Metal matters

A study of an insect prenyltransferase demonstrates that the product specificity of this bifunctional enzyme can be regulated by the presence of different divalent metal cofactors, resulting, for example, in the production of the precursors for either insect defense compounds or developmental hormones.

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Terpenoids are the largest group of natural products, containing more than 55,000 identified compounds that have many essential functions in all domains of life. Examples include the well-known sterols, juvenile sesquiterpene hormones and various monoterpen-, diterpen- and triterpene-derived natural products for defenses in animals and plants. Despite their immense diversity, terpenoids are all ultimately produced from simple C5 linear allylic diphosphate precursors. The mechanisms for induction and regulation of terpenoid biosynthetic pathways are diverse, and many remain obscure. Transcription factors are known to function as key regulators of metabolic pathways1, and the many cases of coexpression of genes from metabolic gene clusters2,3 suggest that chromatin-level regulation of natural product biosynthesis may exist4. A recent study by Frick et al.5 probed the specific influence of various divalent cation cofactors on the bifunctionality of a beetle scIDS (PcIDS1, from *Phaedon cockleariae*). The core of the study consisted of a series of *in vitro* PcIDS1 assays testing a diversity of both allylic substrates (IPP, DMAPP and GPP) and separate divalent cations (Co2+, Mg2+, Mn2+, Ni2+ and Zn2+). In the assays that combined IPP and DMAPP as substrates, maximum PcIDS1 activity was observed with Co2+ as the metal cofactor. A notable additional result from these assays was that the ratios of product accumulation (GPP versus FPP) varied substantially depending on which of the metal cofactors was present (Fig. 1). PcIDS1 produced about 96% GPP from a lepidopteran have indicated that alterations in Mg2+ and Mn2+ concentrations affected the accumulation ratios of scIDS products of different lengths6.

Motivated by these reports, Frick et al.5 probed the specific influence of various divalent cation cofactors on the bifunctionality of a beetle scIDS (PcIDS1, from *Phaedon cockleariae*). The core of the study consisted of a series of *in vitro* PcIDS1 assays testing a diversity of both allylic substrates (IPP, DMAPP and GPP) and separate divalent cations (Co2+, Mg2+, Mn2+, Ni2+ and Zn2+). In the assays that combined IPP and DMAPP as substrates, maximum PcIDS1 activity was observed with Co2+ as the metal cofactor. A notable additional result from these assays was that the ratios of product accumulation (GPP versus FPP) varied substantially depending on which of the metal cofactors was present (Fig. 1). PcIDS1 produced about 96% GPP and only 4% FPP in the presence of Co2+ or Mn2+, whereas it produced 18% GPP and 82% FPP in the presence of Mg2+.

Follow-up assays that varied the relative concentration of these metals indicated that the Mg2+-catalyzed activity of PcIDS1 is abolished as soon as Co2+ reaches its optimal concentration.

Rigorous kinetic studies further bolstered their assertion that PcIDS1 has an energetic preference for Co2+ with DMAPP as an allylic cosubstrate for C10 GPP production but showed that C15 FPP production was favored when Mg2+ was the cofactor. Theoretical modeling of hypothetical reaction energy differences indicated that PcIDS1 has a conspicuously higher affinity for Co2+ than for Mg2+. Cation quantification studies of *P. cockleariae* larval tissues reinforced the physiological plausibility that these organisms may indeed control the product specificity of scIDSs through changes in

**Figure 1** Regulation of terpenoid pathways by metal cofactors. A mustard leaf beetle (*P. cockleariae*) enzyme PcIDS1 (here represented by an avian farnesyl pyrophosphate synthetase, Protein Data Bank code 1UBV) alters its product specificity on the basis of the presence of Co2+ or Mg2+. With Co2+ as a cofactor, PcIDS1 preferentially combines cosubstrates IPP and DMAPP to produce GPP, the precursor for monoterpane metabolism (blue arrow). Monoterpenes such as chrysoamelidial are known to be important in insect chemical defense. With Mg2+ as a cofactor, PcIDS1 preferentially combines cosubstrates IPP and GPP to produce FPP, the precursor for sesquiterpene metabolism (pink arrow), producing sesquiterpene compounds as insect juvenile hormones that regulate many aspects of insect development.

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the local concentrations of these metal ions. Finally, Frick et al.\textsuperscript{5} used size-exclusion chromatography to show that the PcIDS\textsuperscript{1} apo enzyme (lacking a metal cofactor), PcIDS with Co\textsuperscript{2+} and PcIDS\textsuperscript{1} with Mg\textsuperscript{2+} all eluted at different volumes, indicating that the hydrodynamic volume, and thus the quaternary structure of the protein, is altered by the various divalent cofactors. Resolving the three-dimensional structures of PcIDS\textsuperscript{1} with Co\textsuperscript{2+} or Mg\textsuperscript{2+} will be required to characterize the precise chemical mechanism underlying the observed metal cofactor–dependent regulation of product specificity. The discovery of this metal ion concentration–dependent enzyme product specificity reveals a new type of metabolic ‘regulation’. In contrast to alternative splicing mechanisms, which generate multiple gene products from a single genomic locus, this metal ion-dependent regulatory mechanism allows a single enzyme to selectively control the metabolites it produces, thus potentially altering the flow of carbon into separate metabolic pathways. This type of ‘adjustable’ enzyme may afford insects an efficient mechanism for the generation of the chemical diversity that is critical for adaptation to ever-changing ecological contexts. Systematic investigation of the effects of diverse metal cofactors on various metalloproteins may reveal more examples of this regulatory mechanism.

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References

Competing financial interests
The authors declare no competing financial interests.