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Possibility of Tapping NADH Regenerated from Ethylene Glycol Utilization Pathway for Cofactor Regeneration

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Abstract

Although cofactor regeneration is an established system in biocatalysis, work remains in developing new and alternative cofactor regeneration systems with greater efficiency, ease of use, and higher atom economy. In addition, cofactor regeneration system only works if the cofactor regeneration reaction operates at similar kinetics compared to the biotransformation reaction. This meant that only specific cofactor regeneration system is capable of coupling with particular biotransformation reaction. This then leaves open the field for the development of a plethora of alternative cofactor regeneration systems each capable of coupling with different biotransformation reaction of different kinetics. This short write-up examines the possibility of tapping on the NADH regenerated from a two-step ethylene glycol utilization pathway. Current knowledge suggests that this angle has not been explored; thereby, opening up possibilities for future experimental investigations into the feasibility of coupling ethylene glycol utilization pathway with biotransformation reaction as a coupled cofactor regeneration system.

Keywords: NADH cofactor regeneration; ethylene glycol utilization; biocatalysis; atom economy; enzyme kinetics

Subject areas: biochemistry, biotechnology, cell biology, microbiology,

Background analysis

Implementing biocatalytic systems that require redox cofactors such as NADH or NADPH in microbial cells typically require a cofactor regeneration system.[1,2] Such systems avoid the need for provision of stoichiometric amounts of expensive redox cofactor,[3] and switches the cost of the process to that of the co-substrate that regenerates the cofactor using an enzymatic reaction. In whole-cell biocatalytic systems with cofactor regeneration, the main biotransformation reaction consumes the redox cofactor, which is regenerated by a coupled second reaction that consumes a co-substrate such as glucose to produce the redox cofactor. Doing it this way allows a cheap co-substrate to replace a high-cost cofactor that would otherwise be needed in stoichiometric amounts in a reaction. Choice of co-substrate is therefore important. Besides price, simplicity of reaction as well as whether the reaction product of cofactor regeneration could complicate downstream processing are also important considerations. This review focuses on NADH cofactor regeneration, even though there are many other cofactor regeneration systems available such as those for NADPH[4-6] and ATP.[7,8] Currently, glucose dehydrogenase is the most popular NADH regeneration system for biocatalytic reactions in *E. coli*. But, cofactor regeneration systems with better atom economy, simpler reaction setup, and higher productivities are still sought after.[9]

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Conventionally, single enzyme capable of regenerating a particular cofactor are investigated for use in cofactor regeneration, but more recently, the focus has shifted to the use of pathways for regenerating cofactors.[10] In the latter approach, one or more enzymes that participate in the pathway are capable of regenerating a specific cofactor. In general, pathway-based cofactor regeneration would be able to regenerate more cofactors per turn of the pathway compared to single enzyme cofactor regeneration. One approach in this direction is in the use of native pathways present in central carbon metabolism.[11,12] The other approach is in the design, construction and implementation of new heterologous pathways, typically centered on utilizing new unconventional substrates, even though modified improved version of endogenous pathways have also been used.[13] In this vein, exploring the cofactor regeneration capability of ethylene glycol utilization pathway is an important segment of work seeking to develop alternative cofactor regeneration systems.

Ethylene glycol utilization pathway links ethylene glycol to glycolate. This route was first discovered in *E. coli* when mutants were found to grow on propylene glycol.[14] These mutants could also grow on ethylene glycol by first converting it to glycoaldehyde, which was subsequently converted to glycolate that enters central carbon metabolism. In this incarnation of the ethylene glycol utilization pathway, fucO (an L-1,2-propandiol oxidoreductase native to *E. coli*) would catalyze the first step with regeneration of one NADH. This is followed by conversion of the glycoaldehyde produced in the first step to glycolate through the action of aldA (an aldehyde dehydrogenase native to *E. coli*), which regenerates another NADH. Hence, the two-step pathway regenerates 2 NADH per turn of the cycle. Expression levels of endogenous fucO and aldA are low in *E. coli*, and they have to be over-expressed for the purpose of constructing a cofactor regeneration system. Currently, there are no reports detailing the utilization of NADH generated by the ethylene glycol utilization pathway for cofactor regeneration.

In terms of enzyme systems propelling the ethylene glycol utilization pathway, there has been work done on identifying alternative enzymes capable of catalyzing the critical ethylene glycol to glycoaldehyde step. One possibility is the alcohol dehydrogenase from *Gluconobacter oxydans*, which has exhibited highly selective behavior towards terminal hydroxyl group of varied aliphatic and aromatic diols, with no activity towards sec-alcohols.[15] This enzyme offers an option to modify the proposed ethylene glycol utilization pathway in future work once feasibility of the pathway is successfully demonstrated.

Conclusions

NADH can be regenerated by a couple of enzymatic systems such as NADH oxidase (NOX),[15] glycerol dehydrogenase,[16,17] glucose dehydrogenase,[18] ethanol utilization, and, in this report, ethylene glycol utilization. Besides expanding the types of regeneration systems available for the community, work on new cofactor regeneration systems could also offer an opportunity for the system to be integrated in metabolic engineering applications. Overall, this literature review has arrived at the conclusion that ethylene glycol utilization pathway's potential in providing NADH in support of a biotransformation reaction catalyzed by an

oxidoreductase has not been explored, which presents an opportunity for future work. Discovery of an alternative highly selective alcohol dehydrogenase from *Gluconobacter oxydans* that could replace fucO provides more options for improving pathway efficiency and NADH regeneration in future incarnations of the pathway.

Conflicts of interest

The author declares no conflicts of interest.

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