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Modular and selective biosynthesis of gasoline-range alkanes

Micah J. Sheppard [a,1,2], Aditya M. Kunjapur [a,b,2], Kristala L.J. Prather [a,b,*]

[a] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[b] Synthetic Biology Engineering Research Center (SynBERC), Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

Typical renewable liquid fuel alternatives to gasoline are not entirely compatible with current infrastructure. We have engineered *Escherichia coli* to selectively produce alkanes found in gasoline (propane, butane, pentane, heptane, and nonane) from renewable substrates such as glucose or glycerol. Our modular pathway framework achieves carbon-chain extension by two different mechanisms. A fatty acid synthesis route is used to generate longer chains heptane and nonane, while a more energy efficient alternative, reverse-β-oxidation, is used for synthesis of propane, butane, and pentane. We demonstrate that both upstream (thiolase) and intermediate (thioesterase) reactions can act as control points for chain-length specificity. Specific free fatty acids are subsequently converted to alkanes using a broad-specificity carboxylic acid reductase and a cyanobacterial aldehyde decarbonylase (AD). The selectivity obtained by different module pairings provides a foundation for tuning alkane product distribution for desired fuel properties. Alternate ADs that have greater activity on shorter substrates improve observed alkane titer. However, even in an engineered host strain that significantly reduces endogenous conversion of aldehyde intermediates to alcohol byproducts, AD activity is observed to be limiting for all chain lengths. Given these insights, we discuss guiding principles for pathway selection and potential opportunities for pathway improvement.

1. Introduction

The United States relies heavily on gasoline to fulfill transportation needs. The U.S. consumes roughly 4 billion barrels of gasoline annually, which amounts to 40% of total annual domestic petroleum usage and 47% of all gasoline produced worldwide (International Energy Statistics, 2014; Fichman et al., 2012). Vast gasoline infrastructure exists to facilitate its usage, and prevailing renewable liquid fuel alternatives have limited compatibility. Ethanol requires a different distribution system than gasoline because of its hygroscopicity, corrosivity, and biodegradability (Strogen and Horvath, 2013). Due to dissimilar fuel performance characteristics such as energy density and research octane number (RON) (Table S1), renewable gasoline alternatives are blended with gasoline for use in conventional automobile engines. One approach towards addressing the compatibility of renewable fuels is to metabolically engineer a microbe that converts sugars into a product that mimics the composition of gasoline. An added benefit of such a process would be streamlined product separation from the aqueous phase due to increased product hydrophobicity (Table S1).

We began the pathway design process by first looking at published chemical composition studies of gasoline. Typical regular unleaded gasoline is a blend of over 30 aliphatic and aromatic hydrocarbons (Fig. 1A) (Cline et al., 1991; Johansen et al., 1983; Sanders and Maynard, 1968). C3 (propane) to C9 (nonane) are the most common alkane components in gasoline with C5 (pentane) species predominating. With such targets in mind, we next examined previously observed natural and engineered enzymes involved in alkane synthesis. Initial work on microbial alkane production elucidated natural routes to C15–C17 alkanes produced via fatty acid synthesis (FAS) (Schirmer et al., 2010). In cyanobacteria, acyl-acyl carrier protein (acyl-ACP) reductases (AAR) and aldehyde decarbonylases (ADs, also known as aldehyde-deformylating oxygenases or ADOs (Aukema et al., 2013)) convert growing acyl-ACP chains to long-chain alkanes. In a natural progression, fatty acid reductases and ADs have been co-expressed with acyl-ACP thioesterases in order to terminate FAS at medium-chain lengths and convert the free acids to alkanes (Akhtar et al., 2013; Andre et al., 2013; Harger et al., 2012; Howard et al., 2013; Schirmer et al., 2010). The C12 and longer alkanes reported may serve as alternatives for diesel fuels but not for gasoline.

Recent efforts have also targeted the production of short-chain alkanes (< C10). Using a similar FAS termination strategy,

* Correspondence to: Department of Chemical Engineering, 77 Massachusetts Avenue, Room E17-504G, Cambridge, MA 02139, USA. Fax: +1 617 258 5042.
[1] Present Address: Provivi, Inc., 1701 Colorado Avenue, Santa Monica, CA 90404, USA.
[2] These authors contributed equally to this work.

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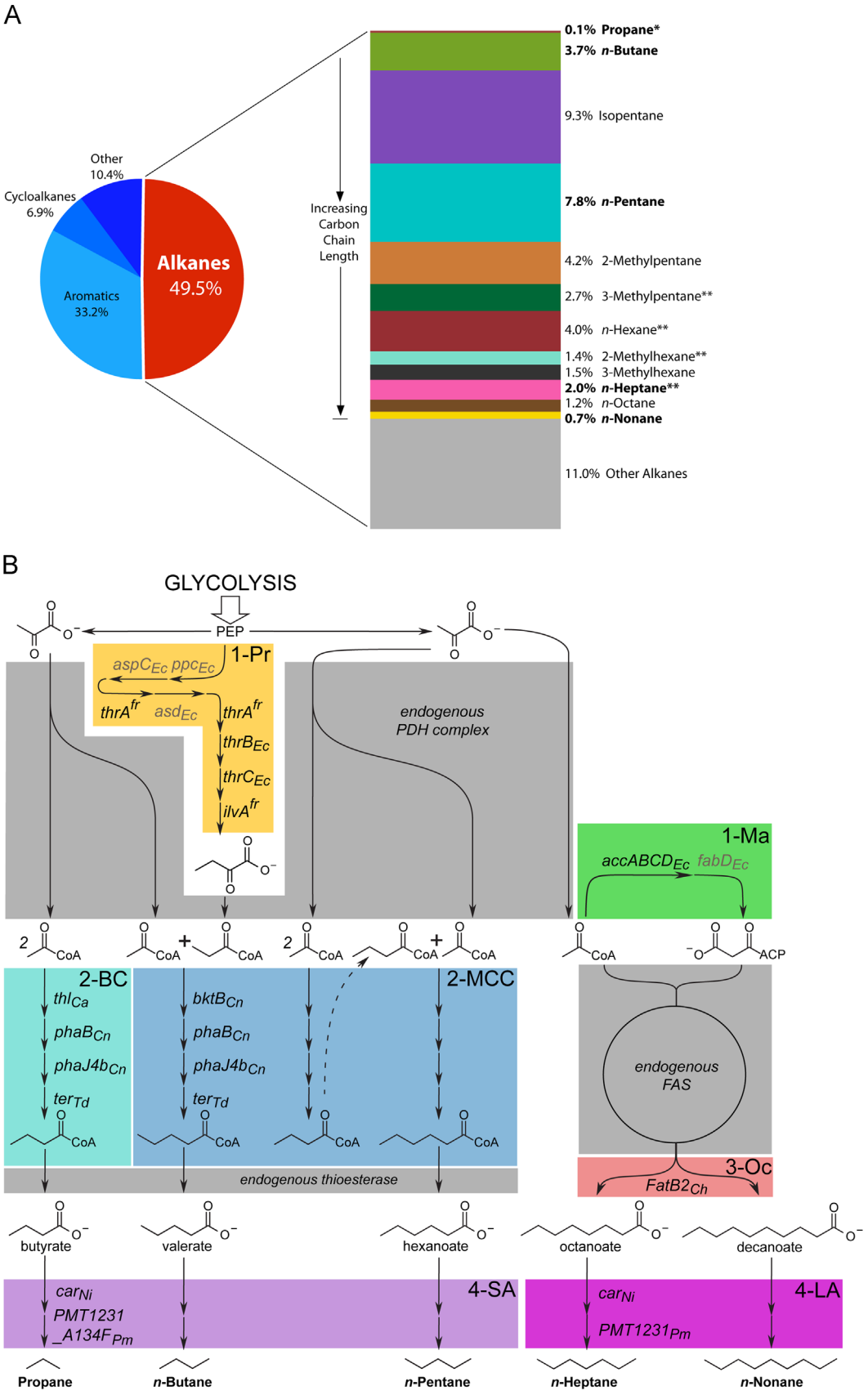

Fig. 1. (A) Composition of typical regular unleaded gasoline displayed in weight percent (wt%) based on the average of values reported by Sanders and Maynard and by Johansen et al. Single asterisk indicates that compounds below 0.5 wt% are not reported by Johansen et al. Double asterisks indicate that wt% includes contribution from trace compounds in Sanders and Maynard. (B) Modular pathway design used for selective synthesis of key gasoline-range alkanes in engineered *E. coli*. Genes in gray within Modules 1-Pr and 1-Ma are native and were not overexpressed, whereas genes in black were overexpressed. Module names are abbreviations for the following: "Pr"=Propionate; "Ma"=Malonyl-ACP; "BC"=Butyrl-CoA; "MCC"=Medium-Chain-CoA; "Oc"=Octanoate; "SA"=Short Alkanes; "LA"=Long Alkanes.