Hydrogenase Enzyme Reactivity Modeling with a Transition-Metal Dihydrogen Complex

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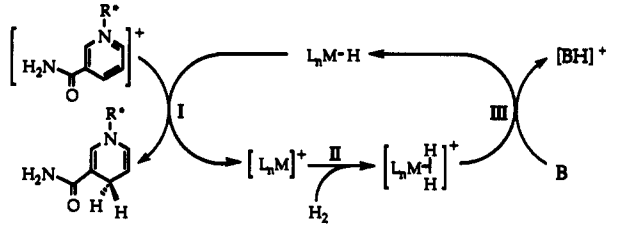
Hydrogenase enzymes catalyze both the "uptake" and formation of hydrogen by microbes.[1] Recent focus on the role of nickel sites in such enzymes has included the speculation that both metal hydrides[2] and metal dihydrogen complexes[3] are intermediates in the activation of H_2 by these systems. Despite controversy with respect to the exact structure and function of the nickel site,[4] a key metabolic role of hydrogenase enzymes is the transfer of reducing equivalents from H_2 to biologically active redox cofactors such as NAD^+. We report herein the first direct catalytic reduction of an NAD^+ model compound with H_2 at ambient pressure and temperature and demonstrate, via isolated stoichiometric reactions, each of the proposed steps of catalysis: hydride transfer, hydrogen coordination, and hydrogen activation (Scheme 1). This catalysis provides the first well-characterized reactivity model illustrating the cooperative roles of a molecular hydrogen complex and a transition-metal hydride as a functional model of hydrogenase enzymes.[5]

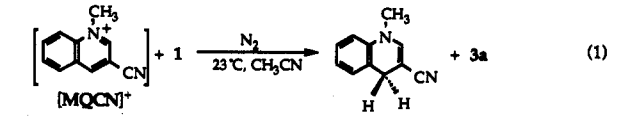
As indicated in Scheme 1, we associate three primary reactions: (I) hydride transfer, (II) H_2 coordination, and (III) H_2 deprotonation with three processes that are catalyzed by hydrogenases: (i) the transfer of reducing equivalents to relevant redox cofactors, (ii) the consumption and/or formation of H_2, and (iii) the exchange of hydrogen isotopes between dihydrogen and water. We have found that Cp*(dppm)RuH (**1**, Cp* = $C_5(CH_3)_5$; dppm = $Ph_2PCH_2PPh_2$) and its related H_2 complex $[Cp^*(dppm)Ru(H_2)]^+$ (**2**)[6] are competent in all of these functions.

A hydride transfer is required to reduce NAD^+ to NADH. Detailed model studies of hydride transfer from dihydropyridines to pyridinium salts have been reported,[8] but little is known about the reaction of metal hydrides with these important substrates.[9] We have observed the quantitative and regioselective reactions of **1** with NAD^+ models such as 3-cyano-*N*-methylquinolinium ($[MQCN]^+$, eq 1)[10] and *N*-methylacridinium ($[MA]^+$, eq 2) salts

Scheme 1

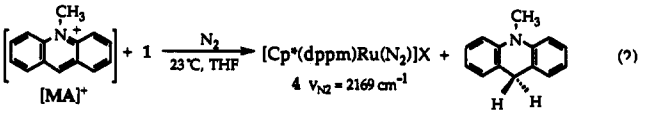


yielding MQCNH and MAH, respectively, and [Cp*(dppm)-Ru(S)][PF_6] (S = CH_3CN (**3a**) eq 1; THF (**3b**)).[11]



(1)

As a hydride abstraction reagent, [MA]PF_6 is a convenient alternative to the trityl cation[12] (it is inexpensive, easy to prepare, and may be stored in air at room temperature for long periods). When the reaction of [MA]PF_6 or the trityl cation with **1** is carried out in THF under N_2, a new Ru(II)–N_2 complex (**4**) can be isolated (eq 2).[13,14] Under an atmosphere of Ar, hydride abstraction yields a labile THF complex (**3b**).



(2)

To test the nature of the hydride transfer in reaction 2, the reduction of [MA]PF_6 with **1** was carried out in the presence of an electron-transfer inhibitor, [MV][PF_6]$_2$ (MV = methyl viologen). Despite a one-electron reduction potential that is lower than that of $[MA]^+$ ($E°(MV^{2+}/MV^+, CH_3CN)$ = –178 mV[16] and $E°(MA^+/MA^\cdot, CH_3CN)$ = –224 mV[17] vs NHE), a 5-fold excess of $[MV]^{2+}$ has no effect on the course of [MA]PF_6 reduction. In addition, the products formed by reaction of **1** with [MA]PF_6 do not change as the relative ratio of [MA]PF_6:**1** is varied. With either an excess of substrate ([MA]PF_6:**1** ≫ 1.0) or an excess of metal hydride ([MA]PF_6:**1** < 1.0), the products are always MAH and **3**. In contrast, when trityl cation is the

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(5) The reaction of H_2 with hydrogenases (uptake activity) is coupled to the formation of NADH or other reduced cofactors (reductase activity), but there is, thus far, no evidence that these functions are performed at a single site. Association of low-potential redox components with hydrogenases[1] implies that electron transfer couples uptake and reductase sites.
(6) An extensive family of H_2 complexes of the general formula $[CpL_2M-(II)]^+$ (M = Fe, Ru, Os) have been developed[7a,b] following the pioneering report of Simpson on the protonation of $Cp(Ph_3P)(t\text{-}BuNC)RuH$.[7c] The synthesis of **1** ($E°$ **1**, Ru(III/II) = +350 mV vs NHE), structural structural studies of **2**, and the acidity of **2** (pK_a(THF) = 9.2) and **2'** (pK_a(THF) = 8.7) have been reported: (a) Jia, J.; Morris, R. H. *J. Am. Chem. Soc.* **1991**, *113*, 875–83. (b) Jia, G.; Lough, A. J.; Morris, R. H. *Organometallics* **1992**, *11*, 161–71. (c) Kooster, W. T.; Koetzle, T. F.; Morris, R. H. *Abstracts of Papers*, 51st Annual Meeting of the American Crystallography Association; Albuquerque, NM, 1993; PE17.
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(10) High regioselectivity was determined by ^1H NMR comparison to borohydride reduction of $[MQCN]^+$, which yields a mixture of 1,2- and 1,4-MQCNH products: Roberts, R. M. G.; Kreevoy, M. M. *J. Org. Chem.* **1983**, *48*, 2053–56.
(11) (a) Addition of dppm to $[Cp^*Ru(CH_3CN)_3]OTf$ also yields **3a**: ^1H NMR (CD_3CN) δ 7.2–7.6 (m, 20 H), 5.18 (dt, *J* = 16.0, 10.1 Hz; 1 H), 4.43 (dt, *J* = 16.0, 11.1 Hz; 1 H), 1.57 (t, *J* = 2.1 Hz; 15 H); $\{^1H\}^{31}P$ NMR δ 10.48; IR(CH_2Cl_2) ν_{CN} 2264. (b) **3b**: ^1H NMR (THF-d_8) δ 7.2–7.6 (m, 20 H), 4.8 (br s, 2 H), 1.68 (s, 15 H); $\{^1H\}^{31}P$ NMR δ 7.3. (c) **3c**: ^1H (THF-d_8) 7.2–7.6 (m, 20 H), 5.55 (dt, *J* = 16.0, 10.1 Hz; 1 H), 4.34 (dt, *J* = 16.0, 11.1 Hz; 1 H), 3.84 (br s, 2 H), 1.55 (s, 15 H); $\{^1H\}^{31}P$ NMR δ 11.2.
(12) (a) Beck, W.; Sünkel, K. *Chem. Rev.* **1988**, *88*, 1405–21. (b) Ryan, O. B.; Tilset, M. *J. Am. Chem. Soc.* **1991**, *113*, 9554–61.
(13) This is the first reported example of an N_2 complex counterpart to an H_2 complex of the $[CpL_2M]^+$ (M = Fe, Ru, Os) family.[7] The ν_{N_2} of **4** (2169 cm^{-1}) is slightly higher than is common for Ru(II) dinitrogen complexes (2060–2150 cm^{-1})[15a,b] but in reasonable agreement with the prediction of a stable H_2 complex based on the ν_{N_2} of its analogous N_2 complex.[15c]
(14) Treatment of a THF solution of **4**, generated by reaction of **1** with [MA]PF_6 under N_2, with Et_2O yields light yellow microcrystals in 85% yield: ^1H NMR (THF-d_8) δ 7.6–7.2 (m, 20 H), 5.57 (m, 1 H), 4.88 (m, 1 H), 1.59 (s, 15 H); $\{^1H\}^{31}P$ NMR δ 3.45; IR(CH_2Cl_2) ν_{N_2} 2169. Anal. Calcd for ($C_{35}H_{37}F_6N_2P_3Ru$): C, 52.98; H, 4.70. Found: C, 52.74; H, 4.38.
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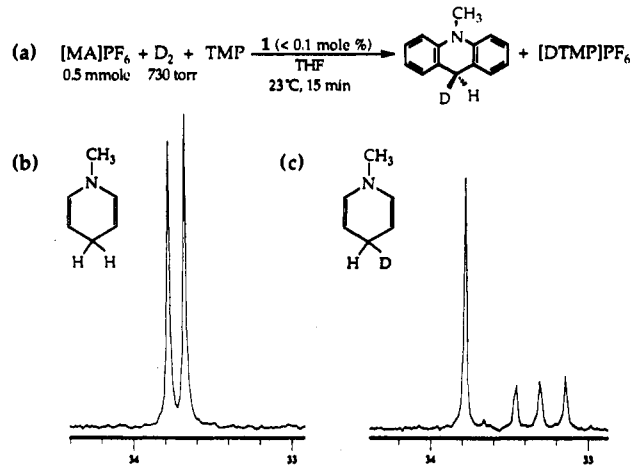


Figure 1. (a) Deuteration of $[MA]PF_6$ catalyzed by **1** and ^{13}C NMR (CD_3CN) spectra with gated decoupling of C-9 in (b) MAH (δ C-9 33.65, N-CH_3 33.80) vs (c) (9-2*H*)-9,10-dihydro-10-methylacridine, MAD (δ C-9 33.31, N-CH_3 33.80).

substrate, net hydride transfer yields tritane and **3** under catalytic conditions ($[Ph_3C]BF_4$:**1** $\gg$ 1) but also trityl dimer and new ruthenium products (including **2** and a dimeric Ru hydride) when excess metal hydride is present ($[Ph_3C]BF_4$:**1** < 1.0). Thus, our evidence supports a single-step hydride transfer from **1** to pyridinium salts and a multistep process for trityl cation, which is a much stronger one-electron oxidant ($E°(Ph_3C^+/Ph_3C^\bullet$, DMSO) = +280 mV).[18] The mode of hydride transfer from **1** is substrate dependent.

The N_2 in **4** is readily displaced by H_2, yielding the **2**/**2'** dihydrogen/dihydride complex equilibrium (eq 3).[19] Although

$$\mathbf{4} + H_2 \xrightarrow[-N_2]{} \underset{\mathbf{2}}{[Cp^*(L_2)Ru(H_2)]X} + \underset{\mathbf{2'}}{[Cp^*(L_2)RuH_2]X} \quad (3)$$

this reaction is irreversible at ambient pressure, the lability of H_2 in **2**/**2'** at 23 °C is revealed by its exchange with D_2 and substitution by CO, yielding $[Cp^*(dppm)Ru(CO)]^+$ (**5**, ν_{CO} = 1977 cm^{-1}).

Hydrogenases not only bind H_2 with facility, they also activate the H–H bond via a reversible cleavage (deprotonation), as shown by catalysis of isotope exchange between D_2 and H_2O (eq 4).[20]

$$D_2 + H_2O \xrightarrow[23\ °C,\ THF]{2\ (1\ mol\ \%)} HD + H_2 + HDO \quad (4)$$

Recent examples,[21] anticipated by Heinekey's discovery of highly acidic H_2 complexes,[22] support the speculation that such intermediates are formed at the H_2-activating site of hydrogenases.[3] We have observed isotope exchange between D_2 and water at ambient conditions catalyzed by **2**.[23] Both labile hydrogen dissociation from **2** and significant acidity are required for it to serve as an active catalyst for this reaction.

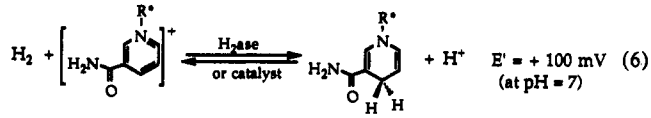
In addition, to be efficient at low partial pressures of hydrogen, the relative binding affinity of the metal must favor hydrogen over water. Water displaces coordinated THF in **3b**, forming a labile aquo complex (**3c**),[11c] and H_2 readily converts **3c** to **2**. The relative affinity demonstrated by $[Cp^*(dppm)Ru]^+$ for a list of key ligands is thus: $CO > CH_3CN > H_2 > H_2O > N_2 >$ THF. The preference for CO over H_2,[24] H_2 over H_2O,[25] and H_2O over N_2 mimics the relative affinity of hydrogenase enzymes for these important small molecules.

The acidity of **2**[6b] is also important in selecting tetramethylpiperidine (TMP) as a noncoordinating base to deprotonate **2**/**2'** (eq 5). This reaction, along with 2 and 3, provides a catalytic cycle as proposed in Scheme 1 and predicts the reduction of hydride acceptors with H_2 catalyzed by "$[Cp^*(dppm)Ru]^+$".

$$TMP + \mathbf{2}/\mathbf{2'} \xrightarrow[23\ °C,\ THF]{N_2} [HTMP]^+ + \mathbf{1} \quad (5)$$

Catalytic reduction of $[MA]PF_6$, as well as $[Ph_3C]BF_4$ and $[Fc]PF_6$, with H_2 is observed in the presence of **1**, **2**, or **4**. This is the first catalyzed reduction of NAD^+ model compounds with H_2 in high yield ([**1**] < 0.1 mol %) at ambient temperature and pressure.[26] A weakly-coordinating counterion and solvent are required as reduction is poisoned by halide, which forms Cp^*(dppm)RuX, or acetonitrile, which blocks coordination of H_2. Simple transfer of deuterium from D_2 to C-9 of $[MA]^+$ yields the monodeuterated product, MAD, without isotope scrambling. The ^{13}C NMR is isolated MAD (1:1:1 triplet for C-9 at δ 33.31) reveals only a trace of MAH (Figure 1).

We conclude that reduction of pyridinium salts by Cp^*(dppm)-RuH are single-step processes and that the catalytic reduction of these NAD^+ model compounds with H_2, at ambient temperature and pressure, is possible with this relatively "hydridic" metal hydride. This catalysis requires a delicate balance of M–H reactivity between a preference for protonation (basicity) and reaction with other electrophiles ("hydridicity"). When a proper balance is struck, hydridic metal hydrides may be generated under mild conditions via the coordination and deprotonation of dihydrogen. The reversible deprotonation of such a hydrogen complex yields a pH-sensitive active site capable of both isotope exchange catalysis and redox cofactor reduction via hydride transfer. This reactivity bears a striking resemblance to that of hydrogenase enzymes. Although in hydrogenase systems hydrogen uptake is coupled to reductase activity by a series of electron and proton transfers, "net hydride transfer" is constrained by the same thermodynamics in all pathways (eq 6). The efficiency of



(6)

energy transduction from H_2 to redox cofactors, in natural and synthetic systems, is limited by the barriers to hydride transfer.

We are currently extending these studies to the development of cofactor-mediated hydrogenation catalysis.

Acknowledgment. We are grateful to the University of Nebraska for startup funds, to the donors of the Petroleum Research Fund, administered by the American Chemical Society (No. 24998-G3), to Nebraska NSF-EPSCoR (OSR-9255225) for their support of this research, and to Colonial Metals, Inc., for a generous loan of ruthenium chloride.

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(25) The same preference in $(i\text{-}Pr_3P)_2(CO)_3W(L)$ shows that a negative ΔS upon coordination of H_2O dictates the relative ligand affinity; at T < –50 °C, H_2O is preferred, and at 23 °C, H_2 is preferred: Kubas, G. J.; Burns, C. J.; Khalsa, R. K.; Van Der Sluys, L. S.; Kiss, G.; Hoff, C. D. *Organometallics* **1992**, *11*, 3390–3404.

(26) Only a single case of NAD model compound reduction with H_2 has been reported, but high pressures (P_{H_2} > 100 atm) were required.[26a] Use of chemical reducing agents or enzymatic or electrochemical methods for NADH formation are nicely reviewed in a recent report.[26b] (a) Okamoto, T.; Yamamoto, S. *J. Mol. Catal.* **1987**, *39*, 219–23. (b) Steckhan, E.; Herrmann, S.; Ruppert, R.; Dietz, E. Frede, M.; Spika, E. *Organometallics* **1991**, *10*, 1568–77.