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Hydrocarbon Synthesis via Photoenzymatic Decarboxylation of Carboxylic Acids

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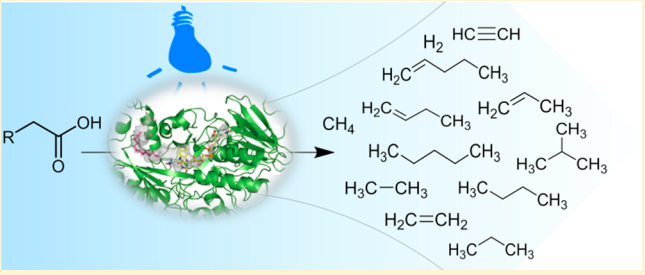
Supporting Information

ABSTRACT: A recently discovered photodecarboxylase from *Chlorella variabilis* NC64A (*Cv*FAP) bears the promise for the efficient and selective synthesis of hydrocarbons from carboxylic acids. *Cv*FAP, however, exhibits a clear preference for long-chain fatty acids thereby limiting its broad applicability. In this contribution, we demonstrate that the decoy molecule approach enables conversion of a broad range of carboxylic acids by filling up the vacant substrate access channel of the photodecarboxylase. These results not only demonstrate a practical application of a unique, photoactivated enzyme but also pave the way to selective production of short-chain alkanes from waste carboxylic acids under mild reaction conditions.



INTRODUCTION

Valorization of biobased feedstock into chemicals[1−4] and fuels[5−7] represents one of the pillars of a more sustainable society. The last few decades have seen an explosion of synthetic methodologies to transform natural products into building blocks for the chemical industry.[1−4]

Catalytic methodologies for the conversion of biobased carboxylic acids, however, are largely limited to esterification or amidation reactions.[8] More recently, chemical[9−12] and biocatalytic[13−17] reduction reactions of carboxylic acids have been developed as well as some approaches for the oxidative decarboxylation into terminal alkenes.[18−21]

In addition, redox-neutral decarboxylation of carboxylic acids into the corresponding alkanes represents an attractive method for the synthesis of alkanes for fuel and synthetic applications. Conventional methods, however, require harsh reaction conditions and high loadings of precious metal catalysts. This implies a significant environmental impact of these reactions and questions their economic feasibility. In addition, the selectivity of the reported methods mostly is poor due to the formation of Kolbe- and Hofer−Moest-by-products.[22,23]

To overcome some of these drawbacks, enzymes have been gaining an increasing interest for the direct formation of hydrocarbons.[24−27] Recently, a new fatty acid photodecarboxylase from the unicellular photosynthetic green microalga *Chlorella variabilis* NC64A (*Cv*FAP) was reported by Beisson and co-workers.[28] *Cv*FAP mediates the blue-light driven decarboxylation of various fatty acids to the corresponding (C1-shortened) alkanes (Figure 1a). *Cv*FAP, however, shows a marked preference for long-chain fatty acids (C16−C17) with its catalytic activity dropping dramatically with shorter chain carboxylic acids. Presumably, shorter substrates are less efficiently stabilized in the long, narrow substrate access channel of the decarboxylase.[28]

A similar phenomenon has been observed with the P450 monooxygenase from *Bacillus megaterium*, which exhibits (almost) no oxyfunctionalisation activity toward small substrate molecules. The groups of Reetz[29] and Watanabe[30] independently proposed to fill up the vacant space left by small substrate molecules with unreactive, perfluorinated fatty acids. Inspired by their work we reasoned that a similar strategy

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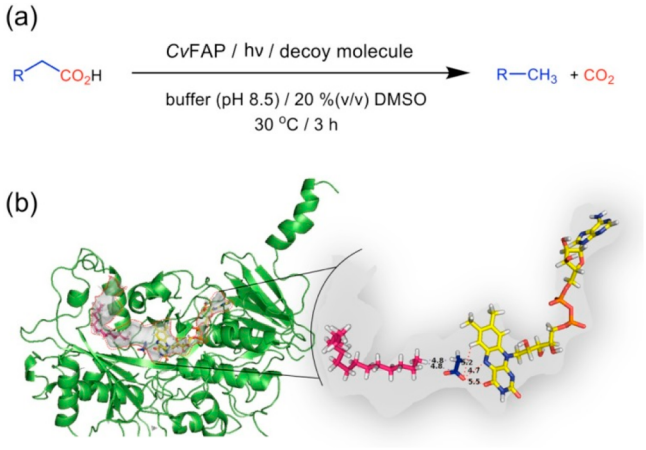

Figure 1. *Cv*FAP-catalyzed photobiocatalytic decarboxylation reaction. (a) general reaction scheme and reaction conditions; (b) schematic representation of acetic acid coordinating to the catalytic flavin group together with tetradecane as decoy molecule filling up the substrate access channel. *Cv*FAP (PDB: 5NCC) is presented as a green cartoon showing the substrate channel as transparent gray surface, while FAD (yellow), acetic acid (blue) and tetradecane (magenta) are represented as sticks. The distances in angstroms from the carboxylate oxygen atom of acetic acid to FAD and from the C1-atom of tetradecane to both carbon atoms of acetic acid are indicated. First, acetic acid was docked into 5NCC, then tetradecane was docked into the minimized result of the first docking using VINA in Yasara (www.yasara.org). The figure was prepared using PyMol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

might be applicable to enlarge the substrate scope of *Cv*FAP (Figure 1b).

We propose using simple alkanes as decoy molecules, as cocatalysts, to accelerate the *Cv*FAP-catalyzed decarboxylation of short-chain carboxylic acids.

RESULTS AND DISCUSSION

We first evaluated *Cv*FAP as a photobiocatalyst for the decarboxylation of acetic acid to methane in the presence of various decoy alkanes (Figure 2). For this the enzyme was overproduced in *Escherichia coli* BL21 (DE3). A typical time course of the reaction is shown in Figure 2a. No methane could be detected in the absence of *Cv*FAP and only minor amounts were formed in the absence of active illumination (possibly due to the incomplete light-insulation of the reaction vessel). Moreover, no background activity was found in crude extracts prepared from *E. coli* cells containing an empty vector. $^{13}CH_3-CO_2H$ was converted into $^{13}CH_4$ (Figure S6). Most interestingly, the rate of methane accumulation was almost doubled in the presence of tridecane as decoy molecule, while also the robustness of the reaction increased significantly leading to more than a 3-fold increase of methane yield. The highest acceleration of methane production was achieved in the presence of tetradecane (Figure 2b). Both in the absence and presence of a decoy molecule, the enzyme reaction rate depended on the concentration of acetic acid (Figure 2c). In both cases half-maximal rates were obtained at comparable acetic acid concentrations (around 50 mM), suggesting that the decoy molecule did not facilitate the binding of the carboxylic acid but rather increased the enzyme reaction rate. When varying the concentration of the decoy molecule (Figure 2d), a saturation-type dependency of the reaction rate was observed. Furthermore, at elevated concentrations of the decoy molecule an inhibitory effect was observed, which may be indicative for an ordered mode of binding of substrate and decoy molecule.

The rate of the photobiocatalytic decarboxylation reaction was linearly dependent on both the enzyme concentration (Figure S7) and the light intensity (Figure S8). The rate of the reaction in D_2O was approximately 1.5 times faster than the same reaction in H_2O (Figure S9). This inverse kinetic isotope effect most probably is due to an increased lifetime of the photoexcited flavin cofactor of *Cv*FAP in D_2O, as well documented previously for other flavoproteins.[31−33] We interpret these findings with the lifetime of the photoexcited flavin of *Cv*FAP being overall rate-limiting.

Encouraged by these promising results, we further evaluated the decarboxylation of a broader range of short-chain carboxylic acids (Table 1). The substrate scope of *Cv*FAP was not limited to straight-chain carboxylic acids as also some branched and unsaturated carboxylic acids were converted at significant rates. Decarboxylation of acrylic acid and propionic acid was only possible with the assistance of decoy molecules. Notably, hydrogen gas was formed from the decarboxylation of formic acid. It also becomes clear from Table 1 (and Figures S11−19) that the most suited decoy molecule depends on the carboxylic acid used. In general the number of carbon atoms of substrate and decoy molecule should sum up to 16, which is in line with the previously reported optimal fatty acid chain length.[28] It is also interesting to note that the accelerating effect of the decoy molecule decreased with the chain length of the substrate carboxylic acids.

The quantum efficiency of the photobiocatalytic decarboxylation reactions was determined. Using acetic acid, butyric acid, pentanoic acid and hexanoic acid, the quantum efficiency (at photon flux 13.8 μE $L^{-1}s^{-1}$) was 0.008%, 1.3%, 3.2% and 2.7%, respectively (Figure S10). It should be noted that a direct comparison with the numbers reported in the initial report is difficult due to the lack of all necessary data.

A further insight into the decarboxylation mechanism was obtained in the framework of the density functional theory (DFT) and time-dependent (TD-DFT) density functional theory (PBE1PBE+D3(BJ)/6-311+G(d,p)+PCM(water)) analysis. These calculations were carried out on a representative model system mimicking the reactive complex of the flavin active site of the FDA and acetate anion as the representative substrate. Our calculations reveal the crucial role of the prearrangement of acetate in the vicinity of the N(5) site of the FAD ($CH_3COO^-\cdots$FAD, Figure S32). This van der Waals complex closely resembles the configuration found from the crystal structure analysis shown in Figure 1. Importantly, in the absence of the confinement caused by the protein residues of *Cv*FAP, an alternative coordination mode dominated by H-bonding between the acetate anion and the N−H moiety of FAD is favored by ca. 20 kJ/mol. However, we were not able to identify a favorable decarboxylation path initiated from this structure, which is in agreement with the lack of decarboxylation activity of the free flavin (Figure S33).

When acetate substrate prearrangement is effective, the decarboxylation path starts from the $CH_3COO^-\cdots$FAD van der Waals complex exhibiting an absorption maximum at 467 nm.[33] Analysis of the respective frontier orbitals (HOMO, LUMO, Figure S32a) shows that photoexcitation of this molecular complex yields a $CH_3COO^{\bullet}\cdots FAD^{\bullet}$ radical pair, from which the decarboxylation of the $CH_3COO^{\cdot}$ proceeds rapidly via a highly favorable and low-barrier noncatalytic

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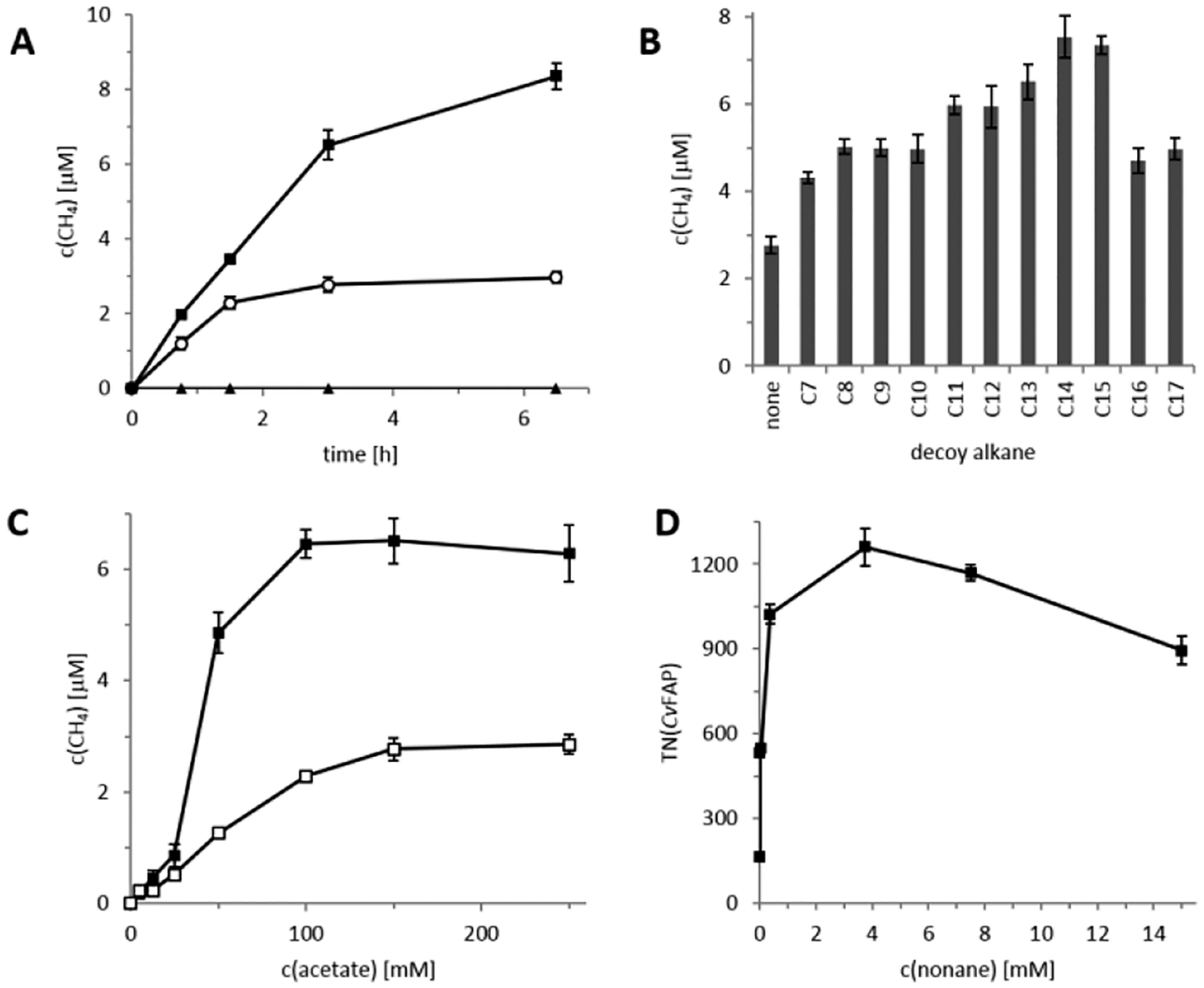

Figure 2. Photoenzymatic decarboxylation of acetic acid to methane (A-C). (A) Time course of methane formation performed with (■) and without decoy molecule tridecane (○), and in the absence of *Cv*FAP (▲). Reaction conditions: [decoy molecule] = 15 mM, [*Cv*FAP] = 6 μM, [acetic acid] = 150 mM, Tris−HCl buffer pH 8.5 (100 mM), 20% DMSO, 30 °C, blue light (450 nm). Methane concentration refers to product in the headspace of the reaction vial (4.0 mL of headspace plus 1.0 mL of liquid). (B) Effect of carbon length of decoy alkane on the methane formation. Reaction time: 3h. (C) The rate-dependency of the methane production rate on the acetic acid concentration with (■) and without tridecane (□). Reaction time: 3 h. (D) Turnover number (TON) of *Cv*FAP in the production of butane using nonane as decoy molecule. TON = $mol_{butane} \times mol_{CvFAP}^{-1}$. The contribution of reaction without using nonane was subtracted. Condition: [*Cv*FAP] = 6 μM, [pentanoic acid] = 150 mM, Tris−HCl buffer pH 8.5 (100 mM), 20% DMSO, 30 °C, 3 h. Error bars indicate the standard deviation of duplicate experiments (n = 2).

process. Computational studies propose that the generated CH_3 radical further binds the anionic flavin semiquinone resulting in the relaxation of the excited state to the original S_0 and the formation of a covalent dearomatized methylquinone flavin adduct. The overall decarboxylation reaction is slightly thermodynamically favorable (ΔG° = −5 kJ·mol^{-1}) allowing thus for a smooth continuation of the catalytic cycle. The flavin adduct intermediate (CH_3−FAD−) is then protonated to release the alkane product and regenerate the oxidized FAD closing the catalytic cycle (Figure S32b). Such a reaction pathway is also in agreement with the observed excellent selectivity of the photobiocatalytic decarboxylation reactions with all substrates used. This high selectivity makes the occurrence of free, C-centered radicals (at least for a prolonged period) highly unlikely.

■ CONCLUSION

Overall, in the present study we have demonstrated the photobiocatalytic decarboxylation of short-chain carboxylic acids using the fatty acid photodecarboxylase from *Chlorella variabilis* NC64A (*Cv*FAP). While the wild-type enzyme to some extent can convert also shorter substrates, the use of so-called decoy molecules significantly accelerates the reaction rate. Admittedly, the present reactions showed modest turnover numbers. However, we expect that engineering of both the enzyme and the decoy molecules will further increase the reaction rate and eventually render the *Cv*FAP-catalyzed decarboxylation of a large scope of (waste) carboxylic acids into a viable approach for the generation of light hydrocarbons. Given the recent success in the photodecarboxylation of long fatty acids,[28,34] this work also demonstrates the broad potential of this photodecarboxylase for the application in biofuel synthesis and thus valorization of organic waste streams.

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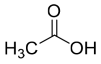
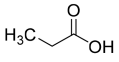
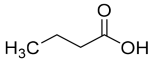
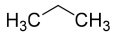
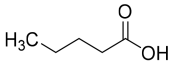
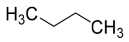
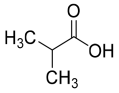
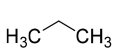
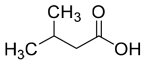
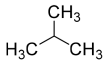
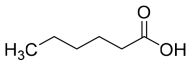
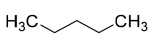
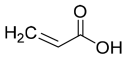
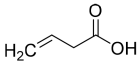
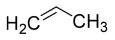
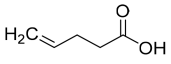
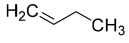
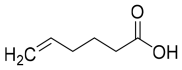
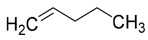
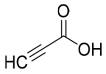
Table 1. Substrate Scope of the Photobiocatalytic Decarboxylation of Short-Chain Carboxylic Acids[a]

Entry	Substrate	Product	Best Decoy molecule	[Product] without decoy (μM)	[Product] with decoy (μM)	Acceleration (-fold)	TON (*Cv*FAP) with decoy	Selectivity (%)[b]
1		H–H	C15	48.8 ± 5.6	291.7 ± 14.2	6.0	194	100
2		CH_4	C14	2.7 ± 0.2	7.5 ± 0.5	2.5	5.0	100
3		$H_3C–CH_3$	C13	103 ± 7.9	347.1 ± 12.7	3.4	289	99.6[c]
4			C12	382.2 ± 9.9	1090.5 ± 28.9	2.9	908.6	99.96[c]
5			C9	860.7 ± 42.5	2440 ± 62.4	2.8	2034	99.99[c]
6			C13	305.15 ± 36.1	1007 ± 57.3	3.3	839	99.8[c]
7			C9	268.6 ± 19.4	940.2 ± 29	3.5	783.6	99.95[c]
8[d]			C14	1601.3 ± 40.1	1987.3 ± 28.7	1.2	1656	100
9		$H_2C=CH_2$	C13	0	2.1 ± 0.3	-	1.6	n.d.
10			C12	1.08 ± 0.1	7.9 ± 0.7	7.3	6.9	100
11			C10	24.27 ± 1.7	162 ± 11.3	6.8	134.5	100
12			C9	188.3 ±1 5.2	502.7 ± 10.9	2.7	418	100
13		HC≡CH	C14	0	9.3 ± 1.2	-	7.1	100

[a]Reaction conditions: [decoy molecule] = 7.5 mM, [*Cv*FAP] = 6 μM, [substrate] = 150 mM, Tris–HCl pH 8.5 (100 mM), 20% DMSO, 30 °C, 3 h, blue light (450 nm), photon flux 13.8 μE/Ls. The standard deviation is based on duplicate experiments (n = 2). The concentration refers to product in the headspace of the reaction vial (4.0 mL of headspace plus 1.0 mL of liquid). [b]Selectivity % = [target product]/([target product]+[other product]) × 100, calculation based on GC. [c]0.4% propane, 0.038% ethane, 0.01% ethane, 0.2% isopentane and 0.05% propane were observed from Entries 3–7, accordingly. [d]Reaction was performed at 40 °C.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b12282.

Experimental details including the preparation of the cell free extracts, photoenzymatic reactions, length of decoy molecule on decarboxylation activity, GC chromatogram and DFC calculations (PDF)

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Author Contributions

[▽]W.Z., M.M. and M.M.E.H. contributed equally.

Notes

The authors declare no competing financial interest.

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