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Mechanistic insights into Diels-Alder reactions in natural product biosynthesis

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Natural enzymes that catalyze Diels-Alder reactions have long been sought after, yet few enzymes have been experimentally confirmed to perform this reaction. In the past five years, several stand-alone enzymes that can catalyze the Diels-Alder reaction had been identified and characterized. Among which, the crystal structures of SpnF, PyrI4 and AbyU have been determined. The structures of PyrI4 and AbyU, which are involved in spirotetronate/spirotetramate biosynthesis, are particularly informative since they shed light on how a natural catalyst captures the flexible substrate and facilitates the intramolecular Diels-Alder reaction through stabilization of the transition state in catalysis. These pioneering studies will inspire the design of artificial catalysts for Diels-Alder reactions.

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Introduction

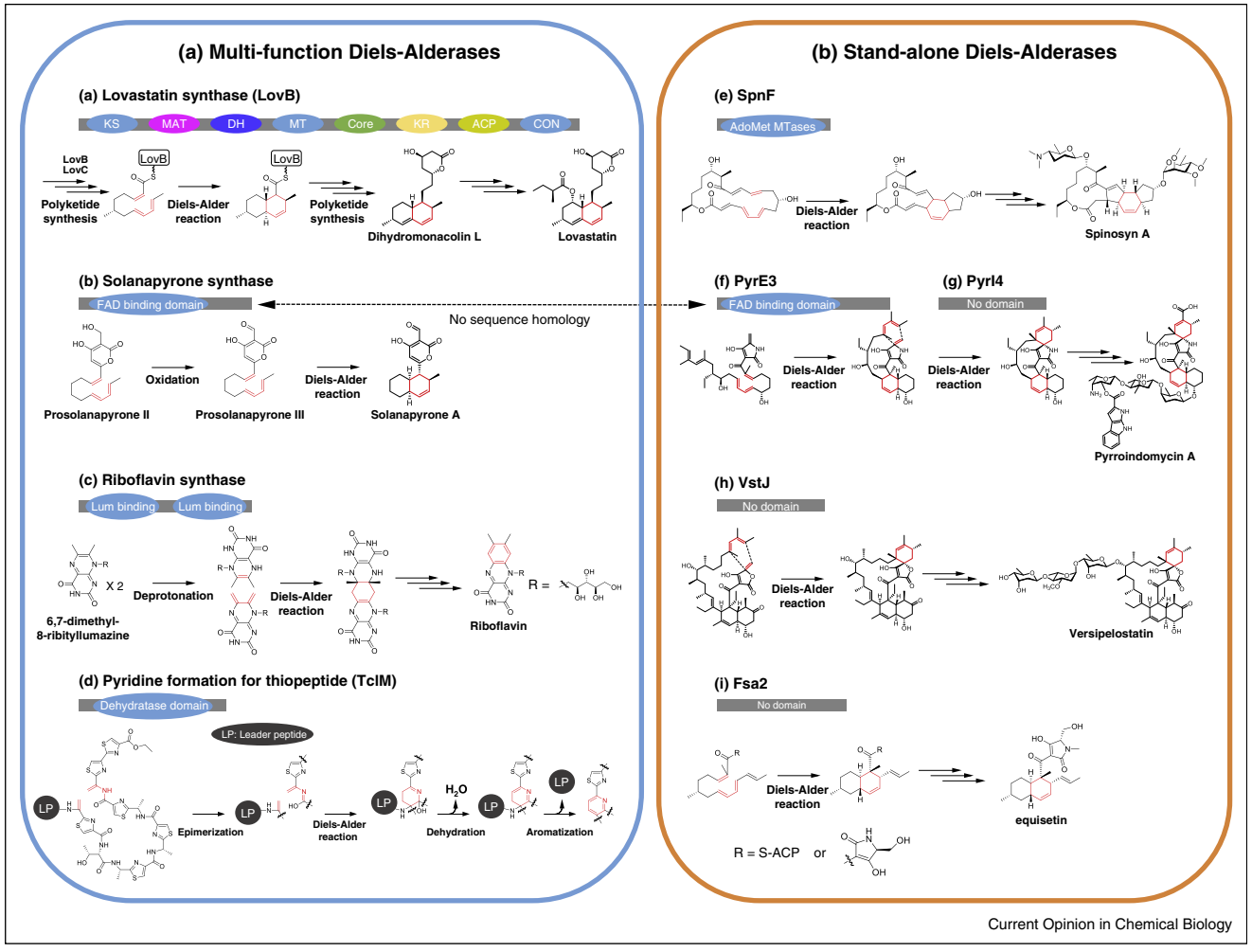
The Diels-Alder reaction is an elegant and perhaps the best-known pericyclic transformation in synthetic chemistry. In this reaction, a 1,3-diene and an alkene (dienophile) cyclize into a cyclohexene through a single transition state [1]. This [4+2]-cycloaddition reaction is a simple yet powerful tool for the construction of C–C bonds and is therefore often utilized for the chemical synthesis of natural products with a transannular cyclohexene ring(s) [2]. More than 400 compounds with this transannular ring structure have been identified among primary and secondary metabolites [3], leading to decades of speculation on the existence of enzymes capable of catalyzing Diels-Alder reactions in the biosynthesis of these compounds [4]. However, only a few such enzymes have been identified, which are often called 'Diels-Alderases' [3–6].

Since the 1980s, some biological catalysts of the Diels-Alder reaction, such as ribozymes [7] and catalytic antibodies [8–10], have been developed using a hapten that mimics the transition state of the target reaction. *De novo*-designed artificial enzymes for Diels-Alder reactions have also been developed [11]. However, artificial catalysts of the Diels-Alder reactions often undergo product inhibition [12], because such catalysts recognize the reaction product as well as the hapten due to their structural similarity. By contrast, natural Diels-Alderases would avoid product inhibition to accomplish the efficient conversion of the substrate to the proper product. Therefore, mechanistic and structural studies of these natural enzymes are expected to reveal a more elaborate underlying mechanism.

Pioneering studies having sought natural Diels-Alderases identified macrophomate synthase (MPS) [4,13], which catalyzes the transformation of 2-pyrone derivatives into the corresponding benzoate analogues. The crystal structure of MPS complexed with pyruvate enolate and Mg^{2+} was solved [14], making MPS the first potential Diels-Alderase with its structure determined. However, later quantum mechanics/molecular mechanics (QM/MM) calculations on the MPS-catalyzed transformation indicated that a two-step Michael-aldol sequence is energetically preferred over a concerted Diels-Alder reaction [15]. Subsequent biochemical studies also supported this conclusion [16,17]. Thus, although MPS is not a *bona fide* Diels-Alderase, a series of studies on MPS has provided significant insights into the concertedness of two-bond formations in Diels-Alderase-catalyzed reactions.

Most enzymes known for their [4+2]-cycloaddition activity catalyze other reactions in addition to the Diels-Alder reaction (Figure 1). For example, solanapyrone synthase (SPS) catalyzes the oxidation of prosolanapyrone II, followed by a Diels-Alder reaction to yield solanapyrone A [18–21]. Lovastatin nonaketide synthase (LovB) is a highly reducing fungal iterative type I polyketide synthase that catalyzes modification reactions as well as carbon chain-elongation reactions. In addition, LovB catalyzes a Diels-Alder reaction during one of its carbon chain-elongation cycles [22–24]. Riboflavin synthase has also been predicted to be a possible Diels-Alderase. This enzyme catalyzes the deprotonation of 6,7-dimethyl-8-ribityllumazine and a subsequent Diels-Alder reaction to yield a pentacyclic intermediate [25]. TclM and its

Figure 1



Diels-Alderase-catalyzed reactions in natural product biosynthesis. **(A)** Multi-function Diels-Alderases. **(a)** Lovastatin synthase (LovB) is a highly reducing fungal type I polyketide synthase. KS: ketosynthase, MAT: malonyl-CoA acyltransferase, DH: dehydratase, MT: methyltransferase, Core: inactive enoyl reductase, KR: ketoreductase, ACP: acyl carrier protein, CON: condensation protein. LovB catalyzes carbon chain elongation. During elongation cycles, LovB catalyzes a single Diels-Alder reaction to yield dihydromonacolin L. **(b)** Solanapyrone synthase (SPS) catalyzes the oxidation of prosolanapyrone II to yield prosolanapyrone III and subsequently catalyzes a Diels-Alder reaction to yield solanapyrone A. **(c)** Riboflavin synthase has two lumazine-binding domains to bind the substrate 6,7-dimethyl-8-ribityllumazine. A pentacyclic intermediate suggests a possible Diels-Alder reaction during catalysis. **(d)** Pyridine formation for thiopeptide antibiotics. Typically, thiopeptide antibiotics have a pyridine ring that acts as a linchpin for the macrocyclic structure. The reaction of TclM during thiocillin biosynthesis is a representative example [27]. The amide bond of dehydroalanine is converted to an imine to generate a nitrogen containing diene. Then, a formal [4+2]-cycloaddition between two dehydroalanine residues occurs. Subsequent dehydroxylation and aromatization form the pyridine ring structure. **(B)** Stand-alone Diels-Alderases. (e, f, g, h, i) SpnF, PyrE3 [37•], PyrI4, VstJ, and Fsa2 [35•] were characterized as stand-alone Diels-Alderases. SpnF shows sequence similarity to methyltransferase, and PyrE3 shows sequence similarity to FAD-binding proteins. SPS and PyrE3 share no sequence similarity with each other, although both enzymes contain an FAD-binding domain.

homologues, which are involved in the biosynthesis of thiopeptide antibiotics, catalyze a Diels-Alder reaction between nitrogen-containing diene and dehydroalanine [26–28]. First, an amide bond is epimerized into a diene, followed by a TclM-catalyzed aza-Diels-Alder reaction. The subsequent dihydroxylation and aromatization are also catalyzed by this enzyme. The multiple functions of these enzymes complicate the detailed analysis of their reaction mechanisms.

In 2011, the discovery of the enzyme SpnF transformed the study of Diels-Alderases, because SpnF was experimentally demonstrated to be the first stand-alone Diels-Aldrase to exclusively catalyze the Diels-Alder reaction. Although SpnF has significant sequence similarity to *S*-adenosylmethionine-dependent *O*-methyltransferase, the enzyme solely catalyzes a [4+2]-cycloaddition reaction during the biosynthesis of spinosyn. SpnF accelerates the reaction rate (k_{cat}/k_{non}) by 500-fold compared with