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Original Research Article

Biological conversion of gaseous alkenes to liquid chemicals

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ABSTRACT

Industrial gas-to-liquid (GTL) technologies are well developed. They generally employ syngas, require complex infrastructure, and need high capital investment to be economically viable. Alternatively, biological conversion has the potential to be more efficient, and easily deployed to remote areas on relatively small scales for the utilization of otherwise stranded resources. The present study demonstrates a novel biological GTL process in which engineered *Escherichia coli* converts C2–C4 gaseous alkenes into liquid diols. Diols are versatile industrially important chemicals, used routinely as antifreeze agents, polymer precursors amongst many other applications. Heterologous co-expression of a monooxygenase and an epoxide hydrolase in *E. coli* allows whole cell conversion of C2–C4 alkenes for the formation of ethylene glycol, 1,2-propanediol, 1,2-butanediol, and 2,3-butanediol at ambient temperature and pressure in one pot. Increasing intracellular NADH supply via addition of formate and a formate dehydrogenase increases ethylene glycol production titers, resulting in an improved productivity of 9 mg/L/h and a final titer of 250 mg/L. This represents a novel biological method for GTL conversion of alkenes to industrially valuable diols.

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1. Introduction

Gas-to-liquid (GTL) technologies convert gaseous substrates, typically methane, into liquid chemicals. Current industrial GTL processes employ catalysts with high heat and pressure (Haynes and Gonzalez, 2014). Heavy capital investment into large, high volume production facilities are required for economic viability, with the corollary requirement that large volumes of gas are constantly available (Haynes and Gonzalez, 2014). Such facilities are full of technical and economic challenges to build and maintain (Fei et al., 2014; Haynes and Gonzalez, 2014). A simpler and potentially cost effective alternative is to use engineered microbes as catalysts for GTL processes (Haynes and Gonzalez, 2014; Hu et al., 2016). A biological GTL system can operate at a smaller scale, at ambient temperatures, and with lower capital costs than conventional GTL systems. This would enable deployment of this technology to remote regions and allow capture of “stranded” gases, which are an underutilized resource (Fei et al., 2014; Haynes and Gonzalez, 2014). Microorganisms can, in principle, achieve higher conversion efficiency (and substrate selectivity) than conventional GTL because of the enzymatic nature of the conversions (Fei et al., 2014). These factors make a biological GTL system potentially more economically and environmentally attractive than a conventional chemical GTL system (Fei et al., 2014; Haynes and Gonzalez, 2014).

Vicinal diols have broad applications as chemical feedstocks and fuels. They are produced industrially, largely from petroleum through a series of steps including cracking to alkenes, alkene epoxidation, and hydrolysis (Sabra et al., 2016). Ethylene glycol is of particular interest due to its many uses such as polyester fibers, PET (polyethylene terephthalate) plastics, and antifreeze. In 2010, 20 million metric tons of ethylene glycol was produced globally, with an estimated 5–10% increase in annual consumption (Yue et al., 2012). Industrially, ethylene glycol production suffers from low specificity (~80%) for epoxidation by O_2, relatively poor conversion to monoethylene glycol (~90%), and high water and energy requirements (Rebsdat and Mayer, 2000). This process presents an opportunity for improvement by biological catalysis, since it can potentially overcome all of these shortcomings. Furthermore, biological production can occur at ambient pressure and temperature and without the requirement of harsh chemical catalysts.

Larger diols (C3–C4) are also valuable (Burgard et al., 2016; Nakamura and Whited, 2003). For example, 1,2-propanediol is used in the food, pharmaceutical, cosmetic, and unsaturated polyester industries (Sullivan, 2000). It is also produced via alkene epoxidation and hydrolysis. Butanediols such as 2,3-butanediol serve as important feedstocks for rubbers, plastics, polymers, pharmaceuticals and insecticides (Gräfje et al., 2000). Ethylene glycol, 1,2-propanediol, 1,3-propanediol, 1,4-butanediol and 2,3-

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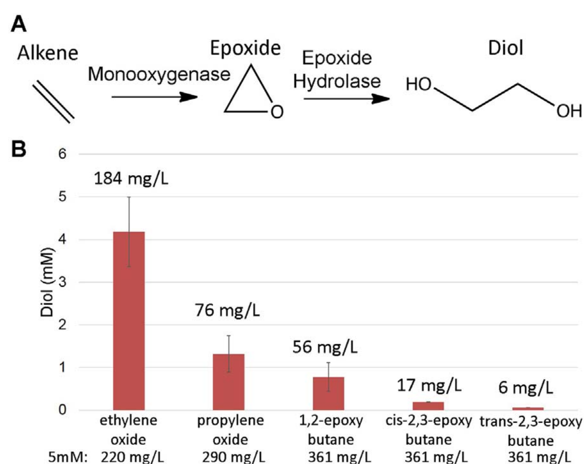

Fig. 1. Characterizing enzymes for diol production. A. Reaction scheme for alkene to diol production with ethylene glycol shown as an example B. *In vivo* conversion of individually fed epoxides to diols. Here, 5 mM of different epoxides were fed to strains expressing the epoxide hydrolase gene. Numbers below epoxides represent mass concentration (mg/L) for the initial 5 mM solution, while numbers above the bars represent diol formation. In the negative control (empty vector), no detectable peaks were observed. N=3; error bars represent standard deviations.

butanediol have been biologically produced from sugars (Burgard et al., 2016; Cam et al., 2015; Chen et al., 2016; Nakamura and Whited, 2003; Pereira et al., 2016, 2015; Sabra et al., 2016).

Some microorganisms naturally metabolize gasses. For example, methanogens generate and methanotrophs catabolize methane, diazotrophs fix nitrogen gas, and photosynthetic organisms fix carbon dioxide (Desai and Atsumi, 2013; Kalyuzhnaya et al., 2015). This study demonstrates that *Escherichia coli* can be engineered to directly metabolize gaseous C2–C4 alkenes to the corresponding diols, which are stable liquids under ambient conditions.

E. coli was chosen as a biological host because it is genetically tractable with many engineering tools readily available. The alkene metabolizing pathway was constructed with two heterologous enzymes. The first enzyme is a monooxygenase (MO) that converts the alkene to an epoxide, which is subsequently converted to a diol by an epoxide hydrolase (EH) from *Agrobacterium radiobacter* AD1 (Rink et al., 1997) (Fig. 1A). This combination of enzymes converts gaseous C2-C4 alkenes (ethylene, propylene, 1-butene, cis-2-butene, and trans-2-butene) into the corresponding diols (ethylene glycol, 1, 2-propanediol, 1, 2-butanediol, *R*,*R*-/*S*,*S*-2,3-butanediol, and meso-2, 3-butanediol).

2. Materials and methods

2.1. Reagents

All enzymes were purchased from New England Biolabs. All synthetic oligonucleotides and DNA sequencing services were provided by Euforins. Chemicals for gas chromatography (GC) and High Performance Liquid Chromatography (HPLC) standards were purchased from Sigma Aldrich. Ethylene gas was purchased from Air Gas. Propene, 1-butene, cis and trans 2-butene, ethylene oxide and propylene oxide were purchased from Sigma Aldrich. 1,2-Epoxybutane was purchased from TCI America. cis and trans 2,3-Epoxybutane were purchased from Acros Organics.

2.2. Plasmid construction

All plasmids and primers are listed in Tables S1 and S2, respectively. The pAL1219 plasmid was constructed using a 300 bp fragment from pAL1354 and a 1.8 kbp fragment from pSA69 (Atsumi et al., 2008) cut with *Aat*II and *Avr*II. All other plasmids were constructed using sequence and ligation-independent cloning (SLIC) (Li and Elledge, 2007). The pAL1220 plasmid was constructed using two fragments; the backbone was amplified from pZE12-luc (Lutz and Bujard, 1997) with primers SD11/YT18 and the gene encoding TOM V106A (Canada et al., 2002) was amplified with primers SD177/YT872. The pAL1293 plasmid was constructed using three fragments; the backbone was amplified from pZE12-luc with primers YT40/YT18 and the gene encoding toluene-4-monoxygenase (T4MO) (Tao et al., 2004) from *Pseudomonas mendocina* KR1 was amplified with primers SD189/SD191 and primers SD187/SD190. The pAL1307 plasmid was constructed similarly to pAL1293 except that the gene encoding T4MO G103S/A107T (Tao et al., 2004) was used. The pAL1354 plasmid was constructed using two fragments amplified from pZE12-luc with primers SD138/SD194 and primers SD12/SD139. The pAL1439 plasmid was constructed using two fragments; the backbone was amplified from pSA69 (Atsumi et al., 2008) with primers YT5/YT6 and the epoxide hydrolase (*echA*) (Rink et al., 1997) gene, which was synthesized with an optimized codon usage for *E. coli* by Life Technologies, was amplified with primers SD178/SD179. The pAL1434 plasmids were constructed using two fragments; the backbone amplified from pZE12-luc (Lutz and Bujard, 1997) with primers YT40/YT18 and the gene encoding P450 BM3 910A (Meinhold et al., 2005) was amplified with primers SD210/SD211. pAL1456 was constructed using two fragments; backbone amplified from pAL1439 with primers SD199/SD200 and *fdh* from *Candida boidinii* (Shen et al., 2011) amplified with primers SD201/SD202. All plasmids are verified by restriction enzyme digest and sequencing.

2.3. NAD^+/NADH assay

The NAD^+/NADH assay was performed by the BioAssay Systems EnzyChrom NAD^+/NADH assay kit (E2ND-100) using the manufacturer's protocol.

2.4. FDH Assay

Cells (6.4 mL) were harvested at 4 °C, 2000 g for 10 min. They were resuspended in 200 μL of 10 mM sodium phosphate buffer containing 100 mM beta-mercaptoethanol (BME). Cells were centrifuged once more, resuspended to a final volume of 200 μL in the same buffer as above and lysed using a mini bead beater 8 (BioSpec Productis, Inc.) by 4 rounds shaking, 45 s each. The crude lysate was centrifuged at 16,000 g for 30 min at 4 °C. The cell lysate was mixed with 10 mM sodium phosphate buffer (pH 7.5), 1.67 mM NAD^+, 167 mM formate, and 100 mM BME and absorbance was immediately read at 340 nm to detect NADH formation. NADH was calculated using an extinction coefficient of 6220 M^{-1} cm^{-1} (Schute et al., 1976).

2.5. Strain preparation

Strains were grown in LB overnight at 37 °C with the appropriate antibiotics: ampicillin (200 μg/mL), kanamycin (50 μg/mL). Production experiments were carried out using a Modified Hutner's Mineral Base (MSB) Medium (Stanier et al., 1966), which consists of the following: 40 mM phosphate buffer, 7.57 mM $(NH_4)_2SO_4$, 0.52 M $N(CH_2COOH)_3$, 1.25 mM KOH, 1.16 mM $MgSO_4$, 0.3 mM $CaCl_2 \cdot 2H_2O$, 0.72 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 4 μM EDTA, 23 μM $FeSO_4 \cdot 7H2O$, 51 μM $MnSO_4 \cdot H_2O$, 1.25 μM $CuSO_4 \cdot 5H_2O$, 0.68 μM $Co(NO_3)_2 \cdot 6H_2O$, 0.23 μM $Na_2B_4O_7 \cdot 10H_2O$. Overnight cultures were inoculated 1% in MSB medium containing 5 g/L yeast extract and 50 g/L glucose. Cells were grown at 37 °C, with shaking

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