Transition-Metal-Catalyzed Regeneration of Nicotinamide Coenzymes with Hydrogen[1]

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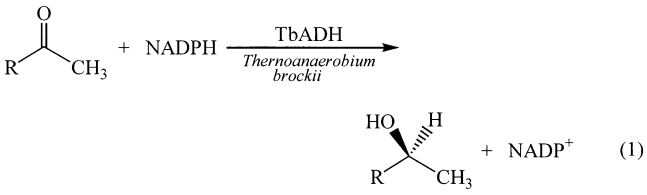
Summary: The nicotinamide coenzymes consumed in alcohol dehydrogenase catalyzed enantioselective reduction of ketones are expensive and thus require recycling. We have demonstrated that ruthenium(II) and rhodium(III) complexes are effective catalysts for the reduction of nicotinamide coenzymes with hydrogen, under conditions that are appropriate for in situ coupling with enzymatic reductions.

The nicotinamide coenzymes NADH and NADPH (Figure 1) belong to a significant class of biomolecules which, in combination with appropriate enzymes, catalyze numerous biochemical oxidations and reductions. These enzymes have attracted widespread interest, due to their potential applications in organic synthesis.[2] For instance, certain enzymatic reductions such as the conversion of simple ketones to chiral alcohols (eq 1) achieve stereoselectivities unmatched by chemical methods.[3,4]



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The nicotinamide coenzymes consumed in stoichiometric quantities in such reductions are very expensive. Practical considerations thus require recycling the oxidized form of the coenzyme to its reduced dihydropyridine counterpart. To be viable, a recycling system must generate reduced coenzyme with very high chemical yield and 1,4-regioselectivity under conditions that are compatible with both the coenzyme and the enzyme. Likewise, a preferred recycling reagent must be inexpensive and not yield byproducts which complicate product purification. Chemical, photochemical, electrochemical, and enzymatic recycling methods have been developed but tend to suffer from cumbersome reaction conditions, expensive reagents, and/or unwanted side products.[5] Historically, enzymatic recycling with formate dehdyrogenase has been preferred for commercial applications,[5b] but recently enzymatic recycling using alcohol dehydrogenase and a sacrificial alcohol has also proven economically viable.[6]

Regenerating NAD(P)H using dihydrogen (H_2) is preferred, because H_2 is the lowest cost reducing agent and it yields no requisite byproducts (i.e. both atoms of hydrogen are delivered to the reduction products). Previous reports on such use of H_2 involve highly air-sensitive hydrogenase enzymes[7] or complex systems in which H_2 first reduces an intermediate "hydride carrier", which subsequently reduces a nicotinamide coenzyme.[8] Herein we present simple transition-metal catalysts for the *direct* reaction between H_2 and $NADP^+$ under conditions, as listed below, appropriate for in situ coupling of NADPH regeneration to an enzymatic reduction: (i) high selectivity for reduction of the nicotinamide coenzyme vs the enzymatic substrate, (ii) high regioselectivity yielding a 1,4-dihydropyridine, (iii) mild reaction temperatures, preferably 25−65 °C, and (iv) reaction in an aqueous medium in a pH range of 6.5−8.5.[9]

We believed that screening catalysts on the basis of whether they catalyze the reduction of the NAD model compound benzyl nicotinamidium chloride, BNACl, under these conditions would be relevant to NAD(P)H regeneration. Reports of the reduction of BNA^+ using H_2,[10] formate,[11,12] dithionite,[13] or photochemical methods[14] exist, but to our knowledge there are no reports on the reduction of BNA^+ with H_2 in aqueous media.

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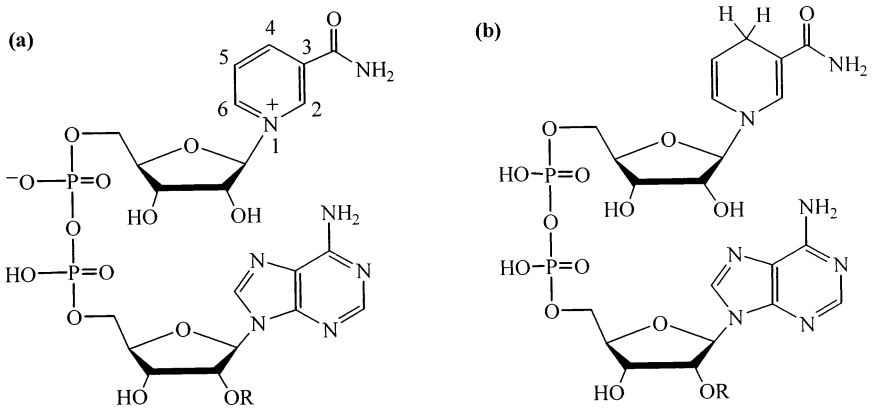
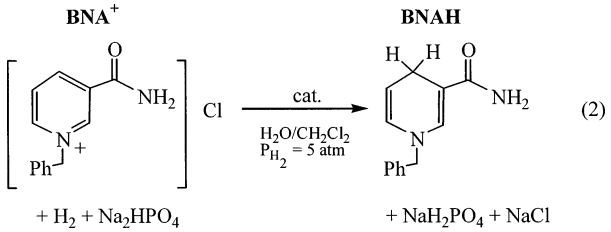


Figure 1. Structures of nicotinamide coenzymes: (a) oxidized forms, NAD^+ (R = H) and $NADP^+$ (R = PO_3H_2), and (b) reduced forms, NADH (R = H) and NADPH (R = PO_3H_2).

One candidate for catalysis of BNA^+ reduction is $[RuCl_2(TPPTS)_2]_2$ (TPPTS = tris(*m*-sulfonatophenyl)-phosphine), a water-soluble catalyst for the reaction of H_2 with carbohydrates under relatively mild conditions.[15] Another candidate is $[Cp^*(bipy)Rh(H_2O)]Cl_2$ (Cp^* = pentamethylcyclopentadienide, bipy = 2,2′-bipyridine), pioneered by Steckhan[16] and Fish[12] for the reduction of nicotinamides by formate. We have found that both of these complexes are active for the aqueous hydrogenation of BNA^+ within the constraints set out above and subsequently that the complexes show analogous behavior with nicotinamide coenzymes.

In a typical BNA^+ reduction a Fisher−Porter bottle was charged with BNACl, phosphate buffer, CH_2Cl_2, and the catalyst. The mixture was degassed, charged with H_2 (5 atm), and stirred at room temperature (eq 2). The purpose of the two-phase (H_2O/CH_2Cl_2) system



(2)

is to segregate the hydrogenation catalyst from the primary reduction product, BNAH. BNACl and the catalyst are water soluble, but BNAH has very limited water solubility. During the reaction, the BNAH is continuously extracted into the organic layer. Buffer is necessary to control the pH by neutralizing the H^+ produced in such a stoichiometric reduction of pyridinium salts. After the mixture was stirred for 4 h, the methylene chloride layer was removed and the aqueous layer extracted once with CH_2Cl_2. After the solvent was removed from the combined organic layers, the residue was analyzed by ^1H NMR spectroscopy. For the case with $[RuCl_2(TPPTS)_2]_2$ as the catalyst, 1,4-BNAH was the major product with detectable amounts of the over-reduced 1,4,5,6-tetrahydropyridine[17] and a third unidentified species.[18] The BNAH accounted for 75% of the total reduced species by ^1H NMR integration, corresponding to 11 turnovers per Ru.[19]

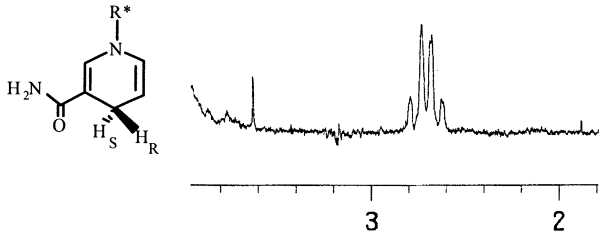


Figure 2. AB quartet (300 MHz ^1H NMR, D_2O) of the C_4 methylene of NADPH produced using H_2 and $[Cp^*Rh(bipy)(H_2O)]Cl_2$. The NADPH yield was determined by integration of this band relative to sodium trimethylsilylpropionate (int. std).

Given the success with BNA^+ under physiological conditions, it seemed reasonable to expect that $NADP^+$ could be reduced in a similar manner without the need for a second phase (NADPH is water soluble due to the dinucleotide−pyrophosphate backbone). As in the BNA^+ reduction, a Fisher−Porter bottle was charged with $NADP^+$ and phosphate buffer and the pH was adjusted to 8.3 with NaOH. Catalyst was added and the mixture degassed, charged with 5 atm of H_2, and stirred for 2−3 h at 40 °C. The characteristic AB quartet of NADPH at δ 2.78 in the ^1H NMR spectrum confirmed the production of NADPH for both catalysts in quantities corresponding to 3−4 turnovers (Figure 2). The rates for both catalysts were similar, but the Ru catalyst trials resulted in slightly more impurities than the trials with the Rh catalyst. Control experiments in the absence of

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(17) The ^1H NMR signals match those for 1,4,5,6-dihydropyridine.[10]

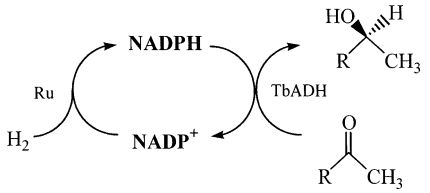
(18) (a) The ^1H NMR of this species shows fine structure similar to that of the 1,4-product. It does not match literature values for 1,6-dihydropyridine,[18b] BNA dimers,[18c] or Dittmer's trimer.[18d] (b) Lovesay, A. C.; Ross, W. C. J. *J. Chem. Soc., Chem. Commun.* **1969**, 192−195. (c) Carelli, V.; Liberatore, F.; Casini, A.; Tortorella, S.; Scipione, L.; Di Rienzo, B. *New J. Chem.* **1998**, 999−1004. (d) Minato, H.; Okuma, K.; Kobayashi, M. *Chem. Lett.* **1977**, 525−528.

(19) The total quantity of the organic residue accounted for 77% consumption of BNACl.

H_2 or catalyst established that both are necessary for coenzyme regeneration.

As an initial test to determine if this system is effective in conjunction with NADPH-dependent enzymatic reductions, we have performed the reduction of 2-heptanone to (*S*)-2-heptanol using the alcohol dehydrogenase from TbADH with a catalytic amount of $NADP^+$ and $[RuCl_2(TPPTS)_2]_2$. After the sample was stirred at 60 °C under 5 atm of H_2 for 4 h, gas chromatography showed that 18% of the 2-heptanone had been converted to 2-heptanol, corresponding to 10 turnovers of both $NADP^+$ and ruthenium. Chiral gas chromatography showed that the 2-heptanol formed after 18 turnovers was 70% *S* and 30% *R* (40% ee). This ee, though lower than the capabilities of the enzyme,[3] demonstrates conclusively that the enzyme is involved in the reduction. Additionally, the turnover numbers, though not impressive, demonstrate that the reduction is catalytic in both $NADP^+$ and ruthenium. Thus, we have demonstrated that this coenzyme recycling system does operate under conditions appropriate to in situ coupling with a reductase enzyme (Scheme 1).

Scheme 1



A discussion of the lower than optimum enantioselectivity is warranted. Control experiments suggest that $NADP^+$ reduction is approximately 3 times faster than the direct reduction of 2-heptanone with the catalyst and hydrogen, suggesting that the majority of 2-heptanol should be formed through the enzymatic path. Some racemic product can result directly from this competition, but additionally, $NADP^+$ likely degrades during the reaction due to over-reduction, compromising the fraction of reduction accomplished by the enzyme as a function of time.

There are two key results here. Foremost is that a simple system for the direct coupling of H_2 to nicotinamide coenzyme regeneration under conditions appropriate for in situ coupling with an oxidoreductase enzyme has been developed. Second is that screening catalyst component mixtures on the basis of their ability to catalyze the reduction of BNA^+ with H_2 under physiological conditions has proven useful in catalyst development. The advantages of H_2 over other reducing agents are significant, namely that all other reagents result in the production of unwanted byproducts and/or the need for continuous addition of protons when coupled to an enzymatic reduction. Dihydrogen, on the other hand, provides both the hydride source and proton source needed for enzymatic reductions. Though the system reported here is not the first to use H_2, its advantage lies in its simplicity.

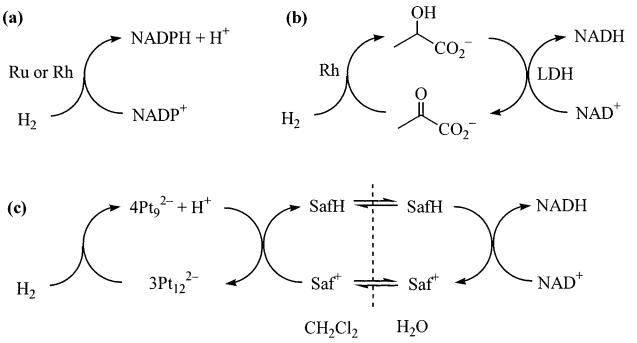


Figure 3. Comparison of coenyme regeneration systems using H_2: (a) this work; (b) ref 8a; (c) ref 8b.

A previously reported coenzyme recycling scheme utilized H_2, a water-soluble Rh catalyst, *and* lactate dehydrogenase (LDH) to regenerate NADH (Figure 3b).[8a] The Rh complex catalyzes the hydrogenation of pyruvate to lactate; then LDH catalyzes the reduction of NAD^+ by lactate. The NADH was then used for the horse liver alcohol dehydrogenase (HLADH) catalyzed reduction of cyclohexanone. The turnover rate of this system is slow and, as the authors state, the system is "not entirely straightforward to operate" and suffers from the instability of the Rh catalyst. Furthermore, the use of cyclohexanone as a substrate precludes a demonstration that this catalyst system can produce chiral alcohols without racemization. Recently Bhaduri et al. have also reported a system using two components to catalyze the reduction of NAD^+ by H_2.[8b] This system activates H_2 with a platinum cluster catalyst partitioned into an organic phase. The reduced cluster transfers redox equivalents to Safranine O, which migrates to the aqueous phase where NAD^+ reduction occurs (Figure 3c).

The simple system reported herein draws attention to a *direct* approach for coupling hydrogen to nicotinamide coenzyme regeneration. The enantioselectivity of the TbADH catalyzed ketone reduction under these conditions is less than perfect and poses a challenge to discover more selective catalysis. The rates of the best systems described to date, vide supra, are too slow to be viable, but that reported herein holds promise due to its simplicity and atom economy. With the wealth of complexes that show reactivity with H_2, it is possible that further study will identify a catalyst that competes with commercial technologies for coenzyme recycling.

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Supporting Information Available: Text giving complete experimental details for the reductions and figures giving the full ^1H NMR spectrum from the $NADP^+$ reduction using $[Cp^*Rh(bipy)(H_2O)]Cl_2$ and comparison spectra of pure $NADP^+$ and NADPH. This material is available free of charge via the Internet at http://pubs.acs.org.

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