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Photochemical Reduction of NAD⁺ to 1,4-NADH without an Enzyme

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 $Rh(terpy)_{2^{3+}}$ and $Rh(bpy)_{3^{3+}}$ (terpy = 2,2'; 6',2"-terpyridine; bpy = 2,2'-bipyridine) catalyse the regiospecific photochemical reduction of NAD⁺ into 1,4-NADH; $Rh(terpy)_{2^{3+}}$ retains its catalytic activity in the photochemical reaction for longer than $Rh(bpy)_{3^{3+}}$.

Many systems for the photochemical reduction of NAD⁺ have been reported recently.¹ Several of these are enzymatic systems. Although enzymes are often highly selective catalysts for reduction at the 4-position (to give 1,4-NADH), they undergo degradation when the system is run for an extended period of time. Therefore, enzyme-free systems are a desirable alternative. Unfortunately, the reduction of NAD⁺ by a high potential photosensitizer² or by electrochemical means³ produces a mixture of dimers (NAD_2) and NADH isomers which are not catalytically active.

Recently, Wienkamp *et al.* proposed that tris(2,2'-bipyridine)rhodium(III), Rh(bpy)₃³⁺, is an effective catalyst forphotochemical or electrochemical reduction.⁵ The utility ofthis catalyst is lessened by a tendency for dissociation of one ofthe bipyridine ligands from the reduced form of the complex.⁶A previous attempt to enhance the stability of this complex



Figure 1. Reverse-phase HPLC analysis of (a) NAD⁺ buffer solution reduced by NaBH₄; (b) 1,4-NADH buffer solution; (c) the deaerated reaction mixture containing TEOA, Ru(bpy)₃²⁺, NAD⁺, and Rh(terpy)₂³⁺ as a catalyst after 90 min irradiation; (d) the same mixture before irradiation. All samples were monitored at 340 nm. The peak assignment of (a) was based on results in ref. 8. Initial conditions [(c) and (d)]; [TEOA] = 250 mM, [Ru(bpy)₃²⁺] = 50 μ M, [NAD⁺] = 4 mM, and [Rh(bpy)₃³⁺] or [Rh(terpy)₂³⁺] = 250 μ M in 4 mI of buffer solution (pH 8.0). Irradiation was carried out with a 500 W Xe-arc lamp fitted with appropriate glass cut-off filters, with light of wavelengths in the range 420—700 nm. Column: radial PAK cartridge C-18, mobile phase. H₂O-0.1 M NH₄HCO₃-MeOH (30:69:1); flow rate 1.5 ml min⁻¹.

has been reported.⁷ We describe herein the catalytic activity of bis(2,2';6',2''-terpyridine)rhodium(III), Rh(terpy)₂³⁺, which we have compared with that of Rh(bpy)₃³⁺. The catalytic systems consist of one of these two catalysts, triethanolamine (TEOA) which acts as an electron donor, Ru(bpy)₃²⁺ as a photosensitizer, and NAD⁺. The products of the reaction were analysed by HPLC and the results are shown in Figure 1. Only 1,4-NADH is produced when the reaction is irradiated [Figure 1(c)]. The additional peak at 19 min which elutes at the same position as 1,2-NADH is due to Ru(bpy)₃²⁺. Significantly, this does not increase in intensity during the course of the reaction. Similar regioselectivity was achieved when Rh(bpy)₃³⁺ was used as a catalyst.

The time course of the reaction revealed the differences between the catalysts (Figure 2). The $Rh(bpy)_3^{3+}$ reaction showed maximal formation of 1,4-NADH after 4 h, whereas the $Rh(terpy)_2^{3+}$ reaction continued to produce further 1,4-NADH after this time. This is presumably due to the



Figure 2. The variation of [NADH] during irradiation of the reaction mixture with (a) $Rh(bpy)_3^{3+}$ and (b) $Rh(terpy)_2^{3+}$ as catalyst. The concentration of 1,4-NADH was determined by HPLC analysis. Experimental conditions are as in Figure 1.

greater stability of the bis(tri-co-ordinate) species over the tris(di-co-ordinate species).

In conclusion, $Rh(terpy)_2^{3+}$ is a good catalyst for the photochemical reduction of NAD⁺ to 1,4-NADH. The details of the mechanism are currently under investigation.

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