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 photosensitizer[2] or by electrochemical means[3] 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), $Rh(bpy)_3^{3+}$, is an effective catalyst for photochemical or electrochemical reduction.[5] The utility of this catalyst is lessened by a tendency for dissociation of one of the bipyridine ligands from the reduced form of the complex.[6] A previous attempt to enhance the stability of this complex

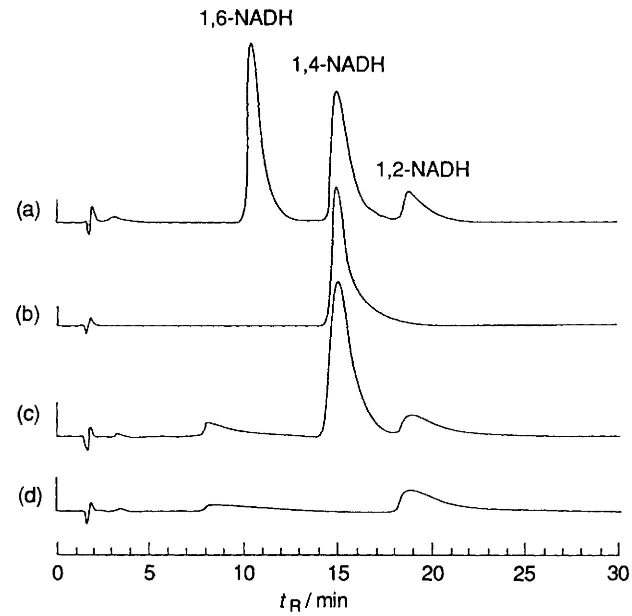

Figure 1. Reverse-phase HPLC analysis of (a) NAD^+ buffer solution reduced by $NaBH_4$; (b) 1,4-NADH buffer solution; (c) the deaerated reaction mixture containing TEOA, $Ru(bpy)_3^{2+}$, NAD^+, and $Rh(terpy)_2^{3+}$ as a catalyst after 90 min irradiation; (d) the same mixture before irradiation. All 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, $[Ru(bpy)_3^{2+}]$ = 50 μM, $[NAD^+]$ = 4 mM, and $[Rh(bpy)_3^{3+}]$ or $[Rh(terpy)_2^{3+}]$ = 250 μM in 4 ml 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. H_2O–0.1 M NH_4HCO_3–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), $Rh(terpy)_2^{3+}$, which we have compared with that of $Rh(bpy)_3^{3+}$. The catalytic systems consist of one of these two catalysts, triethanolamine (TEOA) which acts as an electron donor, $Ru(bpy)_3^{2+}$ 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 $Ru(bpy)_3^{2+}$. Significantly, this does not increase in intensity during the course of the reaction. Similar regioselectivity was achieved when $Rh(bpy)_3^{3+}$ 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 greater stability of the bis(tri-co-ordinate) species over the tris(di-co-ordinate species).

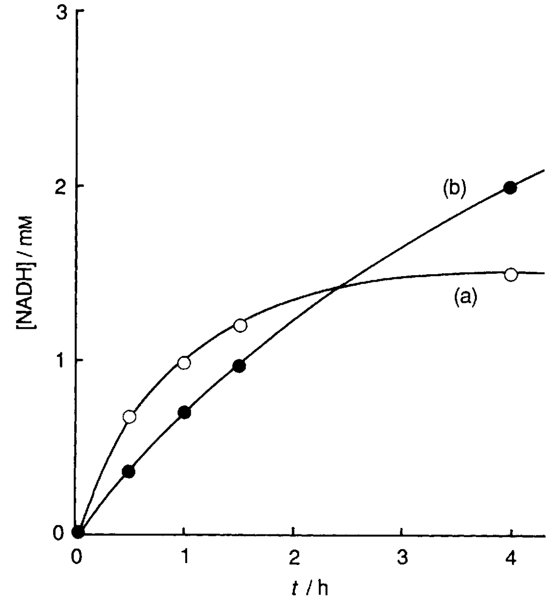

Figure 2. The variation of [NADH] during irradiation of the reaction mixture with (a) $Rh(bpy)_3^{3+}$ and (b) $Rh(terpy)_2^{3+}$ as catalyst. The concentration of 1,4-NADH was determined by HPLC analysis. Experimental conditions are as in Figure 1.

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.

Received, 30th November 1989; Com. 9/05123F

References

1 D. Mandler and I. Willner, *J. Chem. Soc., Perkin Trans. 2*, 1986, 805; 1988, 997; M. Julliard, J. L. Petit, and P. Ritz, *Biotech. Bioenerg.*, 1986, **28**, 1774; Z. Goren, N. Lapidot, and I. Willner, *J. Mol. Catal.*, 1988, **47**, 21; H. K. Chenault and G. M. Whitesides, *Appl. Biochem. Biotech.*, 1987, **14**, 147.
2 J. Kiwi, *J. Photochemistry*, 1981, **16**, 193.
3 F. G. Drakesmith and B. Gibson, *J. Chem. Soc., Chem. Commun.*, 1988, 1493; M. Jensen and P. H. Elving, *Biochim. Biophys. Acta*, 1984, **764**, 310; M. Stundničkova, H. P.-Klukanová, J. Turánek, and J. Kovář, *J. Electroanal. Chem.*, 1988, **252**, 383.
4 R. Wienkamp and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 497.
5 R. Wienkamp and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 782.
6 M. Kirch, J.-M. Lehn, and J.-P. Sauvage, *Helv. Chim. Acta*, 1979, **62**, 1345; S.-F. Chan, M. Chou, C. Creutz, T. Matsubara, and N. Sutin, *J. Am. Chem. Soc.*, 1981, **103**, 369.
7 S. Grammenudi, M. Franke, F. Vögtle, and E. Steckhan, *J. Incl. Phenom.*, 1987, **5**, 695.
8 H. Jaegfeldt, *Bioelectrochem. Bioenerg.*, 1981, **8**, 355.