A Biocompatible Alkene Hydrogenation Merges Organic Synthesis with Microbial Metabolism**

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Abstract: Organic chemists and metabolic engineers use orthogonal technologies to construct essential small molecules such as pharmaceuticals and commodity chemicals. While chemists have leveraged the unique capabilities of biological catalysts for small-molecule production, metabolic engineers have not likewise integrated reactions from organic synthesis with the metabolism of living organisms. Reported herein is a method for alkene hydrogenation which utilizes a palladium catalyst and hydrogen gas generated directly by a living microorganism. This biocompatible transformation, which requires both catalyst and microbe, and can be used on a preparative scale, represents a new strategy for chemical synthesis that combines organic chemistry and metabolic engineering.

Two scientific disciplines, organic chemistry and metabolic engineering, endeavor to produce small molecules using very different techniques. Synthetic organic chemists largely employ non-biological catalysts and reagents to manipulate molecules in multistep processes, while metabolic engineers harness the reactivity of enzymatic catalysts in the context of living organisms to produce molecules directly from fermentations. While organic chemists have been increasingly utilizing enzymes in synthetic efforts such as the industrial-scale synthesis of the diabetes drug sitagliptin (Januvia), efforts to synthesize molecules by incorporating reactions from organic synthesis into biological systems have lagged behind. We envision achieving this goal using biocompatible chemistry: non-enzymatic transformations which directly interface with the metabolism of living organisms, modifying small-molecule metabolites as they are produced and augmenting native biochemistry.

Perhaps the largest obstacle faced in this endeavor is the discordance between the reaction conditions typically required to support life and those often used in organic synthesis (non-aqueous solvents, extreme temperatures, reactive intermediates). For example, the problem of mutual catalyst inactivation can at times complicate efforts to combine transition-metal catalysts with purified enzymes. Additional challenges include the chemical complexity of the cellular and extracellular environments, the typically low concentrations of metabolites, and the potential difficulties associated with accessing intracellular substrates. Encouragingly, similar problems have been surmounted in developing bioorthogonal reactions, which are used to study biological phenomena in living cells and organisms without altering underlying cellular processes. While such transformations illustrate that non-enzymatic chemistry can proceed in the presence of living systems, their application toward small-molecule synthesis has been underexplored.

We identified hydrogen gas as a target metabolite for an initial proof-of-concept reaction: a biocompatible alkene hydrogenation which would directly combine hydrogen generated by living bacteria with a transition-metal catalyst, and could be utilized for preparative scale synthesis. This choice was motivated by previous reports suggesting transition-metal-catalyzed hydrogenation was compatible with living cells. Specifically, unsaturated bacterial membrane lipids can be reduced with transition-metal
catalysts and added hydrogen gas.[12,14] Bacterially produced hydrogen can also directly reduce organic dyes[12b] and ethylene[15,16] on an analytical scale using superstoichiometric amounts of catalyst. Though these examples provided important precedent for the desired chemical reactivity, they did not imply or demonstrate synthetic utility (e.g., preparative scale, broad substrate scope). Beyond its potential use for chemical synthesis, we also envisioned using our hydrogenation to elucidate factors influencing the success of biocompatible reactions.

We began by investigating whether hydrogenation could take place in media complex enough to support the growth of E. coli, our intended source of hydrogen (Table 1). We incubated the water-soluble alkene caffeic acid (1a) with platinum(IV)oxide in two types of growth media under an atmosphere of hydrogen gas (entries 1 and 2) and found that a defined minimal medium (M9 glucose) provided higher conversion than a complex medium (Luria-Bertani (LB) + 0.5% glucose). We then examined the impact of bacteria on these reactions by performing hydrogenations under an atmosphere of hydrogen gas in growth media containing E. coli DD-2 (optical density OD600 = 0.4). This engineered strain produces hydrogen from glucose using an inducible pathway consisting of a pyruvate ferredoxin oxidoreductase, a ferredoxin, and a [Fe-Fe] hydrogenase.[13] We observed little change in conversion with organisms in the reaction mixture (entries 3 and 4). Finally, we incubated E. coli DD-2 under a nitrogen atmosphere, as both metabolites could potentially contribute to the hydrogen and formic acid produced in each reaction (entries 5 and 6). We observed 15% conversion for the reaction run in M9 glucose, thus providing support for our general reaction design.

Our initial optimization efforts focused on varying growth medium and catalyst (Table 1, and see Tables S3 and S4 in the Supporting Information). Based on the increased conversions observed for reactions performed with added hydrogen, we suspected that hydrogen production by E. coli DD-2 was limiting reaction efficiency. We tested various media additives and found that adding either iron or casamino acids to minimal media improved conversion (Table S3). The combination of both additives provided a further increase (Table 1, entry 7). These components may boost hydrogen generation by increasing the amount of functional [Fe-Fe] hydrogenase generated in cells.[14]

We screened a variety of heterogeneous hydrogenation catalysts using our improved reaction media. While most catalysts examined provided no reactivity (see Table S4), the Royer palladium catalyst[15] [2.44% palladium on polyethyleneimine (PEI)/silica gel] proved uniquely effective.[16] Using this catalyst, we could double the concentration of substrate and reduce catalyst loading to 8 mol% without impacting conversion (Table 1, entry 8). Further optimization experiments were carried out with a more challenging substrate (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid (1b) (see Figure S2 and Tables S5–S11). Ultimately, we identified reaction conditions that were readily scaled to hydrogend 9 mmol (1.6 g) of 1a (Table 1, entry 9). The ease with which we could apply this transformation to larger scale reactions is notable, and may indicate that this general approach is suitable for preparative scale synthesis.

We used these optimal reaction conditions to evaluate functional-group compatibility, as it was unclear to what extent the presence of living organisms would impact substrate scope. Overall, the hydrogenation displayed broad utility for preparative-scale reactions of water-soluble alkenes (Scheme 1). An alkyne substrate (1k) also underwent exhaustive hydrogenation to the corresponding alkane. Most notably, 2-hexenedioic acid (1x) and Z,Z-muconic acid (1y) were converted into adipic acid, an important industrial chemical which is produced on a multimillion ton scale annually and has been a frequent but challenging target for metabolic engineering.[17] These results suggest that adipic acid could be obtained directly from fermentations by combining a biocompatible hydrogenation catalyst with organisms that produce hydrogen and an alkene such as 1y, which has already been generated by engineered microbes.[18]

Finally, we investigated how the biocompatible hydrogenation takes place and its impact on E. coli. A series of control experiments delineated the requirements for a successful reaction (Figure 2a and Table S12). We also quantified the hydrogen and formic acid produced in each reaction mixture, as both metabolites could potentially contribute to hydrogenation.[19] No reaction was observed in the absence of catalyst, thus confirming that E. coli cannot reduce 1a. The presence of E. coli was essential, which indicated that the organisms contribute a key reaction component. The low in the presence of E. coli DD-2 under a nitrogen atmosphere, thus relying on bacterial production of hydrogen gas (entries 5 and 6). We observed 15% conversion for the reaction run in M9 glucose, thus providing support for our general reaction design.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Growth medium</th>
<th>Cells/H2 added[a]</th>
<th>Catalyst (mol%)</th>
<th>Conv. [%][b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LB + glucose</td>
<td>no/yes</td>
<td>PtO2 (40)</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>M9 glucose</td>
<td>no/yes</td>
<td>PtO2 (40)</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>LB + glucose</td>
<td>yes/yes</td>
<td>PtO2 (40)</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>M9 glucose</td>
<td>yes/yes</td>
<td>PtO2 (40)</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>LB + glucose</td>
<td>yes/no</td>
<td>PtO2 (40)</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>M9 glucose</td>
<td>yes/no</td>
<td>PtO2 (40)</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>M9CA glucose</td>
<td>yes/no</td>
<td>PtO2 (20)</td>
<td>56</td>
</tr>
<tr>
<td>8</td>
<td>M9CA glucose</td>
<td>yes/no</td>
<td>Royer (8)</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>M9CA glucose</td>
<td>yes/no</td>
<td>Royer (8)</td>
<td>100/89</td>
</tr>
</tbody>
</table>

Reactions were performed at a 5 mmol substrate concentration in 5 mL of growth medium containing ampicillin (50 µg·mL⁻¹), spectinomycin (25 µg·mL⁻¹), chloramphenicol (12.5 µg·mL⁻¹), and isopropyl B-D-1-thiogalactopyranoside (IPTG; 500 µM) under an atmosphere of either hydrogen or nitrogen in 16 mL Hungate tubes with shaking at 190 rpm. [a] E. coli strain DD-2 was used, OD600 = 0.4. [b] Determined by 1H NMR spectroscopy. [c] M9CA glucose + Fe medium contains Fe(NH3)₄(SO4)₂ (50 µM) and casamino acids (5 g·L⁻¹). [d] Royer catalyst is 2.44 wt% palladium on polyethyleneimine/silica gel. [e] Reaction was performed on a 9 mmol scale (1.6 g of 1a) with 8 mol% Royer catalyst at a substrate concentration of 10 mm for 48 h (87% yield upon isolation).

Table 1: Proof-of-concept and reaction optimization experiments.
conversions observed for the IPTG control and a parental E. coli strain which cannot generate hydrogen support our hypothesis that hydrogen gas is the primary metabolite contributing to the transformation.[20] We also assessed the importance of catalyst–cell contact by sequestering the E. coli in dialysis cassettes. Hydrogenation still occurs, albeit with lower conversion, thus indicating that physical interaction is not a requirement (see Table S12, entry 11). However, E. coli is known to adsorb onto PEI, [21] and increased catalyst–cell proximity through this mechanism could contribute to the Royer catalyst/C29s superior utility relative to other heterogeneous catalysts.

The application of non-enzymatic catalysts and reagents in metabolic engineering requires that they do not significantly impede host growth and metabolism. To ascertain whether the E. coli survive the hydrogenation, we performed serial dilutions and plate counts directly from spent reaction mixtures, systematically omitting reaction components to assess biocompatibility (Figure 2b). Remarkably, we observed no significant difference in survival between reactions with and without catalyst. Together with the experiment exploring spatial separation, this result may indicate that the hydrogenation occurs outside of cells. Additional experiments to fully elucidate the factors influencing catalyst activity and compatibility will be the focus of future research.

In summary, this work demonstrates that the metabolic output of living microbes and a biocompatible non-enzymatic transformation may be combined to enable preparative scale chemical synthesis. Although this methodology cannot yet match the efficiency of more established approaches,[22,23] this advance represents a crucial first step in merging the fields of organic chemistry and metabolic engineering, and complements parallel efforts to engineer non-biological reactivity into enzyme scaffolds.[24] Ultimately, full integration of biocompatible reactions with cellular metabolism could provide access to chemical reactivity that would otherwise be out of reach in a cellular setting, and to molecules that could not be made using biological chemistry alone.

Keywords: biotransformations · hydrogenation · metabolism · palladium · synthetic methods
[16] After collecting all of the data described in this Communication, we discovered differences in activity between batches of commercially available Royer catalyst. Consequently we have developed a procedure for reactivating inactive catalyst lots and our own synthesis of this catalyst. For details see the Supporting Information.
[20] A reaction run with catalyst and formic acid (100 μM) in media without cells gave 9% conversion (Table S12, entry 10), thus suggesting that transfer hydrogenation may make a small contribution to conversion under our standard conditions.