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Direct binding of η^5-pentamethylcyclopentadienyl-rhodium(III) to nicotinamide cofactors: a step towards NAD^+/NADH recycling

Alexander D. Ryabov *,*,[a], Dmitri L. Menglet [b] and Mikhail D. Levi [c]

[a] *Division of Chemistry, G.V. Plekhanov Institute of National Economy, Stremyanny per. 28, 113054, Moscow (USSR)*
[b] *Department of Chemistry, Moscow State University, 119899, Moscow (USSR)*
[c] *A.N. Frumkin Institute of Electrochemistry, Academy of Sciences of the USSR, Leninsky pr. 31, 117071, Moscow (USSR)*

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Abstract

The complex $[Cp^*RhCl_2]_2$ reacts with NAD^+ to give $[Cp^*Rh(NAD^+)Cl]^+$ with rhodium(III) bonded to the adenine ring. The metal-bonded cofactor is accepted by two dehydrogenases. Similar reactions with NADH afford NAD^+ and Rh^I compounds.

There is still an urgent need in the efficient recycling of nicotinamide cofactors [1]. In recent years a novel general approach, based on transition metal complex carriers such as 2 e^-/H^+ or hydride became apparent [2]. Rhodium complexes seem now the most promising [3], because of facile, possibly hydride coupled, $Rh^{III} \rightleftharpoons Rh^I$ transitions, that give rise to hydridorhodium intermediates capable of reducing NAD^+. For example, the corresponding hydride, generated from $[Cp^*Rh(dipy)]^+$ ($Cp^* = \eta^5$-C_5Me_5) either electrochemically [3a] or chemically [3b,c], intermolecularly reduces the nicotinamide ring of NAD^+ to give enzymatically active NADH, accepted by lactate dehydrogenase. These facts led us to an idea of how to realize the intramolecular, electrochemical regeneration system, based on $Rh^{I/III}$, applicable both in enzymatic organic syntheses [4] and biosensoring [5]. It was decided to anchor the Cp^*Rh^{III} fragment to the adenine residue of NAD^+ (or NADH) through the corresponding N donor centers, as it gives adducts with many related ligands [6]. Here, we report the complexation of free adenine with $[Cp^*RhCl_2]_2$, the very different reactions of NAD^+ and NADH with the latter, and the "enzymatic" reactivity of coordinated NAD^+.

A dark-purple suspension of $[Cp^*RhCl_2]_2$ (1) reacts in refluxing methanol with a 12.5% excess of adenine to give, after precipitation by ether, a yellow product

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formulated as [Cp*Rh(adenine)Cl]Cl (**2**) *. Rhodium is chelated probably by the N7 and amino nitrogens of the purine ligand. The coordination of N7 is evidenced by the IR peak at 1640 cm^{-1} (C=N) which can be compared with that at 1665 cm^{-1} for free adenine. The Rh–NH_2 bonding is much weaker given the less pronounced changes in the NH_2 region. An indirect evidence for chelation comes from the spectrophotometric study of reaction **1**, in aqueous solution, at 25 °C (pH 4.9). Since complex **1** exists as a trichloro-bridged rhodium(III) dimer (λ_{max} 386 nm) in acidic aqueous solution [7,8], the following stoichiometry might be considered:

$$\underset{\textbf{(1a)}}{[Cp^*Rh(\mu\text{-}Cl)_3RhCp^*]^+} + 2\ \text{adenine} \overset{K_1}{\rightleftharpoons} 2\underset{\textbf{(2)}}{[Cp^*Rh(\text{adenine})Cl]^+} + Cl^- \qquad (1)$$

$$\left(K_1 = [\mathbf{2}]^2[Cl^-]/[\mathbf{1a}][\text{adenine}]^2\right)$$

Obviously, as the equilibrium generates oppositively charged species, the values of K_1 depend very strongly on the ionic strength (0.46 ± 0.03, 0.84 ± 0.03, 2.48 ± 0.09, and 11.3 ± 0.4 at 0.01, 0.02, 0.05, and 0.10 mol dm^{-3} KNO_3, respectively). It can be concluded that the organometallic moiety might be anchored to nicotinamide cofactors through adenine. It is noteworthy that, as is the case with the parent dimer **1** [8], complex **2** is electrochemically active. (Representative cyclic voltammograms are shown in Fig. 1.) With regard to previous reports [3a,8,9], it can be argumented that adenine-bound Rh^{III} and Rh^{I} species might be generated electrochemically.

At higher pH values (> 7), the binding of adenine does not occur readily, because the dimer **1a** is converted into a species with a maximum at 365 nm. (This equilibrium is characterized by the isobestic point at 388 nm.) It could be concluded that the product formed is the trihydroxo-bridged dimer [10]. The quantitative analysis of the corresponding absorbance versus pH plot at 360 nm (not shown) is, however, in a much better agreement with the formation of the μ-chloro-di-μ-hydroxo complex (see eq. 2).

$$\mathbf{1a} + 2\ H_2O \overset{K_2}{\rightleftharpoons} \underset{\textbf{(3)}}{[Cp^*Rh(\mu\text{-}Cl)(\mu\text{-}OH)_2RhCp^*]^+} + 2\ H^+ + 2\ Cl^- \qquad (2)$$

$$\left(K_2 = [\mathbf{3}][H^+]^2[Cl^-]^2/[\mathbf{1a}]\right)$$

Thus the conversion of **1a** into **3** occurs at pH values of 6–7 and the value of K_2 is equal to 5.9 (±0.4) × 10^{-19} mol^4 dm^{-12} (25 °C).

The next step was to bind Cp^*Rh^{III} to NAD^+. To achieve this, a solution of NAD^+ (0.055 mmol) in 2 mL of water was mixed with a suspension of dimer, **1** (0.050 mmol) in 2 mL of H_2O. The mixture was stirred for 30 min, filtered and then lyophilized to give a yellow product **. The corresponding reaction **3** was also studied spectrophotometrically (at 375 nm); equilibrium constant K_3 0.76 ± 0.04 (at 25 °C, pH value of 5 without a supporting electrolyte). This value is very

* Analytical data: Found: C, 40.70, H, 4.54. $C_{15}H_{19}N_5Cl_2Rh$ calcd.: C, 40.65, H, 4.32%. ^1H NMR (CD_3COOD): δ 1.58 (s, 15H, CH_3); 8.60 (m, H^2 and H^3).

** Analytical data: Found: C, 36.18, H, 4.35, N, 8.45. $C_{31}H_{41}N_7O_{14}C_{12}P_2NaRh \cdot H_2O$ calcd.: C, 36.77, H, 4.28, N, 9.68%.

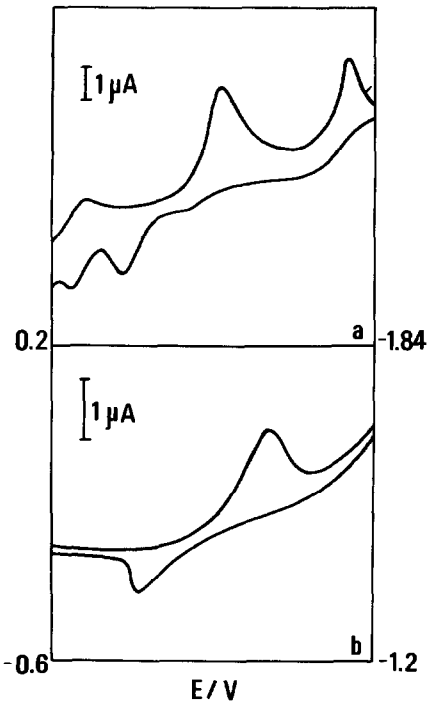

Fig. 1. Cyclic voltammograms of complex **2** obtained in DMSO (a) and in water (b). Conditions: (a) $[\mathbf{2}] = 2.2 \times 10^{-3}$ mol dm^{-3}, scan rate 0.1 V s^{-1}, $[Na_2SO_4] = 0.1$ mol dm^{-3}; (b) $[\mathbf{2}] = 3.1 \times 10^{-3}$ mol dm^{-3}, scan rate 0.025 V s^{-1}, $[Na_2SO_4] = 0.1$ mol dm^{-3}. Stationary mercury electrode, potentials against SCE.

close to that of K_1 at low ionic strength, suggesting identical binding sites in adenine and NAD^+, i.e. N7 and amino nitrogen atoms.

$$\mathbf{1a} + 2\ NAD^+ \overset{K_3}{\rightleftharpoons} \underset{(\mathbf{4})}{2[Cp^*Rh(NAD^+)Cl]^+} + Cl^- \qquad (3)$$

$$\left(K_3 = [\mathbf{4}]^2[Cl^-]/[\mathbf{1a}][NAD^+]^2\right)$$

Complex **4** has one very important feature: its coordinated cofactor is accepted by enzymes. Particularly, enzymatic catalysis is realized in the case of oxidation of formate (more than 75% yield) in the presence of formate dehydrogenase from *Pseudomonas sp. 101* and in that of oxidation of ethanol in the presence of yeast alcohol dehydrogenase. Free or complexed forms of the cofactor may be used in these cases. The former is to be preferred, because the catalytically active cofactor can be produced from the equilibrium 3.

In contrast to all described above, the reduced cofactor, NADH, interacts with **1** quite differently. Instead of a yellow complex, a purple one is formed, that might be the reduced rhodium species. If the reaction is followed spectrophotometrically, decrease in absorbance at ca. 340 nm and an increase at ca. 510 nm, typical of consumption of NADH and formation of rhodium(I) [9b], respectively, can be observed. HPLC analysis showed the formation of NAD^+ in 65% yield. This

interaction can be depicted as shown in eq. 4 [9b]:

$$\left[Cp^*Rh^{III}Cl_2\right]_2 + 2\ NADH \rightleftharpoons 2\left[Cp^*Rh^{I}(NAD^+)\right] + 2\ HCl + 2\ Cl^- \qquad (4)$$

(5)

Note that the oxidized cofactor in **5** is also accepted by the enzymes; the yields are lower, however, when compared with **4**.

Another remarkable feature of reaction **4** is that it does not occur at pH values > 7. The tentative reason is that dimer **1a** only is a reactive species (μ-chloro-di-μ-hydroxo complex **2** does not oxidize NADH). This observation, as well as the basically different behavior of rhodium(III) species toward NADH, suggests a mechanism of oxidation by Rh^{III}.

Because the trichloro-bridged species reacts only, the $Rh(\mu\text{-}Cl)_3Rh$ fragment is probably a gate for NADH to enter the coordination sphere of rhodium. The principle difference of NADH, compared with NAD^+, is the presence of tertiary nitrogen in the reduced nicotinamide ring. The nitrogen atom probably acts as a donor center that cleaves the chloro bridges enabling the intramolecular rupture of the C–H bond, with the hydrogen moving as a hydride, followed by a two-electron transfer to rhodium(III). This C–H cleavage enables the aromatization of the nicotinamide ring as well. There is an analogy with cyclometalation reactions [11] and, hence, the process may be called an oxidative cyclometalation.

In conclusion, the attachment of the Cp^*Rh^{III} moiety to NAD^+ does not eliminate the ability of the latter to being accepted by dehydrogenases. At the same time Cp^*Rh^{III} converts NADH into NAD^+, thereby transforming into a Rh^I species. Because either Rh^{III} or Rh^I species can be generated electrochemically, there are now certain ways to the efficient recycling systems under study.

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