Progress Toward Efficient and Sustainable Hydrogen Production

To Nature and Beyond

Jim

1t of Chemical Engineering

ent of Bioengineering

[FeFe]

Hydrogenase

H2
Sustainable Energy Future? : Planet Earth

- **Solar Energy**
- **Photosynthetic Organisms**
- **Energy: H₂ Economy**
  - Power Plants, Fertilizer, Cement, Automobiles
  - Directly or Indirectly

Our Overall Objective

- **Current H₂ Market**
  - \( \approx \$135\) Billion

Made from CH₄
- \( \approx 1.3\% \) of CO₂ Emissions
Large Potential for Photosynthetic H₂ Production

Current U.S. Consumption Of Liquid Fuels plus Nat. Gas
≈ 60 x 10^{15} \text{ Btu/yr}

Assume: Solar Incidence of 7\text{ kWh/m}^2\text{-day}

and 7.5\% Energy Efficiency (Overall)

Need 35,000\text{ Square Miles} = 5\% of Current Cropland

(But Do Not Need to Convert Current Crop Land)
But in (Bio)Chemical Engineering:
In order to Dream BIG, We have to think SMALL

$2 \ H^+ + 2 \ e^- \rightarrow H_2$

Up to 20,000 Reactions per Second !!

[FeFe] Hydrogenase (CpI)
(from Clostridium pasteurainum)
Can We Engineer An Organism to Make Hydrogen Directly From Sunlight and Water?
Even Better, Can we “Hard-Wire” the Hydrogenase to the Photosystem?
In Addition,

Could We Go From This →

\[ \text{Biomass} \rightarrow \text{Enzymatic depolymerization} \rightarrow \text{Sugars} \rightarrow \text{Enzymatic electron transfer to hydrogenase} \rightarrow \text{H}_2 + \text{CO}_2 \]

To A More Sustainable Process?

Need: High Conversion Efficiency and High Productivity
The Active Site of Fe-Fe Hydrogenases is Complicated: Stabilized by Cysteines, Carbon Monoxide, and Cyanide

An NREL Group* Discovered Three Required Maturases HydE, HydF, and HydG

We Developed an *in vitro* Hydrogenase Maturation Procedure Using Individually Expressed Maturases & Optimized Substrates

- Used ΔiscR Host Strain
- Added GTP
- Added Pyridoxal-P<sub>i</sub>
- Optimized Concentrations
- *E. coli* Extract was Required

Kuchenreuther et al.; PloS ONE 6(5) e20346 – 2011
FTIR Spectroscopy of Apo-Hydrogenase Matured with Isotopically Labeled Tyrosine Confirmed that All the –CO and –CN Was Derived from Tyrosine

These results show that the –CO comes from the -CO$_2$ group on tyrosine and the –CN comes from the alpha carbon and amino nitrogen.

Kuchenreuther et al (Swartz, Cramer, George).; PloS ONE 6(5) e20346 – 2011
Further Work with the Dave Britt Lab at UC Davis Explained the First Steps in Building the All-Important Catalytic Center

The Radical SAM Enzyme HydG Generates an Fe(CO)$_2$(CN) Synthon in the Biosynthesis of the [FeFe] Hydrogenase H-Cluster

Jon M. Kuchenreuther$^{1\dagger}$, William K. Myers$^{1\dagger}$, Daniel L. M. Suess$^1$, Troy A. Stich$^1$, Vladimir Pelmenschikov$^2$, Stacey A. Shiigi$^3$, Stephen P. Cramer$^{1,4}$, James R. Swartz$^{3,5}$, R. David Britt$^{1\star}$, Simon J. George$^{1\star}$

Accepted for Publication in Science
This work also enabled Improvement of Hydrogenase and Maturase Production in *E. coli*

SDS-PAGE Gel of Lysate Supernatant (unpurified)

Yields are at least 30 times higher than any previously reported.

But, Not All of the Hydrogenase is Active

Kuchenreuther, et al. PLoS ONE 2010
We developed an *in vitro* hydrogenase maturation procedure using individually expressed maturases & optimized substrates:

- Used ΔiscR host strain
- Added GTP
- Added pyridoxal-\(P_i\)
- Optimized concentrations

*E. coli* extract was required.

Kuchenreuther et al.; PloS ONE 6(5) e20346 – 2011
The ISC Proteins Were NOT The Magic Factor(s)

Isc proteins were produced and confirmed to be active. They were then evaluated.

No more than 1% activation was observed with ISC proteins alone.
Liliana is Now on a Magic Factor Safari

Cell Extract → Chromatography
- Anion Exchange
- Hydrophobic Interaction
- Size Exclusion

Measure hydrogenase activity over time using methyl viologen reduction assay

Anion Exchange Chromatography Results
Active Fractions

Reducing SDS-PAGE 10% Bis-Tris Gel

CysK

CsyK identified as a candidate protein
- Involved in cysteine metabolism
- Desulfurase activity observed in E. coli

However, She Produced CysK -- NOT the Magic Factor
Back to Business: How Do We Efficiently Convert Sugars into Hydrogen? To Minimize Cost, We Will Use Unpurified Cell Extracts

First Mobilize the 24 Electrons Available from the Glucose Using The Pentose Phosphate Pathway (in Red)
Back to Business: How Do We Efficiently Convert Sugars into Hydrogen? To Minimize Cost, We Will Use Unpurified Cell Extracts

Then Harvest Most of the Electrons to Produce Hydrogen (in Green)

![Diagram of the process](image)

- **Glucose** ($C_6H_{12}O_6$) → **Glucose-6-P** → ATP → ADP
  - Oxidative Phosphorylation
  - Water ($H_2O$) → $\frac{1}{2}O_2$

**Pentose $P_i$ Pathway**

- 6 $H_2O$ → 6 $CO_2$ → 12 NADPH + 12 $H^+$ → 11 NADPH + 11 $H^+$ → 11 NADPH + 11 $H^+$ → 11 $H_2$

**FNR** = Ferredoxin NADPH Reductase

- $Fd^{red}$ = Reduced Ferredoxin
- $Fd^{ox}$ = Oxidized Ferredoxin

**$H_2$ase** = Hydrogenase
But We Need to Phosphorylate the Glucose

Using Oxidative Phosphorylation; We should only need 1 NADPH

Thus, the Conversion Yield Should Exceed 90% Fermentation Yields are Less Than 30%
Proof of Principle: Hydrogen Production from Glucose

- Cell Lysate plus Purified FNR, Fd, & H₂ase Produced H₂
- The FNR/Fd/H₂ase pathway is limiting

Phil Smith (now at BMS)
Franklin Lu
For Commercial Conversion of Glucose (and Xylose) to H₂
Need HIGH Productivity for Distributed Facilities

Published enzyme turnover numbers and expected expression levels suggest possible Fuel Value Productivity of \( \approx 500 \text{ kJ/Liter-hr} \)*

About 10 times higher than current ethanol plants

*Assumes FNR Turnover Rate of 10/sec
We investigated the natural diversity of Ferredoxin NADPH Reductases (FNRs).

![Diagram showing NADPH + H⁺ and H₂ relationships with EcFNR, EcFNR SynFd, EcFNR CpFd, AnFNR CpFd, and RootFNR CpFd.

**Volumetric Productivity** (mmol H₂ L⁻¹ hr⁻¹)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volumetric Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang et al (2007)</td>
<td>1 mmol H₂ L⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>EcFNR SynFd</td>
<td>5 mmol H₂ L⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>EcFNR CpFd</td>
<td>10 mmol H₂ L⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>AnFNR CpFd</td>
<td>15 mmol H₂ L⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>RootFNR CpFd</td>
<td>30 mmol H₂ L⁻¹ hr⁻¹</td>
</tr>
</tbody>
</table>

**Relationships between known FNR sequences**

EcFNR: Ecoli FNR
AnFNR: Anabaena FNR,
SynFd: Synechocystis ferrodoxin,
CpFd: Clostridial ferrodoxin

Franklin Lu

Improving the FNRs by Mutation and Screening

We have observed Sufficient FNR Activity in Several Formats
We are now Modifying the Cell Extracts for More Efficient Conversion
Now, Back to Direct Photosynthetic Hydrogen
Can we “Hard-Wire” the Hydrogenase to the Photosystem?
Purified PSI Complex is Active for H₂ Production

Using the Ferredoxin from *Clostridium pasteurainum* (CpFd) Could Provide a Direct Connection

But Will It Couple with PSI??
Hydrogen Production from the *Clostridial* Ferredoxin: Potential for a “hard-wired” Connection

Stacey Shiigi
We Needed a Device for High Throughput Screen for Hydrogen Production Capability and Oxygen Tolerance

For Calibration, We Inject Hydrogen Gas Below the 96-well plate. Inject Oxygen to test for Oxygen Tolerance

The 96-well Plate Sits on the Frame and is Covered By a Gasket (the hardest part)

A Glass Plate Coated with Paladium and Tungsten Oxide is then Clamped on Top

Jamin Koo and Tim Schnabel
The Screening Device is Working --
We can also Regenerate the Sensor Plates

A Digital Camera Takes Images Over Time and Image Analysis Software Interprets the Rate of Color Formation

Jamin Koo and Tim Schnabel
Conclusions

1. The *in vitro* H$_2$ase maturation system is a valuable tool.

2. Cell-free metabolic engineering offers efficient and highly productive conversion of cellulosic biomass to hydrogen.

3. New Tools and Knowledge suggest feasibility for photosynthetic hydrogen production ----
   BUT, we still need a productive, oxygen-tolerant hydrogenase.

Thanks

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