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A New Class of Enzymes Discovered: A Non-Heme Oxidase Produces Medium-Chain 1-Alkenes**

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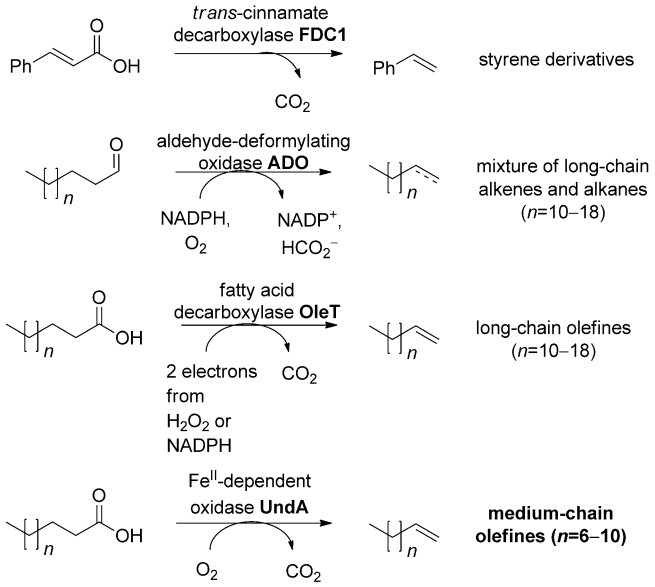
1-alkenes · biotransformations · hydrocarbons · oxidative decarboxylation · renewable resources

Terminal alkenes are very important platform chemicals. Oil refineries produce them on a scale well over 100000 metric tons per year. Their attractiveness as base chemicals results from the versatile alkene group that allows a myriad of derivatizations. Medium- to long-chain olefins are used for the production of detergents, lubricants, and additives. Given their tremendous importance, the foreseeable depletion of fossil resources creates an urgent demand for alternative supply routes. While natural feedstocks offer potential substitutes for many petrol-based chemicals such as ω-amino carboxylic acids and dicarboxylic acids for the polymer industry,[1] nature does not produce significant amounts of 1-alkenes.

The enormous importance of the terminal alkene function for man-made synthesis is contrasted by the fact that it plays only a marginal role in metabolic pathways.[2] Natural metabolism converts carbon dioxide and water into carbohydrates, which are then metabolized via a few key intermediates such as phosphoenolpyruvate, pyruvate, acetyl-CoA, and α-ketoglutarate to amino acids, sugars, and lipids. A few less-specialized, and basically non-essential, roads provide access to secondary metabolites such as terpenes and alkaloids. In none of these pathways are terminal alkenes involved as synthetic intermediates. The low number of natural biosynthesis pathways for olefin synthesis is also reflected in the current biotechnological landscape. While fermentative processes supply amino acids, organic acids, alcohols, and lipids on a million ton scale, no commercially viable process for the production of 1-alkenes exists.

Interestingly, several enzymatic reactions for the synthesis of terminal alkenes have been discovered in the last few years (Scheme 1). Phenolic acid decarboxylases are promising catalysts for the synthesis of styrene derivatives from phenolic acids.[3] The fatty acid decarboxylase OleT from the Gram-positive *Jeotgalicoccus* catalyzes the direct conversion of fatty acids into long-chain 1-alkenes. OleT has been successfully applied in biotransformations and fermentative olefin synthesis.[4] The fatty aldehyde deformylase ADO from *Nostoc punctiforme* produces terminal olefins and allowed the fermentative synthesis of long-chain alkenes in an engineered *E. coli* strain.[5] Unfortunately, OleT and ADO are strictly specific for the generation of long-chain 1-alkenes. Natural catalysts for the synthesis of medium-chain 1-alkenes are rare.



Scheme 1. Biocatalytic synthesis of 1-alkenes. The recently discovered enzyme UndA is a promising catalyst for the synthesis of medium-chain terminal alkenes.

In an outstanding recent contribution, the research group of Wenjun Zhang from the University of California reported a novel oxidase that selectively produces terminal medium-chain alkenes.[6] They investigated the hitherto unknown mechanism of 1-undecene formation in *Pseudomonas aeruginosa* strains.

For the discovery of the biosynthetic gene, Zhang and coworkers chose an in silico as well as a synthetic approach. As a search in *Pseudomonas* genomes did not reveal any homologues of the known enzymatic systems for alkene formation, they chose a comparative genome analysis for the identification of the new enzyme. The analysis, however, yielded several thousand potential gene candidates, thus underlining the limitation of this solely data-driven strategy.

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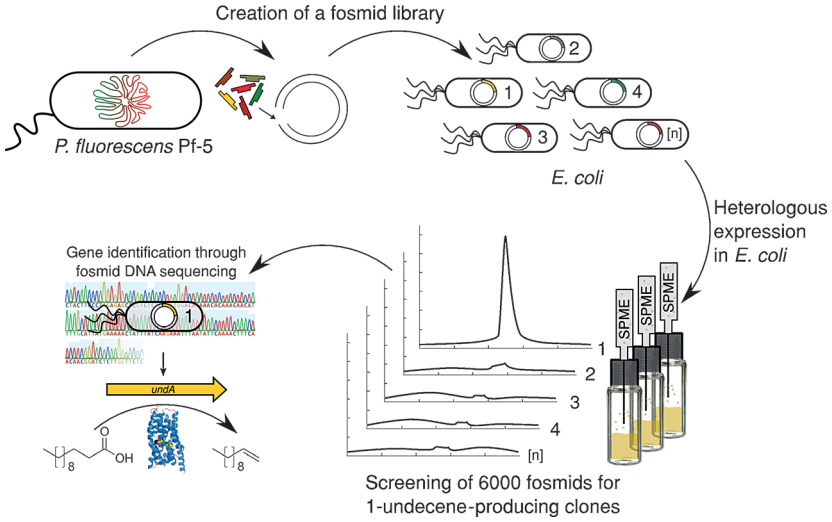
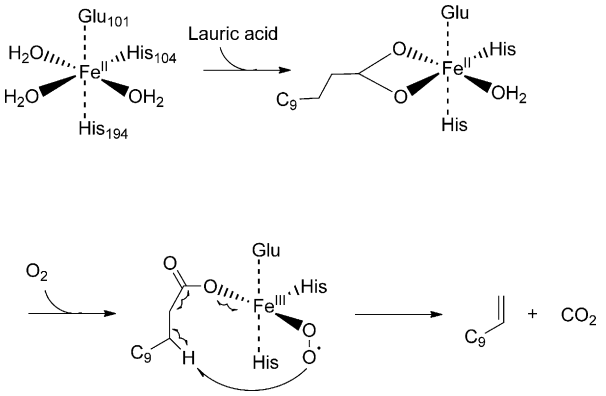
Key to the discovery of the biosynthetic enzyme was highly sensitive analytics for the determination of alkene formation. Headspace solid-phase microextraction coupled with GC/MS allowed the determination of minute ($\approx$1 ng) amounts of the volatile 1-undecene. Zhang and co-workers generated a genome library of the 1-undecene-producing *P. fluorescens* Pf-5. Heterologous expression and biocatalytic characterization of the impressive number of 6000 genome fragments resulted in the discovery of a gene that enabled *E. coli* to produce about 3 $\mu g\,mL^{-1}$ 1-undecene (Scheme 2). Sequencing of this clone revealed that a previously unknown enzyme catalyzes the oxidative decarboxylation of lauric acid to 1-undecene. UndA is a small enzyme of 261 amino acids. The most closely related enzyme with a known function, a bacterial thiaminase, has a sequence identity as low as 13% to UndA.



Scheme 2. Identification of the 1-undecene-producing enzyme UndA through screening of a genomic library of *P. fluorescens* Pf-5.

UndA converts fatty acids with chain lengths of C10, C12, and C14 into odd-numbered 1-alkenes. Interestingly, α-hydroxy fatty acids are converted into the corresponding aldehydes, while β-hydroxyacids are not converted. A biochemical characterization showed that UndA is a non-heme mononuclear Fe^{II}-oxidase. In vitro experiments with purified UndA resulted in single-turnover reactions, thereby indicating that the conversion leaves the enzyme with an inactive oxidized iron species. A comprehensive analysis of several cosubstrates showed that the activity can be reconstituted to some extent by the addition of Fe^{2+} or reducing agents such as ascorbic acid. Continuous in situ generation of oxygen combined with the addition of reducing agents finally resulted in multiple turnovers.

Elucidation of the structure of UndA revealed an octahedral iron center with an Fe^{2+} ion, coordinated by three amino acids and variable sites that are coordinated by oxygen atoms from the substrate. A hydrophobic pocket accommodates the substrate. Its size limits the substrate spectrum to the medium-chain fatty acids with chain lengths between C10 and C14. Zhang and co-workers suggested a radical mechanism for the decarboxylation (Scheme 3), in which the carboxylate group of the alkanoic acid binds to the ferrous iron, which then leads to the coordination of molecular oxygen. A Fe^{III}-superoxide complex abstracts the closely positioned (2.45 Å) β-H atom of the substrate. Single-electron transfer then leads to the formation of the products and an Fe^{IV}=O species that must be reduced by external reducing agents to the initial Fe^{II} ion.



Scheme 3. Suggested mechanism for the UndA-catalyzed oxidative decarboxylation of fatty acids.

In conclusion, Zhang and co-workers reported a novel non-heme oxidase with the capability to produce medium-chain olefins and presented the structure and mechanism of this fascinating enzyme. In the last few years, several new enzymatic mechanisms for the formation of long-chain 1-alkenes have been discovered. With its unique capability to produce medium-chain olefins, UndA complements these enzymes and opens up a new perspective for the biotechnological synthesis of volatile alkenes. The reported yields of the 1-undecene synthesis in *E. coli* are rather far from synthetic applications. Nevertheless, oxidative enzymes can be very efficiently applied for whole-cell biotransformations. A very interesting example is the successful development of a whole-cell process for the synthesis of a building block for polymer production, in which a membrane-bound monooxygenase catalyzes the oxyfunctionalization of lauric acid methyl ester.[1] Several patents demonstrate that the industry has already recognized the tremendous potential of the oxidative decarboxylation of fatty acids as a future supply of bio-based olefins.[4a,f,g] UndA extends this approach towards the synthesis of medium-chain alkenes and represents an important step en route to an industrial synthesis of bio-based olefins, which has tremendous potential to mitigate our dependence on petrol-based hydrocarbons.

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[1] M. Schrewe, N. Ladkau, B. Bühler, A. Schmid, *Adv. Synth. Catal.* **2013**, *355*, 1693 – 1697.
[2] W. Wang, X. Lu, *Synth. Biol.* **2013**, *1*, 10.
[3] R. McKenna, D. R. Nielsen, *Metab. Eng.* **2011**, *13*, 544 – 554.
[4] a) M. F. Alibhai, M. A. Rude, A. W. Schirmer, (LS9 Inc.), WO 2009085, **2009**; b) M. A. Rude, T. S. Baron, S. Brubaker, M. Alibhai, S. B. Del Cardayre, A. Schirmer, *Appl. Environ. Microbiol.* **2011**, *77*, 1718 – 1727; c) Y. Liu, C. Wang, J. Yan, W. Zhang, W. Guan, X. Lu, S. Li, *Biotechnol. Biofuels* **2014**, *7*, 28; d) J. Belcher, K. J. McLean, S. Matthews, L. S. Woodward, K. Fisher, S. E. Rigby, D. R. Nelson, D. Potts, M. T. Baynham, D. A. Parker, *J. Biol. Chem.* **2014**, *289*, 6535 – 6550; e) I. Zachos, S. Gaßmeyer, D. Bauer, V. Sieber, F. Hollmann, R. Kourist, *Chem. Commun.* **2015**, *51*, 1918 – 1921; f) A. Alcasabas, P. I. Darley, P. Pir (Shell Oil Company), US 20140206057A1, **2014**; g) S. Schaffer, J. Gielen, N. Decker, N. Kirchner, T. Haas, M. Pötter, H. Häger (Evonik Degussa GmbH), PCT/EP2012/065933, **2012**.
[5] a) A. Schirmer, M. A. Rude, X. Z. Li, E. Popova, S. B. del Cardayre, *Science* **2010**, *329*, 559 – 562; b) F. Schneider-Belhaddad, P. Kolattukudy, *Arch. Biochem. Biophys.* **2000**, *377*, 341 – 349.
[6] Z. Rui, X. Li, X. Zhu, J. Liu, B. Domigan, I. Barr, J. H. D. Cate, W. Zhang, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 18237 – 18242.

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