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Enzymes for fatty acid-based hydrocarbon biosynthesis

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Surging energy consumption and environmental concerns have stimulated interest in the production of chemicals and fuels through sustainable and renewable approaches. Fatty acid-based hydrocarbons, such as alkanes and alkenes, are of particular interest to directly replace fossil fuels. Towards this effort, understanding of hydrocarbon-producing enzymes is the first indispensable step to bio-production of hydrocarbons. Here, we review recent advances in the discovery and mechanistic study of enzymes capable of converting fatty acid precursors into hydrocarbons, and provide perspectives on the future of this rapidly growing field.

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Introduction

Driven by pressing environmental concerns due to significant increases in atmospheric CO_2 levels, extreme weather events, and mean global temperatures, efforts to create platforms for advanced biofuel production to replace fossil fuel-derived hydrocarbons have recently intensified [1,2]. While bioethanol (primarily corn-derived) has dominated US biofuel production with 13 billion gallons produced annually from approximately 30% of the US corn crop, the low energy density, high hygroscopicity, and chemical incompatibility of ethanol with the current fuel infrastructure have motivated efforts to produce biofuels with chemical and physical properties mirroring those of gasoline, diesel, and jet fuel [3,4].

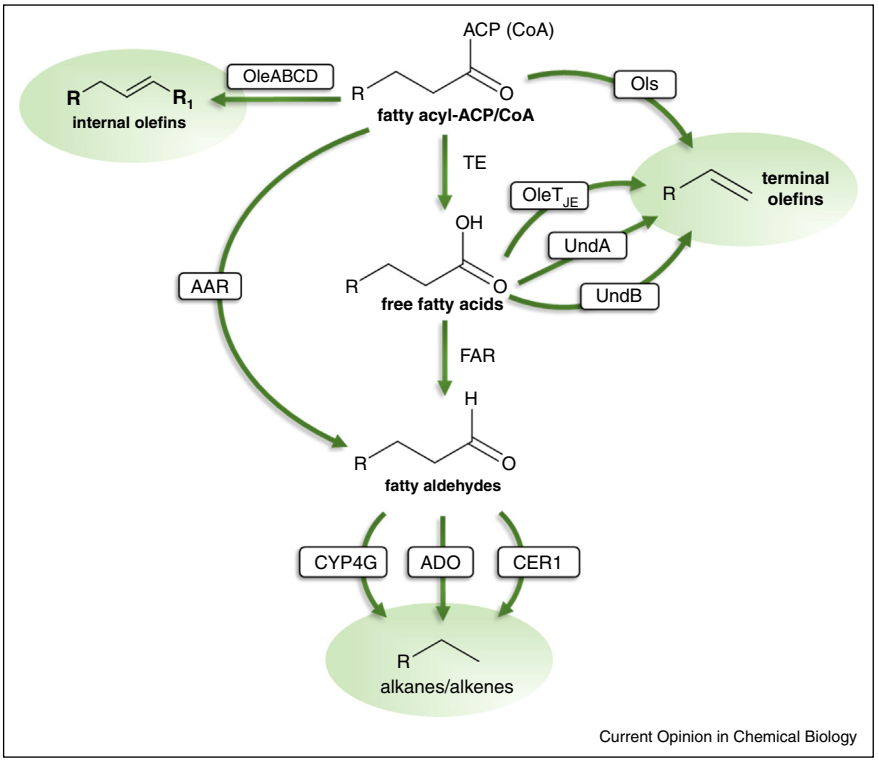
Biosynthesized hydrocarbons (alkanes and alkenes) serve as a convenient solution to this problem given the close chemical similarity of these compounds to petroleum-derived fuels [5]. Despite the importance of these compounds, along with the long-known observations of hydrocarbon production in plants [6], insects [7], microbes [8], and microalgae [9], much of what we know about the identity and function of enzymes able to catalyze hydrocarbon production are based on reports published since 2010 (with the exception of isoprenoid and ethylene synthesis) [10•,11]. Thus, the purpose of this review is to detail recent advances in the discovery and mechanistic study of enzymes involved in hydrocarbon production, with a particular focus on fatty acid-derived alkane/alkene-producing enzymes. We direct readers elsewhere for recent reviews concerning other mechanisms and metabolic engineering efforts for synthesizing hydrocarbon biofuels [3,10•]. Serving as a 'roadmap' for this review, Figure 1 and Table 1 summarize the recently characterized enzymes for fatty acid-based hydrocarbon biosynthesis which we will discuss. The sections that follow then examine these enzymes individually.

AAR (acyl–acyl carrier protein reductase) and ADO (aldehyde deformylating oxygenase)

Since the initial discovery of AAR and ADO from cyanobacteria by Schirmer *et al.* [12], much attention has surrounded this two-step pathway to synthesize terminal alkanes (saturated carbon at the terminal position) from fatty acyl precursors. In particular, these enzymes are capable of converting various chain-length acyl carrier protein (ACP)-bound fatty acids (C_n) to the corresponding C_{n-1} terminal alkanes (primarily C_{15} and C_{17} alkanes when expressed in *E. coli*) with formate as a byproduct [13]. When expressed in *E. coli*, AAR performs the first part of the reaction by reducing the fatty acyl-ACP substrate to an aldehyde intermediate, allowing ADO to complete the reaction with conversion of the fatty aldehyde to the terminal alkane product. Of the two enzymes, ADO has received most of the focus with extensive biochemical, spectroscopic, and structural analyses, given the unusual 'cryptic oxidation' mechanism in catalysis [14–18].

Showing similarity to ferritin-like proteins, ADO is a non-heme di-iron oxygenase requiring molecular oxygen and an external reducing system to provide four electrons (Figure 2). During the catalysis, the di-ferrous cofactor in the enzyme-aldehyde complex binds molecular oxygen in a bridging mode, forming a μ-peroxo-$Fe_2^{III/III}$ intermediate (supported by Mössbauer spectroscopic analysis) [16]. Formation of a peroxy-hemiacetal intermediate is then possible via nucleophilic addition of the previous peroxo-species to the substrate aldehyde carbonyl. With the introduction of an external electron, O–O bond cleavage yields an oxo-$Fe_2^{III/III}$ cluster along with a putative formyl radical intermediate. This radical intermediate is then proposed to undergo fragmentation, inducing a scission of the substrate C1–C2 bond yielding Fe^{III} complexed

Figure 1



Simplified pathways for fatty acid-derived hydrocarbon biosynthesis. Enzymes discussed in this review are shown in white boxes — see Table 1 for additional details. The two generic enzymes not discussed in Table 1 are TE (thioesterase) and FAR (fatty acid reductase), which may be supplied by a number of different homologs depending on the desired chain length specificity. For all species, 'R' represent different chain length and unsaturation level hydrocarbon substituents.

Table 1

Enzymes capable of catalyzing hydrocarbon production discussed in this review. See main text for appropriate references

Enzyme	Description	Proposed substrates[a]	Major products observed[b]	Cofactors	Co-substrates and reducing partners
AAR	Acyl-ACP reductase	MC/LC fatty acyl-ACPs	Fatty aldehydes (C_n)	–	NADPH
ADO	Aldehyde deformylating oxygenase	MC/LC fatty aldehydes	Alkanes/alkenes (C_{n-1}), formate	Fe^{2+}	O_2, Fd/FNR/N
CYP4G	Aldehyde decarbonylase (P450)	LC/VLC fatty aldehydes	Alkanes/alkenes (C_{n-1}), CO_2	Heme	O_2, CPR/NADPH
CER1	Aldehyde decarbonylase	LC/VLC fatty aldehydes	Alkanes/alkenes (C_{n-1}), CO	Fe^{2+}	CYTB5?, others?
OleT$_{JE}$	Fatty acid decarboxylase/ peroxygenase (P450)	MC/LC free fatty acids	α-Olefins (C_{n-1}), CO_2	Heme	O_2, CPR/NADPH or sole H_2O_2
UndA	Fatty acid oxidase/decarboxylase	MC free fatty acids	α-Olefins (C_{n-1}), CO_2	Fe^{2+}	O_2, others?
UndB	Fatty acid oxidase/decarboxylase	MC free fatty acids	α-Olefins (C_{n-1}), CO_2	Fe^{2+}	O_2, others?
Ols	Modular polyketide synthase	LC fatty acyl-ACPs (CoAs)	α-Olefins (C_{n+1}), CO_2	–	Malonyl-CoA, NADPH, PAPS
OleABCD	Thiolase-based system	MC/LC fatty acyl-CoAs	Internal olefins ($C_{n+n'-1}$), CO_2	–	NADPH, ATP?, others?

Abbreviations: ACP, acyl carrier protein; SC, short-chain; MC, medium-chain; LC, long-chain; VLC, very long-chain; fatty acyl-ACPs, which we define here as $<C_8$, C_{10}–C_{14}, C_{16}–C_{24}, and $>C_{24}$, respectively; Fd/FNR/N, ferredoxin/ferredoxin-NADP$^+$ reductase/NADPH; CPR, cytochrome P450 reductase; CYTB5, cytochrome b5 isoform; PAPS, 3′-phosphoadenosine-5′-phosphosulfate.

[a] Approximate substrate chain lengths based on hydrocarbon production of native producers and initial heterologous expression attempts. Substrate chain length specificity may be wider for *in vivo* and/or *in vitro* applications.

[b] Hydrocarbon product chain length based on conversion of substrate with carbon chain length 'n' (C_n). For the OleABCD complex, the internal olefin product is of chain length '$n + n' - 1$' ($C_{n+n'-1}$), where C_n and $C_{n'}$ are the carbon chain lengths of the two fatty acyl-CoA substrates. For all cases, the hydrocarbon product retains any internal desaturations installed prior to catalysis.