Coupling of Catalyses by Carbonyl Clusters and Dehydrogenases: Reduction of Pyruvate to L-Lactate by Dihydrogen

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The unique catalytic potentials of carbonyl clusters have been the subject matter of much current research.[1] Here we describe the use of a platinum cluster for the catalytic reduction of NAD^+ to NADH by dihydrogen. By using the enzyme L-lactate dehydrogenase (L-LDH) the scope of this reaction has been extended to include reduction of pyruvate to L-lactate. Since both NAD^+ and L-LDH are soluble only in water, and the carbonyl cluster is soluble only in organic solvents, a biphasic system consisting of water and dichloromethane has been used. The cluster catalyzes the reduction of a redox active dye, Safranine O (Saf^+, 3,7-diamino-2,8-dimethyl-5-phenylphenazinium), by dihydrogen in the organic phase. The oxidized (Saf^+) and the reduced (SafH) dye shuttles across the phase boundary and facilitates the transfer of two electrons and one proton.

The ability of $[Bu_4N]_2[Pt_{12}(CO)_{24}]$ (**1**) to equilibrate dihydrogen with two protons according to reaction i is well established.[2,3]

$$3[Bu_4N]_2[Pt_{12}(CO)_{24}] + H_2 \rightleftharpoons 6[Bu_4N]^+ + 2H^+ + 4[Pt_9(CO)_{18}]^{2-} \quad \text{(i)}$$

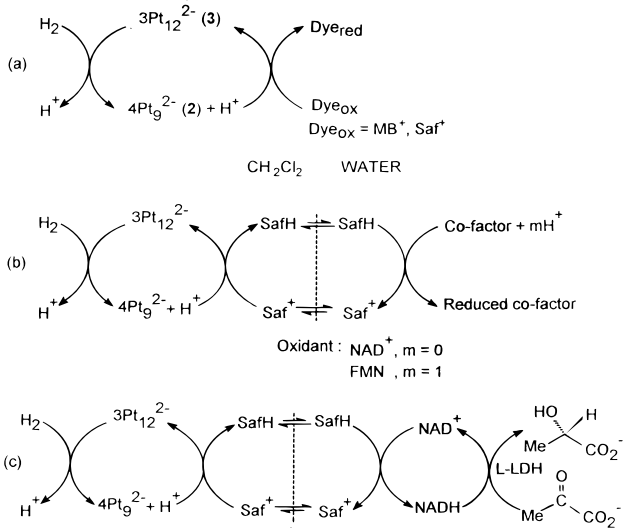
As the aim has been to develop a catalytic system for dihydrogen driven reductions of cofactors such as NAD^+ and FMN, the feasibility of reaction ii has been evaluated.

$$4[Pt_9(CO)_{18}]^{2-} + 2H^+ + A \rightleftharpoons 3[Pt_{12}(CO)_{24}]^{2-} + (2-x)H^+ + AH_x \quad \text{(ii)}$$

$$A = NAD^+,\ x = 1;\ A = FMN,\ x = 2$$

The main problem of reacting $[Pt_9(CO)_{18}]^{2-}$ (**2**) directly with the cofactors is the solubility properties of the cofactors and the platinum cluster. In predominantly aqueous systems (water >95%), with small amounts of a miscible organic component such as DMF or DMSO, the cofactors have some solubility but the sodium or $[Bu_4N]^+$ salt of **2** is unstable. Also, in a biphasic system consisting of water and CH_2Cl_2, reaction ii could not be effected. We also evaluated the catalytic potential of $[Pt_{12}(CO)_{24}]^{2-}$ (**3**), anchored onto biocompatible QAE-sephadex for the reduction of NAD^+ by dihydrogen in water.[4] The results were disappointing in terms of both catalyst lifetime (<120 min) and turnovers (~1 h^{-1}).

Scheme 1. Proposed Catalytic Cycles for the Reduction Mechanism[a]



[a] For clarity "CO" groups of the platinum clusters are not shown. The dashed vertical line is a schematic representation of the phase boundary between the CH_2Cl_2 and water phases.

An alternative strategy based on the concept of a "shuttle carrier" was therefore used. Such carriers play a vital role in the transport of electrons, protons, cofactors, etc. across the biological membranes.[5] It was felt that redox active dyes may function as suitable shuttle carriers. Dyes such as methyl viologen (MV^{2+}), methylene blue (MB^+), Saf^+, and dichloroindophenol sodium salt (Ind^-) have been used as artificial electron acceptors in biological systems.[6] The one-electron $MV^{2+}/MV^{+\bullet}$ couple has in fact been used to effect the reductions of NAD^+.[7] We first established that in a single solvent such as DMF or in a biphasic system reaction iii does take place, and by combining (i) and (iii) a catalytic cycle for dihydrogen driven reduction of Dye_{ox} (Scheme 1a) could be set up.

$$4[Pt_9(CO)_{18}]^{2-} + Dye_{ox} + 2H^+ \rightleftharpoons 3[Pt_{12}(CO)_{24}]^{2-} + Dye_{red} + nH^+ \quad \text{(iii)}$$

(Dye_{ox} = Saf^+, MB^+, $2MV^{2+}$, Ind^-; Dye_{red} = SafH, MBH, $2MV^{+\bullet}$, $IndH_2^-$; n = 1, 1, 2, 0, respectively).

Mixing of DMF solutions of **2** and Dye_{ox} leads to formation of **3** and bleaching of Dye_{ox}, or in the case of MV^{2+}, development of the deep purple color of $MV^{+\bullet}$. In all cases conversion of **2** to **3** is seen by IR, and for MV^{2+}, formation of the radical cation is evident from an isotropic ESR signal at $g = 2.03$. When dihydrogen is passed through a biphasic system, consisting of equal volumes of a solution of **1** in CH_2Cl_2 and a solution of Dye_{ox} in water (Dye_{ox} to **1** molar ratio ≥ 10:1), the water layer is gradually bleached. Spectral recording of the dichloromethane layer at the end of the reaction shows only **2** to be present. Formation of acid with Dye_{ox} = MB^+ or Saf^+ is established by pH measurements

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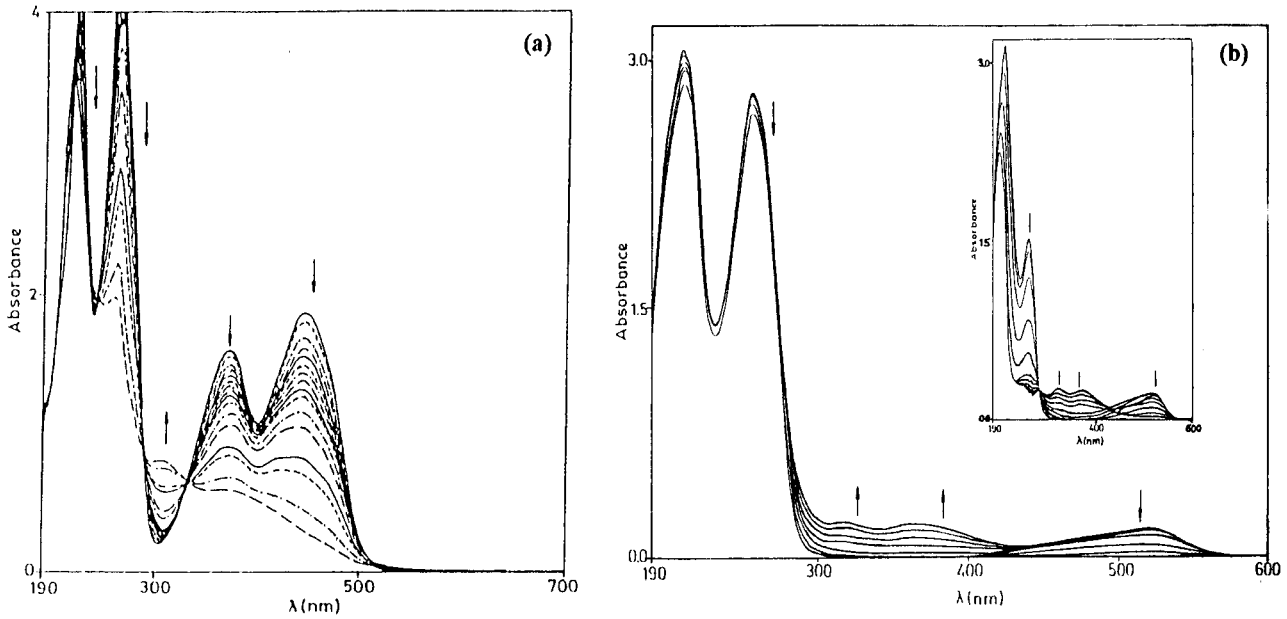

Figure 1. (a) Catalytic reduction of FMN by H_2 in the biphasic system with **4** as the catalyst. (b) Catalytic reduction of NAD^+ by H_2 in the biphasic system with **4** as the catalyst. The band at ~520 nm is due to Saf^+. Inset: Spectral changes for the reduction of BNA^+ to BNAH.

of the aqueous layer. The evidence for selective reduction of the dyes comes from the fact that the bleached aqueous solutions of MBH and SafH, on exposure to air, regain their original colors.[6] This selective catalytic activity of **1** for the reduction of Dye_{ox} is unique. The homogeneous hydrogenation catalyst [Rh(Dppe)-(NBD)]$^+$ and conventional Pt/C tested under identical conditions are found to be inactive.

Among all the Dye_{ox}, Saf^+ has been chosen to be the shuttle carrier for the following reasons. $MV^{+\bullet}$ is a *one*-electron donor while the reduction of NAD^+ requires one proton and *two* electrons, a condition satisfied by SafH and MBH. The half-cell potential of Saf^+/SafH (−0.25 V) makes NAD^+ reduction (NAD^+/NADH, $E°$, −0.34) less unfavorable than reduction by MB/MBH ($E°$, 0.01). Both Saf^+ and SafH have measurable solubilities in water and CH_2Cl_2, a requirement for an effective shuttle carrier. From independent experiments the partition coefficients (C_{H_2O}/$C_{CH_2Cl_2}$) of Saf^+ and SafH in a 1:1 volume mixture of water and CH_2Cl_2 at 30 °C are calculated to be 6.8 and 0.14, respectively.

The salt $[Saf]_2[Pt_{12}(CO)_{24}]$ (**4**) has been synthesized by adopting the literature reported synthetic procedure for **1** with the modification of the use of Saf^+Cl^- in place of $Bu_4N^+I^-$.[8] It is insoluble in water but soluble in dichloromethane. Spectroscopic (IR, UV−visible) monitoring of the reaction of **4** with dihydrogen in CH_2Cl_2 shows complete conversion of **3** to **2** and Saf^+ to SafH according to reaction iv.

$$7H_2 + 3[Saf]_2[Pt_{12}(CO)_{24}] \rightleftharpoons 8H^+ + 6SafH + 4[Pt_9(CO)_{18}]^{2-} \quad \text{(iv)}$$

In the biphasic system the capability of **4** to catalytically reduce NAD^+ and FMN by dihydrogen, as shown by Scheme 1b, was evaluated. The selective reduction of FMN to $FMNH_2$ is evident from clean spectrophotometric isobesticity at ~330 nm, complete bleaching, and full regeneration on exposure to air (Figure 1a). This selectivity is unique, conventional heterogeneous catalysts over-reduce flavin cofactors.[9] The turnover frequency (20 h^{-1}, pH 5.5) shows an inverse relationship with pH within the pH range 5.5−7.5.

For the selective reduction of NAD^+ to NADH, a reaction for which a variety of strategies have been reported in the literature,[7,10] absorption by SafH below the 400 nm region masks the formation of NADH, which has a characteristic absorption at 340 nm (Figure 1b). However, isobesticities and decrease in the 260 nm peaks of the oxidized nicotinamides could be seen for both NAD^+ and BNA^+ (benzyl nicotinamide chloride), a well-established model for NAD^+.[11] Formation of NADH from NAD^+ has also been proved by carrying out enzymatic assays of the reaction product with LDH. The turnover frequencies for NAD^+ and BNA^+ are 13 and 15 h^{-1} at pH 7.0.

The importance of dehydrogenase based enzymatic catalysis in Industry and organic synthesis has been documented.[12,13] The present work is of special relevance to the industrial process for the enantioselective synthesis of L-lactate from pyruvate with *two* enzymes.[13] Extension of the electron-transfer chain as shown in Scheme 1c by using a *single* enzyme has therefore been tested and found to be feasible.

Under the reaction conditions employed, several control experiments establish that in the absence of any one of the components, **4**, L-LDH, or NAD^+, there is no reaction. Also there is no reaction if **1** instead of **4** is used in combination with NAD^+ and LDH. However, complete conversion of pyruvate to L-lactate within 48 h could be obtained with a pyruvate to NAD^+ to **4** molar ratio as high as 600:10:1.

In conclusion, the unique selectivity of the cluster catalyst has enabled us to design, for the first time, a dihydrogen driven, *single* enzyme based catalytic system for the conversion of pyruvate to L-lactate.

Supporting Information Available: Experimental details (1 page, print/PDF). See any current masthead page for ordering and Web access information.

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(8) Analysis of $[Saf]_2[Pt_{12}(CO)_{24}]$. Anal. Calcd for $C_{64}H_{38}N_8O_{24}Pt_{12}$: C, 21.09; H, 1.04; N, 3.07; Pt, 64.25. Found: C, 21.3; H, 1.2; N, 3.1; Pt, 64.35. IR data [ν_{CO}/cm^{-1}] in THF: 2040 (vs), 2030 (sh), 1880 (mw), 1860 (s), 1825 (mw). UV−vis (THF): λ_{max} at 620, 520, and 392 nm.

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