Light-Driven Enzymatic Decarboxylation of Dicarboxylic Acids

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Photodecarboxylase from *Chlorella variabillis* (*Cv*FAP) is one of the three known light-activated enzymes that catalyzes the decarboxylation of fatty acids into the corresponding C1-shortened alkanes. Although the substrate scope of *Cv*FAP has been altered by protein engineering and decoy molecules, it is still limited to mono-fatty acids. Our studies demonstrate for the first time that long chain dicarboxylic acids can be converted by *Cv*FAP. Notably, the conversion of dicarboxylic acids to alkanes still represents a chemically very challenging reaction. Herein, the light-driven enzymatic decarboxylation of dicarboxylic acids to the corresponding (C2-shortened) alkanes using *Cv*FAP is described. A series of dicarboxylic acids is decarboxylated into alkanes in good yields by means of this approach, even for the preparative scales. Reaction pathway studies show that mono-fatty acids are formed as the intermediate products before the final release of C2-shortened alkanes. In addition, the thermostability, storage stability, and recyclability of *Cv*FAP for decarboxylation of dicarboxylic acids are well evaluated. These results represent an advancement over the current state-of-the-art.

1. Introduction

Cascade reactions combining chemo- and biocatalysis are regarded as the most efficient and universal systems to foster significant collaborations between chemists and enzymologists. In the past decade, cascade reactions have been indeed frequently used in biocatalysis together with different types of chemical catalysis for successful performance of multistep syntheses.[1,2] Bornscheuer and the co-workers first subdivided the most intensively studied chemo-enzymatic strategies into three concepts:[3] (1) combination of (transition) metalcatalysis and biocatalysis, which have almost reached the "mature technology" stage, especially in the cases of dynamic kinetic resolution,[4] (2) combination of organo- and biocatalysis, which is complementary, and has been used in a range of unique synthetic cascades. For example, asymmetric organocatalytic C–C bond-forming reactions have been combined with redox enzymatic biotransformation,[5] (3) Combination of photo-/electro-(chemistry and catalysis) and biocatalysis, which is the most attractive approach to promote electron transfer, thus enables the development of green and sustainable chemistry.[6] However, most of the examples combing photo-/electro-and biocatalysis are focusing on the cofactor recycling or cofactor replacement with light irradiation.[7–10] Notably, examples of photoenzymes by direct light activation are quite rare but desirable.

So far, there are only three photoenzymes known today. Protochlorophyllide oxidoreductase is the first example of light-driven enzymes which was described in 2015 and able to catalyze the reduction of the C17–C18 double bond of protochlorophyllide to yield the desired product of chlorophyllide.[11] The formation of chlorophyllide is the challenging step in the chlorophyll biosynthetic pathway.[12] As protochlorophyllide oxidoreductase is light-dependent, it provides essential evidences for the combination of light-activated chemical and biological catalysts. Another known blue-light-activated enzyme is the flavin-dependent DNA-repair enzymes (photolyase), which repairs ultraviolet-induced DNA damage during the process of producing cyclobutene pyrimidine dimers and pyrimidine-pyrimidone photoproducts with the overall quantum yield.[13] Very recently, a unique photoenzyme (*Cv*FAP) was discovered from the microalga *Chlorella variabilis* and shown to catalyze the free fatty acids into the corresponding (C1-shortened) alkanes via decarboxylation by Beisson and the co-authors.[14,15] *Cv*FAP represents an advancement over the current state-of-the-art photoenzyme and must be regarded as door-openers for yet-to-be-expected next steps in organic synthesis.

Indeed, following the footsteps of this contribution, several studies on the application/catalytic mechanism of *Cv*FAP have been performed. For example, Hollmann and co-workers fully assessed the preparative potential of *Cv*FAP for the decarbox-

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ylation of fatty acids.[16,17] Later, *Cv*FAP was used for the production of chiral amines, α-hydroxy acid, and alkenes in different laboratories.[3,18–22] Ma et al. expanded the substrate scope to 'real' substrates of commercially available oils with a bienzymatic cascade comprising a hydrolase and *Cv*FAP.[23] Asymmetric catalysis by an engineered *Cv*FAP was described recently for the kinetic resolution of α-functionalized carboxylic acids[24] and phosphinothricin.[25] Nevertheless, information on the mechanism of *Cv*FAP-catalyzed decarboxylation are very limited.[26,27] Very recently, the pathway about the synthesis of alka(e)nes in *Yarrowia lipolytica* by *Cv*FAP was reported.[28] It is surprising that the preferred substrate for *Cv*FAP is acyl-CoAs, rather than free fatty acids.

These reports on light-dependent *Cv*FAP encouraged us to utilize it for the conversion of dicarboxylic acids, which still represents a chemically very challenging reaction. Although the substrate scope of *Cv*FAP has been altered by protein engineering[21,24] and decoy molecules,[17] it is still limited to mono fatty acids. Therefore, it is of high interest to probe whether the conversion of dicarboxylic acids to alkanes can be accomplished by *Cv*FAP and how broadly it is applicable. The aim of this study was to demonstrate for the first time that dicarboxylic acids can be converted by the newly discovered photoenzyme *Cv*FAP, enlarging the substrate scope of *Cv*FAP.

2. Results and Discussion

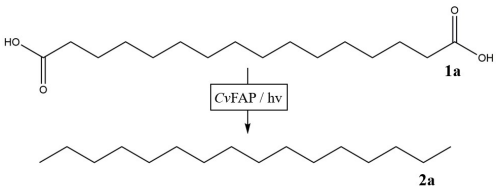
We started our investigation by evaluating the potential of *Cv*FAP as a photobiocatalyst for the decarboxylation of LCDAs to the corresponding C2-shortened alkanes. As a result of its rigid character and wide use as building blocks for polymers, LCDA of C16 chain length (hexadecanedioic acid, **1 a**) was chosen as the main test substrate.[29] According to the previous studies,[15] although there were two variant *Cv*FAP (long-length *Cv*FAP and short-length *Cv*FAP) present in the microalga *Chlorella variabilis*, only short-length *Cv*FAP revealed a good overproduction in *Escherichia coli* (*E. coli*). Thus, short-length *Cv*FAP was used for this study. After heterologous expression in *E. coli* BL21 (DE3), the *E. coli* cells, the cell-free extract (CFE), as well as the purified enzyme (after one-step purification with a HisTrap affinity column) were all used for catalytic activity test. Surprisingly, the overexpressed *E. coli* cells showed a significant activity towards hexadecanedioic acid (**1 a**), as compared to the purified *Cv*FAP or CFE. This is supported by the findings of Scrutton and the co-workers, that "broken cell" operation often leads to significant activity loss of *Cv*FAP.[26] Methods to prevent activity loss of *Cv*FAP during purification step is of great significance. Whereas, whole cells of overexpressed *E. coli* (*Cv*FAP@*E.coli*) were used for this study. In order to increase the solubility of decarboxylated product of alkanes, the catalytic reaction was performed in Tris-HCl buffer with DMSO as a cosolvent (30%, v/v).

The first experiment was performed with 1 mM of hexadecanedioic acid (**1 a**) and 200 mg/mL of wet cells under blue light illumination for 6 h. The desirable product tetradecane (**2 a**) with a concentration of 0.41 mM, corresponding to a yield of 41% was observed (Table 1). An increase of the cell concentration to 300 mg/mL gave no significant change in the conversion, revealing that 200 mg/mL might be a suitable cells concentration. By increasing the substrate concentrations of hexadecanedioic acid (**1 a**) to 5 mM, 10 mM, 13 mM, an almost linear increase in product formation was detected (Table 1). Tetradecane (**2 a**) was obtained with a maximum concentration of 10.28 mM. Notably, the enzyme reaction rate depended on the concentration of hexadecanedioic acid (**1 a**). However, when the substrate concentration continued increasing to 15 mM, a slight decrease in product formation was found, indicating that 13 mM was the maximum substrate concentration for LCDAs decarboxylation. Since the time course, pH and temperature profile of *Cv*FAP had been described previously for the decarboxylation of mono-fatty acids,[16] here we don't want to repeat again those studies. Besides, this study focused on the evaluation of *Cv*FAP as a photocatalyst for the decarboxylation of LCDAs, to enlarge the substrate scope of *Cv*FAP.

It is worth mentioning that background reactions were performed in parallel with experiments of *E. coli* cells containing an empty vector as catalysts, no *Cv*FAP cells, heat-denatured *Cv*FAP cells. No background reaction was taking place, indicating that the decarboxylation of hexadecanedioic acid (**1 a**) to tetradecane (**2 a**) was affected by the active *Cv*FAP enzyme. Thus, dicarboxylic acids were the suitable substrates for *Cv*FAP.

We were pleased that the desired product tetradecane (**2 a**) could be obtained with a maximum concentration of 10.28 mM when 13 mM of hexadecanedioic acid (**1 a**) was loaded. Therefore, the scalability of the developed reaction system was tested by performing a preparative-scale synthesis. The reaction was scaled up to 100 mL using hexadecanedioic acid (**1 a**, 13 mmol) to give the desired tetradecane (**2 a**, 10.5 mmol) in a yield of 81%.

Table 1. Evaluation of the potential of *Cv*FAP as a photobiocatalyst for LCDAs decarboxylation.[a]



[Substrate] [mM]	Catalyst (wet cells)	[Product] [mM][b]	Yield [%]
1	200 mg/mL	0.41	41
5	200 mg/mL	2.88	58
10	200 mg/mL	7.80	78
13	200 mg/mL	10.28	79
15	200 mg/mL	10.03	68
13	*E. coli* cells containing an empty vector	–	–
13	0	–	–
13	denatured cells	–	–

[a] Reaction conditions: [substrate] = given concentrations, [*Cv*FAP wet cells] = 200 mg/mL, Tris-HCl buffer (pH 8.5, 100 mM), 30% DMSO, blue light illumination at 30 °C for 6 hours. [b] Product concentration was determined by GC.

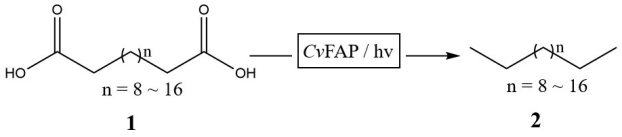
It''s known that wild-type *Cv*FAP preferentially catalyze the decarboxylation of C16~C18 mono-fatty acids, which is not satisfying.[16] The substrate specificity of *Cv*FAP for mono-fatty acids decarboxylation has been improved by protein engineering and decoy molecules. Since the substrates LCDAs in this study are different from the mono-fatty acids shown in previous study[16] in terms of sources, application, chemical/physical properties, we were interested in the scope of *Cv*FAP used as photocatalysts for decarboxylation of LCDAs to alkanes. Eight further LCDAs structurally related to the main test substrate **1 a** were tested. The results are summarized in Table 2.

Generally speaking, *Cv*FAP showed a high activity towards LCDAs of C14~C20, although there was no clear trend towards conversions and chain length. Hollmann and co-workers proposed that in *Cv*FAP-catalyzed mono-fatty acids decarboxylation, the difference in conversions of different substrates was assigned to the distance of the carboxylate group from substrate to the flavin cofactor. Mono-fatty acids with longer chain-length are more easily bound to *Cv*FAP's access channel and the reactive group (–COOH) are more easily exposure to the cofactor (to yield a $CH_3COO^{\bullet}\cdots FAD^{\bullet}$ radical pair), resulting in high conversion and initial rate. LCDAs are different from mono-fatty acids, having two reactive groups of –COOH. Whereas, taking the findings of Hollmann and co-workers and our results in this study into account, it can be proposed that dicarboxylic acids were decarboxylated by *Cv*FAP with mono-fatty acids as intermediate products. In mono-fatty acids decarboxylation, full conversion was observed for 13 mM of substrates in some cases. In LCDAs decarboxylation, 10.28 mM of the desired products were obtained from 13 mM of substrates, as one of the best examples. This phenomenon might be explained by the fact that binding groups shifted from hydrophobic group (–R) in mono-fatty acids to hydrophilic group (–COOH) in LCDAs.

Further proof of the reaction pathway of LCDAs decarboxylation was evidenced by the time course of the reaction. In order to have a clear look at how *Cv*FAP catalyze the dicarboxylic acids into alkanes, heptadecanedioic acid (**1 f**), which was readily decarboxylated by *Cv*FAP, was chosen as the represented substrate. This is to answer a question: two carboxylic acid group were decarboxylated simultaneously to yield alkanes or mono-fatty acids were formed as intermediate products. The reaction was carried out with 13 mM of heptadecanedioic acid (**1 f**) and 200 mg/mL of wet cells under blue light illumination in Tris-HCl buffer (pH 8.5, 100 mM) for the given time. As shown in Figure 1, it is clear that the substrate heptadecanedioic acid (**1 f**) decreased in concentrations readily over time. Palmitic acid (1.05 mM) and trace pentadecane were observed in the reaction mixture after incubation at 30 °C for 5 min, ruling out the simultaneous decarboxylation of the two carboxylic acid moieties, and validating our hypothesis. The amount of palmitic acid (3.30 mM) formed in the reaction system was increasing almost in a triple rate between 5 min to 15 min, with a little amount of pentadecane (**2 f**) (0.46 mM) detected. A clear decrease in the concentration of palmitic acid was observed after incubation from 30 min, while, the concentration of pentadecane (**2 f**) significantly increased at the meantime. Almost all the remaining palmitic acid were completely converted into pentadecane (**2 f**) after 3 h, resulting in a final yield of 87%.

In order to further confirm there exists such a reaction pathway, we docked the substrate (heptadecanedioic acid, **1 f**) into the crystal structure of *Cv*FAP (PDB:5NCC). The molecular docking analyses (Figure 2) clearly showed that only one carboxyl group is positioning to the active site in a correct productive conformation. Combining the results obtained in this study and mechanism of FAP-catalyzed decarboxylation

Table 2. Relative activity of *Cv*FAP towards different LCDAs.[a]



1 **2**

substrate structure	[substrate] [mM]	[product] [mM][b]	relative activity [%]
$HOOCCH_2(CH_2)_{12}CH_2COOH$ (**1 a**)	13	10.28	100
$HOOCCH_2(CH_2)_8CH_2COOH$ (**1 b**)	13	~0.5	~5
$HOOCCH_2(CH_2)_9CH_2COOH$ (**1 c**)	13	~0.5	~5
$HOOCCH_2(CH_2)_{10}CH_2COOH$ (**1 d**)	13	8.74	85
$HOOCCH_2(CH_2)_{11}CH_2COOH$ (**1 e**)	13	5.24	51
$HOOCCH_2(CH_2)_{13}CH_2COOH$ (**1 f**)	13	8.64	84
$HOOCCH_2(CH_2)_{14}CH_2COOH$ (**1 g**)	13	10.28	100
$HOOCCH_2(CH_2)_{15}CH_2COOH$ (**1 h**)	13	7.61	74
$HOOCCH_2(CH_2)_{16}CH_2COOH$ (**1 i**)	13	4.73	46

[a] Reaction conditions: [substrate] = 13 mM, [*Cv*FAP wet cells] = 200 mg/mL, Tris-HCl buffer (pH 8.5, 100 mM), 30% DMSO, blue light illumination at 30 °C for 6 hours. [b] Product concentration was determined by GC.

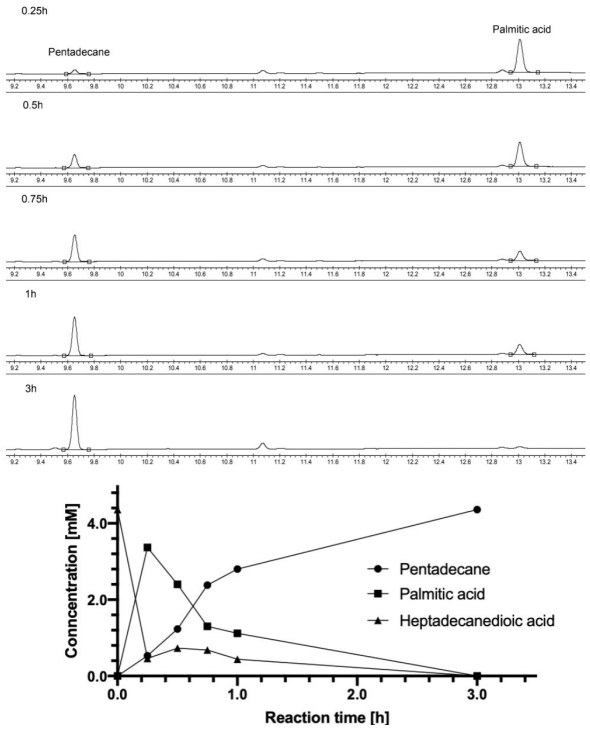


Figure 1. Photoenzymatic decarboxylation of heptadecanedioic acid (**1 f**) into pentadecane (**2 f**), forming palmitic acid as an intermediate product.

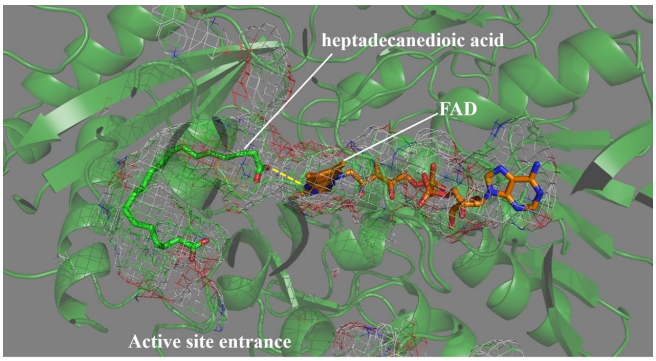


Figure 2. Docking analysis of heptadecanedioic acid (**1 f**) (in dark green) in the active site of *Cv*FAP.

available,[17,26,30] it can be noted that after binding in *Cv*FAP's access channel, heptadecanedioic acid (**1 f**) first positioned one of the carboxylic acid groups towards FAD, to form HOOC$(CH_2)_{15}COO^{\bullet}\cdots FAD^{\bullet}$ radical pair. Consequently, the flavin adduct ($HOOC(CH_2)_{15}$-FAD-) was protonated to release the intermediate palmitic acid ($HOOC(CH_2)_{14}CH_3$). The intermediate product palmitic acid ($HOOC(CH_2)_{14}CH_3$) was ready to enter the next catalytic cycle, which was ended by the final release of alkane product (**2 f**).

The enzymatic conversion of LCDAs to alkanes was established to provide evidence for the recycling of aliphatic polyesters polymers. Thus, the process must be as much efficient as possible, which mostly depended on the enzymes. An enzyme's operational stability and recyclability over a long period of time are considered as the most important characteristics for practical applications.[31,32] The higher stability and recyclability that an enzyme exhibits, the more efficiently a process can be run. Therefore, the thermostability, storage stability and recyclability of *Cv*FAP for the decarboxylation of LCDAs were evaluated.

Experiments were performed to examine the recyclability of *Cv*FAP for the decarboxylation of hexadecanedioic acid (**1 a**) as an example. For the results summarized in Figure 3, every reaction was carried out in 1 mL of Tris-HCl buffer (100 mM, pH 8.5) containing 30% DMSO, 0.2 g of wet cells, 13 mM substrate and shaken at 30 °C under blue light illumination for 6 h. At the end of the reaction, the cells were centrifuged, washed twice with Tris-HCl buffer (100 mM, pH 8.5) and reused for the next cycle under the same reaction setup. Whole cells of overexpressed *E. coli* (*Cv*FAP@*E.coli*) showed no significant changes in activities at the first 3 cycles. From the fourth cycle, enzyme activities started decreasing in a moderate rate, whereas, the cells retained 20% of the initial activity after 9 consecutive cycles. Such rapid deactivation was most likely owing to the massive losses of *Cv*FAP@*E.coli* cells during washing step. Only 50 mg cell were remaining in cycle 9. Immobilization can strongly enhance the stability of *Cv*FAP@*E.coli* cells, and make the catalyst be filtered off easily without washing step, which is currently being implemented in our laboratory.

The thermostability of whole cells of *Cv*FAP@*E.coli* was characterized as well. The cells were first incubated at various temperatures (30–70 °C) for 1 h, respectively, prior to the addition to the reaction mixture. Then, the cells were resuspended to 1 mL Tris-HCl buffer (100 mM, pH 8.5) containing 30% DMSO, 13 mM hexadecanedioic acid (**1 a**). The reaction mixture was shaken at 30 °C under blue light illumination for 6 h. As shown in Figure 4, the cells of *Cv*FAP@*E.coli* maintained approximately 99% of their original activities after 1 h incubation at 30 °C or 40 °C. However, when the temperature was higher than 40 °C, a significant decrease was observed in the activity of the cells over a 1 h period. This is supported by the findings of Beisson and co-workers, who showed that the activity of recombinant *Cv*FAP protein decreased sharply when temperature rose above 35 °C.[13] Methods to enhance the thermostability of *Cv*FAP@*E.coli* cells is of great significance, which is also under currently under way in our laboratory.

To investigate the storage stability of *Cv*FAP, the cells of *Cv*FAP@*E.coli* were stored at room temperature (25 °C) and tested for the activity in decarboxylation of hexadecanedioic acid (**1 a**). Cells were withdrawn at various time intervals (1, 2, 3, 4, 5, 6, 7, 14 days) and added to 1 mL Tris-HCl buffer (100 mM, pH 8.5) containing 30% DMSO, and 13 mM hexadecanedioic

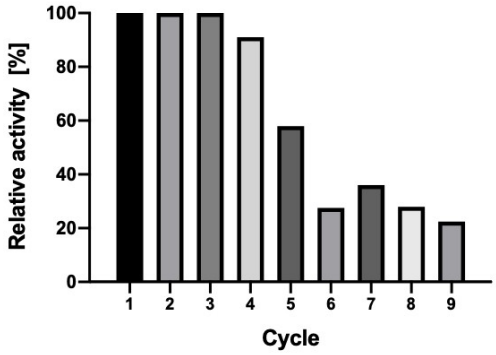


Figure 3. Repeated decarboxylation of hexadecanedioic acid (**1 a**) to tetradecane (**2 a**) under blue light illumination by cells of *Cv*FAP@*E.coli.* Relative activities were determined by GC.

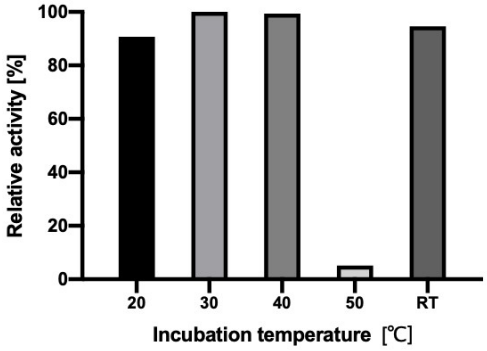


Figure 4. Thermostability of *Cv*FAP@*E.coli* cells for the decarboxylation of hexadecanedioic acid (**1 a**) to tetradecane (**2 a**) under blue light illumination. Cells were incubated at various temperatures for 1 h before substrate was added to initiate reactions.

acid (**1a**) to initiate the reaction. The reaction of the mixture was then shaken at 30 °C under blue light illumination for 6 h. Gratifyingly, in each case, the cells maintained almost 100% of the initial activities. That is, the *Cv*FAP@*E.coli* cells were stable at room temperature (25 °C) within the measured periods (2 weeks) without any activity loss (data not shown).

3. Conclusion

The annul global production of plastics has exceeded 359 million tons; 90% of these are derived from fossil feedstock, while, only ~14% are collected for recycling. This causes a huge waste of fossil resources. Besides, most of the plastics are remarkably persistent in the environment, thus becoming a critical environment threat to the ecological systems. Especially, the negative, hazardous effects of microplastics have induced increasing concerns since they can enter the food chain and impose serious problems. Degradable plastics from renewable resources is one of the most potential alternatives. Nevertheless, there are very few reports on the synthesis of degradable plastics from bio-based materials. Having both degradable and sustainable properties in one type of plastics is still challenging. For example, one of the most competitive biodegradable polymers commercialized up to now is aliphatic polyesters, while, their monomers (LCDAs) are mainly produced from petrochemical alkanes by fermentation with *C. tropicalis* in China so far.

To date, numerous microorganisms and/or enzymes have been identified to hydrolyzing aliphatic polyesters plastics into monomers, LCDAs and diols. The development indeed could provide a viable bioremediation strategy to recycle and reuse plastic waste, however, the loop of the circular plastics economy is not yet closing. If the starting material (petrochemical alkanes) could be isolated and reused, this could significantly reduce the consumption of petrol-based feedstock, thereby contributing towards the concept of a circular degradable plastics economy. To isolate alkanes from monomer (LCDAs), the key step involves the decarboxylation of LCDAs, which still represents a chemically very challenging reaction.

Thus, a straightforward light-driven route for the decarboxylation of LCDAs to alkanes was established, employing recombinant cells of a recently discovered photodecarboxylase from *Chlorella variabillis* (*Cv*FAP@*E.coli*). *Cv*FAP readily decarboxylated a series of LCDAs with a concentration of 13 mM into the corresponding (C2-shortened) alkanes in good yields under blue light illumination. The reaction was scaled up to a 100 mL scale to determine the scalability of the light-driven enzymatic decarboxylation of hexadecanedioic acid with *Cv*FAP@*E.coli*, and 10.5 mM of the desired product tetradecane were achieved after 24 h. Our study suggested that mono-fatty acids were formed as an intermediate product before the final release of C2-shortened alkanes. Thermostabilities studies showed that *Cv*FAP@*E.coli* cells maintained 99% of the original activity during 1 h of incubation at 40 °C, while lost almost all activities when temperature rose above 50 °C. *Cv*FAP@*E.coli* cells retained 99% of the initial activity after storage at room temperature (~ 25 °C) for 14 days. Batch experiments showed that *Cv*FAP@*E.coli* cells could be reused for 3 cycles without significant loss of activity. After 9 consecutive cycles, the cells retained 22% of the initial activity. In summary, the newly discovered photoenzyme *Cv*FAP was able to catalyze the decarboxylation of LCDAs to the corresponding (C2-shortened) alkanes under mild conditions. This approach will hopefully provide concepts and solutions in reducing the need of petrol-based materials for the production of aliphatic polyesters, making degradable and sustainable plastics possible.

Experimental Section

Materials and Methods

Vector pET28a(+) was purchased from Novagen (Merck Millipore, Amsterdam, The Netherlands). All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany) and were used without further purification unless otherwise specified. The culture media components were obtained from BD (Becton, Dickinson and Company, Breda, The Netherlands).

Conversion of substrates and yield of products were quantified by GC using calibration lines. GC analysis of alkanes was followed with a Scion GC 456 system equipped with an Agilent J&W GC Columns (60 m × 0.53 mm × 2.5 μm) using N_2 as the carrier gas. The following conditions were used for the dicarboxylic acids/alkanes separation: injector 260 °C, detector (FID) 280 °C, FID hydrogen 30 oxygen 300, column flow 20 mL/min, maximum temp: 255 °C, temperature program: start 110 °C, hold time 2 min, rate 25 °C/min to 190 °C hold time 2 min, rate 30 °C/min to 280 °C hold time 5 min, rate 30 °C/min to 310 °C hold time 2 min. The retention time was shown as below: *n*-tetradecane (**2a**) = 8.78 min, *n*-decane (**2b**) = 4.86 min, *n*-undecane (**2c**) = 5.85 min, *n*-dodecane (**2d**) = 6.85 min, *n*-tridecane (**2e**) = 7.86 min, *n*-pentadecane (**2f**) = 9.65 min, *n*- cetane (**2g**) = 10.45 min, *n*-heptadecane (**2h**) = 11.24 min, *n*- octadecane (**2i**) = 11.97 min.

Heterologous Expression

The recombinant plasmids were received from the laboratory of Prof. Frank Hollmann as a gift.[22] The recombinant plasmids were subsequently transformed into *E. coli* BL21 (DE3) cells. Expression was performed in LB medium containing 50 μg/mL kanamycin at 30 °C. When OD_{600} reached 0.5–0.6, the production of the recombinant *Cv*FAP was induced by addition of isopropyl thio-β-D-galactoside (IPTG) to a final concentration of 0.1 mM. For the determination of the optimal expression conditions, cultures were grown after induction between 17 and 30 °C, and assayed after a period of 2 days. *E. coli* pET28a(+) empty was cultivated and induced with the same system as control experiment. Cells were harvested by centrifugation (11000 *g*, 10 min, 4 °C) and washed two times with Tris-HCl buffer (pH 8.0, 50 mM, NaCl, 100 mM). Harvested cells were stored at −80 °C. When needed, the wet pellets were freeze dried overnight and collected as lyophilized cells.

General Procedure for Decarboxylation

Reactions were carried out in 5 mL screw-capped glass vials to prevent evaporation of substrate/product. The blue light-driven *Cv*FAP-catalyzed decarboxylation reaction was carried out in a total volume of 1.0 mL of Tris-HCl buffer (pH 8.5, 100 mM) containing 30% DMSO as a co-solvent at 30 °C for 6 h. The system contains 200 μL 65.5 mM dicarboxylic acids (DMSO as solvent), 100 μL pure

DMSO, 200 mg whole-cell *Cv*FAP@*E.coli* mixed in 700 μL Tris-HCl buffer (pH 8.5, 100 mM). The transparent glass bottle was sealed and exposed to a blue LED light under slight magnetic stirring. The final conditions of the reaction are: [dicarboxylic acids] = 13.1 mM, [*Cv*FAP] = 0.2 g/mL in Tris-HCl buffer (pH 8.5, 100 mM), 30% DMSO. For the blank reaction the setup was the same but heat denaturated cells (90 °C, 30 min) were used. For work-up, the cells were removed by centrifugation and 1 mL of the supernatant was saturated with NaCl followed by extraction with 0.5 mL (×2) of ethyl acetate by shaking for 5 min. The combined organic layer was dried over Na_2SO_4 and measured with GC for yield.

Evaluation of the Potential of *Cv*FAP as a Photobiocatalyst for Hexadecanedioic Acid (1 a) Decarboxylation

Samples for the evaluation of the potential of *Cv*FAP as a photobiocatalyst for hexadecanedioic acid decarboxylation were prepared and analysed as described above in General procedure for decarboxylation. Reactions were performed using hexadecanedioic acid (**1 a**) as a substrate with various concentrations ranging from 1 to 15 mM.

General Procedure for Substrate Screening

Reactions were carried out as described in the General procedure for decarboxylation using the same concentration (13 mM) for each substrate. After extraction with ethyl acetate (2×0.5 mL) samples were dried over Na_2SO_4 and crude samples were analysed by GC for yield determination.

Scale up Experiment

The experiment of decarboxylation of long chain dicarboxylic acids to alkanes was scaled up from 1 mL to 100 mL. In this reaction, 20 mL of 65.5 mM DMSO containing hexadecanedioic acid (**1 a**), 10 ml of pure DMSO, and 70 mL of Tris-HCl buffer (Ph 8.5, 100 mM) containing 10.0 g of wet whole-cell *Cv*FAP were added to the transparent glass container. The container was added to the stir bar and sealed, stirred gently at 30 °C, under blue light illumination overnight (24 h). The final reaction conditions of this reaction were: [hexadecanedioic acid] = 13.1 mM, [wet whole-cell *Cv*FAP] = 0.1 g/mL, 30 °C. The workup was the same as described above for the General procedure for decarboxylation.

Time Study to Indicate the Presence of Mono-Acids

Samples for the time study were prepared and analysed as described above in the General procedure for decarboxylation. Sample was taken from the reaction mixture after given times and measured by GC after proper workup.

Docking of Heptadecanedioic Acid (1 f)

Molecular docking analyses were performed employing AutoDock Vina algorithm.[33] The crystal structure of CvFAP (PDB:5NCC) was used as rigid receptor. Ligand structures were prepared using AutoDock Tools setting a free torsions for all the C–C bonds.

General Procedure for Recyclability

Reactions were carried out with substrate **1 a** (13 mM) in 1 mL of Tris-HCl buffer (pH 8.5, 100 mM) containing 30% DMSO, and 200 mg/mL of *Cv*FAP@*E.coli* cells, shaken at 30 °C under blue light illumination for 6 h. At the end of the reaction, cells were centrifuged at 4 °C, 13000 *g* for 10 min to be separated from the reaction mixture, then washed twice by Tris-HCl buffer (pH 8.5, 100 mM), and resuspended in 1 mL of the same buffer containing the same substrates. The reaction mixture (1 mL of supernatant separated from cells) was saturated with NaCl and then extracted with 2×0.5 mL of ethyl acetate by shaking for 5 min. The combined organic phases were dried over Na_2SO_4 and crude samples were analysed by GC.

Thermostability

*Cv*FAP@*E.coli* cells were first incubated at various temperatures (20–50 °C) for 1 h, prior to the addition to the reaction mixture. Then, the cells (200 mg) were resuspended to 1 mL Tris-HCl buffer (pH 8.5, 100 mM) containing 30% DMSO and 13 mM hexadecanedioic acid (**1 a**). The reaction mixture was shaken at 30 °C under blue light illumination for 6 h. Work-up and analysis were performed as described above.

Storage stability

*Cv*FAP@*E.coli* cells were stored at room temperature (~25 °C) for given periods and tested for the activity in decarboxylation of hexadecanedioic acid (**1 a**). 200 mg cells were withdrawn at various time intervals (1, 2, 3, 4, 5, 6, 7, 14 days) and added to 1 mL Tris-HCl buffer (pH 8.5, 100 mM) containing 30% DMSO and 13 mM hexadecanedioic acid (**1 a**). The reaction mixture was shaken at 30 °C under blue light illumination for 6 h. Work-up and analysis were performed as described above.

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Conflict of Interest

The authors declare no conflict of interest.

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