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Bioinduced Room-Temperature Methanol Reforming**

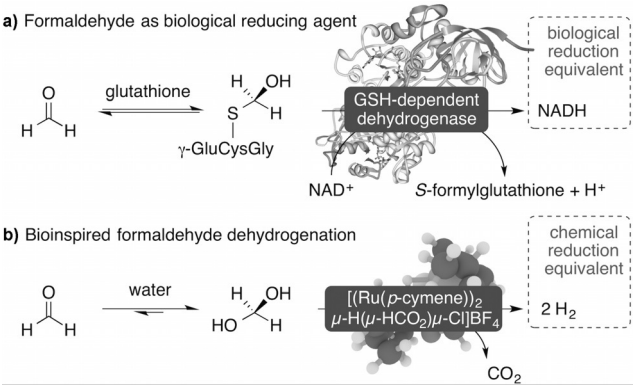
Leo E. Heim, Daniel Thiel, Christian Gedig, Jan Deska, and Martin H. G. Prechtl**

Dedicated to Professor Luis A. Oro on the occasion of his 70th birthday

Abstract: *Imitating nature's approach in nucleophile-activated formaldehyde dehydrogenation, air-stable ruthenium complexes proved to be exquisite catalysts for the dehydrogenation of formaldehyde hydrate as well as for the transfer hydrogenation to unsaturated organic substrates at loadings as low as 0.5 mol %. Concatenation of the chemical hydrogen-fixation route with an oxidase-mediated activation of methanol gives an artificial methylotrophic in vitro metabolism providing methanol-derived reduction equivalents for synthetic hydrogenation purposes. Moreover, for the first time methanol reforming at room temperature was achieved on the basis of this bioinduced dehydrogenation path delivering hydrogen gas from aqueous methanol.*

In light of anthropogenic global warming and the depletion of ecologically and economically reasonable sources for fossil fuels, in recent years, one-carbon molecules have emerged as highly attractive renewable hydrogen carriers.[1–11] Owing to difficulties in the selective activation of methane at low temperatures and the rather poor energy balance of formic acid, methanol is currently discussed as most promising C_1 unit.[12] Combining a high hydrogen content (12.5 wt %) with the convenience of a liquid fuel, catalytic methods for the dehydrogenation of methanol as well as its production from CO_2 and H_2 have attracted major interest from the scientific community over the past decade to establish a carbon-neutral methanol economy.[13] However, despite a number of successful examples on the methanol-to-H_2 conversion, in particular this process is still in great need for further improvement. Herein we present a conceptually unprecedented strategy for the hydrogen generation from aqueous methanol based on a multicatalytic system implementing enzyme catalysis as new player in the field.[14] The interplay of methanol-activating biocatalysts with H_2-liberating metal complexes is opening up new opportunities en route to ambient-temperature hydrogen-production systems or enzyme-driven hydrogen fuel cells.

In contrast to the recent history of ex vivo approaches utilizing the C_1 feedstock, millions of years of evolution have provided solutions to the problems of the activation and use of organic one-carbon entities by creating well-defined low-temperature pathways for methane- or methanol-feeding organisms.[15,16] Interestingly, as opposed to the chemical objective aiming for full dehydrogenation processes (e.g. aqueous methanol yielding the maximum three equivalents of H_2), in aerobic methylotrophic yeasts and bacteria, formaldehyde is found to play a central role in the cells' metabolism. Once formed by oxidase/catalase-catalyzed formal oxygenation of methanol, formaldehyde hydrate serves both as source of reduction equivalents, such as NAD(P)H, as well as the actual carbon feedstock for the generation of carbohydrates.[17] Dehydrogenation generally proceeds by nucleophilic activation of formaldehyde through enzyme cofactors, such as pterins (e.g. tetrahydrofolate) or mercaptanes (e.g. glutathione (GSH)).[18] In particular, the mechanism of GSH-dependent formaldehyde dehydrogenation is found as highly conserved motif even in higher eukaryotes playing a key role in the detoxification systems.[19,20] In this case, GSH adds reversibly to formaldehyde to generate *S*-(hydroxymethyl)-glutathione.[21,22] Tight binding of the hemithioacetal GSH-



Scheme 1. Nucleophile-activated formaldehyde dehydrogenation: a) biotic methylotrophic NADH formation and b) artificial ruthenium-catalyzed H_2-generation from tetrahedral formaldehyde conjugates.[25]

[*] M.Sc. L. E. Heim, Dipl.-Chem. D. Thiel, C. Gedig, Priv.-Doz. Dr. J. Deska,[+] Priv.-Doz. Dr. M. H. G. Prechtl
Department Chemie, Universität zu Köln
Greinstrasse 4-6
50939 Cologne (Germany)
E-mail: martin.prechtl@uni-koeln.de
Homepage: http://www.catalysislab.de

[+] Current address: Department of Chemistry, Aalto University
Kemistintie 1, 00076 Espoo (Finland)
E-mail: jan.deska@aalto.fi

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conjugate to the zinc-dependent dehydrogenases results in Meerwein–Ponndorf–Verley-type transfer hydrogenation forming NADH and *S*-formylglutathione (Scheme 1 a), with the *S*-formylglutathione being subsequently hydrolyzed liberating formic acid to serve as the second reduction equivalent.[23,24] While biogenic formaldehyde transfer hydrogenation requires complex cofactors to facilitate the reduction of $NAD(P)^+$, much more simplistic settings are easily conceivable in an abiotic environment allowing for nucleophiles as simple as water to be involved in the formaldehyde activation. In aqueous solution, formaldehyde is fully hydrated as methanediol with an equilibrium constant of $K = 2 \times 10^3$.[25] With the development of air- and water-stable transition-metal complexes acting on formaldehyde hydrate, this highly energetic, yet widely unheeded, small molecule ought to become an important player within the family of low-molecular-weight organic hydrogen equivalents. Most recently, we demonstrated that methanediol (formalin) can indeed be considered a high-potential H_2 source as dehydrogenation in presence of $[\{Ru(p\text{-cymene})Cl_2\}_2]$ (**1**) as the precatalyst provides two equivalents of pure hydrogen gas along with one equivalent of CO_2 (Scheme 1 b).[25] In this bioinspired approach, apparently formaldehyde enables water splitting via methanediol formation delivering H_2 which originated both from H_2O and HCHO. A major advantage of formalin in comparison to formic acid or isopropanol is particularly its superior hydrogen content accounting for 8.4 wt % H_2 as opposed to 4.4 wt % (HCO_2H) and 3.3 wt % (*i*PrOH), respectively, a result of the feasibility to deliver two equivalents of hydrogen from a one-carbon entity.

In analogy to the natural model in which formaldehyde-derived NAD(P)H serves as general reducing agent within the metabolism, we envisioned that in the presence of suitable organic acceptor molecules direct or indirect transfer of hydrogen from formalin should occur. In our previous mechanistic investigations about the composition of the liquid and gaseous phase in ruthenium-mediated formalin reforming, we found that $[\{Ru(p\text{-cymene})Cl_2\}_2]$ (**1**) is readily converted into $[(Ru(p\text{-cymene}))_2\mu\text{-}H(\mu\text{-}HCO_2)\mu\text{-}Cl]Cl$ (**2**-Cl) in presence of formalin or formic acid. Both complexes are active for the dehydrogenative decomposition of methanediol into H_2 and CO_2, and most likely complex **2**-Cl plays a key role in the catalytic cycle for dehydrogenation and hydrogenation processes.[25] In our studies about the decomposition of methanediol using the ruthenium dimers **1** and **2**-X (X = Cl or BF_4), we found that this approach is also rather generally applicable for transfer hydrogenation reactions. In an adaptation of our hydrogen generation approach using methanediol, formalin-derived hydrogen can be smoothly transferred to, for example, acetophenone by the same catalyst, and most notably, 1-phenylethanol is formed even at room temperature in concentrated as well as in diluted aqueous formalin (Supporting Information, Table S1). To elucidate the underlying processes, kinetic studies on the dehydrogenation and hydrogenation steps were conducted in a decoupled fashion in separate reaction vessels (Figure 1 a). Under the assumption that methanediol decomposition proceeds initially at normal pressure, H_2-formation was measured in the absence of the

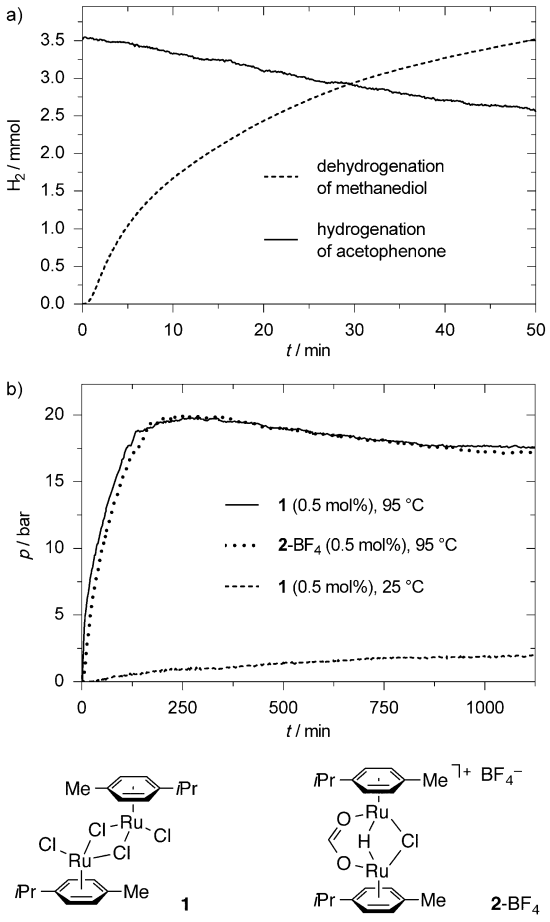
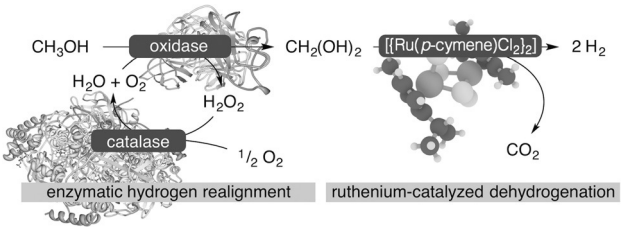


Figure 1. Gas-phase analysis: a) decoupled kinetics of hydrogen formation and consumption using precatalyst **1** and b) pressure profiles of transfer hydrogenations of acetophenone using **1** or **2**.

ketone acceptor in a flow-setup providing rates of dehydrogenation with an initial turnover frequency of 31.5 min^{-1}. Additionally, rates of hydrogen consumption were obtained by the hydrogenation of acetophenone in the absence of formalin under 12 bar H_2 atmosphere resembling the partial hydrogen pressure observed in coupled transfer hydrogenations. The lower turnover frequency of 2.6 min^{-1} of this process is also reflected in the pressure profile of the transfer hydrogenations (Figure 1 b). Both with the neutral complex **1** as well as the cationic species **2**-BF_4, a sharp pressure increase was observed cause by the fast decomposition of formalin to H_2 and CO_2 towards a maximum pressure of 20 bar. From here, with the lower rate of acetophenone reduction, the pressure decreases in a shallow slope reaching a plateau at 17 bar after 18 h. These results for the transfer hydrogenation indicate that dehydrogenation and hydrogenation are operating as two independent processes rather than a Noyori-type direct hydrogen-transfer reaction.[26] Strikingly, the constant increase in pressure even at room temperature caused by formation of H_2 and CO_2 underlines the high potential of this simple catalytic arrangement particularly in dehydrogenative processes making it a very promising system in the context of C_1-to-H_2-reforming.

Although homogeneously catalyzed direct methanol reforming is no longer beyond reach, current processes are still hampered by operational handicaps, such as excessive base-loading or high-temperature requirements.[6–9] On the other hand, nature does not only teach us how to circumvent such obstacles and perform C_1-dehydrogenation under physiological conditions. With the enormous progress in the field of biotechnological protein production, natural tools are becoming broadly available and enzyme-catalyzed transformations gradually enter the chemist's toolbox.[27,28] We envisioned that the lack of reactivity of **1** and **2** towards methanol at ambient temperature could be compensated in a chemoenzymatic approach combining biocatalytic methanol activation with the use of the thus formed methanediol in subsequent metal-catalyzed dehydrogenative processes. In this case, alcohol oxidases from methylotrophic organisms would provide the activity for the room-temperature dehydrogenation. In combination with a catalase, methanol is directly converted into formaldehyde hydrate under net consumption of one atom of aerial oxygen. After successful methanediol accumulation, four hydrogen atoms would be available a) for transfer hydrogenation to organic substrates by precatalyst **1** resulting in an artificial methanol metabolism or b) for acceptorless dehydrogenation resembling formal methanol reforming on the basis of an enzyme–metal-coupled catalytic cascade (Scheme 2).[29]



Scheme 2. Chemoenzymatic interpretation of the methanol metabolism found in methanol-feeding microorganisms.

To identify potential biocatalysts for the activation of methanol, four commercial hydroxy oxidases were evaluated with regard to their activity in the methanediol formation. Aiming for a practical low-dilution overall process, a high methanol concentration (2.0 M) was chosen as the critical parameter to validate the biocatalysts' performance (Figure 2). The two oxidases tested, which act on sugars as their native substrate, failed, galactose oxidase (*Dactylium dentroides*) exhibited only marginal activity with $v_{max} = 2.3\ nmol\,min^{-1}\,mg^{-1}$ while glucose oxidase (*Aspergillus niger*) did not provide any measurable turnover. Short-chain alcohol oxidases on the other hand converted methanol at rates that were higher by several orders of magnitude.[30] The good activity of oxidase from *Candida boidinii* ($v_{max} = 1.7\ \mu mol\,min^{-1}\,mg^{-1}$) was impressively surpassed by *Pichia pastoris* oxidase with an initial rate of $29.1\ \mu mol\,min^{-1}\,mg^{-1}$. However, on a preparative scale, in combination with catalase from *Corynebacterium glutamicum* inducing disproportionation of the liberated hydrogen peroxide, oxidase from *C. boidinii* proved to be more robust and reliable reaching

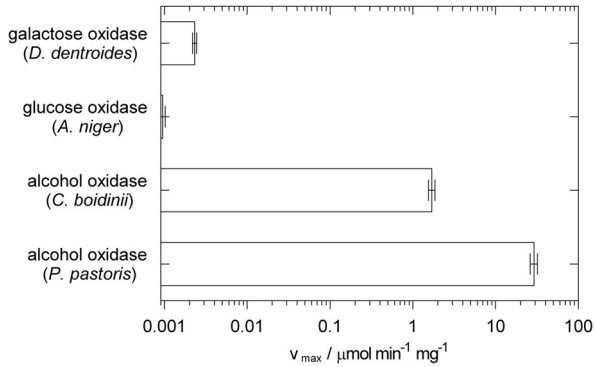


Figure 2. Activity screening of commercial oxidases under substrate stress conditions: Initial rates of methanol conversion at high concentration (2.0 M MeOH) were recorded photospectrometrically using horseradish peroxidase/ABTS as a reporter system.

a final methanediol concentration of 0.53 M (± 0.04 M) after 24 h while formalin production using *P. pastoris* oxidase already terminated at 0.08 M concentration.

To our delight, the biocatalytically produced formalin could also be used as reducing agent in the Ru-catalyzed transfer hydrogenation of acetophenone. However, direct use of the untreated protein solution resulted in low conversion (18% after 48 h), owing to catalyst deactivation in presence of protein at elevated temperature (95 °C). While the development of more robust (de)hydrogenation catalysts will be an important requirement on the longer run, simple protein removal by means of membrane-based techniques would allow for the generation of more catalyst-friendly biogenic methanediol. Incubation of aqueous methanol employing the previously identified biocatalytic cocktail in a membrane reactor followed by ultrafiltration (MWCO = 10 kDa) gave rise to an easily processed formalin-enriched solution. Subsequent hydrogen transfer using ruthenium dimer **1** (5 mol%) at 95 °C was not only successful for acetophenone (72% conv.) but even also in the reduction of cyclooctene where full conversion was observed (Supporting Information, Scheme S1).

Currently, the prime limitations of a bioinduced transfer hydrogenation lies in the relatively poor hydrogenation abilities of the dehydrogenation precatalyst **1**, which result in the need for high catalyst loading and elevated temperatures. We speculated that the acceptorless dehydrogenation of the enzymatically derived methanediol should be more feasible even with a lower energy input, thus providing a room-temperature pathway for the catalytic methanol reforming. In a first experiment, untreated aqueous methanol was added to complex **1** (5 mol%) and the solution was stirred at 25 °C in a sealed autoclave for 24 h, but no change in gas composition or internal pressure was detected (Figure 3a). In contrast, using the same solution after preincubation with the oxidase/catalase-system, gas formation commenced instantaneously as indicated by a steady increase in pressure. Headspace GC-TCD analysis of the gas phase confirmed the generation of hydrogen in substantial amounts (Figure 3b). To our delight, not only the decomposition of the membrane-filtered formalin generated a hydrogen-enriched

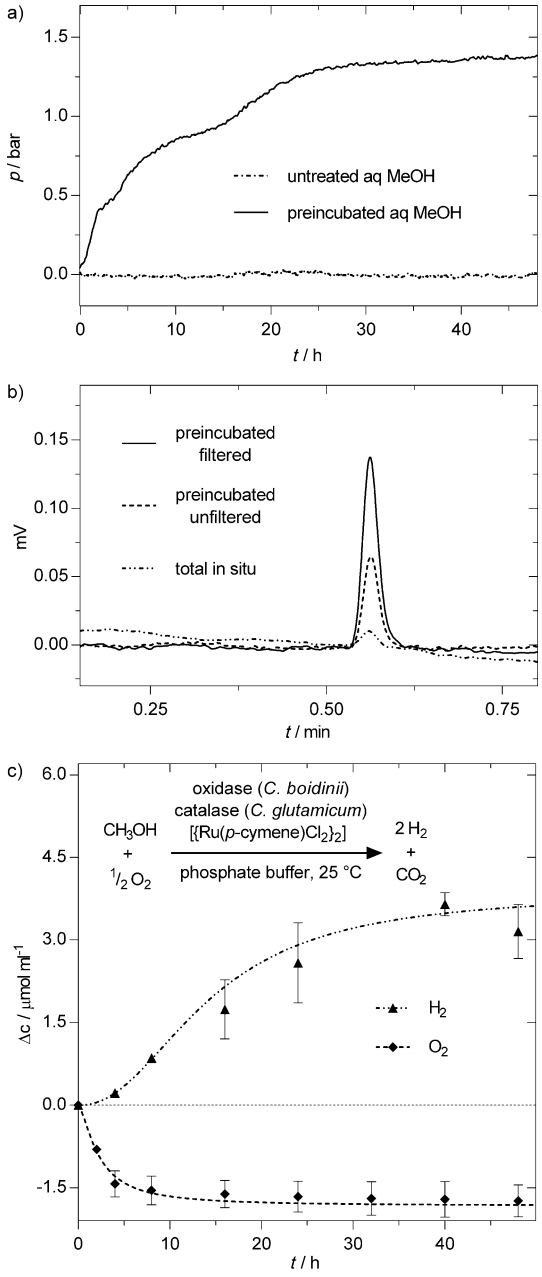


Figure 3. Bioinduced methanol dehydrogenation: a) pressure profile of the enzyme-driven metal-catalyzed methanol decomposition. b) Chromatographic traces of headspace GC-TCD for the quantification of the generated hydrogen gas from preformed formalin (filtered and unfiltered) and one-pot in situ formed formaldehyde solution. c) Time-resolved monitoring of the hydrogen and oxygen content in the gas phase in the one-pot chemoenzymatic methanol reforming.

gas phase (15.3 μmol mL^{-1} H_2), the experiments further revealed that also treatment of enzyme-containing aqueous methanediol with precatalyst **1** led to effective dehydrogenation (8.7 μmol mL^{-1} H_2) (Figure 3c). Most remarkably, even co-incubation of oxidase, catalase, and [{Ru(*p*-cymene)Cl_2}$_2$] in aqueous methanol at room temperature resulted in detectable amounts of hydrogen in the headspace (3.2 μmol mL^{-1} H_2), unequivocally demonstrating that chemoenzymatic methanol reforming is feasible in a one-pot fashion. In a closed aerial atmosphere, time-resolved monitoring of the gaseous constituents showed fast consumption of oxygen as expected for the oxidase-catalyzed methanol oxygenation. At lower rates of dehydrogenation, metal-induced hydrogen liberation is recorded proving a catalyst lifetime of more than 24 h (Figure 3c), although inhibitory effects still prevent quantitative conversions (see also Supporting Information, Figure S16). Regarding compatibility issues, modern immobilization and protein-hybrid techniques will be exploited in future studies to further improve the efficiency of this enzyme–metal-coupled system.[31,32]

Bringing together enzymatic and metal catalysis, we were able to construct the first effective system to perform methanol reforming at room temperature in aqueous media. Identification of suitable biocatalysts for the activation of methanol in combination with a novel bioinspired ruthenium-catalyzed dehydrogenation method employing the in situ formed methanediol as the active hydrogen species proved to be the key to overcome the high-temperature constraint of purely metal-based approaches. As a proof-of-principle study, this conceptual work will serve as a foundation for the development of more efficient chemoenzymatic H_2-generating systems exploiting other modes to couple bio- and metal catalysts to cope with the current formaldehyde-related concentration limitations.

Keywords: biocatalysis · bioinspired reactions · chemoenzymatic reactions · hydrogen · ruthenium

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