Thermal and Photochemical Regeneration of Nicotinamide Cofactors and a Nicotinamide Model Compound Using a Water-soluble Rhodium Phosphine Catalyst

Itamar Willner,* Ruben Maidan, and Michal Shapira
Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

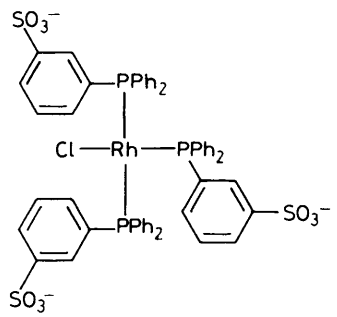
Chlorotris[diphenyl(*m*-sulphonatophenyl)phosphine]rhodium(I) acts as a homogeneous catalyst in the thermal and photoinduced regeneration of natural nicotinamide adenine dinucleotide cofactors, NAD(P)$^+$, and of a model compound 1-benzylnicotinamide. The thermal regeneration system uses formate as the hydride donor. The kinetics of the system has been studied and shows enzyme-like characteristics. The photoinduced regeneration system with $Ru(bpy)_3^{2+}$ as the photosensitizer and ascorbate as the electron donor proceeds through formation of a rhodium–hydrido active species. The photoregenerated cofactors are subsequently coupled to the enzymatic reduction of acetaldehyde to ethanol, or to the model enzymatic reduction of benzil to benzoin.

Regeneration of dihydronicotinamide cofactors is of substantial importance in numerous biocatalytic transformations.[1,2] The high cost of the nicotinamide cofactors requires their *in situ* regeneration in the biocatalytic processes. Thermal,[1,3] electrochemical,[4] and photochemical[2,5] means for the regeneration of nicotinamide adenine dinucleotide cofactors, NAD(P)H, have been developed in recent years. Chemical regeneration of NAD(P)H has been accomplished with a variety of biocatalysed reactions which utilize a substrate and the respective NAD(P)$^+$-dependent enzyme: *i.e.* alcohol and alcohol dehydrogenase,[6] formate and formate dehydrogenase,[7] glucose and glucose dehydrogenase,[8] or hydrogen and hydrogenase.[9] Electrochemical[4,10] regeneration of NAD(P)H cofactors was accomplished through electrochemical generation of the *N,N'*-dimethyl-4,4'-bipyridinium radical, $MV^{+\bullet}$ (methyl viologen radical), which acts as an electron carrier for NADH and NADPH regeneration in the presence of lipoamide dehydrogenase and ferredoxin NADP-reductase, respectively. Photochemical regeneration of NAD(P)H cofactors was accomplished in various photosystems which included biocatalysts. Homogeneous photosensitizers such as tris(bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$, *meso*-tetramethylpyridinium zinc(II) porphyrin (Zn-TMPyP^{4+}), tris-(bipyrazine)ruthenium(II) [$Ru(bpz)_3^{2+}$] or heterogeneous semiconductor powders and colloids *i.e.* CdS or TiO_2, have been used for the light-induced production of $MV^{+\bullet}$ and subsequent regeneration of NADH and NADPH in the presence of lipoamide dehydrogenase and ferredoxin reductase, respectively.[5]

Non-enzymatic regeneration of NAD(P)H cofactors requires the regioselective transfer of two electrons and a proton to the cofactor [equation (1)]. In early studies[11] dithionite, $S_2O_4^{2-}$,

$$NAD(P)^+ + 2e^- + H^+ \rightleftharpoons NAD(P)H \qquad (1)$$

was employed as a reducing agent for the cofactor NAD^+. However, the turnover numbers of the regeneration process are limited due to non-selective reduction of the cofactor and dimerization of the intermediate radicals, NAD$^\bullet$. Previously, various rhodium complexes have been applied as catalysts for the regeneration of NAD(P)H cofactors. In these systems hydrido-rhodium species are assumed to be the active catalytic moiety in the regeneration of NAD(P)H. Tris(bipyridine)-rhodium(III),[12] and tris(5-sulpho-2,2'-bipyridine)rhodium(III)[13] were used for the electrocatalysed and photocatalysed regeneration of these cofactors, yet the turnover numbers in such non-enzymatic processes were poor. $Rh^I(C_5Me_5)(bpy)$ has also been utilized as a catalyst for the regeneration of NADH using formate as the hydride donor.[14]



(1)

Recently,[15] we applied chlorotris[diphenyl(*m*-sulphonatophenyl)phosphine]rhodium(I), (**1**), as a homogeneous catalyst in the photoinduced hydrogenation and hydroformylation of unsaturated substrates, by *in situ* generation of hydrido-rhodium species. Here we report on the application of (**1**) as a catalyst for the thermal and photochemical regeneration of NAD(P)H cofactors, as well as the regeneration of a cofactor model compound for the natural system, *i.e.* 1-benzyl-1,4-dihydronicotinamide (BNAH). We have demonstrated that these systems can be coupled to subsequent synthetic processes, but, nevertheless, we find that the artificially catalysed NAD(P)H regeneration cycles are substantially less effective than the respective biocatalysed transformations.

Experimental

Absorption spectra were recorded with an Uvikon-860 (Kontron) spectrophotometer equipped with a thermostatted cell holder including a magnetic stirring motor. The irradiations were performed with a 150 W Xenon lamp, with the light filtered through a 400 nm cut-off filter. Product formation was analysed by GC (Hewlett–Packard 5890), and by HPLC (Merck–Hitachi) with a RP-18 column and UV detector. Chemicals were obtained from Aldrich; enzymes and cofactors from Sigma. Compound (**1**),[16] 1-benzylnicotinamide[17] and DCO_2Na[18] were prepared according to the literature.

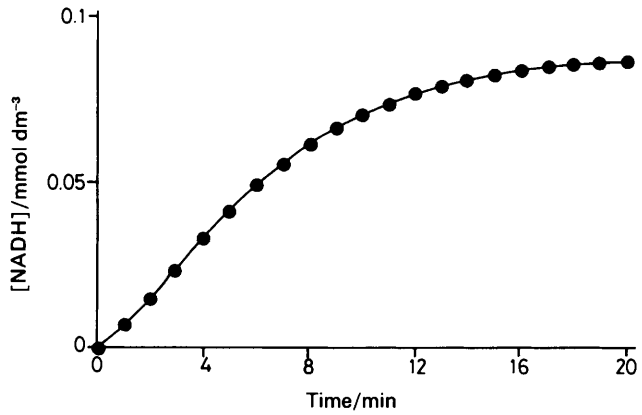


Figure 1. Thermal formation of NADH as a function of time, using formate as hydride donor. $[HCO_2^-]$ = 1.0 mol dm^{-3}, $[NAD^+]$ = 1.0 × 10^{-3} mol dm^{-3}, [(1)] = 5.0 × 10^{-5} mol dm^{-3}, pH = 7.0 (0.1 mol dm^{-3} phosphate buffer), T = 70 °C.

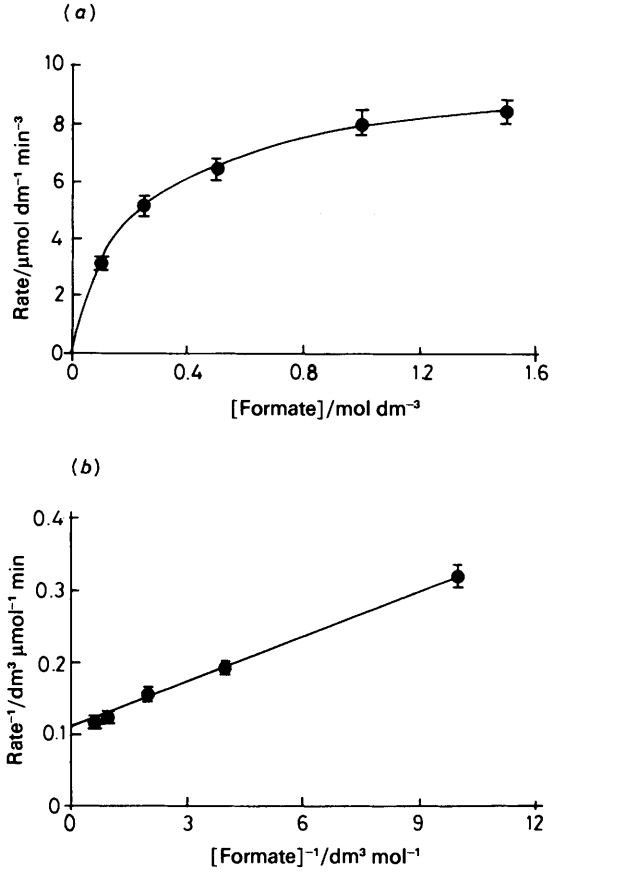


Figure 2. (*a*) Initial rates of NADH thermal formation as a function of substrate (HCO_2^-) concentration. (*b*) Graphic analysis of the experimental saturation curve. In all experiments $[NAD^+]$ = 1.0 × 10^{-3} mol dm^{-3}, [(1)] = 5.0 × 10^{-5} mol dm^{-3}, pH = 7.0 (0.1 mol dm^{-3} phosphate buffer), T = 70 °C.

All experiments were conducted in glass cuvettes (4 cm^3) equipped with a micro stirrer and serum stopper. All samples were deaerated with repeated evacuations and oxygen-free argon flushings.

A typical dark (thermal) reduction experiment was performed on a 3 cm^3 aqueous sample, pH = 7.0 (0.1 mol dm^{-3} phosphate buffer), containing NAD^+ (1.0 × 10^{-3} mol dm^{-3}) and sodium formate as the reductant (usually 0.5 mol dm^{-3}). The reaction was initiated by injection of (**1**) (final concentration 5.0 × 10^{-5} mol dm^{-3}) into the thermostatted cell and the kinetics of NADH formation were followed by the increase in absorbance at λ = 340 nm (ε = 6 200 dm^3 mol^{-1} cm^{-1}) as a function of time.

The photochemical NAD(P)H formation system was composed of an aqueous phosphate buffer (pH = 6.6, 2.5 cm^3) that contained tris(bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$ (1.4 × 10^{-3} mol dm^{-3}) as the photosensitizer, ascorbate (5.0 × 10^{-2} mol dm^{-3}) as the electron donor, the rhodium catalyst (**1**) (2.0 × 10^{-4} mol dm^{-3}), and either of the oxidized cofactors NAD^+ or $NADP^+$ (3.0 × 10^{-4} mol dm^{-3}). NAD(P)H formation was followed spectroscopically between the irradiations. The coupled photosensitized NADH regeneration system was composed of 2.5 cm^3 aqueous buffer (pH = 6.8) solution containing $Ru(bpy)_3^{2+}$ (1.4 × 10^{-3} mol dm^{-3}) as the photosensitizer, ascorbate (5.0 × 10^{-2} mol dm^3) as the electron donor, the rhodium catalyst (**1**) (6.5 × 10^{-5} mol dm^{-3}), NAD^+ (3.0 × 10^{-4} mol dm^{-3}), alcohol dehydrogenase (horse liver, HLADH, 2 units), and acetaldehyde (1.8 × 10^{-2} mol dm^{-3}). The system was irradiated and the ethanol formation was followed by GC on a Porapak T column.

Identification of the NAD^+ reduction product as the 1,4-dihydro isomer was conducted by HPLC using 0.1 mol dm^{-3} KH_2PO_4 buffer pH = 7.1 containing 10% methanol as the eluant and detection wavelength λ = 340 nm.

Benzil reduction was performed on a biphasic system. The aqueous phase, 2 cm^3 (phosphate buffer, pH = 6.8) included ascorbate (1.9 × 10^{-2} mol dm^{-3}), $Ru(bpy)_3^{2+}$ (1.4 × 10^{-3} mol dm^{-3}), catalyst (**1**) (2.0 × 10^{-4} mol dm^{-3}), 1-benzylnicotinamide, BNA^+ (5.0 × 10^{-3} mol dm^{-3}), and $Mg(ClO_4)_2$ (5.0 × 10^{-5} mol dm^{-3}). The organic phase included benzil (5.0 × 10^{-3} mol dm^{-3}) dissolved in methylene chloride (1 cm^3). The combined phases were irradiated and stirred. At certain time intervals the stirring was interrupted, the phases allowed to separate, and 50 mm^3 of the organic layer were withdrawn. The sample was evaporated and the residue dissolved in 100 mm^3 methanol, the benzoin formation was analysed by HPLC using methanol/water (70/30) as the eluant and detection at λ = 254 nm.

Results and Discussion

Thermal Regeneration of NADH using Formate as the Hydride Donor.—Addition of catalyst (**1**) to a solution of formate containing NAD^+ results in the formation of NADH. Figure 1 shows the accumulation of NADH as a function of time; the initial rate of NADH formation corresponds to 8.1 × 10^{-6} mol dm^{-3} min^{-1}. In the absence of (**1**), no NADH is formed, implying that the complex acts as a homogeneous catalyst for the formation of NADH. The maximum rates of NADH generation as a function of formate concentration in the system have been examined and are displayed in Figure 2(*a*). It is evident that as the concentration of formate increases, the rate of NADH formation is enhanced, and at a concentration of 1 mol dm^{-3} formate the rate of NADH formation reaches a plateau. Such saturation effect in the (**1**)-catalysed regeneration of NADPH suggests that the catalyst provides an active site for the production of NADH; when this active site is saturated the maximum rate of NADH formation is observed. As will be discussed later, catalyst (**1**) provides a site for the production of an intermediate hydrido-rhodium complex, which is the active species in the reduction of NAD^+. Assuming that (**1**) provides sites for the generation of hydrido-rhodium species [equation (2)] which will be subsequently utilized in NADH formation [equation (3)], the kinetics of NADH formation should follow the Michaelis–Menten kinetic model of an enzyme–substrate active site. Under such conditions, the relationship outlined in equation (4) must be fulfilled. Figure 2(*b*) shows the analysis of

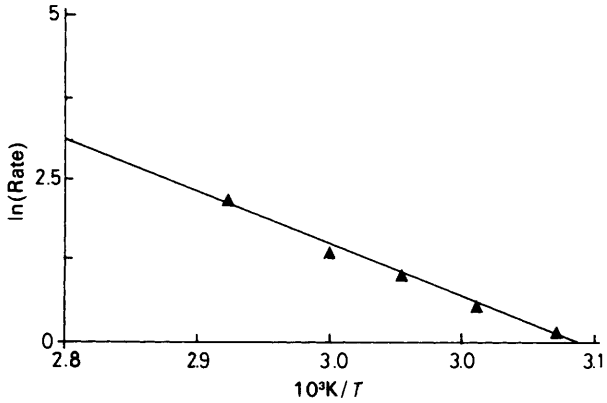


Figure 3. Arrhenius plot for the thermal formation of NADH, $[HCO_2^-] = 0.5$ mol dm^{-3}, $[NAD^+] = 1.0 \times 10^{-3}$ mol dm^{-3}, $[(1)] = 5.0 \times 10^{-5}$ mol dm^{-3}, pH = 7.0 (0.1 mol dm^{-3}, phosphate buffer).

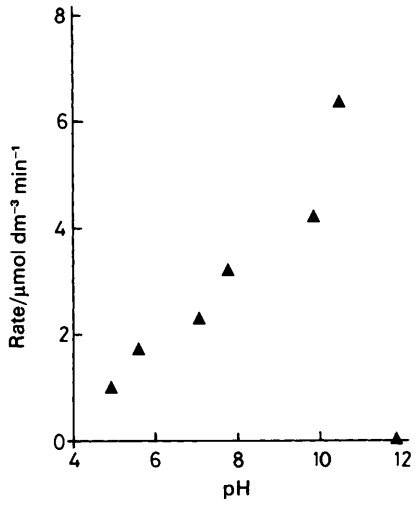


Figure 4. Effect of pH on the initial rate of NADH formation, $[HCO_2^-] = 0.5$ mol dm^{-3}, $[NAD^+] = 1.0 \times 10^{-3}$ mol dm^{-3}, $[(1)] = 5.0 \times 10^{-5}$ mol dm^{-3}, $T = 65$ °C.

$$RhCl(X)_3^{3-} + HCO_2^- \rightleftharpoons H\text{-}RhCl(X)_3^{4-} + CO_2 \quad (2)$$
[*i.e.* (1)]

$$H\text{-}RhCl(X)_3^{4-} + NAD^+ \longrightarrow RhCl(X)_3^{3-} + NADH \quad (3)$$
[X = $P(Ph)_2(C_6H_4SO_3^-)$]

$$\frac{1}{V_0} = \frac{K_m}{v_m[S]} + \frac{1}{V_m} \quad (4)$$

the experimental saturation curve [Figure 2(*a*)], where a linear relationship between the reciprocal of the initial rate (V_0) *vs.* the reciprocal of the substrate concentration is obtained.[19] Thus, the kinetics of NADH formation follows the sequential active site model given by [equation (2)] and [equation (3)]. The values of the Michaelis–Menten constant, $K_m = 0.2$ mol dm^{-3} and the maximum rate of the process $V_{max} = 9.4 \times 10^{-6}$ mol^2 dm^{-6} min^{-1}, are derived from this plot, for $[NAD^+] = 1.0 \times 10^{-3}$ mol dm^{-3}.

We have also examined the kinetic isotope effects and the activation barrier for the regeneration of NADH by formate. Substitution of HCO_2^- by DCO_2^- results in a substantial retardation in the formation of the reduced cofactor, $k_{HCO_2^-}/k_{DCO_2^-} = 3.2$. In turn, no effect on the formation of NADH is observed in the presence of D_2O instead of H_2O. Thus, the rate-limiting step in the formation of NADH involves the cleavage of the C–H bond of formate and generation of the hydrido-rhodium species [equation (2)]. The Arrhenius plot for the production of NADH is displayed in Figure 3. From this plot an activation barrier for NADH formation corresponding to $E_a =$ 20 kcal mol^{-1} * is calculated.

Further studies have examined the variation of the medium pH on the rate of NADH formation (Figure 4). It is evident that as the pH of the aqueous solution increases, the rate of NADH formation is enhanced. At pH = 10.5 the initial rate of NADH formation corresponds to 6.4×10^{-6} mol dm^{-3} min^{-1}. At pH = 11.9, however, a sharp decline in the rate of NADH formation is observed, and the system activity is entirely blocked. The drop in the activity of the system at pH = 11.9 is attributed to an irreversible deactivation of the catalyst (**1**). In an independent experiment, the catalyst was exposed to an aqueous solution at pH = 12, and after adjusting the pH of the system to pH = 10 the catalyst failed to regenerate NADH upon addition of NAD^+ and formate. This result suggests that the catalyst is indeed deactivated at pH = 12.

In nature, the enzyme formate dehydrogenase catalyses the oxidation of formate by NAD^+ [equation (5)]. Here we

$$HCO_2^- + NAD^+ \xrightarrow[\text{Dehydrogenase}]{\text{Formate}} CO_2 + NADH \quad (5)$$

demonstrate that (**1**) acts as an artificial catalyst for the same process.

Photochemical Regeneration of NAD(P)H using (**1**) *as the Catalyst.*—Illumination ($\lambda > 400$ nm) of the photosystem composed of tris(bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$, as the photosensitizer, ascorbate as the electron donor, and (**1**) as the catalyst, in the presence of NAD^+ or $NADP^+$, results in the formation of NADH and NADPH respectively. The quantum yield for NAD(P)H formation corresponds to $\varphi = 5 \times 10^{-3}$. Control experiments show that all of the components are essential to effect the reduction of $NAD(P)^+$, and exclusion of either (**1**) or ascorbate prohibits the formation of NAD(P)H. The photophysical properties of excited $Ru(bpy)_3^{2+}$ in the presence of ascorbate are well documented. The participation of a hydrido-rhodium species in the reduction of $NAD(P)^+$, as discussed in the thermal regeneration process, allows us to propose a mechanism for the photochemical regeneration of NAD(P)H [equations (6)–(9)]. The primary step in-

$$Ru(bpy)_3^{2+} \xrightarrow{h\nu} {}^*Ru(bpy)_3^{2+} \quad (6)$$

$${}^*Ru(bpy)_3^{2+} + HA^- \longrightarrow Ru(bpy)_3^{+} + HA^{\bullet} \quad (7)$$

$$Ru(bpy)_3^{+} + Rh^{I}Cl(X)_3^{3-} + H^+ \longrightarrow$$
[*i.e.* (1)]
$$Ru(bpy)_3^{2+} + Rh^{II}HCl(X)_3^{3-} \quad (8)$$

$$2Rh^{II}HCl(X)_3^{3-} \longrightarrow Rh^{I}HCl(X)_3^{4-} + Rh^{I}Cl(X)_3^{3-} + H^+ \quad (9)$$
[X = $P(Ph)_2(C_6H_4SO_3^-)$]

volves the reductive electron-transfer quenching of the excited photosensitizer by ascorbate (HA^-), $k_q = 2.0 \times 10^7$ dm^3 mol^{-1} s^{-1}. The resulting reduced photoproduct, $Ru(bpy)_3^+$, reduces the catalyst (**1**), and the generated hydrido species, $Rh^{II}HCl(X)_3^{3-}$[X = $P(Ph)_2(C_6H_4SO_3^-)$] undergoes dispro-

* 1 cal = 4.184 J.

Table. Efficiencies of NADH/BNAH regeneration systems in reduction processes.

System	Substrate	Product	Illumination time/h	Conversion (%)	Total turnover numbers (1)	Cofactor/model	Enzyme[a]
A	Acetaldehyde	Ethanol	10	65	180	39	1 740
B	Benzil	Benzoin	30	40	5	20	20

[a] HLADH for A, Mg^{2+} for B.

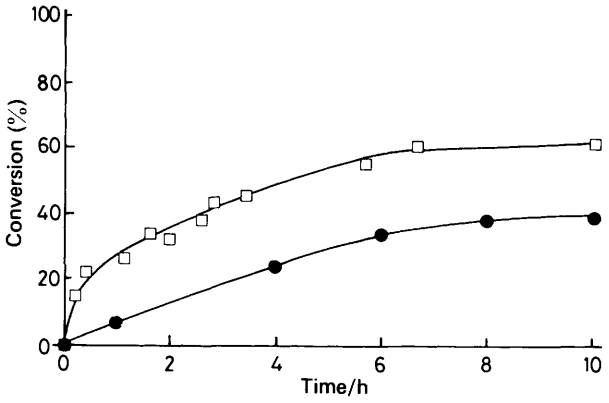


Figure 5. Rates of photoinduced conversion of acetaldehyde into ethanol (□) and of benzil into benzoin (●) using the NADH cofactor and BNAH model-cofactor regeneration systems. The ethanol producing system was composed of 2.5 cm^3: $[Ru(bpy)_3^{2+}]$ = 1.4×10^{-3} mol dm^{-3}, [ascorbate] = 5.0×10^{-2} mol dm^{-3}, [(1)] = 6.5×10^{-5} mol dm^{-3}, $[NAD^+]$ = 3.0×10^{-4} mol dm^{-3}, alcohol dehydrogenase 2 units, $[CH_3CHO]$ = 1.8×10^{-2} mol dm^{-3}, pH = 6.8 (0.1 mol dm^{-3} phosphate buffer). The benzoin-producing system contained an aqueous phase (2 cm^3): $[Ru(bpy)_3^{2+}]$ = 1.4×10^{-3} mol dm^{-3}, [ascorbate] = 1.9×10^{-2} mol dm^{-3}, [(1)] = 2.0×10^{-4} mol dm^{-3}, $[BNA^+]$ = 5.0×10^{-3} mol dm^{-3}, $[Mg(ClO_4)_2]$ = 5.0×10^{-5} mol dm^{-3}, pH = 6.8 (0.1 mol dm^{-3} phosphate buffer) and an organic phase (methylene chloride 1 cm^3): [benzil] = 5.0×10^{-3} mol dm^{-3}.

portionation resulting in $Rh^{I}HCl(X)_3^{4-}$, which reduces the cofactor [equation (3)]. Similar disproportionation processes have been suggested with other intermediary Rh^{II} species.[20] The photogenerated hydrido-rhodium catalyst has also been suggested as the active species in the photoinduced H_2-evolution,[21] hydrogenation[15,22] and hydroformylation processes featuring (1) as primary homogeneous catalyst.

A further aspect to consider is the extent of regioselective reduction of the natural cofactors in the biologically active 1,4-positions.[23] HPLC analysis of the photochemically reduced cofactor, NADH, reveals that the biologically active 1,4-dihydronicotinamide adenine dinucleotide is formed with regioselectivity exceeding 90%. The high regioselectivity observed in the reduction of NAD^+ to the biologically active NADH cofactor, suggests that the regenerated cofactor can be coupled to subsequent biocatalysed transformations. Surprisingly, coupling of the (1)-catalysed NAD(P)H regeneration system to various NAD(P)H dependent enzymes *e.g.* the reduction of pyruvic acid with lactate dehydrogenase or the reductive amination of pyruvic acid with alanine dehydrogenase, result in low conversion levels of substrate to product, and the activity of the system persists only for short periods of time. A detailed examination of the origin of this catalytic failure reveals that the free ligand, $P(Ph)_2(C_6H_4SO_3^-)$, inhibits most of the enzymes, but the catalyst, (1), does not. The inhibition of enzymes occurs only after the system is irradiated to regenerate the cofactor. Thus, photoinduced dissociation of the ligand, $X = P(Ph)_2(C_6H_4SO_3^-)$, in equilibrium with the complex $RhCl(X)_3^{3-}$, produces the enzyme inhibitor. To overcome this difficulty, the concentration of the catalyst, $RhCl(X)_3^{3-}$, in the system which is subsequently coupled to a biocatalyst, is lowered (as described in the Experimental section). The photosystem for the regeneration of NADH under these conditions was coupled to the reduction of acetaldehyde in the presence of horse liver alcohol dehydrogenase (HLADH). Illumination of this system produces ethanol; the rate of formation at different time intervals of irradiation is displayed in Figure 5, and the initial rate of product formation corresponds to $(2 \pm 0.5) \times 10^{-3}$ mol dm^{-3} h^{-1}. Control experiments confirm that all the components are essential to effect the reduction of acetaldehyde to ethanol. The total turnover numbers for the various components included in the system are provided in the Table (System A). It is evident that all of the components are effectively recycled in the system. The turnover number of NAD^+ in this system, where a homogeneous artificial catalyst is applied for the regeneration of the cofactor, is noteworthy and improved by a factor of four as compared with the best result reported previously with a homogeneous photocatalyst.[13]

Photoreduction of a Nicotinamide Cofactor Model Compound.—Model compounds for nicotinamide adenine dinucleotide cofactors have been examined thoroughly as a means of mimicking the functions of the naturally occurring and active cofactors.[24–27] Various aspects that have been studied with nicotinamide model compounds include chiroselective reduction by chiral dihydronicotinamides, determination of structure and reactivity of substrates reduced by the model cofactor compound, and the participation of electron carriers in the production of the reduced cofactor. Special emphasis was directed towards the organization of assemblies which include the reduced cofactor and divalent ions such as Mg^{2+} or Zn^{2+}. With such systems, catalytic effects on the reduction of carbonyl compounds have been observed, and the assemblies were considered as simple models for the active sites of dehydrogenation enzymes which are coupled to the cofactor unit. The catalytic activity of the metal ions has been attributed to the activation of the carbonyl function by the metal ions acting as Lewis acids.[28]

The photoinduced reduction of 1-benzylnicotinamide, BNA^+, was examined by studying the reaction in a similar photocatalytic system to that used for the regeneration of NAD(P)H, *i.e.* $Ru(bpy)_3^{2+}$ as sensitizer, ascorbate as electron donor, and (1) as hydride transfer catalyst. Irradiation of the system ($\lambda > 400$ nm) results in the reduction of BNA^+ to 1-benzyl-1,4-dihydronicotinamide, BNAH, $\varphi = 2.0 \times 10^{-2}$. Figure 6 shows the accumulation of BNAH in the system at various time intervals of irradiation. It is evident that BNAH is formed at a rate corresponding to 5.5×10^{-5} mol dm^{-3} min^{-1}. In contrast to the natural cofactors $NAD(P)^+/NAD(P)H$ which are water soluble in their oxidized and reduced forms, the model compound exhibits opposite hydrophilic–hydrophobic properties in its oxidized and reduced forms. Therefore, while BNA^+ is hydrophilic and soluble in an aqueous phase which is in contact with organic solutions, the reduced product BNAH is extracted from the water phase in contact with organic solu-

tions such as dichloromethane or ethyl acetate. Furthermore, divalent ions such as Mg^{2+} or Zn^{2+} are extracted into the organic phase through association with BNAH. These properties of BNAH allow the transport of the reductant and the divalent ions, acting as activation sites, into those organic solutions which solubilize water-insoluble organic substrates. As a result, reduction of organic substrates in a water–oil two-phase system, where BNAH is photogenerated in the water phase, seems feasible.[28]

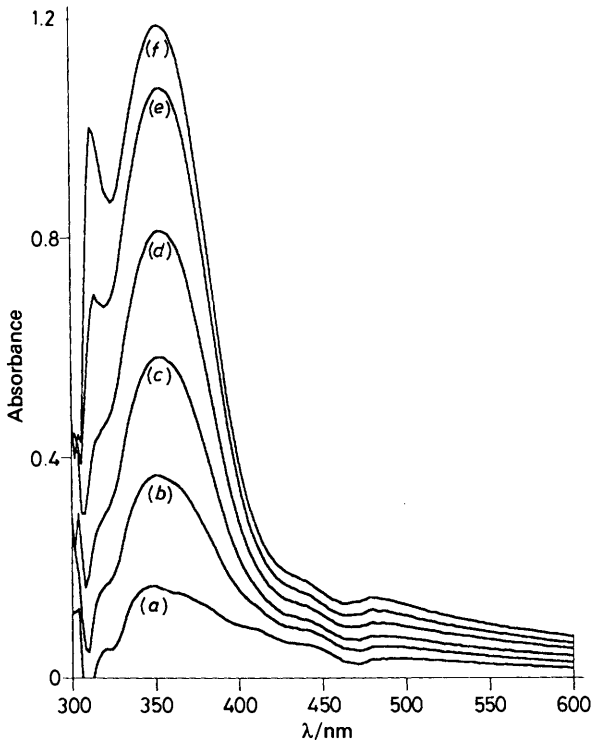


Figure 6. Photoinduced formation of BNAH at time intervals of illumination. (*a*) 1 min; (*b*) 2 min; (*c*) 3 min; (*d*) 4 min; (*e*) 6 min; (*f*) 10 min. $[Ru(bpy)_3^{2+}] = 1.4 \times 10^{-3}$ mol dm^{-3}, [ascorbate] = 1.9×10^{-2} mol dm^{-3}, [(**1**)] = 2.0×10^{-4} mol dm^{-3}, $[BNA^+] = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 6.8 (0.1 mol dm^{-3} phosphate buffer).

We have examined the photosensitized reduction of benzil, (**2**), solubilized in dichloromethane which is in contact with a water phase containing the photosystem for regeneration of BNAH. This latter includes the photosensitizer $Ru(bpy)_3^{2+}$, the catalyst (**1**), ascorbate as sacrificial electron donor, and $Mg(ClO_4)_2$. Irradiation of the system ($\lambda > 400$ nm) results in the reduction of benzil (**2**) to benzoin (**3**). Figure 5 shows the rate of benzoin formation as a function of irradiation time; the initial rate of benzoin formation corresponds to 3×10^{-4} mol dm^{-3} h^{-1}. Control experiments reveal that all the components of the system are essential to effect the reduction of benzil; exclusion of any of the components precludes formation of (**3**). The total turnover numbers for the various components involved in the photoreduction of benzil are summarized in the Table (System B). It is evident that all of the components are recycled in the system.

Figure 7 describes a schematic representation for the photosensitized reduction of benzil in the two phase system using BNAH as a cofactor model compound. The photosensitized process in the aqueous phase generates the hydrido-rhodium intermediate which mediates the reduction of BNA^+ to BNAH. The reduced nicotinamide is associated with Mg^{2+} and is transported into the organic phase due to its hydrophobic character. Activation of the carbonyl substrate by the Mg^{2+} ions allows the subsequent hydride reduction of (**2**) to benzoin by BNAH.

Conclusions

We have shown that (**1**) can be applied as a homogeneous catalyst for the thermal and photochemical regeneration of NAD(P)H cofactors, and for the regeneration of an artificial model compound for the natural cofactor, BNAH. The thermally catalysed regeneration of NAD(P)H, using formate as the hydride source, mimics the natural process catalysed by

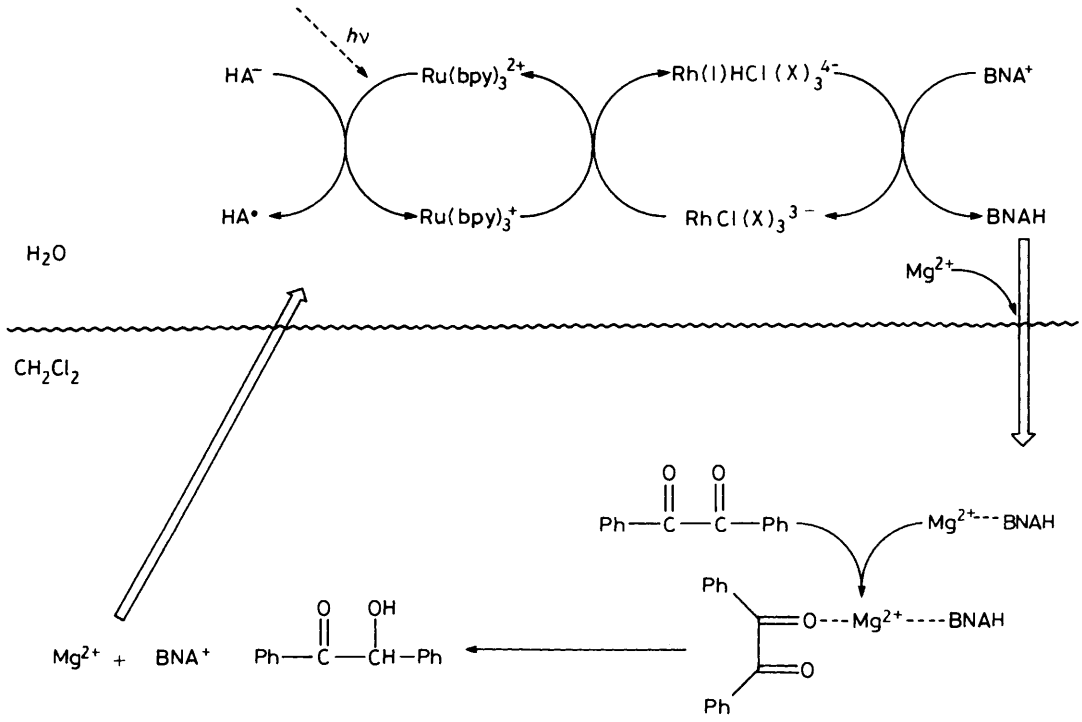


Figure 7. Schematic representation of the biphasic photosensitized reduction of benzil using BNAH as the cofactor model; X = $P(Ph)_2(C_6H_4SO_3^-)$.

formate dehydrogenase. In the photochemical regeneration processes, electrons provided by the photosensitized electron transfer reactions and protons from water lead to the formation of the intermediate hydrido-rhodium catalyst that mediates the production of NAD(P)H and BNAH. Previous studies[21] have indicated that (**1**) acts as a homogeneous H_2-evolution catalyst in acidic aqueous media, (pH = 5), through the intermediate formation of the hydrido species. In more basic environments, pH > 6.5, the H_2-evolution is retarded and hydride transfer to $NAD(P)^+$ or BNA^+ is feasible.

We find, however, that the application of artificial catalysts for the regeneration of NAD(P)H cofactors is not free of limitations: the rates of NAD(P)H regeneration and the extent of regioselectivity in the formation of the reduced cofactors are far below those of the biocatalysed regeneration process. Furthermore, ligand dissociaton of the artificial catalyst introduces inhibition phenomena to subsequent biocatalysed transformations utilizing the regenerated cofactors. Thus, design of improved homogeneous catalysts for the regeneration of naturally active cofactors remains a future challenge.

Acknowledgements

The support of the project by BMFT, West Germany, is gratefully acknowledged.

References

1 (*a*) H. K. Chenault and G. M. Whitesides, *Appl. Biochem. Biotech.*, 1987, **14**, 147; (*b*) G. M. Whitesides and C.-H. Wong, *Aldrichim. Acta*, 1983, **16**, 27.
2 I. Willner and D. Mandler, *Enzyme Microb. Technol.*, 1989, **11**, 467.
3 H. Dugas and C. Penney, in 'Bioorganic Chemistry,' Springer Verlag, New York, 1981, p. 395.
4 (*a*) R. M. Kelly and D. J. Kirwain, *Biotechnol. Bioeng.*, 1977, **19**, 1215; (*b*) Z. Shaked, J. J. Barber, and G. M. Whitesides, *J. Org. Chem.*, 1981, **46**, 4100; (*c*) H. Simon, J. Bader, H. Gunther, S. Newmann, and J. Thanos, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 539.
5 (*a*) D. Mandler and I. Willner, *J. Am. Chem. Soc.*, 1984, **106**, 5352; (*b*) D. Mandler and I. Willner, *J. Chem. Soc., Perkin Trans. 2*, 1986, 805; (*c*) D. Mandler and I. Willner, *J. Chem. Soc., Chem. Commun.*, 1986, 851; (*d*) Z. Goren, N. Lapidot, and I. Willner, *J. Mol. Catal.*, 1988, **47**, 21.
6 S. S. Wang and C. K. King, *Adv. Biochem. Eng.*, 1979, **12**, 119.
7 (*a*) Z. Shakad and G. M. Whitesides, *J. Am. Chem. Soc.*, 1980, **102**, 7104; (*b*) R. Wichmann, C. Wandrey, A. F. Buckmann, and M-R. Kula, *Biotechnol. Bioeng.*, 1981, **13**, 2789.
8 C.-H. Wang, D. G. Drueckhammer, and H. M. Sweers, *J. Am. Chem. Soc.*, 1985, **107**, 4028.
9 A. M. Klibanov and A. V. Pugliski, *Biotechnol. Lett.*, 1980, **2**, 445.
10 (*a*) R. Dicosimo, C.-H. Wong, L. Daniels, and G. M. Whitesides, *J. Org. Chem.*, 1981, **46**, 4622; (*b*) S. Chao and M. S. Wrighton, *J. Am. Chem. Soc.*, 1987, **109**, 5886.
11 O. Warburg, W. Christian, and A. Griese, *Biochem. Z.*, 1935, **282**, 157.
12 (*a*) R. Wienkamp and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 782; (*b*) R. Weinkamp and E. Steckhan, *ibid.*, 1983, **22**, 497.
13 M. Franke and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 265.
14 R. Rupert, S. Herrmann, and E. Steckhan, *J. Chem. Soc., Chem. Commun.*, 1988, 1150.
15 I. Willner and R. Maiden, *J. Chem. Soc., Chem. Commun.*, 1988, 876.
16 A. F. Borowski, D. J. Cole-Hamilton, and G. Wilkinson, *Nouv. J. Chim.*, 1978, **2**, 137.
17 E. M. Kosower and P. E. Klinedinst, *J. Am. Chem. Soc.*, 1956, **78**, 3493.
18 G. A. Rapp and C. E. Nelton, *J. Am. Chem. Soc.*, 1958, **80**, 3509.
19 I. H. Segel, 'Enzyme Kinetics,' Wiley, New York, 1975.
20 M. Kirch, J. P. Sauvage, and J.-M. Lehn, *Helv. Chim. Acta*, 1979, **62**, 1345.
21 S. Oishi, *J. Mol. Catal.*, 1987, **39**, 225.
22 F. Joo, E. Csuhai, P. J. Quinn, and L. Vigh, *J. Mol. Catal.*, 1988, **49**, L1.
23 K. Dalziel, in 'The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1975, vol. 11, p. 1.
24 D. M. Stout and A. I. Meyers, *Chem. Rev.*, 1982, **82**, 223.
25 (*a*) R. H. Abeles, R. F. Hutton, and F. H. Westheimer, *J. Am. Chem. Soc.*, 1957, **79**, 712; (*b*) T. J. van Bergen, D. M. Hedstrand, W. H. Kruizinga, and R. M. Kellog, *ibid.*, 1985, **107**, 3981.
26 (*a*) D. J. Creighton and D. S. Sigman, *J. Am. Chem. Soc.*, 1971, **93**, 6314; (*b*) R. A. Gase, G. Boxhorn, and U. K. Pandit, *Tetrahedron Lett.*, 1976, 2889.
27 (*a*) Y. Ohnishi, M. Kagami, and A. Ohno, *Tetrahedron Lett.*, 1975, 2437; (*b*) Y. Ohnishi, M. Kagami, and A. Ohno, *J. Am. Chem. Soc.*, 1975, **97**, 4766; (*c*) S. Fuzukumi, K. Hironaka, and T. Tanaka, *Chem. Lett.*, 1982, 1583.
28 (*a*) K. Nakamura, A. Ohno, S. Yasui, and S. Oka, *Tetrahedron Lett.*, 1978, 4815; (*b*) P. Dan and I. Willner, *Nouv. J. Chim.*, 1984, **8**, 719.

Paper 9/02110H
Received 19*th May* 1989
Accepted 6*th November* 1989