Biosynthesis of hydrocarbons in microalgae

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Research objectives

• Study mechanisms of solar energy conversion and storage by photosynthetic micro-organisms (microalgae, cyanobacteria)

• Explore the potential of these organisms for the production of energy-rich (hydrogen, starch, lipids) or high-value compounds

Héliobiotec plateform

- Gas ($H_2$, $O_2$, $CO_2$) and isotope ($D$, $^{18}O$, $^{13}C$) exchange measurements by mass spectrometry

- Instrumented 1L photobioreactors to evaluate strain performances

- Controled chambers for algal cultivation

- Screening of strains (flow cytometer, GC-MS/FID)

- Lipidomics (LC-MS/MS)
The microalgal lipid factory

Water
Nutrients

Membrane lipids

CO₂

Fatty Acid Synthase

Calvin cycle

Fatty Acids

Storage lipids (oil)

Chloroplast

Microalgal cell

Linear hydrocarbons
(n-alkanes/n-alkenes)
Alkanes are major components of fossil fuels

- Kerosene: C10-C13
- Diesel: C12-C20
- Petrol (gasoline): C5-C10

Cetane (n-hexadecane)

Iso-octane (2,2,4-trimethyl-pentane)
Alkenes are used for a variety of chemical syntheses

- Ethers: ROH, H₂SO₄
  - ROH, Hg(OAc)₂, then NaBH₄ (oxymercuration)
- BH₃ then H₂O₂, NaOH ("Hydroboration")
- H₂O, H₂SO₄ ("Hydration")
- H₂O, Hg(OAc)₂, then NaBH₄ ("Oxymercuration")

- Carboxyls via oxidative cleavage (ketones, aldehydes, carboxylic acids)
  - e.g. O₃, then Zn, H⁺ ("Ozonolysis")
  - product(s) depend on alkene and on reaction conditions

- Alkyl Halides
  - e.g. H=Br
  - [also works with H–Cl, H–I]
  - also: H–Br, ROOR

- Alkanes
  - Pd–C, H₂ ("Hydrogenation")

- Vicinal Diols
  - OsO₄ ("Dihydroxylation")

- Cyclopropanes
  - Zn–Cu, CH₂I₂
  - CHCl₃, KOH ("Dichlorocyclopropanation")

- Epoxides
  - RCO₂H (e.g. mCPBA) ("Epoxidation")

- Halohydrins
  - e.g. Cl₂, H₂O
  - [also works with Br₂, I₂, NCS/NBS/NIS]
Synthesis and excretion of hydrocarbons would bypass costly steps

From algal biomass to biofuels and oleochemicals

1. Algal Strain Selection
2. Strain Cultivation
3. Biomass Harvest
4. Lipid Extraction
5. Chemical Processing
6. Ready to use molecule

Hydrocarbon Synthesis and Excretion

Adapted from Delrue et al. Bioresource Technol. 2013
Alka(e)ne-forming enzymes have been identified in plants, cyanobacteria and insects

Fatty Acids

\[
\text{HO} \overset{\text{Reductase}}{\rightarrow} \text{Reductase} \overset{\text{« Decarbonylase »}}{\rightarrow} \text{Linear hydrocarbons} \quad (n\text{-alkanes}/n\text{-alkenes})
\]

Issues:

Low efficiency of « decarbonylase »
Inhibition by co-products
Fatty aldehyde intermediate
Synthesis of hydrocarbons by microalgae has been mostly studied in *Botryococcus braunii*

- Botryococcenes
- Fatty acid-derived: Alkadienes and Alkatrienes
1) Do other microalgae synthesize alka(e)nes?

2) What is the alka(e)ne biosynthetic enzyme(s)?
Chlamydomonas and Chlorella synthesize alka(e)nes

(Sorigué et al., Plant Physiology 2016)
Long chain alka(e)nes in microalgae: a summary

- Alka(e)nes \((C_{15}-C_{17})\) are found in both green and red lineages
- These alka(e)nes are produced from fatty acids
- Amount to a few % of total fatty acids
- Major product is 7-heptadecene deriving from \(cis\)-vaccenic acid
- Synthesis is strictly dependent on light
- In Chlamydomonas and Chlorella genomes: no homologs to known alka(e)ne-forming enzymes

(Sorigué et al., Plant Physiology 2016)

- There must be a novel alkane synthase in these microalgae
Purification of the alkane-synthesizing activity

- Solubilization of activity from the microsomal fraction using detergents
- Partial purification of activity

(Activity assay: $D_{31}$-labeled palmitate, SPME and GC-MS)

SDS-PAGE
A candidate for the alkane synthase was identified by proteomics

Number of proteins detected in each of 3 partial purifications (A,B,C)

- **A**: 927 proteins detected
- **B**: 48 proteins detected
- **C**: 80 proteins detected

Venn diagram showing the overlap:
- **Intersection A & B**: 10 proteins detected
- **Intersection B & C**: 18 proteins detected
- **Intersection A & C**: 5 proteins detected
- **Unique A**: 917 proteins detected
- **Unique B**: 48 proteins detected
- **Unique C**: 75 proteins detected

List of detected proteins:
- **Putative oxidase**
- Ferredoxin--NADP reductase
- Heat shock protein 70
- ATP synthase subunit alpha
- Glycerol-3-phosphate dehydrogenase
- Malate synthase
- Carbohydrate kinase
- Transketolase
- Putative glycolate dehydrogenase
- Isocitrate lyase
Expression of the Chlorella putative oxidase in E. coli results in alkane production

Sorigué et al. « New fatty acid decarboxylase and its uses » (EP16305583, May 2016)
The purified recombinant alkane synthase acts on C12-C22 free fatty acids

C18 unsaturated fatty acids are also substrates
The co-product of the reaction is $CO_2$ (i.e. the alkane synthase is a fatty acid decarboxylase)

Purified recombinant Chlorella alkane synthase incubated with $^{13}C$-labeled palmitate

Detection of $CO_2$ by GC-MS
The Chlorella alkane synthase is a photoenzyme

- Purified Chlorella alkane synthase incubated with $1^{-13}C$ palmitate
- Detection of $^{13}CO_2$ by membrane inlet mass spectrometry (MIMS)

(Sorigué et al., unpublished)
Crystal structure of the Chlorella alkane synthase

Data: P. Arnoux and D. Pignol, CEA Cadarache
Conclusions

Microalgae produce C15-C17 alkanes

An alkane-forming enzyme was identified in Chlorella

Converts fatty acids to alkanes in a single step

The Chlorella alkane synthase is light-driven (only a handful of photoenzymes in nature)
On-going work

Biotechnological aspects:
- Overexpression of Chlorella alkane synthase in bacteria, yeast and microalgae
- Explore stability and substrate specificity:
  - of homologs in other algae
  - of mutants

Fundamental aspects:
Mechanism? Localization? Partner proteins?
Function of alka(e)nes in microalgae?
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