Reductive Glycine Pathway : A Versatile Route for One-Carbon Biotech

Trends in Biotechnology

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https://doi.org/10.1016/j.tibtech.2021.02.005

cultures. Novel strategies to control the ecosystem inside reactors and cope with outdoor conditions must be developed at the strain engineering stage, hand-in-hand with optimization of scale-up cultivation. To develop managed stable cultivation systems, synthetic consortia [13], or media/strain systems that exclude growth of other organisms should be systematically evaluated [14]. Robust thermophilic and halophilic strains such as *Synechococcus* PCC 7002 may be less prone to contamination and could be ideal hosts for future engineering efforts that are amenable to scale.

The holistic approach of PHOTOFUEL towards engineered fuel secretion from light and CO_2 enabled a targeted and critical discourse to steer engineering efforts at all levels. Genetic engineers took feedback from industrial culture experts, who interacted with process design specialists to develop cultivation strategies for secreted hydrocarbons. Engine testing and technological challenges in product separation from aqueous cultures influenced genetic engineering strategies, and market and perception analysis impacted business case and scale planning. Although comprehensive studies are needed to identify optimal systems for cost-effective commercial biomanufacturing of fuels, PHOTOFUEL has set the foundation for their development and outlined some of the key issues to be addressed in the future.

Acknowledgments

The authors would like to thank Dr. Michael Bippes for scientific advice regarding engine tests. This work was supported by the EU Horizon 2020 research and innovation program Photofuel (grant No 640720).

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https://doi.org/10.1016/j.tibtech.2021.01.003

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Special Issue: Bioconversion of C1 Products and Feedstocks

Spotlight

Reductive Glycine Pathway: A Versatile Route for One-Carbon Biotech

Nico J. Claassens[1,*,@]

Hong *et al.* heterologously expressed the metabolic core of the reductive glycine pathway (rGlyP) as a sink for the anaerobic conversion of glycerol. This recent study concludes several reports in 2020 on the ATP-efficient, one-carbon-assimilating rGlyP. Its engineering in diverse hosts could help the transformation toward renewable, one-carbon-based bioproduction.

The reductive glycine pathway (rGlyP) is a synthetic pathway for formate assimilation [1]. This soluble one-carbon molecule has been suggested as a promising, sustainable substrate for future biotechnology [2]. In rGlyP, formate is first activated with the co-factor tetrahydrofolate into formyl-THF, at the expense of one ATP (Figure 1A). Next, formyl-THF is reduced to methylene-THF, which is then condensed and reduced with CO_2, NH_3, and NADH into glycine. This formation of glycine is catalyzed by the glycine cleavage/synthase system, a reversible four-component enzyme, which can catalyze the synthesis of glycine under elevated CO_2 concentrations. The overall conversion of formate and CO_2 into glycine can be defined as the core module of rGlyP. Glycine can be further assimilated into biomass and product precursors via several routes, such as by the

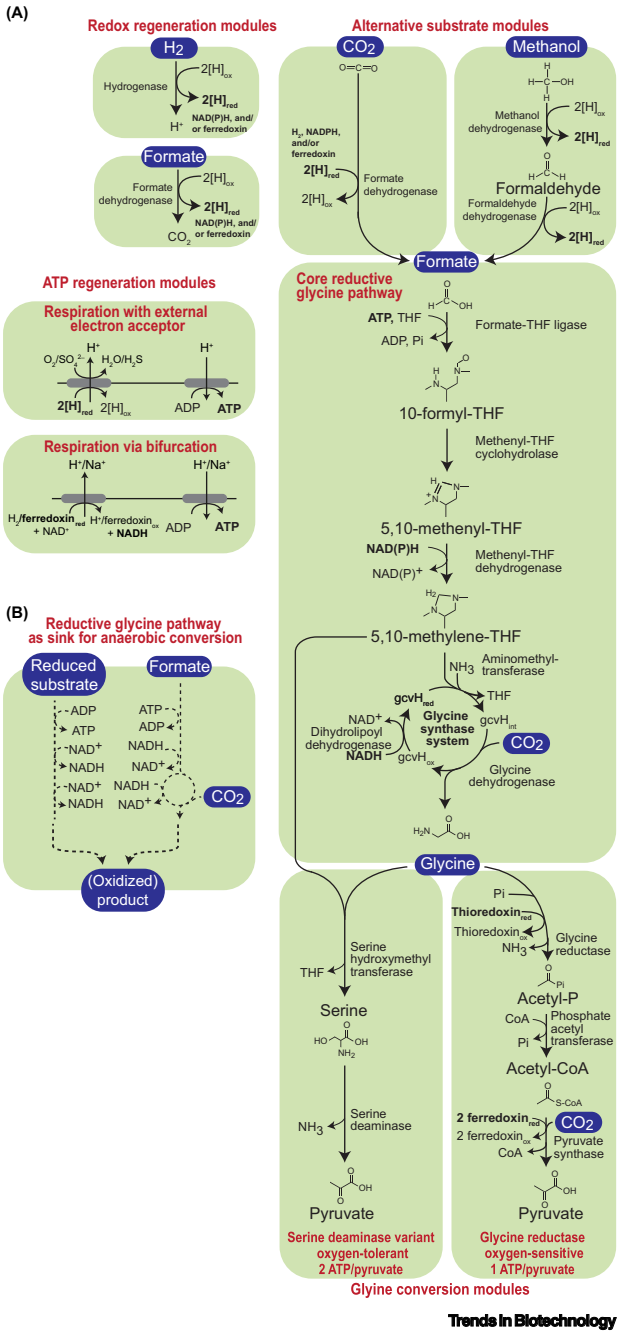

Figure 1. Metabolic Scheme of Reductive Glycine Pathway and Related Modules. (A) The core metabolic module of the reductive glycine pathway (formate to glycine) and additional metabolic modules for energy supply, the conversion of alternative substrates to formate, and the conversion of glycine to the central metabolic intermediate pyruvate. Note that more modules for glycine conversion to pyruvate can be envisioned with varying ATP costs, such as a 'wasteful' variant observed in *Desulfovibrio desulfuricans* for the fixation of CO_2 via the pathway [7]. In this case, acetyl-phosphate formed by glycine reductase is first converted to acetate by a kinase and, subsequently, to acetyl-CoA by acetyl-CoA synthetase, costing overall 2 ATP/pyruvate. (B) Simplified metabolic scheme depicting the reductive glycine pathway as a sink for electrons during the anaerobic oxidation of a reduced substrate, such as the fermentation of glycerol, as proposed by Hong *et al.* [10].

addition of another methylene-THF generated from formate (costing another ATP) to generate serine. The latter C3-amino acid can be deaminated into pyruvate to enter central metabolism. Alternatively, in anaerobic conditions, the oxygen-sensitive glycine reductase complex converts glycine into acetyl-phosphate, which can be further converted to acetyl-CoA and carboxylated to pyruvate, potentially without additional ATP costs.

The aerobic assimilation of formate into pyruvate costs only 2 ATP, making this a highly energy-efficient route, enabling higher product yields than other aerobic routes for formate assimilation [3]. The anaerobic variant of rGlyP via glycine reductase can work with only 1 ATP/pyruvate and, hence, can rival the highly energy-efficient Wood–Ljungdahl pathway. Its ATP efficiency and simple linear structure make the rGlyP an attractive synthetic route for metabolic engineering. One year ago, the lab of Arren Bar-Even published a milestone in the engineering of this pathway: formatotrophic growth of an engineered *Escherichia coli* strain via rGlyP in an 8-h doubling time [4]. This work also provided the proof-of-principle for methylotrophic growth of *E. coli* via rGlyP by adding a metabolic module for oxidation of methanol into formate. Further studies also reported the engineering of rGlyP in aerobic organisms for formate assimilation, including full pathway implementation in the bioplastic-producer *Cupriavidus necator* [5], and demonstration of the core module of rGlyP from formate to glycine in *Saccharomyces cerevisiae* [6].

Another recent study showed that a variant of rGlyP can operate as a natural CO_2 fixation route in *Desulfovibrio desulfuricans* G11, which grows autotrophically on hydrogen in anaerobic conditions (via glycine reductase) [7]. Here, CO_2 is first reduced to formate via the reversible formate dehydrogenase. This study confirmed the rGlyP as the seventh CO_2 fixation pathway known in nature, after a previous study suggested this based on metagenomics [8]. Among the known CO_2 fixation routes, rGlyP is also one of the most

ATP-efficient pathways, only rivalled again by the Wood–Ljungdahl pathway and the reductive tricarboxylic acid cycle, which also both require ~1–2 ATP/pyruvate.

The low ATP costs of the rGlyP also make it a potentially promising production route for anaerobic industrial conditions, in which ATP is more limited. However, in anaerobic conditions, ATP is still required to drive rGlyP. In anaerobic CO_2 fixation via rGlyP by *D. desulfuricans*, ATP is regenerated via respiration with sulfate as an electron acceptor. However, for most anaerobic biotech processes, the use of limitedly available electron acceptors, such as sulfate or nitrate, is undesired. Hence, ATP-regeneration mechanisms other than anaerobic respiration are required. Anaerobic acetogens utilizing the Wood–Ljungdahl pathway operate energy-conserving mechanisms to produce ATP via the activity of bifurcating enzymes. Some bifurcating respiration enzymes can generate an ion gradient across the cell membrane to drive ATP synthase, using the energetic difference between higher and lower potential redox carriers (e.g., ferredoxin and NADH). The recent discovery of rGlyP operating in parallel to the Wood–Ljungdahl pathway in the acetogen *Clostridium drakei,* as well as the functional heterologous expression of rGlyP into the acetogen *Eubacterium limosum*, suggest that these bifurcation mechanisms can also support rGlyP [9]. Both the Wood–Ljungdahl pathway and rGlyP can allow for highly efficient anaerobic conversion of formate (or H_2/CO_2) into products such as acetate, ethanol, and butanol. However, many products cannot be made using these pathways under anaerobic conditions, because too little ATP is available for their biosynthesis.

A recent study by Hong and colleagues [10] proposes an alternative application for rGlyP in anaerobic conditions. They suggest rGlyP as a sink for electrons under anaerobic conditions when a highly reduced substrate, such as glycerol, is used (Figure 1B). In fact, during the 1980s, the role of rGlyP as an electron sink was described in anaerobic degraders of highly reduced purines [11]. A potential biotechnological application could be the co-fermentation of glycerol with formate in a bacterium harboring rGlyP to enable efficient conversion into products that are more oxidized than glycerol. On the road to establish the rGlyP in an anaerobic, glycerol-fermenting bacterium, Hong and colleagues equipped *Clostridium pasteurianum* with the missing glycine synthase system by expressing from a plasmid the four genes encoding the glycine synthase system from the purine-degrader *Gottschalkia acidurici*. Unfortunately, the heterologous expression of these genes caused a high burden, eliminating growth of this organism on glycerol and formate. Still, the authors observed increased uptake of formate, and some physiological impacts of formate addition in the presence of glucose, suggesting that the heterologous glycine synthase system is active. Thus, further studies are needed to optimize the expression of this enzyme complex and the rest of the pathway in *C. pasteurianum*. Another recent study in which the rGlyP was established by engineering and lab evolution in *C. necator* showed that proper balancing of the expression of the glycine synthase system is crucial to achieve sufficient rGlyP activity, while preventing a detrimental expression burden of this system [5]. I expect that further engineering of rGlyP, including improved understanding and optimization of the glycine synthase system kinetics [12], will enable future sustainable biotech applications using various one-carbon substrates in diverse hosts and conditions.

Acknowledgments

I am grateful to Arren Bar-Even (1980–2020) for inspiring and supporting me and many others to work on the reductive glycine pathway. I thank Ari Satanowski and Steffen Lindner for critical reading of this manuscript. I acknowledge support from a Veni Fellowship of the Dutch Science Organisation (NWO) (VI.Veni.192.156).

Declaration of Interests

None declared by author.

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https://doi.org/10.1016/j.tibtech.2021.02.005

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