A Journal of the Gesellschaft Deutscher Chemiker

Angewandte

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Authors: Sofiene Abdellaoui, Florika Macazo, Rong Cai, Antonio de Lacey, Marcos Pita, and Shelley Minteer

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201712890
Angew. Chem. 10.1002/ange.201712890

Link to VoR: http://dx.doi.org/10.1002/anie.201712890
http://dx.doi.org/10.1002/ange.201712890

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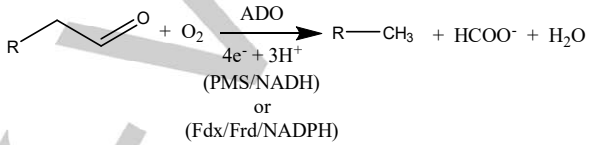
COMMUNICATION

Enzymatic Electrosynthesis of Alkanes via Bioelectrocatalytic Decarbonylation of Fatty Aldehydes

Sofiene Abdellaoui,[a] Florika C. Macazo,[a] Rong Cai,[a] Antonio L. De Lacey,[b] Marcos Pita,[b] and Shelley D. Minteer*[a]

Abstract: An enzymatic electrosynthesis system is created by combining an aldehyde deformylating oxygenase (ADO) from cyanobacteria catalyzing the decarbonylation of fatty aldehydes to alkanes plus formic acid with an electrochemical interface. This system is able to produce a range of alkanes (octane to propane) from aldehydes and alcohols. The combination of this bioelectrochemical system with a hydrogenase bioanode yields a H_2/heptanal enzymatic fuel cell (EFC) able to simultaneously generate electrical energy with a maximum current density of 25 μA cm^{-2} at 0.6 V while producing hexane with a faradaic efficiency of 24 %.

Renewable biofuels are alternatives to petroleum-based fuels. However, the large-scale production of biofuels involves a massive production of food crops requiring the utilization of large quantities of water, fertilizer, and land.[1] As an alternative, researchers have tried to exploit fatty acid catabolism pathways of eukaryotic and prokaryotic organisms to biochemically synthesize hydrocarbons – the major constituents of gasoline, diesel, and other fuels.[2] Cyanobacteria, having characteristics of both plants and prokaryotes and being easy to genetically manipulate, offer the most advantage for this purpose, especially for industrial applications.[3] Two key enzymes involved in cyanobacteria-mediated hydrocarbon biosynthesis include an acyl carrier protein reductase (AAR), which catalyzes the reduction of fatty acyl-ACP (acyl carrier protein) to fatty aldehydes, and an aldehyde decarbonylase (AD), which catalyzes the decarbonylation of fatty aldehydes to alkanes and formic acid.[4] However, the main drawbacks of microbial production of "bio-alkanes" involve complex and costly extraction/purification techniques, as well as production of side/end products that can be toxic to the cells. Therefore, an *in vitro* system working with isolated enzymes, such as ADs, could overcome these problems. ADs belong to the ferritin-like diiron-carboxylate oxygenase and oxidase (FDCOO) protein structural superfamily.[5] In cyanobacteria, AD is a soluble non-heme di-iron protein catalyzing the production of formic acid and alkane from aldehyde (Scheme 1).[4a, 6] This enzyme was recently designated as an aldehyde-deformylating oxygenase (ADO), since it has been shown that it requires oxygen to catalyze a cryptic redox oxygenation reaction to form alkanes and formic acid from a broad range of aldehydes (heptadecanal to butanal).[7] Like most of the FDCOOs, the ADOs require an external auxiliary reducing system to supply four electrons per catalytic cycle, such as NADPH combined with ferredoxin (Fdx) and a ferredoxin reductase (Fdr).[8] Artificial chemical reductants can also be used to supply electrons to ADOs such as the redox couple NADH/PMS (phenazine methosulfate).[6]



Scheme 1. *ADO-mediated enzymatic conversion of aldehyde to alkanes and formic acid.*

Numerous oxidoreductases have been interfaced with an electrode to achieve a bioelectrocatalytic reaction in the context of biosensors and enzymatic biofuel cells (EFC).[9] The heterogeneous electron transfer between redox enzymes and electrodes can take place either directly or *via* mediated electron transfer (MET) using redox mediators. Enzyme-modified electrodes were recently used for enzymatic electrosynthesis of several molecular targets, whereby the high chemo-, regio- and stereoselectivity of enzymes are used to circumvent the necessity for harsh reaction conditions in traditional chemical synthesis.[10] Milton et al. have demonstrated the coupling of a hydrogenase bioanode with a nitrogenase biocathode resulting in EFC able to simultaneously produce electrical energy and NH_3.[11]

Herein, we demonstrate the use of toluidine blue O (TBO) as a redox mediator to achieve the bioelectrocatalytic decarbonylation of an aldehyde to an alkane using the recombinant ADO from *Prochlorococcus marinus* (PmADO). We employ TBO to mediate the electron transfer between a carbon electrode and the active site of PmADO to produce alkanes ranging from octane (C8) to propane (C3). In addition, we combine PmADO with a NAD-dependent alcohol dehydrogenase to achieve an enzymatic cascade reaction yielding alkanes from a range of short chain fatty alcohols. Finally, we construct a H_2/heptanal EFC employing a hydrogenase at the anode and PmADO at the cathode, which is able to simultaneously generate electrical energy and hexane as the final product.

It was shown that ADOs require four electrons per catalytic cycle to reduce the cofactor $Fe_2^{III/III}$ form to the O_2-reactive active form (μ-peroxo-$Fe_2^{II/II}$), which result in the reductive cleavage of an intermediate $Fe_2^{II/II}$-peroxyahemiacetal formed with substrates. [7, 12] In order to achieve bioelectrocatalysis with PmADO by MET on carbon electrodes, we chose TBO as a cofactor regeneration mediator, since it can easily be chemically reduced by NADH and

[a] Department of Chemistry, University of Utah, 315 S 1400 E, Salt Lake City, UT, 84112 (USA)
E-mail: minteer@chem.utah.edu
[b] Instituto de Catalisis y Petroleoquimica, CSIC, C/ Marie Curie 2, L10, 28049, Madrid (Spain)

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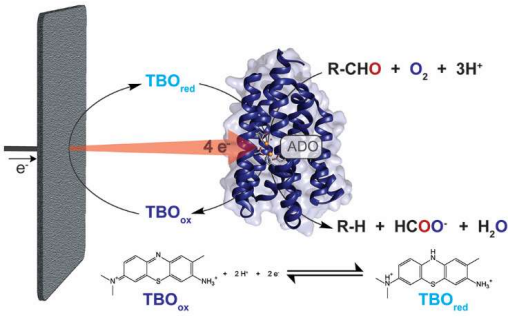
electrochemically reduced/oxidized at the electrode surface.[13] For this prospect, we initially evaluated the activity of PmADO using the redox couple TBO/NADH with a range of different aldehyde substrates. The turnover number (k_{cat}) for each substrate was calculated by detecting and quantifying the alkanes formed using gas chromatography (GC) coupled with a flame ionization detector (FID) (Table 1). The calculated values indicate that PmADO has slow turnover with the highest activity observed with heptanal. These values are close to the k_{cat} values reported for ADO/PMS-mediated assays.[14] Khara and Al reported a k_{cat} value of 0.0031 min^{-1} for the decarbonylation of butanal to propane by PmADO using PMS/NADH, which is close to the value we obtained with TBO (0.0024±0.0004 min^{-1}).[14] Furthermore, the k_{cat} for heptanal (1.31±0.17 min^{-1}) is close to the highest value described in the literature (~ 1 min^{-1}).[7]

Table 1. Comparison of PmADO k_{cat} with NADH/TBO.

Substrate[a]	k_{cat} (min^{-1})
Nonanal	0.13 ± 0.03
Octanal	0.50 ± 0.02
Heptanal	1.31 ± 0.17
Hexanal	0.47 ± 0.03
Pentanal	0.0029 ± 0.0003
Butanal	0.0024 ± 0.0004

A carbon electrode was then used to build a biocathode with PmADO and TBO as a redox mediator (Scheme 2). This system was investigated via cyclic voltammetry (CV) using heptanal substrate. Since TBO is sensitive to O_2, all measurements were carried out in a sealed system prepared under argon atmosphere. Of note, the stock solution of heptanal was prepared under aerobic conditions in order to introduce some O_2 required for the enzymatic reaction. The voltammogram in Figure 1 reveals the reversible redox peaks of TBO (black solid curves), with a reduction and oxidation peaks at -0.26 V and -0.22 V vs. SCE, respectively. To demonstrate enzymatic activity, 10 mM heptanal was added and a bioelectrocatalytic wave was observed, indicating that electrocatalytic reduction of heptanal has occurred. A control using denatured PmADO shows no catalytic signal in the presence of heptanal. Moreover, new redox peaks can be seen at ~ -0.06 V vs. SCE after the addition of heptanal in the presence of the native or denaturated enzyme, suggesting that heptanal is potentially affecting the redox properties of TBO. This could be attributed to the hydrophobicity of the substrate that induces the formation of TBO aggregates in the bulk.[15]
To illustrate substrate concentration dependence, we carried out a series of CVs in the presence of increasing concentrations of heptanal to 10 mM. The catalytic current densities at -0.50 V vs. SCE were then extracted and plotted against the concentration of heptanal (Figure 2). It is evident from Figure 2 that the current density, and hence, the enzymatic electrocatalytic activity, increases as the concentration of heptanal increases. After 5 mM heptanal, the signal decrease due to low solubility and possible inhibition processes. These results demonstrate that a combination of PmADO, TBO and a carbon electrode can constitute a system that is able to bioelectrocatalyze the decarbonylation of aldehydes to alkanes.



Scheme 2. *Schematic of the PmADO biocathode.*

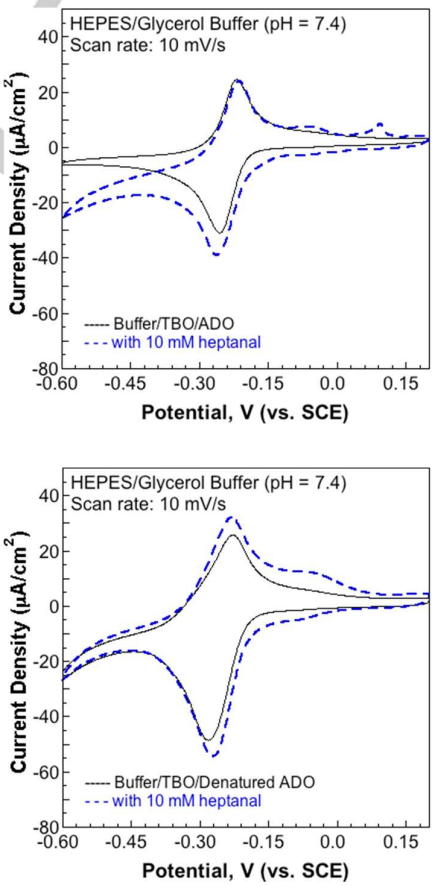


***Figure 1**. Catalytic reduction of heptanal by TBO-mediated PmADO (scan rate: 10 mV/s^{-1}). (top) Catalytic CV's obtained for a Toray electrode immersed in buffer/TBO/PmADO solution (black solid curve) and in the presence of 10 mM heptanal (blue dashed curve). (bottom) CV obtained in a solution containing buffer/TBO/Denatured PmADO (black solid curve) and in the presence of 10 mM heptanal (blue dashed curve).*

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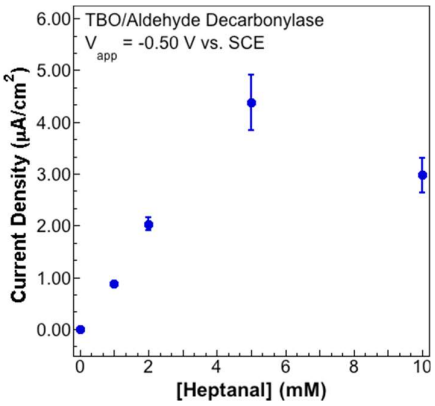


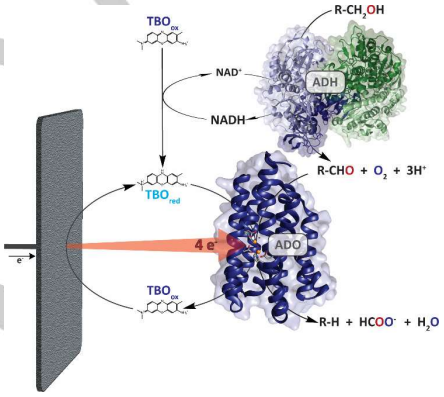
Figure 2. *Heptanal-induced changes in the catalytic reduction current by TBO-mediated PmADO with a -0.5V applied potential.*

Since we have already established the bioelectrocatalytic activity of PmADO, we then sought to demonstrate its ability as an enzymatic electrosynthesis system to produce a range of alkanes from octane to propane. For that, bulk electrolysis in the presence and absence of PmADO was performed at -0.5 V vs SCE using six aldehydes shown in Table 1. These experiments were carried out in sealed vials prepared anaerobically (O_2 < 1 ppm) (Figure S1) and the electrosynthesis was initiated by the addition of substrates and 10 mL of air to introduce a small amount of O_2 required for the reaction. After an hour of electrolysis, alkanes were detected and quantified by GC/FID (chromatograms shown in Figure S2). The quantities of alkanes and the faradaic efficiencies were calculated and summarized in Table 2. The faradaic efficiencies are generally low, with the highest value obtained for the conversion of octanal to heptane (13.02 %). This can be attributed to the electro-reduction of O_2 competing with the electro-decarbonylation of the aldehydes understudy (Figure S3).

The PmADO-modified electrode was then combined with a NAD-dependent alcohol dehydrogenase (ADH) to achieve an enzymatic cascade reaction sequentially converting fatty alcohols to aldehydes by enzymatic oxidation, and aldehydes to alkanes by bioelectrocatalytic decarbonylation (Scheme 3). We aimed to produce octane to propane from fatty alcohols, which are more sustainable than aldehydes for practical applications. Prior to testing this enzymatic cascade reaction, the ADH activity with these alcohols was evaluated by UV-Vis (Figure S4.). The results show significant activities towards butanol, pentanol, hexanol and heptanol and low activities with octanol and nonanol. These alcohols were then used as substrates for the enzymatic electrosynthesis of alkanes monitored by GC/FID (Figure S5). After electrolysis, the alkanes produced were detected and quantified (Table 3). The slow conversions of nonanol and octanol to octane and heptane, respectively, are due to the low activity of ADH towards those substrates, while the slow production of propane (0.003mg$L^{-1}h^{-1}$) is due to the low activity of PmADO with butanal. However, the production of hexane from heptanol is 50 to 100 times higher, with a rate of 1.353mg$L^{-1}h^{-1}$. This burst of production rate could be explained by the combination of the high activities of PmADO with heptanal and ADH with heptanol. The combination of ADO with other enzymes, such as an acyl-CoA synthase and acyl carrier protein reductase, which catalyze the reduction of fatty acyl-CoA to aldehydes, will allow for the production of alkanes from fatty acids.

Table 2. Quantification of alkanes and faradaic efficiencies obtained from the enzymatic electrosynthesis of alkanes from aldehydes.

Product	Quantity (nmol h^{-1})	Faradaic efficiency (%)
Octane	1.966	2.18
Heptane	8.666	13.02
Hexane	29.570	7.32
Pentane	6.549	4.28
Butane	3.026	0.46
Propane	0.6289	1.16



Scheme 3: Illustration of the bioelectrochemical system combining PmADO and ADH.

Table 3. Rate of production of alkanes from alcohols by enzymatic electrosynthesis.

Substrate → Product	mg L^{-1} h^{-1}
Nonanol → Octane	0.017
Octanol → Heptane	0.024
Heptanol → Hexane	1.353
Hexanol → Pentane	0.014
Pentanol → Butane	0.020
Butanol → Propane	0.003

Finally, a H_2/heptanal EFC resulting from the combination of a hydrogenase bioanode with a PmADO biocathode was evaluated. The Ni-Fe hydrogenase (from *Desulfovibrio gigas*)[16] at the anode facilitates the electro-oxidation of H_2 to H^+ using methyl viologen ($MV^{2+}/MV^{\cdot+}$) as a redox mediator and subsequently supplies electrons for the bioelectrocatalytic decarbonylation of heptanal to hexane (Figure 3). This EFC operating with H_2 as a fuel and heptanal as an oxidant yielded an OCP of 597±5 mV and generated a maximum current density (J_{max}) and power density

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(P_{max}) of 25±3 μA cm^{-2} and 4.7±0.5 μ cm^{-2}, respectively. In order to probe the effect of O_2 reduction, a control was performed without PmADO in the presence of O_2 and heptanal, which yielded a J_{max} of 13±2 μAcm^{-2} and a P_{max} of 2.9±0.1 μWcm^{-2}. These results show that the reduction of oxygen itself contributes to about half of the current and power density obtained for the H_2/heptanal EFC. A J_{max} of 13±3 μAcm^{-2} and a P_{max} of 3.6±0.2 μW cm^{-2} were obtained with a second control carried out in the absence of oxygen and in the presence of PmADO and heptanal. It was shown previously that PmADO are also able to achieve a slow decarbonylation of aldehyde in the absence of O_2 through an oxygen-independent hydrolytic mechanism,[6, 17] thus, the signal obtained with the second control could be induced by this side enzymatic reaction. Finally, hexane produced from EFCs was quantified after the passage of 40 mC, yielding 22±1 nmol of hexane with a faradaic efficiency of 24±4%. These results demonstrate that the H_2/heptanal EFC is able to simultaneously generate electrical energy and produce hexane from heptanal.

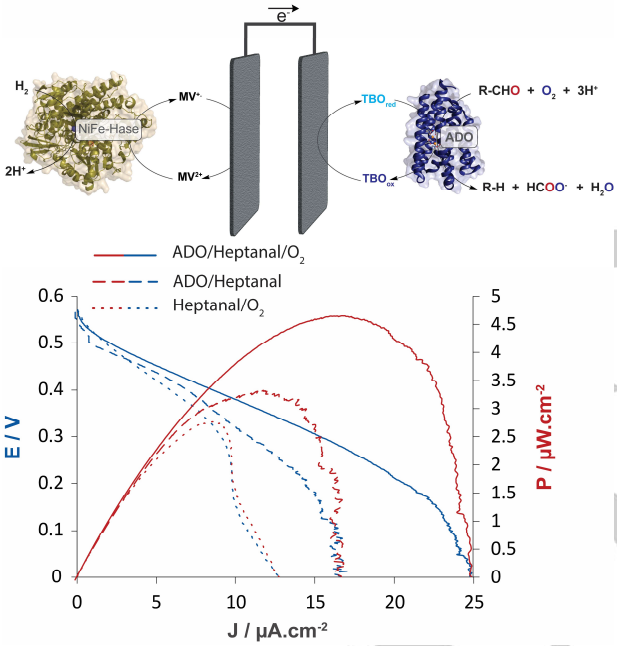

Figure 3: *Representative power (red lines) and polarization curves (blue lines) for H_2/heptanal fuel cells (solid lines); a control fuel cell without ADO in the presence of O_2 and heptanal (dotted lines), and a control with ADO in the presence of heptanal without O_2 (dashed lines). The measurements were performed by linear sweep polarization at 0.2 mV s^{-1} with vigorous stirring.*

Acknowledgements

The authors would like to thank the Army Research Office MURI grant (#W911NF1410263) and the Spanish Economy Ministry (MINECO/FEDER CTQ2015-71290-R grant) for funding this work.

Keywords: bioelectrochemical catalysis, aldehyde-deformylating oxygenase, alkane, enzymatic electrosynthesis

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10.1002/anie.201712890

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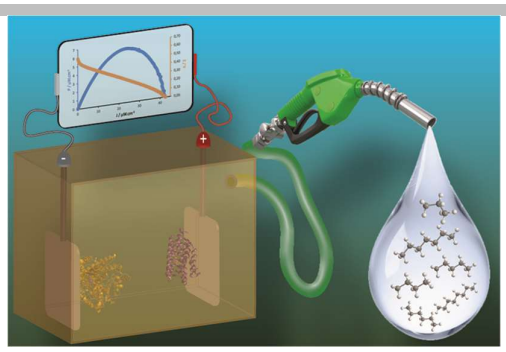
An H_2/aldehydes enzymatic fuel cell (EFC) able to simultaneously generate electrical energy and produce alkanes



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[a] Department of Chemistry, University of Utah, 315 S 1400 E, Salt Lake City, UT, 84112 (USA)