bed. However, when mixed in drinking water, the earthy aroma that heralds the approaching growing season leaves one with the unpleasant taste of mud on the tongue. This 'earth smell', known as geosmin, assembles in several soil- and waterborne micro-organisms and emerges from the C₁₅ isoprenoid building block farnesyl diphosphate (FPP). FPP perches at the juncture of primary and secondary metabolism in nearly all organisms. Until now, the molecular under-pinnings surrounding the biosynthesis of the C₁₂ bicyclic tertiary alcohol geosmin from a C₁₅ precursor remained a sometimes confusing, fascinating and unfolding mystery^{1,2}. In this issue of *Nature Chemical Biology*, Jiang *et al.*³ convincingly demonstrate that this earth smell forms in two distinct metabolic steps on the bifunctional enzyme germacradienol-geosmin synthase (GGS). In a fascinating example of natural selection, GGS arose harboring two discrete, independently folded but structurally related sesquiterpene (C₁₅) synthase catalytic domains. Quite unexpectedly, the C-terminal domain of these fraternal Siamese twins, which was previously thought to be a dead look-alike of the N-terminal domain, is alive and well catalyzing an unprecedented Mg²⁺-dependent transmutation of the neutral sesquiterpene alcohol germacradienol to the C₁₂ alcoholic product geosmin (Fig. 1). From a practical standpoint, the clear linkage of a single conserved gene with geosmin biosynthesis and the elucidation of the unexpected chemical mechanism accompanying FPP turnover offer new avenues for remediation of 'soiled' water supplies. From a fundamental standpoint, this discovery alters our preconceived notions of how nature evolved the machinery to produce, diversify and exploit the complexity of ecologically active isoprenoid metabolites⁴.

Typically, terpene synthases of microbial origin span ~350 amino acid residues and include two motifs, **DDXX(D/E)** and **(N/D)DXX(S/T)XXXE**, separated in primary sequence and responsible for binding the catalytically essential Mg²⁺ necessary for initial pyrophosphate ionization that launches one or more rounds of electrophilic cyclization⁴. Strikingly, GGS

COMPETING INTERESTS STATEMENT

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spans ~730 residues, with two equal proteinaceous portions having considerable sequence similarity to each other and to a host of microbial sesquiterpene synthases. Though sesquiterpene synthases characterized so far predictably catalyze the biosynthesis of large amounts of hydrocarbon products through electrophilic polyene-type cyclizations sometimes involving 'reactivation' of bound neutral intermediates by protonation⁴, minor amounts of sesquiterpene alcohols sometimes form presumably through adventitious capture of reactive carbocation intermediates by water. In rare cases, such as for GGS's N-terminal domain, sesquiterpene synthases promote water capture to yield sesquiterpene alcohols as major products^{2,5}. Moreover, the minor product distributions of terpene synthases often offer important clues about the mechanism underlying the formation of bioactive compounds. Finally, these minor products sometimes serve as chemical vestiges of the evolutionary history of extant sequences⁶ or as harbingers of future biosynthetic pathways⁷.

Using an elegantly simple series of *in vitro* analyses, Jiang *et al.*³ establish that the N-terminal domain of GGS, independent of its C-terminal fraternal twin, catalyzes the Mg^{2+} -dependent formation of germacradienol and minor amounts of germacrene D and the C_{12} hydrocarbon octalin (Fig. 1). Addition of recombinantly produced C-terminal domain restored geosmin production in a concentration-dependent fashion. Unexpectedly, addition of a mixture of germacradienol and germacrene D containing negligible amounts of octalin to recombinant GGS C-terminal domain resulted in cleavage of the 2-propanol moiety lost as acetone from germacradienol, thereby forming geosmin. Surprisingly, this latter transformation absolutely required the inclusion of Mg^{2+} . Finally, catalytic impairment of GGS's N-terminal domain by mutation of its first Mg^{2+} -binding motif resulted in the accumulation of the bicyclic sesquiterpene hydrocarbon isolepidozene. This neutral intermediate, which contains an atypical cyclopropyl unit, is poised for acid-catalyzed ring opening and subsequent quenching by water capture. Its presence establishes the posited intermediacy of isolepidozene in germacradienol biosynthesis.

It remains to be seen whether the minor amounts of the C_{12} octalin intermediate produced by the recombinant GGS N-terminal domain observed by Jiang *et al.*³ result from the spontaneous breakdown of the C_{15} germacradienol intermediate or the low-probability turnover of this sesquiterpene alcohol by the N-terminal domain. Curiously, previously published *in vivo* studies conducted on the *Streptomyces coelicolor* GGS¹ (studied *in vitro* by Jiang *et al.* as reported here³) showed that *S. coelicolor* mutants carrying only the coding sequence for GGS's N-terminal domain still produce geosmin. The *in vivo* accumulation of geosmin in *S. coelicolor* GGS C-terminal domain deletion mutants¹ may be explained by the presence of N-terminal-domain-generated octalin and by the retention of vestigial levels of geosmin synthase activity *in vivo* by the still-present N-terminal domain. Alternatively, the previously reported *in vivo* results¹ may point to redundant catalytic activity in *Streptomyces* species, or to the spontaneous proton-initiated activation and water-mediated turnover of N-terminal-domain-generated octalin.

The startling discovery that the conversion of germacradienol to geosmin by the GGS C-terminal domain absolutely depends on Mg^{2+} even though the neutral substrate lacks a pyrophosphate handle suggests that we have much to learn about the structure, mechanism and evolution of terpene synthases. Is it possible that Mg^{2+} has a structural role in organizing a catalytically competent C-terminal domain active site, or, through available coordination sites, does the Mg^{2+} ion sequester and properly orient the hydroxyl moiety of the germacradienol substrate or the water molecule necessary for octalin turnover? While the biochemical surgeons have successfully separated the GGS fraternal Siamese twins, the earthy odorant geosmin will remain fertile ground for future gentle-person farmers.

References

1. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. Proc. Natl. Acad. Sci. USA 2003;100:1541–1546. [PubMed: 12563033]
2. Jiang J, He X, Cane DE. J. Am. Chem. Soc 2006;128:8128–8129. [PubMed: 16787064]
3. Jiang J, He X, Cane DE. Nat. Chem. Biol 2007;3:711–715. [PubMed: 17873868]
4. Christianson DW. Chem. Rev 2006;106:3412–3442. [PubMed: 16895335]
5. Deguerry F, et al. Arch. Biochem. Biophys 2006;454:123–136. [PubMed: 16970904]
6. Greenhagen BT, O'Maille PE, Noel JP, Chappell J. Proc. Natl. Acad. Sci. USA 2006;103:9826–9831. [PubMed: 16785438]
7. Yoshikuni Y, Ferrin TE, Keasling JD. Nature 2006;440:1078–1082. [PubMed: 16495946]

Figure 1.

Schematic depiction of the bifunctional germacradienol-geosmin synthase. Each half of the protein (N-terminal domain, dark blue; C-terminal domain, light blue) adopts the α -helical fold commonly observed in terpene synthases⁴. The functionally independent domains are joined by a ~50-amino-acid-residue linker. The dark blue N-terminal domain converts FPP into germacradienol, which then diffuses into the active site of the light blue C-terminal domain, whereupon germacradienol undergoes transformation to geosmin. Various postulated and identified mechanistic intermediates are shown.