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## The Bifunctional Acyltransferase/Decarboxylase LnmK as the Missing Link for-Alkylation in Polyketide Biosynthesis

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Both  $\alpha$ - and  $\beta$ -alkylations contribute to the vast structural diversity displayed by polyketide natural products (Figure 1A).<sup>1</sup> While the  $\alpha$ -alkyl branches are typically derived from the extender units, the choice of which is dictated by the acyltransferase (AT) domain of modular polyketide synthases (PKSs),<sup>1</sup> the  $\beta$ alkyl branches often result from the activities of hydroxymethylglutaryl-CoA (HMG-CoA) synthase homologs (HCSs).<sup>2</sup> For a  $\beta$ methyl branch, HCS catalyzes condensation of acetyl-S-acyl carrier protein (ACP) with the  $\beta$ carbonyl group of the PKS-ACP-tethered growing polyketide intermediate to afford an HMG-S-ACP intermediate, which is subsequently dehydrated and decarboxylated by two enoyl-CoA hydratase homologs (ECH1 and ECH2) sequentially to afford a  $\beta$ methylated intermediate in either olefinic form (Figure 1B). This pathway has been experimentally confirmed for the biosynthesis of bacillaene (**1**),<sup>3</sup> curacin (**2**),<sup>4</sup> and myxovirescin A (also known as TA) (**3**),<sup>5</sup> and a dedicated set of three proteins - an ACP, an AT, and a ketosynthase homolog (KS) - has been identified that derives acetyl-S-ACP from malonyl-CoA for this pathway (Figure 1C).<sup>2-5</sup>

A parallel pathway replacing acetyl-S-ACP with propionyl-S-ACP could be envisaged for  $\beta$ ethyl branch introduction, and this proposal has been supported for **3** using chemoenzymatically prepared propionyl-S-ACP as a substrate (Figure 1B).<sup>5b</sup> However, counterparts for propionyl-S-ACP biosynthesis from methylmalonyl-CoA, such as the AT and KS enzymes required to generate acetyl-S-ACP from malonyl-CoA, are absent from gene clusters known to encode biosynthesis of polyketides with  $\beta$ ethyl branches (Table 1); the origin of propionyl-S-ACP remains unknown.<sup>2-5</sup>

Leinamycin (LNM, **4**), a potent antitumor antibiotic, possesses a  $\beta$ -branched C3 unit, which is a part of its unique five-membered 1,3-dioxo-1,2-dithiolane moiety. We have previously cloned, sequenced, and characterized the *lnm* biosynthetic gene cluster from *Streptomyces atroolivaceus* S-140.<sup>6</sup> Close examination of the *lnm* cluster revealed a subset of four genes - *lnmL*, *lnmM*, *lnmF*, and *lnmK* - encoding an ACP (LnmL), an HCS (LnmM), an ECH1 (LnmF), and a protein of unknown function (LnmK). Counterparts of LnmL, LnmM, and LnmF are present in biosynthetic clusters of polyketides with both  $\beta$ -methyl and  $\beta$ ethyl branches,<sup>2-6</sup> supporting the proposal that the C3  $\beta$ branch of **4** is likely installed by LnmL/LnmM/LnmF in a mechanistic analogy to the  $\beta$ methyl branch for **1**, **2** and **3**. Homologs of LnmK however can only be found in gene clusters encoding the biosynthesis of ethyl branch-bearing polyketides,

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**Supporting Information Available:** Full experimental details, Figures S1, S2, Table S1 are available free of charge via the Internet at <http://pubs.acs.org>.

suggesting LnmK as a candidate for propionyl-S-ACP biosynthesis (Table 1) (Figure 1C). Here we report the characterization of LnmK as a bifunctional acyltransferase/decarboxylase (AT/DC) that derives propionyl-S-ACP from methylmalonyl-CoA. Hence, LnmK represents a new family of AT/DC enzymes supplying a key substrate for  $\beta$ -alkylation in polyketide biosynthesis.

We first overproduced both LnmL and LnmK in *Escherichia coli* BL21(DE3) and purified them to near homogeneity (Figure S1). The purified LnmL was eluted as a single peak upon HPLC analysis (Figure 2A, panel I) and confirmed to be in its apo-form by ESI-MS analysis (Table S1). In vitro phosphopantetheinylation was carried out by incubating apo-LnmL with CoA in the presence of the known promiscuous phosphopantetheinyltransferase Svp,<sup>7</sup> and the resultant holo-LnmL was confirmed by HPLC (Figure 2A, panel II) and ESI-MS (Table S1) analyses.

We then established that LnmK is a bifunctional AT/DC catalyzing the formation of propionyl-S-LnmL. Holo-LnmL was incubated with [1-14C]acetyl-, [1-14C]propionyl-, [1,3-14C<sub>2</sub>] malonyl- or [1,3-14C<sub>2</sub>]methylmalonyl-CoA in the presence of LnmK, and the reaction mixtures were subjected to SDS-PAGE and phosphorimaging. LnmK specifically and efficiently catalyzed the loading of methylmalonyl-CoA to holo-LnmL, and no loading was observed with the other acyl-CoAs tested (Figure 2B). To verify the molecular identity of the acyl-S-LnmL species, the reaction was repeated with cold methylmalonyl-CoA, and the resultant product was subjected to HPLC and ESI-MS analyses. A distinct new product was formed (Figure 2A, panel III), ESIMS analysis of which remarkably revealed it as propionyl-S-LnmL (Table S1); LnmK apparently acts as bifunctional AT/DC, catalyzing both methylmalonyl transfer to form the methylmalonyl-S-LnmL intermediate and its subsequent decarboxylation to yield propionyl-S-LnmL (Figure 1C).

We finally determined the precise timing of acyl transfer and decarboxylation events catalyzed by LnmK. The fact that LnmK cannot decarboxylate methylmalonyl-CoA and only loads methylmalonyl-CoA, but not propionyl-CoA, to holo-LnmL, indicates that decarboxylation most likely occurs on methylmalonyl-S-LnmL. To directly verify this mechanism, we prepared methylmalonyl-S-LnmL via in vitro phosphopantetheinylation by incubating apo-LnmL with methylmalonyl-CoA in the presence of Svp.<sup>7</sup> Methylmalonyl-S-LnmL formation was monitored by HPLC (Figure 2A, panel IV) and confirmed by ESI-MS (Table S1) analyses. Incubation of methylmalonyl-S-LnmL with LnmK allowed us to investigate LnmK's DC activity directly. LnmK catalyzes specific and efficient decarboxylation of methylmalonyl-S-LnmL to yield propionyl-S-LnmL whose identity was confirmed by HPLC (Figure 2A, panel V) and ESI-MS (Table S1) analyses. Taken together, these results unambiguously established that LnmK first transfers methylmalonyl from methylmalonyl-CoA to holo-LnmL to form methylmalonyl-S-LnmL and then decarboxylates the latter to form propionyl-S-LnmL (Figure 1C).

LnmK homologs are known but to date were all annotated as hypothetical proteins (Figure S2).<sup>2-5</sup> We now propose LnmK to represent a new family of AT/DC enzymes supplying substrates for  $\beta$ -alkylation in polyketide biosynthesis. To further probe the catalytic mechanism of this newly discovered family of AT/DC enzymes, LnmK was incubated with [1,3-14C<sub>2</sub>] methylmalonyl-CoA in the absence of holo-LnmL, and the reaction mixtures were subjected to SDS-PAGE and phosphorimaging. Specific and efficient loading of [1,3-14C<sub>2</sub>] methylmalonyl-CoA onto LnmK was observed (Figure 2C), indicative of a transient acyl-LnmK intermediate in LnmK catalysis. This is reminiscent of ATs with Ser at their active sites,<sup>8</sup> although no conserved AT or DC active site motif is apparent in LnmK (Figure S2).

In summary, LnmK has been characterized as a bifunctional AT/DC that catalyzes the formation of propionyl-S-ACP from methylmalonyl-CoA, accounting for the missing link for

the  $\beta$ -ethyl or propionyl branch in polyketide biosynthesis. LnmK therefore could be exploited by combinatorial biosynthesis methods to engineer novel polyketides, especially those with  $\beta$ -alkyl branches. LnmK also represents an emerging family of novel AT/DC enzymes.

## Supplementary Material

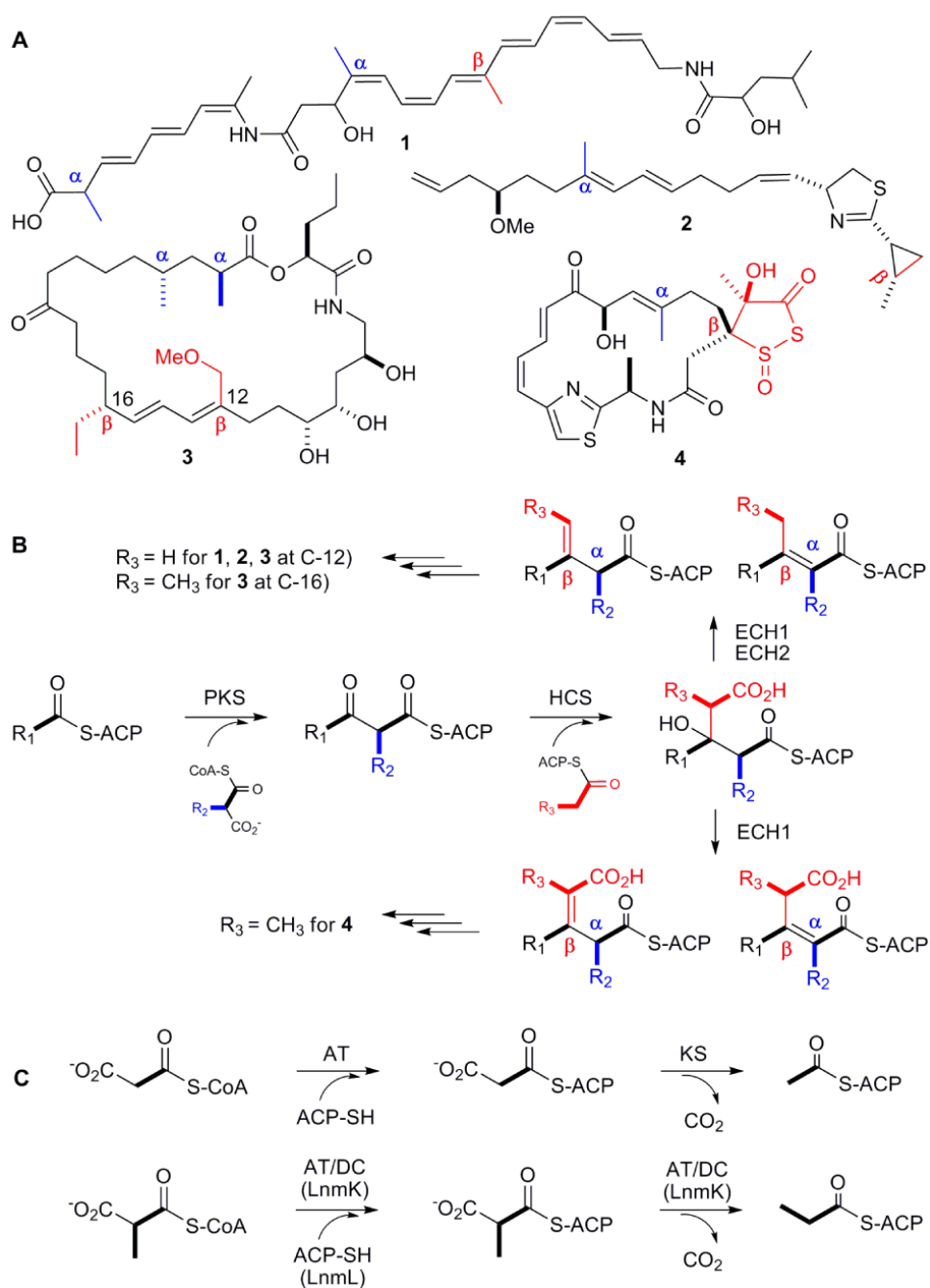
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## Acknowledgment

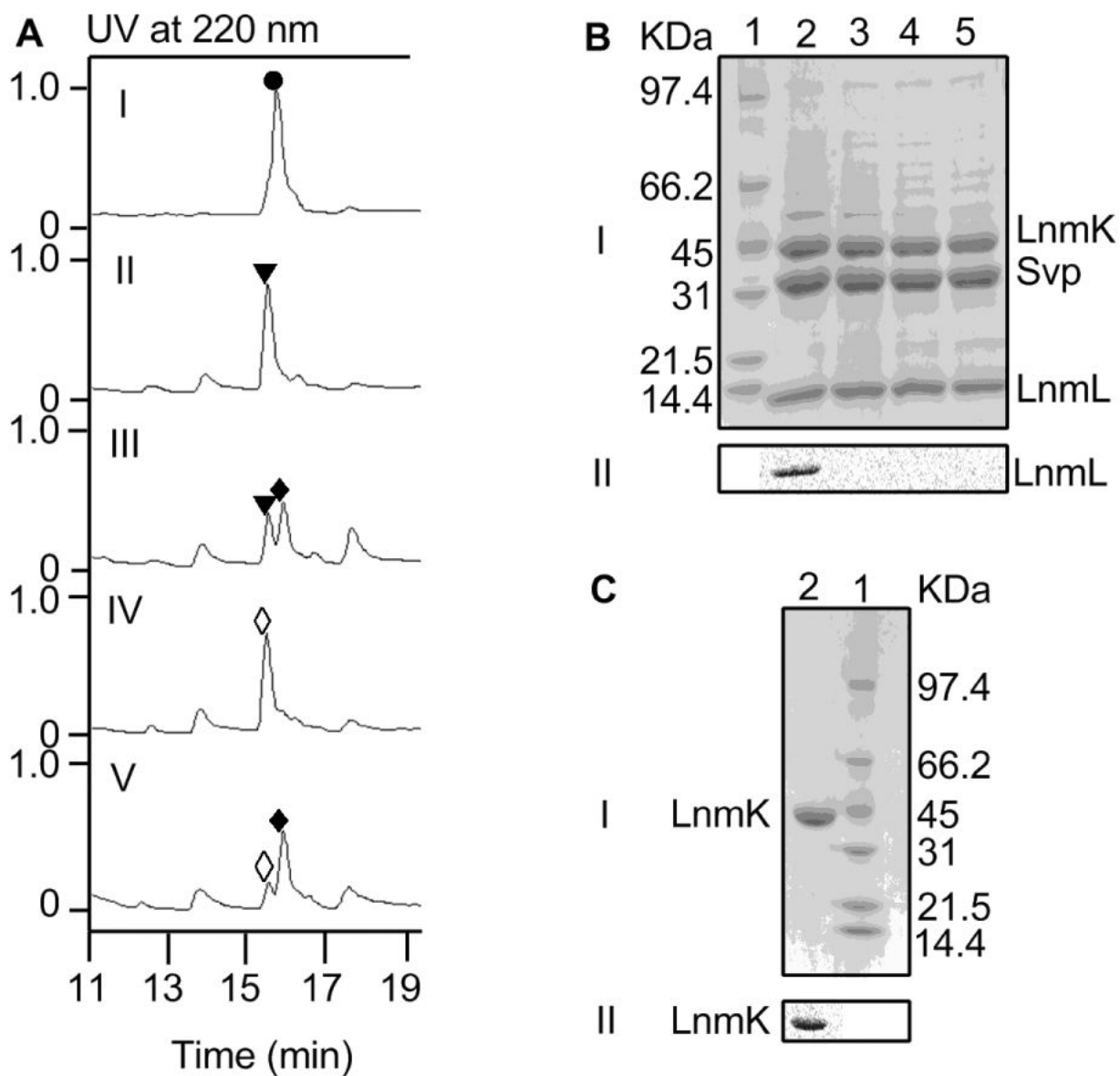
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## References

1. (a) Staunton J, Weissman KJ. Nat. Prod. Rep 2001;18:380–416. [PubMed: 11548049] (b) Fischbach MA, Walsh CT. Chem. Rev 2006;106:3468–3496. [PubMed: 16895337]
2. Calderone CT. Nat. Prod. Rep 2008;25:845–853. [PubMed: 18820753]
3. Calderone CT, Kowtoniuk WE, Kelleher NL, Walsh CT, Dorrestein PC. Proc. Natl. Acad. Sci. U.S.A 2006;103:8977–8982. [PubMed: 16757561]
4. Gu L, Jia J, Liu H, Hakansson K, Gerwick WH, Sherman DH. J. Am. Chem. Soc 2006;128:9014–9015. [PubMed: 16834357]
5. (a) Simunovic V, Müller R. ChemBioChem 2007;8:497–500. [PubMed: 17330904] (b) Simunovic V, Müller R. ChemBioChem 2007;8:1273–1280. [PubMed: 17583882] (c) Calderone CT, Iwig DF, Dorrestein PC, Kelleher NL, Walsh CT. Chem. Biol 2007;14:835–846. [PubMed: 17656320]
6. (a) Cheng Y, Tang G, Shen B. J. Bacteriol 2002;184:7013–7024. [PubMed: 12446651] (b) Cheng Y, Tang G, Shen B. Proc. Natl. Acad. Sci. U.S.A 2003;100:3149–3154. [PubMed: 12598647] (c) Tang G, Cheng Y, Shen B. Chem. Biol 2004;11:33–45. [PubMed: 15112993] (d) Cheng Y, Tang G, Shen B. J. Nat. Prod 2006;69:387–393. [PubMed: 16562841] (e) Tang G, Cheng Y, Shen B. J. Bio. Chem 2007;282:20273–20282. [PubMed: 17502372]
7. Du L, Shen B. Chem. Biol 2001;6:507–517. [PubMed: 10421758]
8. (a) Reeves CD, Murli S, Ashley GW, Piagentini M, Hutchinson CR, Mcdaniel R. Biochemistry 2001;40:15464–15470. [PubMed: 11747421] (b) Ridley CP, Lee HY, Khosla C. Proc. Natl. Acad. Sci. U.S.A 2008;105:4595–4600. [PubMed: 18250311]

**Figure 1.**

(A) Selected polyketides bacillaene (**1**), curacin (**2**), myxovirescin A (**3**), and leinamycin (**4**) with  $\alpha$ - (blue) or  $\beta$ -alkyl (red) branches; (B) a unified pathway for  $\beta$ -alkylation utilizing both acetyl-*S*-ACP and propionyl-*S*-ACP as substrates; and (C) distinct pathways for acetyl-*S*-ACP and propionyl-*S*-ACP biosynthesis.

**Figure 2.**

(A) HPLC analysis of LnmK-catalyzed formation of propionyl-S-LnmL: (I) apo-LnmL (•), (II) holo-LnmL (▼), (III) holo-LnmL and propionyl-S-LnmL (◆), (IV) methylmalonyl-S-LnmL (◇), (V) propionyl-S-LnmL. (B) LnmK-catalyzed loading of acyl-CoAs to holo-LnmL and (C) LnmK-catalyzed self-acylation as judged by (I) 4-15% SDS-PAGE and (II) autoradiogram: lane 1, molecular weight standards; lane 2, [1,3-<sup>14</sup>C<sub>2</sub>]methylmalonyl-CoA; lane 3, [1,3-<sup>14</sup>C<sub>2</sub>]malonyl-CoA; lane 4, [1-<sup>14</sup>C]propionyl-CoA; lane 5, [1-<sup>14</sup>C]acetyl-CoA.

**Table 1**

Enzymes that generate acetyl-S-ACP and propionyl-S-ACP and incorporate them into polyketides with  $\beta$ -alkyl branch (methyl for **1**, **2**, and **3** at C-12 or ethyl for **3** at C-16 and propionyl for **4**).<sup>2-6</sup>

Compd	AT/DC	AT	KS	ACP	HCS	ECH1	ECH2
<b>1</b>	-	PksC	PksF	AcpK	PksG	PksH	PksI
<b>2</b>	-	-	CurC	CurB	CurD	CurE	CurF
<b>3</b> (C-12)	-	TaV	TaK	TaB	TaC	TaX	TaY
<b>3</b> (C-16)	TaD	-	-	TaE	TaF	TaX	TaY
<b>4</b>	LnmK	-	-	LnmL	LnmM	LnmF	-