Very Efficient Reduction of NAD(P)$^+$ with Formate catalysed by Cationic Rhodium Complexes

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Cationic rhodium complexes catalyse very efficiently the reduction of pyridine coenzymes by sodium formate.

Reduced nicotinamide cofactors (NADH and NADPH) play an important role in a variety of enzyme-catalysed reactions. Unfortunately, their generation and regeneration by electrochemical means or by powerful reducing agents leads to non-selective reduction of the oxidised cofactor. Several effective enzymatic,[1] indirect (redox catalysis) electrochemical,[2] or photochemical[3] regeneration systems have been described in recent years. The only chemical reducing agent which selectively and effectively reduces NAD(P)$^+$ to 1,4-NAD(P)H is dithionite.[4]

Recently, we described a regeneration system in which NAD$^+$ was selectively reduced to the enzyme-active form and *in situ* coupled to an enzymatic reduction by a (C_5Me_5)(2,2'-bipyridine)rhodium(I) complex generated at a cathode.[5] With an appropriate working potential, the single electron reduction of NAD$^+$ could be almost totally suppressed.

We now propose a more efficient, purely homogeneous reduction system, in which the reducing equivalents (2 electrons and a proton) are supplied by formate as described in Scheme 1.

Results of the reduction of NAD$^+$ and NADP$^+$ using various rhodium pentamethylcyclopentadienyl complexes are shown in Table 1 and indicate the following.

(i) This system provides the first example of a chemical reduction of nicotinamide coenzymes catalysed by metal complexes. The reduction occurs under very mild conditions (pH 6—10; 20—40 °C; formate as ultimate electron donor).

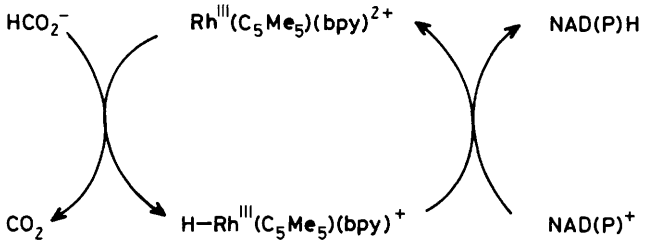
(ii) This system can be used to reduce both NAD$^+$ and NADP$^+$ in contrast with enzymatic reduction where different enzymes are needed.

(iii) A pronounced temperature effect was observed (compare experiments at 25 and 38 °C) and an activation energy of about 21 kcal/mol (cal = 4.184 J) was calculated from the experimental data.

(iv) The formation of NAD(P)H depends linearly on time (see Figure 1). This indicates that the reaction rate is independent of the NAD(P)$^+$ and NAD(P)H concentration. As a consequence, and in contrast with the enzymatic reduction where inhibition is observed with increasing NAD(P)H concentration [for example, the inhibition constant for formate dehydrogenase is known: K_I (NADH) = 0.09 mM],[6] the reaction can be run until all the NAD(P)$^+$ present is reduced (99 ± 1%).

(v) In the absence of these rhodium complexes, no reduction of NAD(P)$^+$ is observed.

By varying the formate concentration, Michaelis type kinetic behaviour is observed (Figure 2). This may indicate that a pre-equilibrium, with formation of a complex between Rh^{III} and formate, is the initial step. A K_M value of 140 mM can be determined (for comparison the Michaelis constants of the enzyme formate dehydrogenase for formate and NAD$^+$ are 43 and 0.03 mM[6] respectively). The subsequent step of the reaction must be formation of the rhodium(III) hydride with extrusion of CO_2. This is probably the rate limiting step of the overall reaction. The pronounced isotopic effect observed when DCO_2D was used instead of HCO_2H supports this hypothesis (see Figure 1), while use of D_2O instead of H_2O had only a limited influence on the overall reaction rate. The reactive rhodium hydride formed subsequently reacts rapidly with the coenzyme, regenerating the starting rhodium(III) complex (see Scheme 2).



Scheme 1

Table 1. Reduction of NAD(P)$^+$ with formate catalysed by the rhodium complexes [RhCl(C_5Me_5)L]$^+$.[a]

Ligand L	Turnover frequency/h^{-1} [b] bpy[c]	4,4'-Me_2bpy	6,6'-Me_2bpy
NAD$^+$ ⟶ NADH			
25 °C	19.2	17.8	15.1
38 °C	81.5	76.4	60.9
NADP$^+$ ⟶ NADPH			
38 °C	81.9	73.4	58.4

[a] A degassed buffer solution (1.5 ml; sodium phosphate, 0.1 M; sodium formate, 0.5 M; pH 7) containing NAD(P)$^+$ (4.2×10^{-4} M) was thermostated in a spectrophotometer. After addition of the rhodium complex (7.5 µl of a 5×10^{-3} M stock solution), the formation of NAD(P)H was followed by monitoring the absorbance at 340 nm. [b] The turnover frequency is defined as the ratio of the moles of NAD(P)H formed per hour to the moles of rhodium complex introduced. [c] bpy = 2,2'-bipyridine.

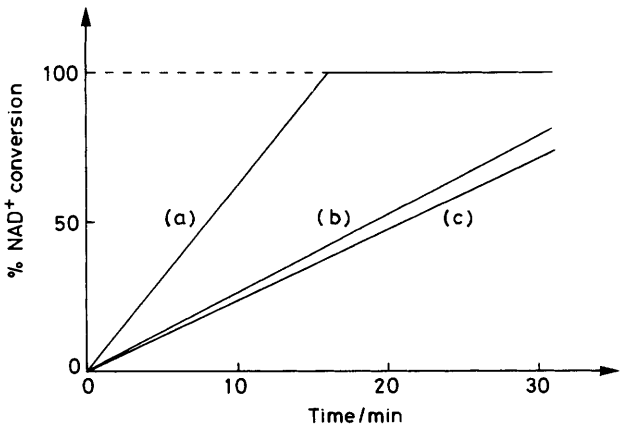


Figure 1. Conversion of NAD$^+$ with [RhCl(C_5Me_5)(bpy)]$^+$ as a function of time at 38 °C. (a): with HCO_2H in H_2O; (b): with DCO_2O in H_2O; (c): with DCO_2D in D_2O.

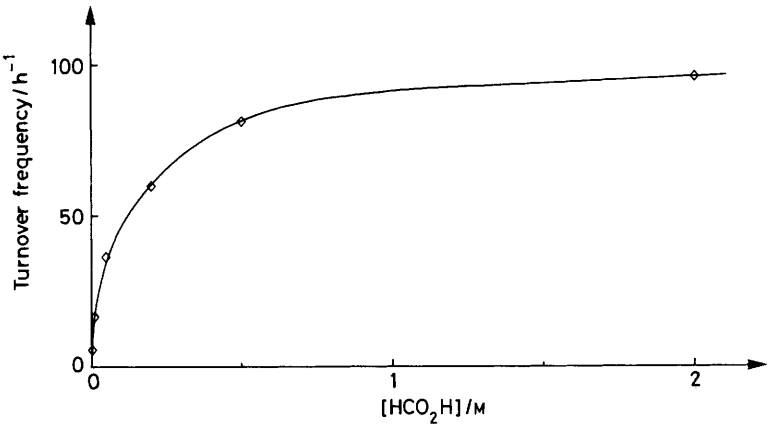


Figure 2. Turnover frequency as a function of formate concentration at 38 °C (pH 7:0.1 M sodium phosphate buffer); with [RhCl-(C_5Me_5)(bpy)]$^+$.

$$[Rh^{III}]^{2+} + HCO_2^- \rightleftharpoons [Rh^{III}(O_2CH)]^+$$

$$[Rh^{III}(O_2CH)]^+ \longrightarrow [H\text{-}Rh^{III}]^+ + CO_2 \quad \text{rate limiting}$$

$$[H\text{-}Rh^{III}]^+ + NAD(P)^+ \longrightarrow [Rh^{III}]^{2+} + NAD(P)H \quad \text{fast}$$

$$Rh^{III} = Rh(C_5Me_5)(bpy)$$

Scheme 2

In contrast with the electrochemical regeneration system, where reduction of rhodium(III) to rhodium(I) was the initial step, in this case no formation of this complex was observed when the variation of the u.v.–visible spectrum of a degassed solution of rhodium and formate with time was monitored. A broad absorption band (around 610 nm) slowly appears in the absence of coenzyme [the rhodium(I) complex has been described previously[7] and shows an absorption maximum at 512 nm].

By using this type of cationic rhodium complexes or even the water-soluble form of Wilkinson's catalyst (with sulphonated phosphines), the coenzymes can also be hydrogenated under atmospheric pressure. However, the rates of reduction are slow compared to the formate regeneration system.

In conclusion, the use of cationic complexes with aromatic diimines allows the selective reduction of nicotinamide coenzymes to the enzyme-active form to occur. The mechanism of this reaction is currently being studied.

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