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Previews

A Fine Balancing Act of Type III Polyketide Synthase

In this issue of *Chemistry & Biology*, a novel Aldol-Switch mechanism is proposed for the biosynthesis of type III polyketides, which include many antioxidants found in colorful fruits [1]. Based on structural and mutagenesis studies, the Aldol-Switch mechanism suggests that electronic effects balance between two competing cyclization specificities in Type III polyketide synthases. A novel hypothesis is also used to explain stilbenecarboxylate biosynthesis.

Type III polyketides (Figure 1), such as chalcone, stilbene, resveratrol, and pterostilbene, recently received much attention due to their biological activities [2]. Abundant in colorful fruits such as grape and blueberry, these polyketides play beneficial medical roles as antioxidants, platelet aggregation inhibitors, anti-inflammatories and anti-cancer agents. Recently, they have been found to bind the PPAR, a family of proteins involved in lowering cholesterol and other blood fats [3]. Studies showed that the consumption of colorful fruits, such as grapes and blueberries, can be beneficial to health due to the abundance of these type III polyketides [4]. In fact, resveratrol is believed to be one of the major reasons why moderate consumption of red wine ("the French paradox") is beneficial to cardiovascular health [5].

Type III polyketides belong to the highly diverse family of polyketide natural products. Polyketides are one of the most important families of natural products that exhibit a wide variety of bioactivities. Polyketides have served as antibiotics, anticancer drugs, antifungal agents, immunosuppressants, and insecticides [6]. The chemical structures of polyketides are as diverse as their pharmaceutical activities. However, the biosynthetic pathways of all polyketides share a common theme: they are produced from shorter acyl-CoA extender units (such as malonyl-CoA) that are coupled together by polyketide synthase (PKS). In theory, if we can control the selection of starter or extender units, as well as the polyketide chain length and chain modification, billions of possible polyketide analogs can be produced by combinatorial biosynthesis [7]. Moreover, many polyketide compounds are very difficult to obtain by organic synthesis, whereas their biosyntheses by PKS offer much higher yields [6, 7]. Therefore, it is of great interest to understand the actions of PKSs, especially for enzyme features that are important to polyketide diversity.

Depending on the overall architecture, PKS can be categorized into at least three or more classes. Type I and type II PKSs consist of many subunits and active sites. On the other hand, type III PKSs are structurally simple, homodimeric enzymes that catalyze repeated chain elongation between a CoA-linked starter unit (usually an aromatic CoA) and acetyl units (derived from malonyl-CoA). Following chain extension, the linear polyketide intermediate is cyclized in the same active site cavity. Chalcone and stilbene are both produced by the same chain-elongation reaction, which involves the coupling of p-coumaroyl-CoA with three malonyl-CoAs (Figure 1). Subsequently, differential aldol-cyclization of the same linear polyketide intermediate, either C6→C1 or C2→C7, results in chalcone or stilbene, respectively (Figure 1). During the past decades, thanks to a combination of structural and functional studies [2], the enzymatic features of type III PKS that mediate starter unit selection and chain elongation are largely understood. However, the control of aldol-cyclization specificity, which is the key event that differentiates the production of chalcone and stilbene, remains a mystery.

Previously, the alfalfa chalcone synthase (CHS) structure implied that proper orientation of the polyketide intermediate by the active site residues is sufficient to promote the appropriate cyclization reaction [2]. Although sequence comparisons indicated no major difference for these active site residues between CHS and stilbene synthase (STS), it was presumed that a steric reshaping of the active site cavity may direct the divergent C2→C7 aldol-cyclization in STS. While the steric model provides an explanation for the different aldol-cyclization patterns observed between chalcone and stilbene, it could not account for the additional thioester hydrolysis, dehydration and decarboxylation activities that are necessary for STS to produce stilbene. Apparently, an important step in unraveling this mystery is solving the crystal structure of STS.

In this issue, Austin et al. report the first crystal structure of STS and present a novel mechanism that not only explains the alcol-cyclization specificity between chalcone and stilbene production, but also accounts for the additional STS activities [1]. It is worth mentioning that this structure was solved amid great technical difficulties. Further, a comparison of the active site cavity between CHS and STS revealed very minor differences in topology. Therefore, unless the crystal structures deviate greatly from the protein solution geometry, steric reshaping of the active site cannot account for the alternate polyketide cyclization observed in STS.

Next, Austin et al. undertook a heroic, extensive mutagenic effort that successfully converts the CHS to a functional STS after mutating 18 residues of CHS (referred to as the 18× CHS). Based on a detailed structural comparison between CHS and STS, four areas were targeted for mutagenesis using a quasi-combinatorial strategy. The mutagenesis identified important areas (areas 1–3) and residues for stilbene synthesis. Both the 18× and 8× CHS mutants predominantly resulted in stilbene production with very little derailment products, indicating a smooth, one-step transition from C6→C1 to C2→C7 aldol-cyclization. The structures of apo and resveratrol-bound 18× CHS were subsequently solved. Resveratrol binding does not induce any significant conformational change. Again, this observation argues

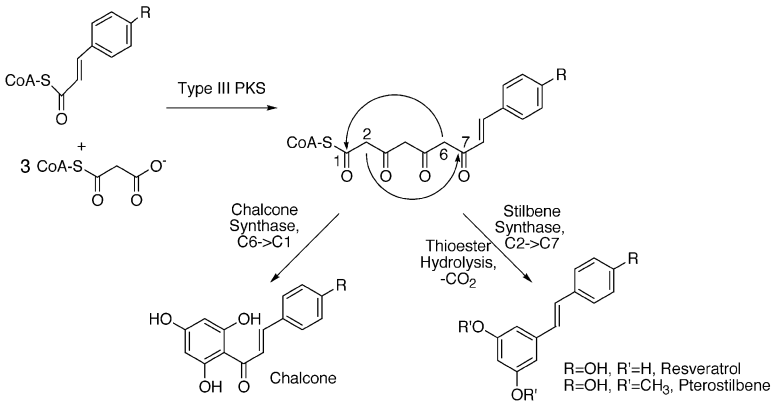

Figure 1. The Biosyntheses of Chalcone and Stilbenes by Type III PKS Involve a Regio-Specific Cyclization, which May be Controlled by the Aldol-Switch Mechanism Proposed in this Issue of *Chemistry & Biology*

against the likelihood of drastic reorientation within the enzyme active site cavity upon substrate binding.

So if steric interactions do not control the C6→C1 to C2→C7 transition, what other features may cause the different cyclization? With such an extensive mutagenesis effort, a very detailed comparison becomes possible. As a result, several residues (including Thr132) were identified due to their connection to a protein-stablized water molecule. It forms a water network in the STS-like active site cavity. Remarkably, this newly identified hydrogen bonding network is very similar to that of a type II thioesterase [8], corresponding to the thioester hydrolysis activity that is essential for STS but not CHS. This leads to the hypothesis that electronic effects, mediated through the emergent hydrogen-bonding network, may be responsible for the additional STS thioesterase and decarboxylase activities, which in turn may result in the C2→C7 aldol-cyclization pattern. Based on this hypothesis, additional mutations were made to disrupt the hydrogen-bonding network of the 18× CHS mutant. Indeed, these 18×(+1) CHS mutants exhibit increased chalcone production at the expense of stilbene production. These results suggest that the aldol-cyclization specificity is electronically mediated and closely related to the thioesterase activity that involves the repositioning of Thr132 to a water molecule. This rearrangement is called "the Aldol Switch."

The next question concerns the order of the five necessary reactions to produce stilbene: hydrolytic cleavage of the thioester bond, aldol cyclization, decarboxylation, dehydration, and aromatization. Based on the above results and previous model studies, Austin et al. offer a detailed discussion on the order of the five reactions. Briefly, based on the Aldol Switch mechanism and solution chemistry studies, hydrolysis of the thioester bond may occur, followed by cyclization, decarboxylation, and dehydration. Some of the steps may occur in either a stepwise or concerted fashion. Finally, using the insights from this work and solution chemistry studies, a novel mechanistic hypothesis is proposed to explain the biosynthesis of stilbenecarboxylate, in which the reopening of the cyclic lactone results in the non-enzymatic C2→C7 cyclization product stilbenecarboxylate. The readers are urged to enjoy these scholarly discussions in the original text by Austin et al.

In conclusion, thanks to the current progress in structural and functional studies of natural product biosynthesis, we now have a much better understanding of Nature's intricate molecular networks that result in the tremendous diversity of natural products. Steric effects have been the basis of many structure-based hypotheses; however, Nature's approach may be much more subtle than direct steric contacts, and long-range electronic effects may be just as important. The fine balance act of type III polyketide synthase offers a nice demonstration of such subtlety.

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Selected Reading

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