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Mendeleev Commun., 1995, 5(5), 187–189

Mendeleev Communications

The Effect of Chloride on the Electroreduction of NAD$^+$ in the Presence of [Cp*Rh^{III}]$^{2+}$ Species

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A cyclic voltammetry study of the η^5-pentamethylcyclopentadienylrhodium (Cp*Rh) complexes $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ and $[Cp^*Rh^{III}{}_{aq}]^{2+}$ at pH 6.05 in the absence and in the presence of nicotinamide cofactors indicates that the chloro complex is more advantageous for electrochemical reduction of NAD$^+$ into NADH; the reverse oxidation of NADH by $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ is insufficiently selective for practical applications according to the ^{1}H NMR data.

We have previously reported that rhodium(III) chloro-bridged $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ dimer (Cp* = pentamethylcyclopentadienyl) interacts with oxidized nicotinamide dinucleotide (NAD$^+$) to form a compound of the formula $[Cp^*Rh(NAD^+)Cl]^+$, reaction (1).[1,2] The reduced cofactor NADH is oxidized into NAD$^+$ under the same conditions with a concomitant formation of the red rhodium(I) species, reaction (2).

$$[(Cp^*Rh)_2(\mu\text{-}Cl)_3]Cl + 2NAD^+ \rightleftharpoons 2[Cp^*Rh(NAD^+)Cl]^+ + 2Cl^- \qquad (1)$$

$$[(Cp^*Rh)_2(\mu\text{-}Cl)_3]Cl + 2NADH \rightarrow 2[Cp^*Rh^I(NAD^+)Cl] + 2H^+ + 2Cl^- \qquad (2)$$

Reactions (1) and (2) are interesting because of the potential utility of the rhodium complexes in the regeneration of nicotinamide cofactors.[3,4] We assumed that NAD$^+$ is coordinated to rhodium(III) *via* adenine nitrogen N7 and the NH$_2$ group.[1,2]† However, the binding mode was not unequivocally proved. In addition, it has recently been demonstrated that the corresponding aqua species $[Cp^*Rh^{III}{}_{aq}]^{2+}$, which is generated from $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ by chloride abstraction with silver ion, interacts with adenine derivatives and related nuclear bases to afford unusual polynuclear compounds.[5–7] In the case of 9-methyladenine, a trimer is formed as a result of N–H bond cleavage of the amino group by Rh^{III}. The trimer structure was confirmed by X-ray crystallography.[5] As far as reaction (2) is concerned, the chloride-free species seems to be more electrophilic compared to the aquated ones with a higher potency for C–H bond activation. This could be advantageous in oxidation of NADH into NAD$^+$ and related processes. In order to verify this suggestion we investigated the electrochemical behaviour of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ and $[Cp^*Rh^{III}{}_{aq}]^{2+}$ in the presence of

Table 1 Peak potential for the reduction/oxidation of Cp*Rh complexes in the absence and in the presence of nicotinamide cofactors. For conditions, see legends to Figs. 1 and 2.

Compound	$-E$/V *vs.* SCE			
	p1	p2	p3	p4
$[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$	0.69	0.84	0.28	0.16[a]
$[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ + NAD$^+$	0.69	0.93	0.7	0.34
$[Cp^*Rh_{aq}]^{2+}$	0.76	1.03	0.69	*ca.* 0.3[b]
$[Cp^*Rh_{aq}]^{2+}$ + NAD$^+$	0.73	0.97	n.o.[c]	0.33
$[Cp^*Rh_{aq}]^{2+}$ + NADH	0.76	0.94	0.64	0.46

[a] Adsorptive peak. [b] Very broad. [c] Not observed.

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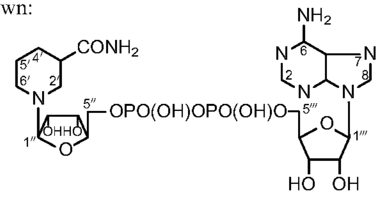
† Atom numbering in nicotinamide cofactors used in this paper is as shown:



‡ *Experimental details.* All rhodium(III) complexes used in this work were prepared as described previously.[9,10] NAD$^+$ and NADH were purchased from Serva and used as received. Electrochemical measurements were performed with EG&G equipment (potentiostat 173 with IR compensation, programmer 175). NMR data were obtained on a Varian Unity 500 instrument.

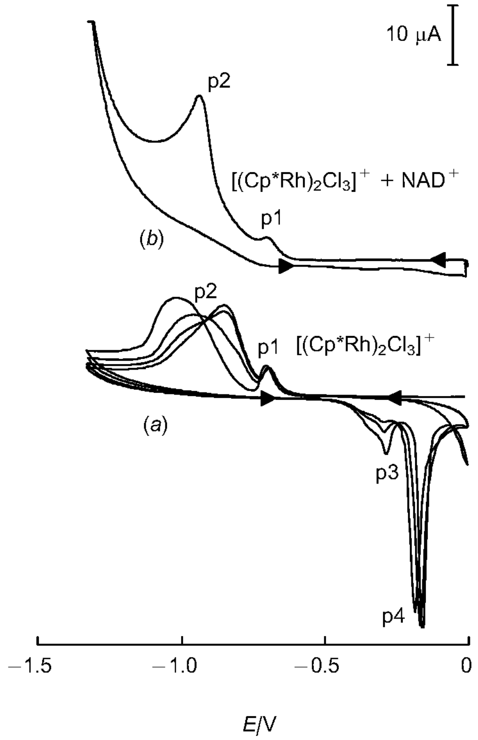


Fig. 1 Cyclic voltammograms of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ in the absence (*a*) and in the presence (*b*) of NAD^+. [Rh] = 5×10^{-4} M, $[NAD^+]$ = 1.5×10^{-3} M; pH 6.05 (0.1 M phosphate), scan rate 50 mV s^{-1}, potentials are *versus* SCE; 22 ± 2 °C.

NAD^+ and NADH. A cyclic voltammetry technique on a dropping mercury electrode was used in this work.‡

The principal electrochemical results of this study are presented in Figs. 1 and 2, as well as in Table 1. Repetitive scans of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ in buffered aqueous solution (pH 6.05) are shown in Fig. 1(*a*). There are two well defined reduction and oxidation peaks in each voltammogram. Interestingly, the most negative reduction peak p2 observed at −1.01 V during the first scan moves progressively to positive potentials and ends up at −0.84 V. There is a pseudo-isosbestic point around −0.91 V which is indicative of the rearrangement of one species into another in the course of repetitive scans. The effect is explained by substitution of OH^- for Cl^- in one of the low oxidation states to form a variety of mixed μ-OH,Cl species. By varying potential ranges, it was confirmed that the reductions at p1 and p2 are related to oxidation peaks p3 and p4, respectively. The sharpest oxidation peak p4, the potential of which is also slightly scan-dependent, is adsorptive in origin. In general, such a behaviour is typical of the two-electron reduction of

$$Rh^{III} \underset{-e\ (p4)}{\overset{+e\ (p1)}{\rightleftharpoons}} Rh^{II} \underset{-e\ (p3)}{\overset{+e\ (p2)}{\rightleftharpoons}} Rh^{I}$$

Scheme 1

Rh^{III} species[8–10] and might be accounted in terms of the simplified Scheme 1.

The simplification concerns mostly the redox half-reaction $Rh^{II} + e \rightleftharpoons Rh^{I}$. In fact, conversion of Rh^{II} into Rh^{I} occurs predominantly *via* disproportionation reaction (3).[9]

$$2Rh^{II} \rightarrow Rh^{III} + Rh^{I} \quad (3)$$

The presence of NAD^+ provides noticeable changes in the system, Fig. 1(*b*). These are, first, almost complete elimination of the oxidation peaks. Only minor oxidative hills are seen at the former potential of p3 and p4 around −0.7 and −0.35 V. Second, the height of the p2 reduction peak is about twice that of Fig. 1(*a*). Both observations are in good accord with the EC′ electrocatalytic mechanism,[11] in which a reduced rhodium species is capable of reduction of NAD^+. As a result, there is a channel for regeneration of rhodium(III) and thus elimination of the oxidation peaks. The reduction of NAD^+ by Rh^I is likely to be an intermolecular process since Rh^I is not expected to be bound strongly to NAD^+.

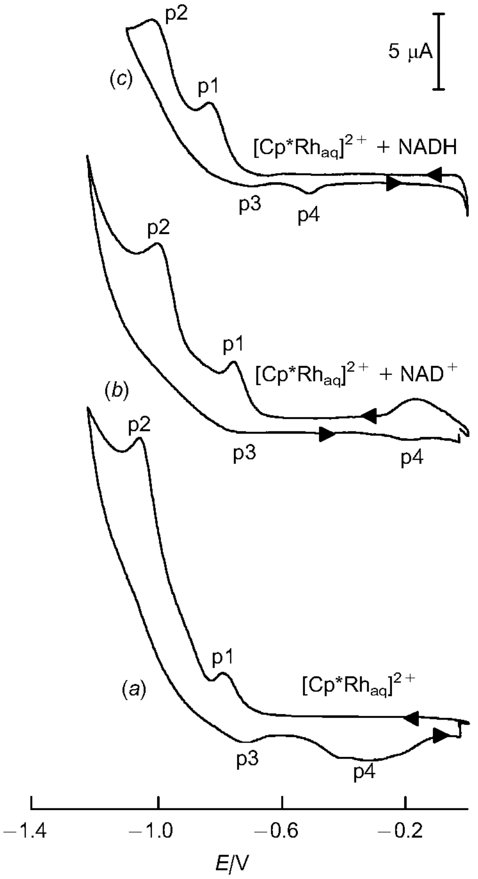


Fig. 2 Cyclic voltammograms of $[Cp^*Rh_{aq}]^{2+}$ in the absence (*a*) and in the presence of NAD^+ (*b*) or NADH (*c*). [Rh] = 5×10^{-4} M, $[NAD^+]$ = 1.8×10^{-3} M; [NADH] = 6.44×10^{-4} M; other details as in Fig. 1.

The main difference between $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ and $[Cp^*Rh_{aq}]^{2+}$ as metal centres for binding with NAD^+ is that $[Cp^*Rh_{aq}]^{2+}$ binds more strongly to the ligand and the binding site, *viz.* the deprotonated amino group N6 and N7 of the purine ring, is known.[5] Besides differences in electrophilicity, the electrochemical properties of the two species deserves comparison under identical conditions. The representative cyclic voltammograms are presented in Fig. 2. In the absence of cofactors [Fig. 2(*a*)], $[Cp^*Rh_{aq}]^{2+}$ is characterized by two reductions at −0.76 and −1.03 V and two broad oxidation peaks at −0.69 and −0.3 V. In contrast to $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$, there are no adsorptive peaks. Addition of NAD^+ causes (*i*) anodic shift of p2 by *ca.* 0.06 V, (*ii*) a decrease in the p2 peak current and (*iii*) almost complete disappearance of the oxidation peaks p3 and p4. These observations could be rationalized by a pronounced binding of $[Cp^*Rh_{aq}]^{2+}$ to NAD^+ through adenine N7 and activated NH_2.[5] Since a heavy ligand is now attached to the redox active rhodium species, its molecular mass increases and, hence, the diffusion coefficient decreases resulting in a decrease in the peak current on the cyclic voltammogram in Fig. 2(*b*). The current drop must be a factor of *ca.* 1.2, if only diffusion effects are taken into account based on the fact that

$i \sim D_0^{1/2}$ and $D_0 \sim M^{-1/3}$, where i, D_0 and M are the peak current, diffusion coefficient and molecular weight, respectively. It is also clear that there is no catalytic current in the case of the $[Cp^*Rh_{aq}]^{2+}$–NAD^+ pair as opposed to the above $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ case. Hence, a stronger binding of the redox centre to the adenine ring of NAD^+ is inefficient for the electrocatalysis. This observation sheds some light on the mechanism of NAD^+ reduction by Cp^*Rh^I species in terms of the intra- *versus* intermolecular reaction involving the adenine moiety as an anchor. Since weaker binding results in better catalysis, the intermolecular mode seems more advantageous.

The peak current of $[Cp^*Rh_{aq}]^{2+}$ in the presence of NADH is the lowest, Fig. 2(*c*). It is probably due to the binding as in the case of NAD^+, as well as to the slow reduction of Rh^{III} into a red Rh^I species by NADH. In other words, the behaviour of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ and $[Cp^*Rh_{aq}]^{2+}$ are similar. Both are reduced by NADH into rhodium(I) species. It seems, however, that the reaction in the case of $[Cp^*Rh_{aq}]^{2+}$ is slower compared to $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$. The reason may be identical to the case discussed above, *viz.* better binding decreases reactivity.

An attempt has been made to follow the NADH oxidation with $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ by ^1H NMR operating at 500 MHz in D_2O as solvent. The main goal was to check how selective the reaction is. The signal assignments were made in accordance with the current knowledge.[12] In the δ range 6–9 the spectrum of NADH consists of three sharp singlets at δ 8.34, 8.08 and 6.80 assigned to protons H8, H2 and H2′, respectively. Two doublets at δ 2.64 and 2.54 ($^2J_{HH}$ 18 Hz) comprise the AB quartet from the H4′ protons. The low-field part of the NAD^+ spectrum contains three sharp singlets at δ 9.30, 8.46 and 8.27 from H2′, H8, and H2, respectively, doublets at δ 9.15 and 8.81 from H6′ and H4′, respectively, and a doublet of doublets at δ 8.16 from H5′. There are no signals upfield of 4 ppm. Based on these observations it was anticipated that by following the spectral changes of NADH in the presence of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ it would be possible to draw conclusions about the selectivity of reaction (2). The ^1H NMR spectrum of the mixture $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ and NADH recorded within 1 h after mixing contains a new singlet at δ 2.21 together with a group of signals in the range of 1.25–1.65. These are usually ascribed to methyl resonances from the Cp* ligand of different species present in solution. For instance, this is at δ 1.65 for $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ [9] and at 2.26 for $[Cp^*Rh(\eta^5\text{-pyrrole})]PF_6$.[13] This fact indicates that several rhodium species may be present in solution. In accord with this, a very broad signal is noticed instead of the clear AB quartet from H4′. This observation, as well as a colour change of the solution from yellow to pink, suggests a reaction between Rh^{III} and NADH. Recorded after 24 h, the spectrum has weaker signals in the δ range 2.5–2.9 together with a group of new resonances in the down-field part of the spectrum which are noticeably more complicated compared to those expected in the case of selective conversion of NADH into NAD^+. This might be a crucial obstacle for utilization of the Cp*Rh-promoted catalytic cofactor regeneration in practically important, biocatalytic reactions.[14,15]

In conclusion, better binding did not provide better reactivity, *i.e.* $[Cp^*Rh^{III}{}_{aq}]^{2+}$ has no advantages compared to $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ as a starting material in electrochemical reduction of NAD^+. The reduction of $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$ by NADH is characterized by a low selectivity and, therefore, there is no true reversibility in the reaction $Rh^{III} + NADH \rightleftharpoons Rh^I + NAD^+ + H^+$.

The research described in this publication was made possible in part by financial support from the Alexander von Humboldt Foundation for ADR and grant no. ML2000 from the International Science Foundation.

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Received: Moscow, 25th April 1995
Cambridge, 7th June 1995; Com. 5/02735G