Catalytic reduction of redox-active co-factors and proteins by dihydrogen with Sephadex supported platinum clusters as catalysts

Sumit Bhaduri* and Krishna Sharma

Alchemie Research Centre, PO Box 155, Thane-Belapur Road, Thane 400 601, Maharashtra, India

The platinum carbonyl cluster $[Pt_{15}(CO)_{30}]^{2-}$, anchored onto QAE-SEPHADEX anion exchanger, is an effective catalyst for the reduction of flavin co-factors, lipoamide dehydrogenase and $C_{yt}C_{ox}$.

Catalytic reduction of redox-active co-factors and proteins by dihydrogen is an attractive research target.[1–3] Such reactions may have potential applications in asymmetric catalysis, biosensors *etc.* Conventional heterogeneous catalysts, such as platinum supported on carbon, are unsatisfactory for this purpose since the use of such catalysts results in over reduction and/or denaturation of the biomolecules. In this communication we report our preliminary findings on the use of a polynuclear platinum carbonyl anion, supported on a commercially available Sephadex-based anion exchanger {diethyl(2-hydroxypropyl)aminoethyl–SEPHADEX), as a catalyst for the reduction of selected redox-active co-factors and proteins by dihydrogen.

The ability of the Chini clusters $[Pt_3(CO)_6]_n^{2-}$ (n = 4–6) to equilibrate dihydrogen and protons according to eqn. (1) is well known.[4,5]

$$H_2 + (n-1)\,[Pt_3(CO)_6]_n^{2-} \rightleftharpoons 2H^+ + n\,[Pt_3(CO)_6]_{n-1}^{2-} \quad (1)$$

The reduced clusters in turn could be converted back to the oxidised derivatives with an oxidiser such as quinone (Q). Indeed homogeneous catalytic hydrogenation of quinone to dihydroquinone (H_2Q) with the platinum clusters as catalysts, *i.e.* combination of eqns. (1) and (2), has been reported by us.[5]

$$n[Pt_3(CO)_6]_{n-1}^{2-} + 2H^+ + Q \rightarrow H_2Q + (n-1)\,[Pt_3(CO)_6]_n^{2-} \quad (2)$$

Due to the total lack of solubility of the platinum clusters in water, a similar approach for the reduction of redox-active biomolecules cannot be adopted. Although the anionic clusters could be easily anchored onto commercially available, polystyrene based anion exchangers such as IRA 401, the resultant material is *not* compatible with biomolecules.[6] Proteins such as $C_{yt}C_{ox}$ and lipoamide dehydrogenase are rapidly denatured when brought into contact with such a resin.

Table 1 Catalytic reductions of redox-active co-factors and proteins by dihydrogen with Sephadex supported platinum cluster[a]

Substrate	t/min	Conv. (%)	$t_{turnover}$/min
FAD	100	100[b–d]	2.5
FMN	100	100[b–d]	2.5
NAD^+	90	20[b,c,e,f]	45
$C_{yt}C_{ox}$	2	100[b,g]	1.5
Lipoamide dehydrogenase	30	80[c,e,h]	>15
Glucose oxidase	30	0[e,i]	
	30	80[e,j]	>15

[a] All reactions carried out at 20 °C with 15 mg of **1** (*ca.* 0.75 mg platinum) in phosphate buffer solution. For FAD and FMN the approximate mole ratio of cluster to substrate is 1:40, for NAD^+ 1:10, for $C_{yt}C_{ox}$ 1:3 and for the others <1:1. [b] Conversion monitored by spectrophotometry. [c] pH 7.4. [d] Full regeneration of original spectrum on autoxidation. [e] Conversion monitored by fluorometry. [f] Confirmation of NADH by assay with lactate dehydrogenase. [g] pH 7.0 and 0.1 mol dm^{-3} $NaClO_4$. [h] Regeneration (>90%) of original spectrum on autoxidation. [i] pH 5.9. [j] Regeneration (>75%) of original spectrum on autoxidation.

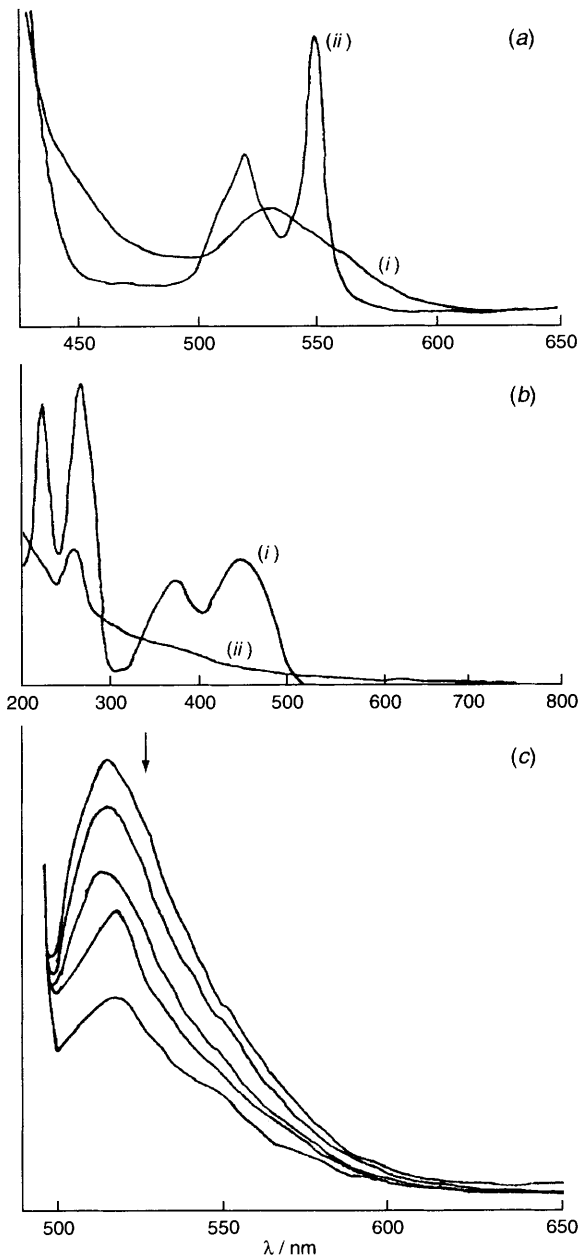
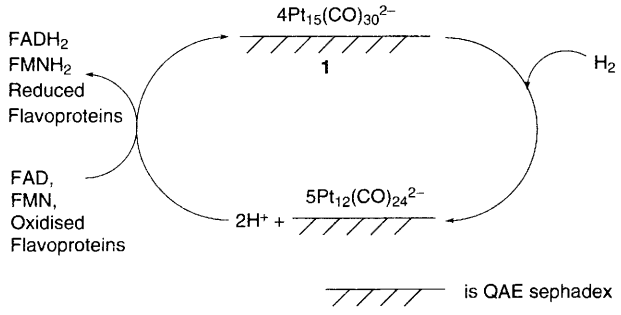


Fig. 1 UV–VIS spectra for (*a*) $C_{yt}C_{ox}$, (*i*) before and (*ii*) after reaction with H_2; (*b*) FAD, (*i*) before and (*ii*) after reaction with H_2; (*c*) lipoamide dehydrogenase reduction with H_2 monitored by emission spectra, taken at 5 min intervals (experimental conditions are those described in Table 1)

To overcome these problems we chose a bio-compatible anion-exchange resin, QAE-SEPHADEX (Sigma), as the support material. The cluster anion $[Pt_{15}(CO)_{30}]^{2-}$ can be easily anchored onto the resin by simply treating a methanolic solution of the sodium salt of the cluster with the resin for *ca.* 2 h. The resultant material **1** has the characteristic UV–VIS and IR bands[5,6] of $[Pt_{15}(CO)_{30}]^{2-}$ **2**. The platinum content, as measured by atomic absorption spectroscopy, is about 5%.

The reaction conditions and relative ease of reductions for a variety of biomolecules, as measured by the approximate time taken for one turn-over, are summarised in Table 1. The reductions of the biomolecules have been monitored by spectrophotometry or fluorometry (Fig. 1). The selective reduction of flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN) to $FADH_2$ and $FMNH_2$ has been confirmed by ensuring that the reduced products could be autoxidised to regenerate the original spectra of FAD and FMN. Over reduced products, obtained by using conventional heterogeneous catalysts, do not undergo such reversible autoxidation.[7]

The flavoproteins, lipoamide dehydrogenase and glucose oxidase exhibit similar behaviour with the exception that regeneration of the original emission spectra on autoxidation are to the extent of >90% and >75% respectively. Also the rates of reduction are much slower than that of the free flavin co-factors. Reduction of glucose oxidase is observed only at neutral pH. Under acidic pH, a condition under which the enzyme is normally assayed, there is no reduction. This is in accordance with eqn. (1); under acidic conditions the platinum clusters cannot be reduced by dihydrogen.



Scheme 1 Proposed catalytic cycle for the reduction mechanism

For the first four substrates of Table 1, the rates of reduction as judged by the time per unit turnover are interestingly in the same order as their reduction potentials. Both glucose oxidase (M > 150 000) and lipoamide dehydrogenase (M > 100 000) are high-molecular weight proteins containing two FAD prosthetic groups.[8] These redox-active sites are probably less accessible for electron transfer than in the case of free FAD. It should be noted that for the flavoproteins the reduction is stoichiometric since substrate : **2** mol ratios are slightly less than unity.

The proposed mechanism of reduction is shown in Scheme 1. According to eqn. (1) the anchored cluster anion **2** is reduced to $[Pt_{12}(CO)_{24}]^{2-}$ **3** by dihydrogen. Similar behaviour of the platinum clusters on IRA 401 was reported by us.[6] In mixed solvent systems such as dimethyl formamide–water (>90% water), the extent of reduction of **3** to $[Pt_9(CO)_{18}]^{2-}$ **4** is insignificant and cannot be spectroscopically observed.[9] Participation of **4** in the catalytic cycle is therefore unlikely. The reduced cluster **3**, is oxidised back to **2** by the biomolecule regenerating **1** and completing the catalytic cycle. For the flavin co-factors the last step invovles addition of two protons plus two electrons. For $C_{yt}C_{ox}$ however, addition of only one electron is involved. We are currently evaluating the applicability of **1** for the reduction of other redox-active biomolecules by dihydrogen.

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