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Bioorganometallic chemistry Part 12. Reaction of $[Cp^*Rh(H_2O)_3](OTf)_2$ with nicotinamide adenine dinucleotide in water: synthesis, structure, and a pH-dependent ^{1}H-NMR and voltammetric study of the cyclic trimer product, $[Cp^*Rh(\mu\text{-}\eta^1(N1):\eta^2(N6,N7)\text{-}9\text{-}(5'\text{-ribose pyrophosphate-}5''\text{-ribose-}1''\text{-nicotinamide})adeninato]_3(OTf)_3$

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Abstract

The reaction of $[Cp^*Rh(H_2O)_3](OTf)_2$ (**1**) with nicotine adenine dinucleotide (NAD^+, **2**), an important co-factor in enzymatic reactions, was studied utilizing ^{1}H-NMR spectroscopy, electrospray ionization mass spectroscopy (ESI/MS), cyclic voltammetry (CV), and isolation techniques, as a function of pH. The product was formulated from the above-mentioned spectroscopic data as the well-known Cp*Rh cyclic trimer structure, $[Cp^*Rh(\mu\text{-}\eta^1(N1):\eta^2(N6,N7)\text{-}9\text{-}(5'\text{-ribose pyrophosphate-}5''\text{-ribose-}1''\text{-nicotinamide})adeninato]_3(OTf)_3$, **3**, which forms via a self-assembly mechanism as the pH is increased from 3 to 6 (^{1}H-NMR). We also compared **3** with the putative one reported that formed via reaction with $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ and was tentatively assigned the formula, [Cp*Rh(NAD)Cl](Cl). In fact, both Cp*Rh synthons provide the same cyclic trimer product at pH 6, while a presumed mixture of [Cp*Rh(NAD)] and Cp*Rh aqua intermediates (at least eight Cp*Rh ^{1}H-NMR signals are evident) were formed at pH 3.0. A full analysis of the CV data reveals that some Cp*Rh aqua complexes are electroactive at potentials around -1.2 V versus Ag | AgCl, but probably not the cyclic trimer, complex **3**. Unfortunately, we were not able to utilize complex **3** in an *intramolecular*, regioselective reduction reaction, with sodium formate as the hydride source, to provide the corresponding biologically active 1,4-dihydro derivative. Published by Elsevier Science S.A.

Keywords: Nicotinamide adenine dinucleotide; Organorhodium–NAD complex; ^{1}H-NMR and CV analysis

1. Introduction

Recently, we have been studying the reactions of $[Cp^*Rh(H_2O)_3](OTf)_2$ (**1**) with a wide variety of nucleobases, nucleosides, and nucleotides, all in water as a function of pH [1]. Moreover, we have also been particularly intrigued by a common cyclic trimer structure that occurs with all nine-substituted adenine derivatives we have perused [1a,c]. This cyclic trimer structure, $[Cp^*Rh(\mu\text{-}\eta^1(N1):\eta^2(N6,N7)\text{-}9\text{-substituted adeninato}]_3^{3+}$, we believe, forms via a self-assembly mechanism and provides the thermodynamically favored product, where the *N*-9-adeninato substituent was alkyl, ribose, or a ribose phosphate ester.

An adenine derivative that recently sparked our interest was nicotinamide adenine dinucleotide (NAD^+, **2**) an important co-factor in enzymatic react ions that utilizes its reduced form, 1,4-NADH, as a hydride source in chiral reduction reactions [2]. More importantly, recent publications by Ryabov and coworkers have focused on the reactions of a dimer, $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$, and of Cp*Rh aqua spe-

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cies with NAD^+ and it was proposed that each Cp*Rh synthon mentioned provides a different [Cp*Rh(NAD)] compound [3].

This latter proposal was primarily based upon the observation of different electrochemical responses of the $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ and the Cp*Rh aqua complexes upon addition of NAD^+ and 1,4-NADH to the solutions. In particular, catalytic reduction of NAD^+ was reported to be strong in the presence of $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ and absent with the Cp*Rh aqua species. This effect was attributed to the stronger binding of NAD^+ to the Cp*Rh aqua species, and it was further concluded, that intermolecular reduction catalysis of NAD^+ was more favored over a competing intramolecular pathway [3]. However, no indication of the reduction product, 1,4-NADH, was provided experimentally.

It is important to note that no structures were actually assigned to either product, but they speculated that $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ gave a 1:1 complex with NAD^+ having a formula [Cp*Rh(NAD)Cl](Cl), from primarily, we presume, the elemental analysis data [3a]. Therefore, we have sought to identify the structure of the Cp*Rh complex formed in the reaction of the known synthon, **1** [4] with NAD^+ utilizing ^{1}H-NMR spectroscopy, electrospray ionization mass spectroscopy (ESI/MS), cyclic voltammetry (CV), and isolation techniques, as a function of pH, and then to compare this product with the putative one reported that formed via reaction with $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ [3]. We also wanted to delineate the role, if any, of the complex formed between **1** and **2** in an *intramolecular* co-factor regeneration; i.e. catalyzed *intramolecular*, regioselective reduction of the nicotinamide nucleus to its biologically active 1,4-dihydro form.

2. Results and discussion

2.1. ^{1}H-NMR spectroscopy titration experiments: reaction of complex 1 with NAD^+

The ^{1}H-NMR spectroscopy titration study (Fig. 1, D_2O) of **1** and **2** shows very unambiguously that as you increase the pH from 3.0 to 6.0, two diastereomeric compounds are evident (Cp* signals at 1.670; 1.673 ppm, pH 6.0), and that these diastereomers, complex **3**, have a very narrow stability range, i.e. at pH values less than 6.0, there is a presumed mixture of [Cp*Rh(NAD)] and Cp*Rh aqua intermediates (at least eight Cp*Rh signals are evident) being formed, while at pH values greater than 6.0, complex **3** appears to be decomposing to NAD^+ hydrolysis products and the known $[(Cp^*Rh)_2(\mu\text{-}OH)_3](OTf)$ (**4**) [2,4]. Furthermore, the diagnostic chemical shifts that we have observed previously for all the enantiomeric/diastereomeric, cyclic trimer $[Cp^*Rh(\mu\text{-}\eta^1(N1):\eta^2(N6,N7)\text{-}9\text{-substituted adeninato}/5'\text{-adenosinato}/5'\text{-adenosinatomonophosphate methyl ester}]_3^{3+}$ derivatives we synthesized [1a,c] are evident in the ^{1}H-NMR spectrum of **3** at pH 6.0 (Fig. 2). Thus, the H8 proton (8.28 ppm, spectrum a) of the adenine nucleus of free NAD^+ moves downfield ($\Delta\delta = 0.35$ ppm; 8.63, 8.64 ppm, spectrum b) upon formation of **3**, while the free NAD^+ adenine H2 proton (8.01 ppm, spectrum a) moves dramatically upfield ($\Delta\delta = 0.48$ ppm; 7.54, 7.52 ppm, spectrum b). These diagnostic ^{1}H-NMR spectroscopy results allow us to unequivocally assign a cyclic trimer structure to complex **3**; if complex **3** had a mononuclear structure, [Cp*Rh(η^2(N6, N7)-9-(5′-ribose pyrophosphate-5″-ribose-1″-nicotinamide)adeninato], the H8 and H2 pro-

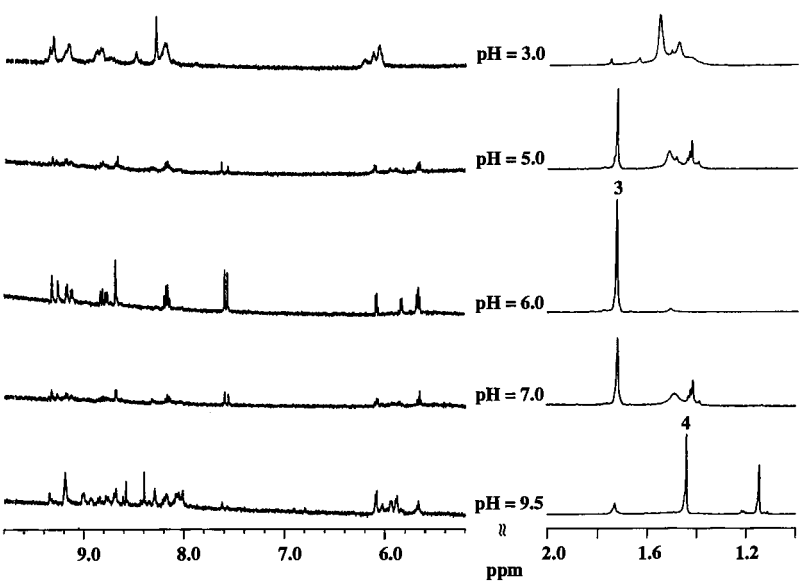


Fig. 1. ^{1}H-NMR titration profile in the formation of **3**.

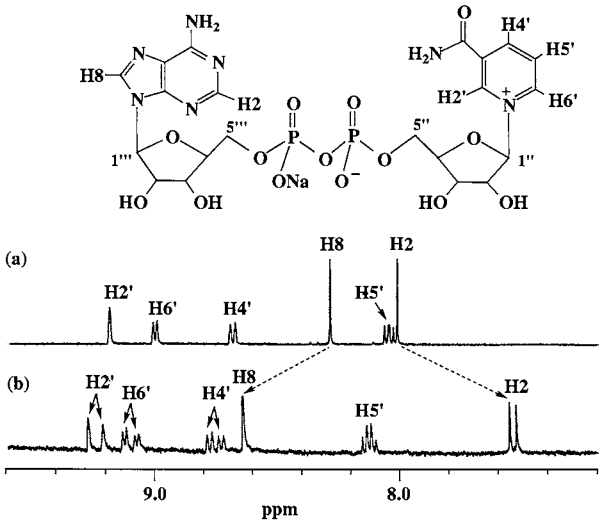
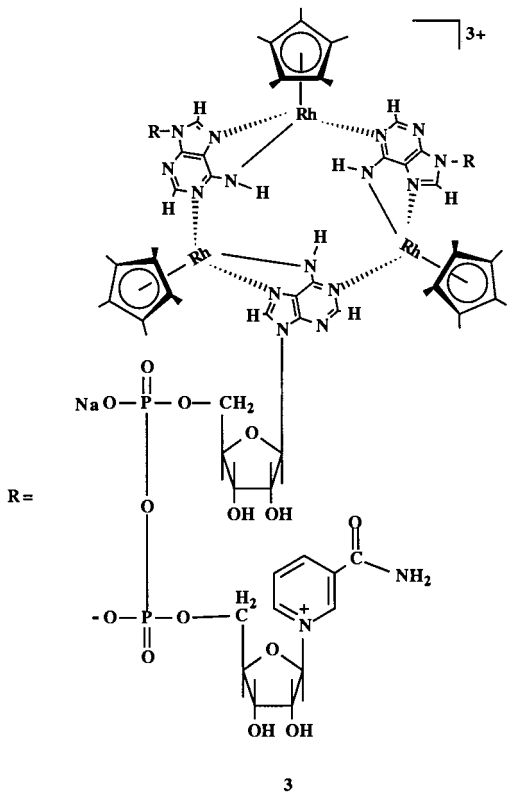


Fig. 2. (a) ^{1}H-NMR spectrum of NAD^+; (b) $[Cp^*Rh(NAD)]_3(OTF)_3$, **3**. Both spectra obtained at pH 6.0.

tons would *both be downfield shifted* and no diastereomers would be evident, while the supramolecular, cyclic trimer structure of **3** provides a deshielding effect of H8 (downfield shift) and a shielding effect on H2 (upfield shift) with the presence of diastereomers (C3 symmetry) [1a]. Interestingly, the nicotinamide protons also show two diastereomers with all pyridinium and ribose protons being doubled (Fig. 2).



3

2.2. Electrospray ionization mass spectrometry of complex *3*

We attempted to further define the cyclic trimer structure of **3** using the soft ionization technique, ESI/MS [5]. However, we found that the steric bulk of the *N*-9 substituent on the adenine nucleus has an effect on the stability of the cyclic trimer structure *during the ESI/MS process*. For example, we observed the ESI/MS generated a singly charged, high intensity, molecular ion for a cyclic trimer analog, $[Cp^*Rh(2'\text{-deoxyadenosine})]_3(OTf)_3$, **5**, at m/z 1762 ($I = 1\%$), but we found that a similar molecular ion was not evident for **3**. Moreover, **5** provided a doubly charged ion at m/e 807 ($I = 60\%$), and a triply charged monomer ion, $[Cp^*Rh(2'\text{-deoxyadenosine})]^+$, at m/e 488 ($I = 50\%$ in the range m/e 100–3000). Therefore, as shown in Fig. 3(a), the positive-ion ESI mass spectrum

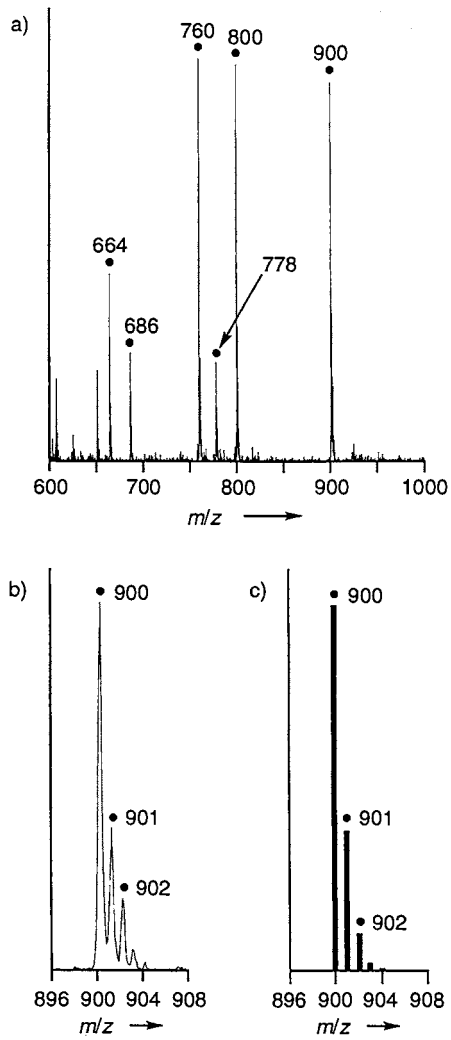


Fig. 3. (a) The positive-ion ESI mass spectrum of an aqueous solution (at pH 6.0) of **3** in the range of m/z 600 to 1000. (b) The signal at m/z 900. (c) The calculated isotropic distribution for $[Cp^*Rh(NAD)-Na+H]^+$.

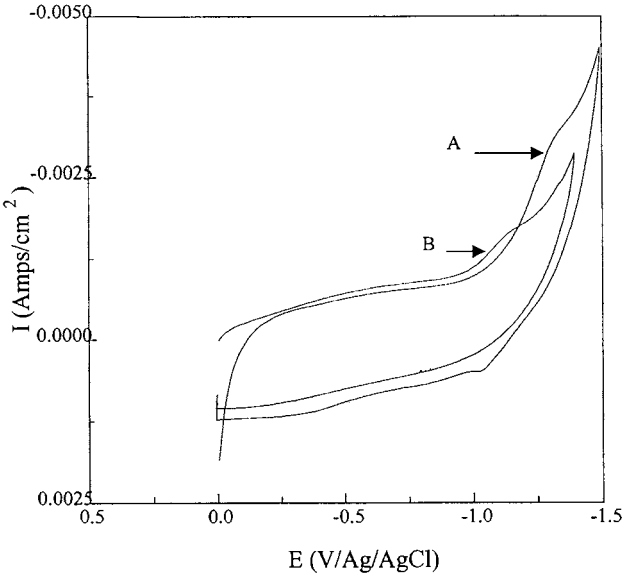


Fig. 4. CV of **3** (B, 0.5 mM) and **5** (A, 0.5 mM) in phosphate buffer (pH 6.0) at a glassy carbon electrode ($v = 50$ mV s^{-1}).

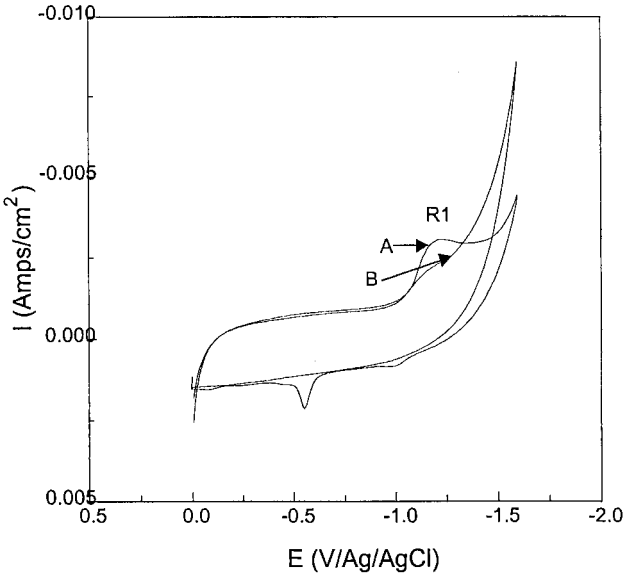


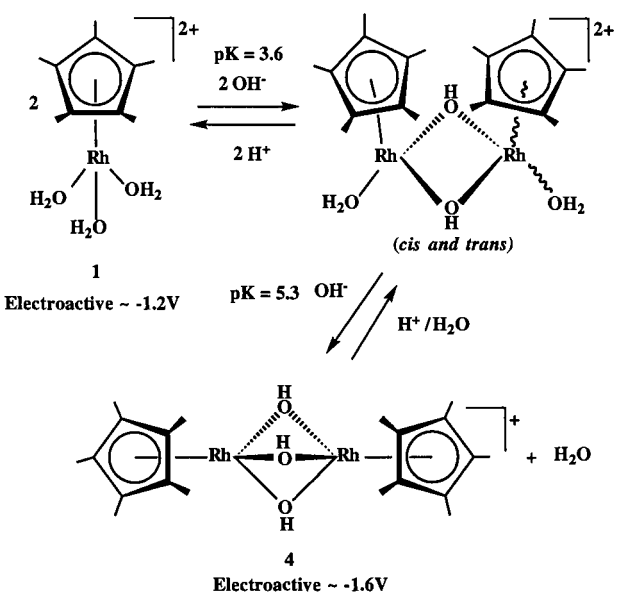
Fig. 5. CV of **4** (0.5 mM) in phosphate buffer (pH 6.0) at a glassy carbon electrode ($v = 50$ mV s^{-1}), in the absence (A) and presence of NAD+ (B, 0.5 mM).

of an aqueous solution (pH 6.0) of **3** in the range of m/z 600 to 1000 provides three prominent signals at m/z 900 ([Cp*Rh(NAD)–Na + H]$^+$, relative intensity (I) = 93%), m/z 800 ([Cp*Rh(NAD)–nicotinamide]$^+$, I = 98%), and m/z 760 ([Cp*Rh(NAD)–nicotinamide–Na + H–H_2O]$^+$, $I = 100\%$) as well as middle-intensity signals, such as m/z 778 ([Cp*Rh(NAD)–nicotinamide–Na + H]$^+$, $I = 25\%$), m/z 686 ([Cp*Rh(NAD)–nicotinamide–ribose + 2H]$^+$, $I = 27\%$), and m/z 664 ([Cp*Rh(NAD)–nicotinamide–ribose–Na + 3H]$^+$, $I = 46\%$). The signal at m/z 900 has a characteristic distribution of isotopomers that matches extremely well with the calculated isotopic distribution (Fig. 3(b,c)).

2.3. Cyclic voltammetry experiments with complexes *3* and *4* at pH 6

Since Ryabov et al. [3] carried out extensive CV studies on Hg electrodes (both stationary and dropping) with their putative [Cp*Rh(NAD)] complex(es), as well as [(Cp*Rh)$_2$(μ-Cl$_2$)Cl$_2$], and Cp*Rh aqua complexes at pH 6.05, we carried out similar studies using both a hanging mercury drop electrode and a glassy carbon disk electrode. The CV behavior of diastereomeric complex **3**, the major aqua complex at pH 6.05, complex **4**, and [(Cp*Rh)$_2$(μ-Cl$_2$)Cl$_2$] (at pH 6.05 this complex is hydrolyzed to a mixture of Cp*Rh aqua/chloride complexes) were all examined; the latter two complexes in the presence and absence of NAD$^+$ [4]. Use of a hanging mercury electrode failed to reproduce the same CV results reported by Ryabov et al., particularly with regard to the occurrence of adsorption or surface reactions. A glassy carbon electrode gave similar results to mercury, but avoids possible complications with mercury and chloride reactions. Thus, we observed (pH 6.0, phosphate buffer, Fig. 4) *one* irreversible reduction wave for **3** at −1.18 V (vs. Ag | AgCl, glassy carbon electrode), and also show for comparison the CV of the analog of **3**, complex **5** (reduction peak at −1.35 V, Ag | AgCl, glassy carbon electrode), the reduction peak of NAD$^+$ is −1.39 V under the same conditions.

Furthermore, it was reported that a Cp*Rh aqua complex (pH 6.05) forms a product in situ with NAD$^+$, as was interpreted from the CV studies [3b]. One crucial synthetic point that we found is that complex **3** *cannot* be prepared in situ directly at pH 6.0; we conducted this



Scheme 1. [Cp*Rh(H_2O)$_3$]$^{2+}$ equilibria as a function of pH.

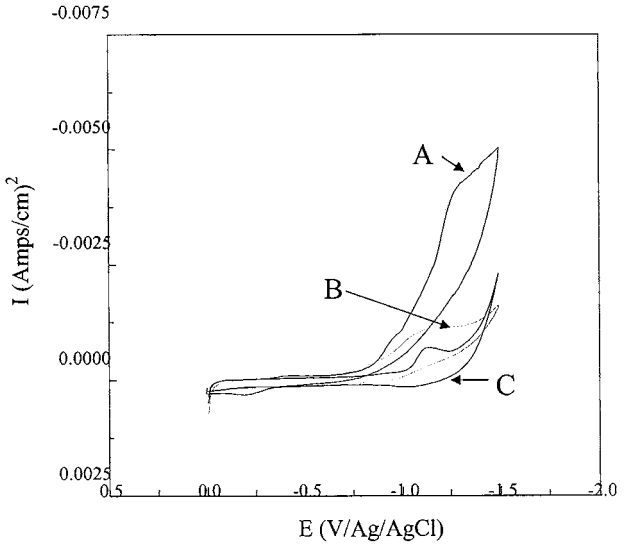


Fig. 6. Cyclic voltammograms of $[Cp^*Rh(H_2O)_3]^{2+}$ at pH 1.68 (A), 3.0 (B) and 5.0 (**C**). $V = 10$ mV s^{-1}, and the Cp*Rh concentration was 0.5 mM.

control experiment and believe that this reaction does not occur appreciably because aqua complex **4** dominates at this pH (vide infra), and does not readily react with NAD^+ as does **1** at pH 3–4 (Fig. 1). Therefore, **1** reacts readily with NAD^+ at pH 3–4 (presumed initial η^1(N7) binding), followed by η^2(N6, N7) bonding, and then self assembly of the proposed mononuclear [Cp*Rh(NAD)] complex to form cyclic trimer **3**, upon an increase of the pH from 3.0 to 6.0 (Fig. 1). More importantly, if we very carefully purified and isolated **3** via Sephadex G-25 chromatography, using a pH 6.0 phosphate buffer solution as the eluent, we were able to redissolve the isolated solid **3** back into a pH 6.0 phosphate buffer and observe that it maintained its integrity (^{1}H-NMR spectroscopy).

The CV of Cp*Rh aqua complex, **4**, at pH 6.0 (Fig. 5) *shows a decrease in the height of the irreversible reduction peak* (-1.20 V vs. Ag | AgCl, glassy carbon electrode) upon the addition of a molar equivalent of NAD^+, in agreement with the report of Koelle and Ryabov [3b] no reduction peak was observed for NAD^+, further suggesting a strong interaction between the Cp*Rh metal center and NAD^+ [3]. However, to reiterate, ^{1}H-NMR spectroscopy shows no evidence for the formation of a complex between **4** and NAD^+ if you carry out this reaction directly at pH 6.0 instead of pH 3, followed by raising the pH to 6.0. Thus, caution is necessary in the interpretation of electrochemical data under non-equilibrium conditions as demonstrated by the apparent discrepancies between our ^{1}H-NMR results and the previously reported electrochemical data [3b].

Scheme 1 illustrates the various equilibria that have been elucidated previously describing the reactions of the Cp*Rh aqua complexes in solution, as a function of pH [4]. It is conceivable that the equilibria at pH 6 between **1**, **4**, and the plausible intermediate for these aqua complexes, $[(Cp^*Rh)_2(\mu\text{-}OH)_2(H_2O)_2]^{2+}$, are perturbed by the electrochemistry, via a classical CE type of mechanism. Therefore, complicated interactions between electron (E) and ligand transfer (C) reactions in organometallic and inorganic electrochemistry are well known to produce voltammetric responses that require considerable care upon interpretation of the results [6].

Furthermore, it has been shown by Koelle [7] that complex **4** has a very negative reduction potential in CH_2Cl_2 (-1.6 V vs. SCE). This indicates that the electroactive species at -1.2 V is likely to be complex **1**, although a simple calculation of concentrations based on the thermodynamic equilibrium constants indicates that $\sim 1\%$ of the Cp*Rh aqua complex, **1**, will be present at pH 6. Indeed, the reduction potential measured by Koelle et al. [8] for a Cp*Rh aqua complex in methanol/water (10:1) at $\sim$pH 2 is -1.155 V versus SCE, in good agreement with these studies; the meaning of pH in this medium is unclear, but generally results in a more basic medium than the pH value indicates [9]. Therefore, it is reasonable to propose that the voltammetric response of the Cp*Rh aqua species in Fig. 5 involves reduction of **1** at the electrode and that the response is limited by the rates of response of the equilibria shown in Scheme 1. It is important to note that the theoretical aspects for such CE systems have been well described [9,10], and, indeed, the shape of the curve in Fig. 5 fully fits that theoretical description [10,11]. Further confirmation was provided by measuring the voltammetric response of **1**, as a function of pH, where it can be observed (Fig. 6) that the current dramatically decreases with increasing pH, i.e. the equilibrium is shifting from Cp*Rh aqua complex **1** to complex **4** (Scheme 1) [4].

The decrease in current observed in Fig. 5 upon addition of NAD^+ may then be attributed to the reaction of the adenine group with **1**, to form some of the intermediates observed in the ^{1}H-NMR spectra shown in Fig. 1. These intermediates then disturb the equilibrium in a way that reduces the supply of electroactive species in solution and results in lower currents. Such a proposal is also quite consistent with the very low currents and catalytic wave-shapes observed in Fig. 4 for **3** and **5**. *Therefore, the cyclic trimer complexes, **3** and **5**, are not likely to be electroactive, but in their equilibria to form cyclic trimers, electroactive Cp*Rh aqua complexes are present to account for the observed electrochemistry*. This plausible explanation reconciles the evidence from the ^{1}H-NMR spectra in Fig. 1 with our reported electrochemistry, as well as the CV data previously reported by Ryabov et al. [3]; further studies on the equilibrium constants and redox

potentials for these reactions are in progress to comprehend fully these results.

2.4. Reaction of $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ with NAD^+: ^{1}H-NMR spectroscopy titration and CV experiments as a function of pH

As stated, we also wanted to verify whether the purported 1:1 [Cp*Rh(NAD)Cl](Cl) complex [3] was different structurally from cyclic trimer **3**, and thus, conducted an ^{1}H-NMR titration experiment from pH 3 to 10. Not surprisingly, we found that the only NAD^+ complex with $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ at pH 6.0 had a similar ^{1}H-NMR spectrum to **3**; however, in comparison with the reaction of **1** with NAD^+, the rates of formation were very slow. Therefore, it is our contention that the novel, diastereomeric, cyclic trimer complex, **3**, is a common product at pH 6.0 whether you start with the more reactive **1** or the less reactive $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$, and that *a complex mixture of Cp*Rh compounds exists at lower pH values with both Cp*Rh synthons* (Fig. 1).

Furthermore, the voltammetric behavior of the complex(es) formed from $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ in phosphate buffer (pH 6.0) is very complicated, in accordance with the reported aqueous chemistry studied by NMR spectroscopy. Contrary to the reports of Koelle and Ryabov [3b], we were unable to observe catalytic reduction of NAD^+ by $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ in phosphate buffer solution at pH 6. Again, to avoid complications due to interactions of chloride and mercury, a glassy carbon electrode was employed. Fig. 7 shows the voltammograms that result from $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ alone, and then from the addition of NAD^+ in phosphate buffer at pH 6. The response of the Cp*Rh complex

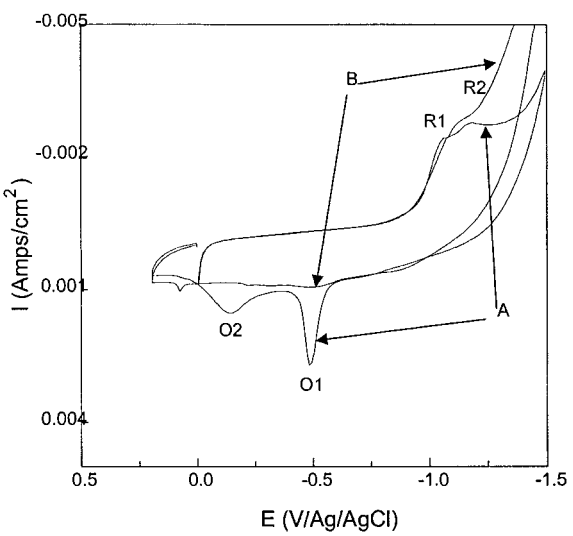


Fig. 7. CV of $[(Cp^*Rh)_2(\mu\text{-}Cl)_2Cl_2]$ in phosphate buffer (pH 6.0) at a glassy carbon electrode in the absence (A) and the presence of one molar equivalent of NAD^+ (B), where $v = 20$ mV s^{-1} and the Cp*Rh concentration was 0.5 mM.

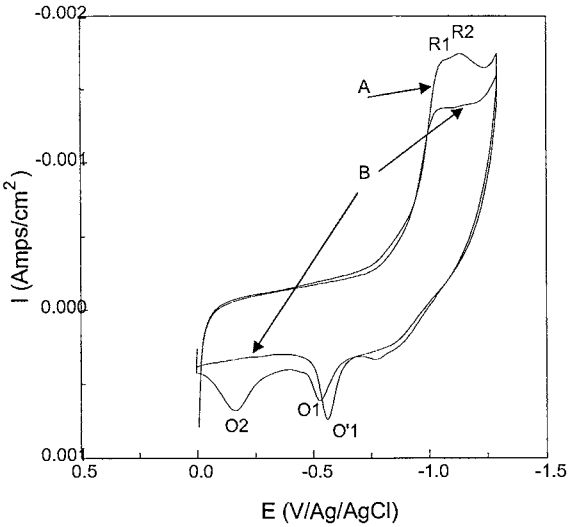


Fig. 8. CV of $[(Cp^*Rh)_2(\mu\text{-}Cl)_2Cl_2]$ in phosphate buffer (pH 6.0) at a glassy carbon electrode in the absence (A) and the presence of one molar equivalent of 2′-deoxyadenosine (B), where $v = 20$ mV s^{-1} and the Cp*Rh concentration was 0.5 mM.

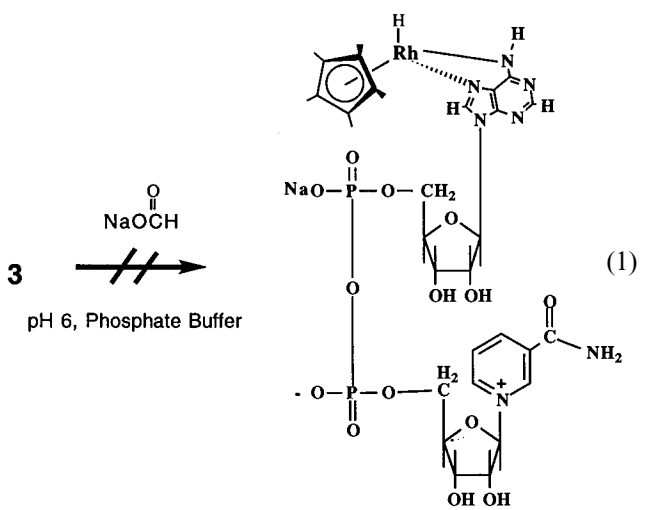
alone shows the presence of more than one species from the two reduction peaks at R1 and R2, and these are connected with the two oxidation peaks, O1 and O2, which display behavior that is consistent with adsorption of products from the reduction peaks. The multiple reduction peaks are plausibly consistent with the formation of the μ-chloro dimer, $[(Cp^*Rh)_2(\mu\text{-}Cl)_3]^+$, as well as hydrolysis products to form various μ-hydroxo-chloro/complexes **1** and **4**, as suggested by Ryabov et al. [3]. Upon addition of the NAD^+, the two reduction peaks, R1 and R2, are replaced with a single wave, while the oxidation peaks, O1 and O2, disappear. Continued addition of NAD^+ up to a 4-fold excess, with respect to Cp*Rh, results in an increase in the current by about 30% at -1.1 V, with the appearance of a reduction wave for NAD^+ at -1.39 V, indicating at most, weak catalysis if any.

Thus, this type of reactivity is most likely consistent with ligand exchange, but not necessarily catalysis. It is conceivable that the apparent catalysis reported by Koelle and Ryabov [3b] was affected by the use of mercury electrodes (e.g. streaming maxima phenomena due to adsorption) and some differences in the actual composition of the solution. Similar behavior was observed upon addition of 2′-deoxyadenosine to $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ (Fig. 8), which could result in the formation of **5**, but cannot participate in any catalytic NAD^+ reduction reaction, due to the lack of an nicotinamide group. The reduction of the current upon addition of 2′-deoxyadenosine may conceivably be attributed to the kinetically faster binding to the Cp*Rh aqua complex and thermodynamically more stable cyclic trimer product in comparison with NAD^+. *These overall results appear to indicate that many of the variations in the observed CV results were more*

likely due to the interactions of the ligand transfer equilibria with the electron transfer processes. For example, addition of chloride ion to a solution of **1** in phosphate buffer (pH 6) results in changes in the voltammogram, which was possibly consistent with formation of μ-chloro/hydroxo complexes. Furthermore, addition of bipyridine immediately gives rise to the voltammogram of $[Cp^*Rh(bipy)Cl]^+$, which is known to catalyze NAD^+ reduction to 1,4-NADH while exhibiting a stronger catalytic wave in CV experiments [12].

*2.5. Attempted reaction of cyclic trimer complex **3** with sodium formate at pH 6*

Co-factor regeneration, NAD^+ to 1,4-NADH, is an important process for many enzymatic reduction reactions and it was shown previously in an intermolecular reaction that $[Cp^*Rh(bipy)H]^+$, generated in situ, was able to catalyze this conversion using sodium formate as the hydride source [12]. Therefore, we thought to use complex **3** as a precursor for the possible formation of a mononuclear complex, [Cp*Rh(NAD)H]. Unfortunately, all our attempts to use complex **3** to induce intramolecular hydride reduction of the N-9-nicotinamide group of the adeninato nucleus, via a putative monomeric [Cp*Rh(NAD)(H)] complex, plausibly formed in situ by chemical reaction of **3** with sodium formate [7], failed to show (1 h at ambient temperature) any 1,4-NADH product as analyzed by UV–vis spectroscopy (Eq. (1)). This is also consistent with our interpretations of the CV results with **3**, which involves no catalytic reactivity. Thus, cyclic trimer **3** appears to play no apparent role in any conceivable intramolecular co-factor regeneration process [3b]. However, it is important to note that in recent studies, we found that the driving force for intermolecular regioselective reduction of NAD^+ to 1,4-NADH with in situ formed $[Cp^*Rh(bipy)H]^+$ was a consequence of coordination of the amide carbonyl of NAD^+ to a ring-slipped Cp*Rh metal ion center, η^5 to η^3-Cp*, in a possible concerted process with hydride transfer to carbon 4 of the nicotinamide nucleus [13]. Therefore, it might also be plausible that the intramolecular reduction reaction was not successful due to conformational effects that prevent or encumber intramolecular carbonyl coordination to the Cp*Rh metal ion center.



(1)

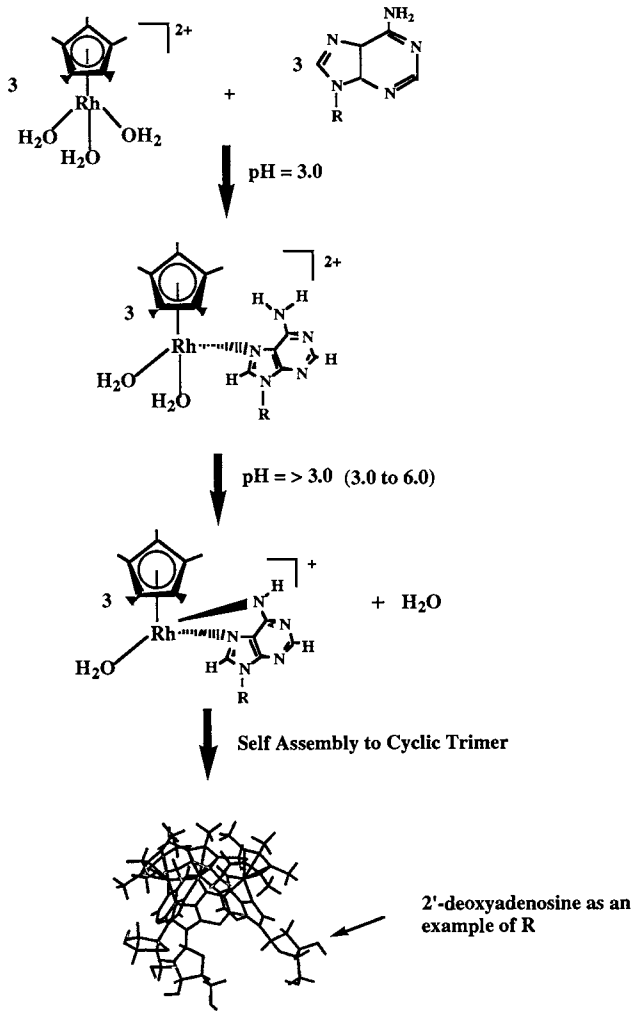
3. Conclusions

In conclusion, as far as we have been able to discern, this is the first organometallic complex of NAD^+ whose structure was clearly identified by spectroscopic techniques (unfortunately, **3** was too unstable to get suitable crystals for X-ray analysis). Moreover, we have been able to show by ^{1}H-NMR that mononuclear [Cp*Rh(NAD)] complexes (presumed initial η^1(N7) adenine binding) form at low pH under equilibrium conditions, and as the pH is raised from 3 to 6, we presume that η^2(N6, N7) adenine bonding occurs, followed by self assembly to the cyclic trimer structure (μ-η^1(N1):η^2(N6, N7)) at pH 6 (Scheme 2). Both synthons, $[Cp^*Rh(H_2O)_3](OTf)_2$ (**1**), and $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$, provide similar pH-dependent ^{1}H-NMR spectra upon reaction with NAD^+ from pH 3 to 10 to form complex **3**. We have also provided clear evidence that complex **3** is not electroactive, while the Cp*Rh aqua complex, **1**, appears to be the most electroactive species in solution. As the pH is raised from pH 2 to 9 in the CV experiments of complex **1**, a dramatic decrease in the current is observed as the equilibrium shifts from Cp*Rh aqua complex **1** to that of **4**. Similarly, this decrease in current phenomena also occurs when complex **4** or $[(Cp^*Rh)_2(\mu\text{-}Cl_2)Cl_2]$ was reacted with NAD^+ at pH 6. Future studies will focus on the reactivity of complex **3** in host–guest, molecular recognition chemistry [1h].

4. Experimental

4.1. Methods and instrumentation

Chemicals were purchased from Aldrich Chemical Co. and from Sigma. The 400 MHz ^{1}H-NMR spectroscopy and ESI/MS were performed in the Department of Chemistry, University of California, Berkeley. The cyclic voltammetry experiments were performed under argon in a three-electrode cell. The reference electrode was Ag | AgCl separated from the solution by a bridge compartment filled with the same buffer as that used in the cell. The platinum electrode was a platinum grid. The working electrode was constructed



Scheme 2. Plausible generic mechanism for formation of Cp*Rh–adeninato cyclic trimers.

from a cross section of carbon wire (diameter 2.5 mm). An EG&G PAR 173 Potentiostat with a 276 interface and CorrWare/CorrView software were used to record the current and potentials. The working electrode was a glassy disk (Bioanalytical, diameter 2.5 mm; $S = 5 \times 10^{-6}$ cm^2) and a mercury electrode ($S = 0.25 \times 10^{-6}$ cm^2) prepared by hanging a mercury drop on to a platinum disk sealed in glass. The phosphate buffer was obtained from $NaH_2PO_4.H_2O$, NaOH and distilled water (Burdick & Jackson). Solution volumes were typically 10–25 ml.

4.2. Synthesis of **3**

To a solution of $[Cp^*Rh(H_2O)_3](OTf)_2$ (59.0 mg, 0.1 mmol) [4] in H_2O (10 ml) was added β-nicotinamide adenine dinucleotide, sodium salt (68.5 mg, 0.1 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at ambient temperature (pH 4.1) for 30 min, and then the pH of the solution was adjusted to 6.0 by the addition of 0.65 M NaOH. Upon evaporation of the solvent, an orange residue was obtained (100 mg). The residue was dissolved in 2 ml of pH 6.0 phosphate buffer solution (prepared from $NaH_2PO_4 \cdot H_2O$ and NaOH), and then it was purified via Sephadex G-25 chromatography (column diameter: 25 mm; height: 400 mm) using the pH 6.0 phosphate buffer as the eluent. The first yellow fraction (4 ml) from the Sephadex column was collected, and then the solvent was evaporated and the yellow powder was dried in vacuo (yield: 30%). ^{1}H-NMR (400 MHz, D_2O, 23°C, reference to the residual protons of D_2O, 4.67 ppm, see Fig. 2 for NAD^+ proton designation) d 9.27, 9.21 (s, 1H, H2′), 9.13, 9.09 (d, 1H, H6′), 8.79, 8.74, (d, 1H, H4′), 8.64, 8.63 (s, 1H, H8), 8.14, 8.12 (dd, 1H, H5′), 7.54, 7.52 (s, 1H, H2), 6.03, 5.78 (d, 1H, H1‴), 5.64, 5.62 (d, 1H, H1″), 4.50–3.90 (m, 4H, H5″ and H5‴), 1.67, 1.673 (s, 15H, Cp*). ESI/MS (pH 6.0 phosphate buffer) m/z 900 ($I = 94\%$). UV–vis (pH 6.0 buffer): λ 208 ($\varepsilon = 11\,847$), λ 228 ($\varepsilon = 10\,211$), λ 260 nm ($\varepsilon = 5000$). The analytical C, H, and N data for **3** could not be obtained due to its purification with pH 6.0 phosphate buffer.

4.3. UV–vis experiment with **3** and sodium formate

A degassed solution of complex **3** (4.4 mg, 1.4×10^{-3} mmol) in 10 ml of a pH 6.0 phosphate buffer was transferred to a modified cuvette with a serum cap (under N_2) and the UV–vis spectrum recorded (pH 6.0 buffer): λ 208 ($e = 11\,847$), λ 228 ($e = 10\,211$), λ 260 nm ($\varepsilon = 5000$). Addition of a degassed solution of sodium formate (1.1 mg, 14×10^{-3} mmol) in 2 ml of phosphate buffer (pH 6.0) caused no change in the UV–vis spectrum of complex **3** over a 1 h period, where the 1,4-dihydro derivative would be expected to have an absorbance at 340 nm [12].

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