Engineering Enzymes for Hydrogen Production from Biomass

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Introduction

Hydrogen is a potential energy carrier to replace hydrocarbon fuels and reduce emissions produced by their consumption.

Challenges with current hydrogen production:
- Released CO_2 accounts for over 1% of global annual CO_2 emissions
- Some clean production methods are expensive, e.g. wind electrolysis

Our Target:
→ Efficient use of biomass to produce hydrogen:
- Renewable pathway with costs competitive with fossil sources
- Abundant supply of biomass from crop residues
- Nearly Carbon neutral

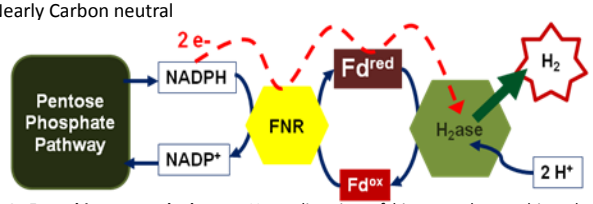

Fig 1. From biomass to hydrogen. Upon digestion of biomass, the resulting glucose metabolite enters the Pentose Phosphate Pathway in crude cell lysates. The flow of two electrons (red dotted arrow) from NADPH to protons (H^+) drives the production of biohydrogen. The required enzyme Ferredoxin-NADPH Reductase (FNR) is derived from *Anabaena variabilis* (AnFNR), while both the [FeFe] Hydrogenase (H_2ase) and the electron transfer protein Ferredoxin (Fd) are from *Clostridium pasteurianum*.

Goal:
The electron transfer downstream from FNR in the biomass to hydrogen pathway was shown to be rate-limiting. In this study, we examine the capability of CpI H_2ase–*Anabaena* FNR fusion proteins to increase the electron flux rates for improved hydrogen productivity.

Methods

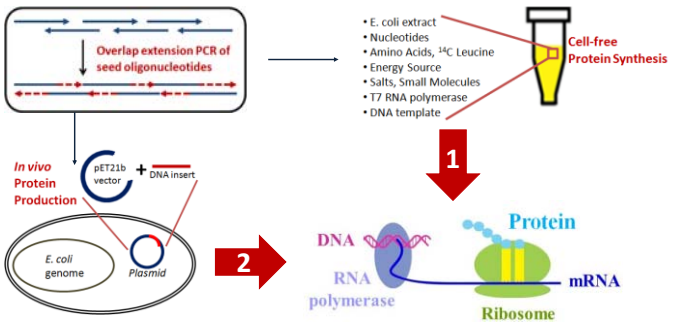

Fig. 2. Schematic of fusion protein production. The fusion CpI-AnFNR gene was assembled by overlap extension PCR. The protein was produced by: (1) *CFPS*: Cell-free protein synthesis which allows direct modification of the protein expression and folding environment; (2) *In vivo* expression in *E.coli*. Synthesized proteins were then assayed for activities.

Hydrogenase-FNR Fusion

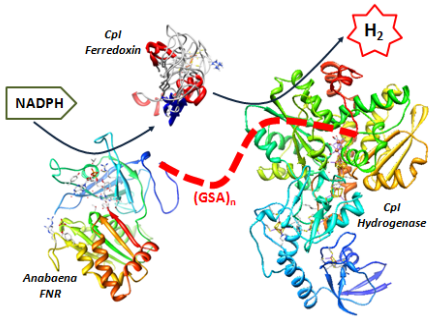

Fig. 3. CpI-AnFNR fusion protein. Shown to have higher hydrogen productivity, the C-terminus of CpI H_2ase is tethered to the N-terminus of AnFNR by a peptide linker (in bold red dotted line). The linker consists of GSA (Glycine-Serine-Alanine) repeats with varying lengths.

Results

Cell-free protein synthesis (CFPS) yields of CpI-AnFNR fusion at varying GSA (Glycine-Serine-Alanine)$_n$ linker lengths

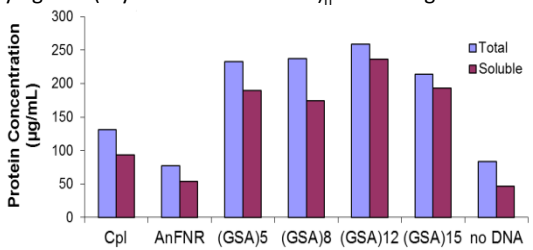

Fig. 4. CFPS protein yields quantified by the incorporated radioactive C^{14}-Leucine. Total protein yields are based on the total cell-free reaction product mixture, while soluble yields are measured in the supernatant after centrifugation. The reactions were conducted anaerobically at 25°C for 14 hours.

Effects of DNA template concentrations on CFPS yields

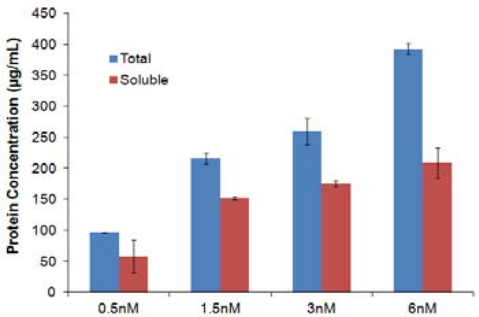

Fig. 5. CFPS protein yields from varied DNA template concentrations. The anaerobic reactions were conducted at 25°C for 17h. Higher DNA template concentrations increased total yields, but did not give a proportional increase in the soluble yields.

Hydrogen evolution using cell-free synthesized CpI-AnFNR fusion proteins (A) and *in vivo* fusion lysate (B)

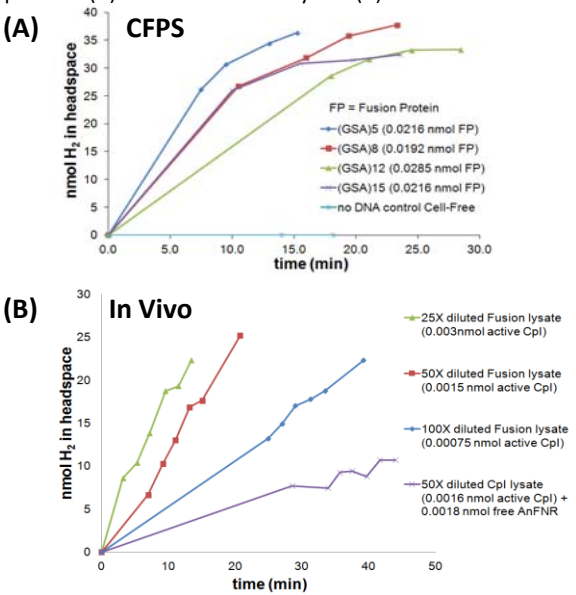

Fig. 6. H_2 production of CpI-AnFNR fusion protein measured over time: (A) From CFPS. The reaction was started by adding 2mM NADPH into the 2X diluted CF reactants with 50µM Cp ferredoxin. The fusion protein with n=5 GSA linker contains 0.05µM active CpI and gives the highest volumetric productivity of 6.98mmol H_2 L^{-1} hr^{-1} (0.039 nmol H_2 (nmol active CpI)$^{-1}$ s^{-1}); **(B) From *in vivo* fusion lysate with n=5 GSA linker**. The reaction was started by adding 2mM NADPH into different dilutions of lysate mixed with 50µM Cp ferredoxin. The 25X diluted lysate gives a volumetric productivity of 2.69mmol H_2 L^{-1} hr^{-1} (0.075 nmol H_2 (nmol active CpI)$^{-1}$ s^{-1}). At 50X dilution, fusion lysate gives a 5-fold increase in H_2 productivity compared to the CpI lysate with equimolar free AnFNR.

Conclusions

- Fusion CpI-AnFNR cell-free protein synthesis gives higher yields than for CpI and AnFNR synthesis. Higher cell-free DNA template concentration does not give a proportional increase in soluble protein yields.
- Shorter GSA linker lengths (n=5) in CpI-AnFNR fusion gives the highest H_2 volumetric productivity, suggesting an effective linear distance of 7.5 nm between CpI and AnFNR.
- *In vivo* cell lysate with the CpI-AnFNR fusion protein shows a 5-fold increase in H_2 productivity compared to that of CpI lysate supplemented with free AnFNR.

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