Biosynthesis of Ethylene Glycol

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1. Introduction

Fossil fuels are currently the primary source of energy around the world, with the biggest consumers being the transportation and industrial sectors. However, fossil fuels are finite and their rising prices along with their contribution to global warming has prompted research toward finding alternative forms of energy that are both sustainable and renewable. The development of biosynthetic routes that can produce target biofuels from biomass have been used as an avenue toward this end. This method also involves the design of microbial cell factories by which organisms are constructed to express particular pathways to generate a biofuel of interest. One such target is ethylene glycol (EG). It is used as an antifreeze and coolant for automobiles and a desiccant for natural gas production, but its primary consumer is the polyester fiber production industry. Presently, the large portion of EG is produced via a synthetic route in which dimethyl oxalate obtained from non-renewable fuels undergoes hydrogenation. In an effort to provide a means by which the increasing demands for EG can be met despite the dwindling supply of nonrenewable energy sources, we propose the use of a biosynthetic scheme that utilizes a carboxylic acid reductase (CAR) to reduce glycolate to glycoaldehde. Glycoaldehyde can be reduced to ethylene glycol through glycoaldehyde reductase (Fig 1.2). We are further motivated by research that has already shown the breadth of specificity displayed by *Nocardia sp* CAR and *M. marinum* CAR though the native substrate of CARs is benzoate (Fig 1.3). These CARs can reduce a wide range of substrates from aromatic to aliphatic compounds respectively. Our short-term goal is to identify a CAR variant with a high catalytic efficiency toward glycolate by testing CAR candidates from both reported and putative CARs of several organisms. Finally, through protein engineering we hope to evolve the best CAR candidate into a CAR with highly improved catalytic efficiency toward glycolate.

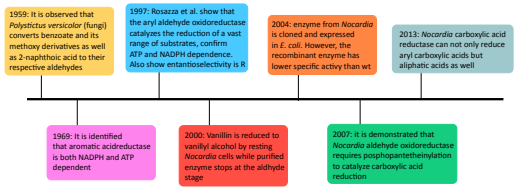


Fig 1.1 Seminal papers on CAR

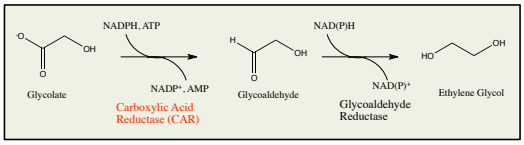


Fig 1.2 Proposed biosynthetic scheme for biosynthesis of EG

Applied and Environmental Microbiology, **2004**; 70:1874-1881
Biotechnology Journal, **2014**; 9, 822-843
Computational and Structural Biotechnology, **2014**; 11:91-99
Chemical Reviews, **2013**; 113, 4611-4632
PNAS, **2103**; 110:87-92

2. CAR Requires Phosphopantetheinyl Transferase (sfp) for Catalytic Activity

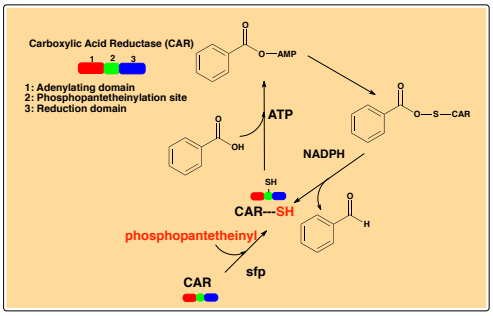
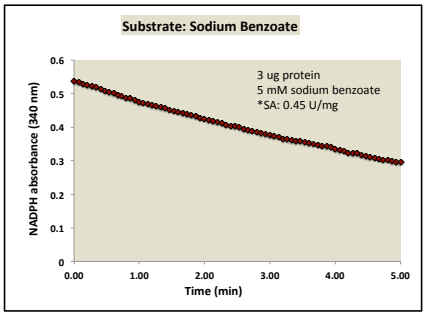


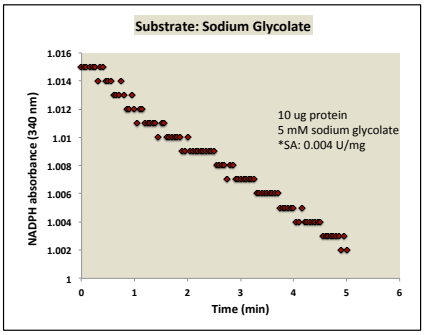
Fig 1.3 *Nocardia* sp. CAR (first reported CAR) scheme for catalysis of benzoate

5. Cloning and Expressing CAR Candidates

CARs were cloned into pET30b-sfp vectors with a His-tag at the C-terminal. The *sfp* encodes a phosphopantetheine transferase enzyme from *Bacillus subtilis* because previous has research has demonstrated that this post-transitional modification is necessary for CAR enzymes to become catalytically active (Fig 1.3). Plasmids were transformed into BL21(DE3) and induced for protein expression in Luria-Bertani media with 0.5 mM IPTG. Cells were lysed by sonication and cell debris was removed via centrifugation. The supernatant was loaded onto a column of Ni-sepharose resin, where the CAR-His-tag generally eluted with 5-10 mM imidazole.

3. *Nocardia* sp. CAR Activity on Benzoate and Glycolate





The activity of *Nocardia* sp CAR on glycolate was low compared with its native substrate benzoate. Therefore, we sought to identify other CAR variants with improved catalytic efficiency.
*SA: Specific Activity

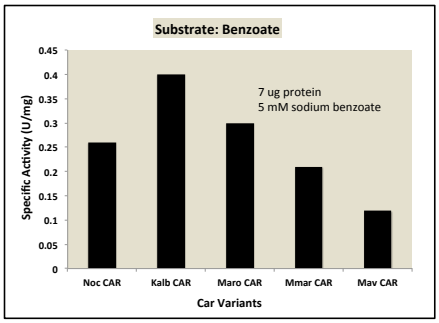
4. CAR Variants from NCBI Database

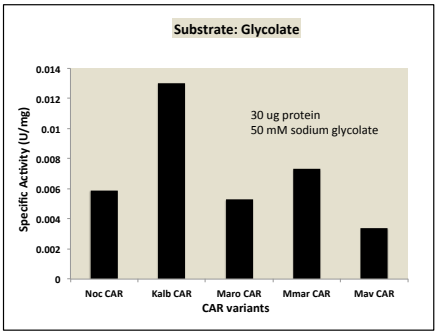
A BLAST search of the NCBI database was performed using the sequence of *Nocardia* sp. CAR. Hits were selected using a sequence identity >60% as the cut off for a total of 6 CARs (some reported and some putative):

1. **Nocardia sp. – 100%*
2. **M. marinum – 62%*
3. *M. avium – 61%*
4. *M. aromaticivorans – 61%*
5. *R. wratislaviensis – 62%*
6. *K. Albida – 67%*

*Previously reported CARs

6. Activity of CAR Candidates on Benzoate and Glycolate





Preliminary data showing the activity of various CARs on benzoate glycolate

7. Conclusion

So far, we have tested the catalytic activity of CARs from 5 of the 6 candidates on glycolate. K. albida CAR is by far the most promising candidate and can catalyze the reduction of benzoate and glycoate. As far as we know, it is also the first CAR to have been reported from this strain.

8. Future Goals

We are colaborating with Dr. Mark Wilson to obtain the crystal structure of CAR so that we can use rational design to directly evolve the best CAR variant to have a high catalytic efficiency. We have also designed an unnatural amino acid to replace S689 – the residue that is modified with posphopantetheinyl prosthetic group. This will allow the CAR to continually be in an "active" state and thus increase the rate of glycoate catalysis by CAR.

9. Acknowledgments

I acknowledge the Nebraska Center for Energy Sciences Research for funding and the University of Nebraska – Lincoln for the opportunity to work on this project . I would like to especially thank Dr. Guo and Dr. Niu for their guidance in my research.