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Advances in metabolic engineering and synthetic biology have enabled the biomanufacturing of diverse sustainable products from various carbon feedstocks. Recently, there has been increasing interest in using one-carbon compounds such as CH$_4$ or CO$_2$ as substrates because of their abundance and their role in the greenhouse effect. However, relevant carbon fixation pathways do not naturally exist in industrial microorganisms such as *E. coli* and *Saccharomyces cerevisiae*, and incorporating heterologous enzymes is challenging in regard to generating efficient pathways in a new host. To address such difficulties, Siegel *et al.* have constructed an artificial formate assimilation pathway aided by a computationally designed enzyme.

A total of nine one-carbon fixation pathways have been discovered in nature. The CO$_2$-based pathways include the Calvin-Benson cycle, the reductive tricarboxylic acid (TCA) cycle, the reductive acetyl-CoA pathway, the 3-hydroxypropionate cycle, the 3-hydroxypropionate--4-hydroxybutyrate cycle and the dicarboxylate--4-hydroxybutyrate cycle. These pathways require energy input from sunlight or hydrogen, and they are limited by low catalytic rate, oxygen sensitivity and complicated regulatory mechanisms. Because formate can be electrochemically...
generated from CO₂, biological conversion of formate presents an efficient approach to biofuel production from C1 sources. All three natural formate pathways involve the activation of formate into methylenetetrahydrofolate (methylene-THF). However, these pathways present their own limitations: for the ribulose 4-phosphate cycle and the xylulose 5-phosphate cycle, the spontaneous cleavage of the desired product, methylene-THF, into its precursor, formaldehyde, presents a kinetic barrier to conversion. For the serine cycle, the biomass yield is relatively low according to flux balance analysis. Further, establishing new metabolic pathways across species is often challenging, and attempts to implement fully functional one-carbon fixation pathways in heterologous hosts have so far not been successful.

To avoid the intrinsic limitations of natural pathways, Siegel et al. applied computational design to construct the formolase pathway, the first synthetic biological route for formate assimilation (Fig. 1). The critical step is the carboligation of three C1 formaldehyde molecules into one C3 dihydroxyacetone (DHA) molecule. Prior to this work, no enzyme had been reported to catalyze two rounds of carbon-carbon coupling reaction. However, the authors envisioned that benzaldehyde lyase (BAL), whose natural function is to catalyze the aldol condensation of two benzaldehyde molecules into benzoin, could couple three molecules of formaldehyde together if a second alddehyde hydrogen were available. Interestingly, they discovered that wild-type BAL could actually catalyze the coupling of three formaldehydes to form DHA—the formicase reaction—although the catalytic efficiency (kcat/Km) of this reaction was 36,000-fold lower than for the reaction with benzaldehyde. The next obvious step was to improve the enzyme’s specificity and activity toward formaldehyde. Thus, the authors used the computational modeling tools RosettaDesign and Foldit to redesign the binding pocket of BAL. After examining a total of 121 designs in four iterations, they identified a BAL mutant named Des1, with four amino acid mutations, that had a 26-fold higher activity on formaldehyde than did wild-type BAL. Modeling of the active site indicated that the G419N mutation enabled the formation of a hydrogen bond with the DHA transition state, and A28I and A480W created packing interaction with the carbon backbone of DHA. Further computationally guided site-directed mutagenesis and error-prone PCR introduced three additional mutations that further improved the substrate binding affinity. This new enzyme, formolase, has catalytic efficiency of 4.7 M⁻¹ s⁻¹, about 100-fold higher than that of the wild-type BAL. Moreover, no detectable activity was found for formolase in the benzoin-formation reaction, indicating a 10-million-fold specificity switch between BAL and formolase.

With the success in designing formolase, the authors were able to assemble a full biosynthetic pathway to convert formate into dihydroxyacetone phosphate (DHAP) (Fig. 1). Formate is first turned into formyl-CoA by acetyl-CoA synthase (ACS), then to formaldehyde by acetyl-CoA dehydrogenase (ACDH). Formolase enables the carboligation of three formaldehyde molecules to generate DHA, which can be phosphorylated into DHAP in a reaction catalyzed by dihydroxyacetone kinase (DHAK). The entire pathway utilizes solely formate as both carbon and NADH regeneration source. To probe the pathway’s activity in vitro, Siegel et al. incubated either purified enzymes (formate dehydrogenase (FDH), ACS, ACDH, formolase and DHAK) or cellular lysate of their formolase pathway–expressing E. coli strain with [¹³C]formate substrate. They observed [¹³C]DHAP product, and its concentration increased linearly over time. Without formolase, no [¹³C]DHAP was detected. These results confirm the successful development of the formolase pathway. However, at this stage the artificial pathway does not support cellular growth on formate because of the low kinetic efficiency of the computationally designed formolase.

The formolase pathway has several advantages over the nine known natural C1 fixation pathways. It requires only five reaction steps catalyzed by well-expressed enzymes. The pathway has favorable chemical driving force (>3 kcal/mol), which can lead to high production rate and support a high biomass yield. Although the artificial pathway still requires further directed evolution to optimize its efficiency, this elegant work represents a potential game changer for engineering C1 metabolism to assimilate CO₂, methane, formate and other C1 compounds. If the formolase pathway could be optimized sufficiently, the entire bioproduct industry would be able to shift its carbon feedstocks from sugars to cheaper C1 substrates for the production of fuels, chemicals and materials. This work also demonstrates a new strategy for developing synthetic metabolic pathways: recruiting non-natural biochemical reactions enabled by computationally designed enzymes. With
this vision, it is possible to redesign central metabolic pathways such as glycolysis, the pentose phosphate pathway, the TCA cycle and, potentially, a variety of secondary metabolic pathways. This approach opens up a very large metabolic space that is currently inaccessible, providing a green approach to meeting future chemical demand that has positive social, environmental and economic impacts.

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Competing financial interests
The authors declare no competing financial interests.