Biosynthesis of Ethylene Glycol

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1. Introduction

Fossil fuels are currently the primary source of energy, 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 biofuels from biomass has 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 antifreeze and coolant for automobiles and as a deicer 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 ethylene oxide undergoes hydration. 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 glycoaldehyde. Glycoaldehyde can be reduced to ethylene glycol through glycolaldehyde 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. avium CAR through the native substrate of CARs in 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 that are both reported and native. Finally, through protein engineering we hope to evolve a CAR with highly improved catalytic efficiency toward glycolate.

2. CAR Requires Phosphopantetheinylation (sfp) for Catalytic Activity

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.

3. Nocardia sp, CAR Activity on Benzoate and Glycolate

CARs were cloned into pET30a(-) vectors with an His-tag at the C-terminal. The sfp encodes a phosphopantetheine transferase enzyme from Rhodobacter capsulatus, which has been shown to be promiscuous in its phosphopantetheinyl conjugation of substrates (Fig 1.3). Plasmids were transformed into the BL21(DE3) strain 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-NTA sepharose resin, where the CAR-His was eluted with imidazole.

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 native).

5. Cloning and Expressing CAR Candidates

Our CAR research progress

6. Activity of CAR Candidates 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.

7. Conclusion

Based on the activity assay, CARs from M. avium is by far the most promising candidate and can catalyze the reduction of both benzoate and glycolate. This invokes its use in a biocatalytic pathway for the generation of ethylene glycol. As far as we know, it is also the first CAR to have been reported from this strain.

8. Future Goals

We will test the conversion of glycolate in vitro in E. coli using Mavi CAR. We are generating a library of CARs by recombination of the sfp gene forming domain with the intent of generating CAR variants that enhance the conversion of glycolate into glycoaldehyde. We are also collaborating with Dr. Mark Wilson to obtain the crystal structure of CAR so that we can use rational design to directly evolve a catalytically efficient CAR variant.

9. Acknowledgments

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10. References

*Previously reported CARs

Fig 1.1 CAR research progress

Fig 1.2 Proposed biosynthetic scheme for biosynthesis of EG

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