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Coupling Molecular Photocatalysis to Enzymatic Conversion

Alexander K. Mengele,[a] Gerd M. Seibold,[b] Bernhard J. Eikmanns[b] and Sven Rau*[a]

Abstract: A heterobinuclear dyad containing a ruthenium polypyridyl moiety bound via an aromatic bridging ligand to an organometallic catalytic center has been used for the light driven reduction of BNA^+, NAD^+ and $NADP^+$ yielding the two electron reduced analog. Direct coupling with enzymatic conversion could be proven by means of UV-vis spectroscopy as well as liquid chromatography, showing cofactor recycling and enzymatic conversion with a turnover number of 350 per photocatalyst. First insights into the complex behavior of the catalytic system under irradiation points towards multiple prerequisites on the molecular as well as on the macroscopic level in order to generate highly efficient semiartificial photobiocatalytic systems for future energy storage applications.

Introduction

The storage of light energy in chemical bonds is one of the most promising approaches to the global energy shortage by supplying mankind with renewable green fuels.[1] Among a variety of different methods, photoredox catalysis using multimetallic architectures is a promising concept to achieve this goal.[2] To date a variety of such systems has been reported mainly focusing on proton[3] and CO_2[4] reduction as well as on water oxidation.[5] On the other side, coupling of light harvesting systems with biocatalysts appears highly relevant as well.[6] It allows the light induced powering of mild and selective enzymatic transformations of potentially rather inert or structurally complex substrates. Besides the direct light driven regeneration of reduced flavins,[7-9] the reduction of oxidized nicotinamide cofactors is of particular interest as well, since an effectively working catalyst could provide the reducing equivalents to an even larger number of oxidoreductases, simultaneously saving resources by directly recycling the consumed biological hydride donor.

For this purpose $[(N,N)Rh(Cp^*)X]^{n+}$ compounds with Cp* = pentamethylcyclopentadienyl, N,N = N,N-chelating ligand and X = Cl, H_2O have been shown to be highly active in selectively reducing meta functionalized pyridinium ions to the 1,4-dihydro form using either formate[10-12] or phosphite[13] as reducing agent. Applying $NAD(P)^+$ as the substrate for the selective $[(N,N)Rh(Cp^*)X]^{n+}$ mediated generation of the biologically active reduced cofactor, a variety of coupled systems for the efficient reductive enzymatic synthesis of chiral compounds has been described.[14-17] Moreover it has been shown that even biomimetic cofactor analogs not bearing the naturally occurring ribose containing N-substituents can be recognized by certain enzymes enabling less expensive and even more effective enzymatic stereoselective reactions.[15,18-20] In addition these Rh catalysts can also play the role of an efficient redox mediator in electrochemical NAD(P)H regeneration systems by lowering the required potential, simultaneously inhibiting unselective side reactions arising from cofactor radical chemistry.[21-23]

To date photocatalytic reduction of nicotinamide cofactors is mainly tackled using multicomponent systems.[24] Interestingly, an intermolecular system, where the formation of a bimodular aggregate by reversible coordination of Eosin Y to $[(bpy)Rh(Cp^*)(H_2O)]^{2+}$ (bpy = 2,2´-biypyridine) was proposed, reached very high turnover frequencies (TOFs) by facilitating the charge transfer onto the catalytic center.[25] In addition a polymer compound linked to a similar rhodium catalyst has been shown to drive enzymatic substrate conversion upon irradiation with visible light.[26]

As other studies proved oligonuclear photocatalysts performing superior in comparison to intermolecular systems as well,[27,28] we were interested in efficient nicotinamide cofactor recycling using a structurally well-defined bimetallic dyad, enabling fast photoinduced electron transfers to the $[(N,N)Rh(Cp^*)X]^{n+}$ catalytic center. In addition, decreasing the chance of forming a highly reactive photoreduced chromophoric subunit by eliminating diffusion controlled redox processes between the chromophore and the catalyst might furthermore diminish the unproductive radical chemistry of nicotinamide cofactors upon one electron reduction. This process is known to generate biologically inactive dimers, which could impede efficient photobiocatalytic systems.[29,30] Therefore the well-known mononuclear Ru complex $[(tbbpy)_2Ru(tpphz)]^{2+}$ (**Rutpphz**; tbbpy = 4,4´-di-tert-butyl-2,2´-bipyridine, tpphz = tetrapyrido[3,2-a:2´,3´-c:3´´,2´´-h:2´´´,3´´´-j] phenazine) was chosen as chromophoric subunit, since it has been under extensive investigation in terms of photocatalytic hydrogen production as well as intramolecular sub-ns electron transfers using Pd or Pt moieties as the catalytic centers.[31,32] Thus we sought to examine the light driven reduction of artificial as well as biologically utilizable NAD-like cofactors employing the previously described hydrogen evolving photocatalyst $[(tbbpy)_2Ru(tpphz)Rh(Cp^*)Cl]Cl(PF_6)_2$ (**RutpphzRhCp***),[33] whose molecular structure is depicted in scheme 1.

Results and Discussion

In order to test the photocatalytic activity of **RutpphzRhCp*** for nicotinamide cofactor reduction, initial experiments were performed using BNA^+ (N-benzyl-3-carbamoylpyridinium cation) as convenient structural analog for the biologically active compounds. Irradiation (λ = 470 nm) of a solution containing **RutpphzRhCp***, BNA^+, triethylamine (TEA) and NaH_2PO_4 in degassed H_2O:MeCN (1:1, v:v) under an argon atmosphere led

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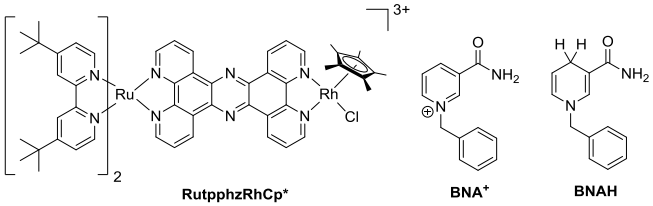


Scheme 1. Molecular structures of the heterobinuclear photocatalyst **RutpphzRhCp*** as well as the oxidized (BNA^+) and the 1,4-reduced (BNAH) nicotinamide cofactor analogs.

to the spectral changes shown in Fig 1. The raising band at 355 nm is characteristic for the formation of a reduced species derived from BNA^+.[24a] After 3.5 h a conversion of nearly 80% was obtained, calculated by the increase of absorbance at 355 nm relative to the initial spectrum (ε^{355nm}(BNAH) = 7240 L mol^{-1} cm^{-1}, taken from literature[24a]). This correlates to a TON (turnover number) of 39 after 3.5 h with a maximum TOF (turnover frequency) of almost 15 h^{-1}.

Moreover an induction phase is observed (Fig. S1) and the catalyst´s TOF reaches its maximum after 90 minutes with a subsequent slight slope. This could be interpreted as formation of the active catalyst at the first minutes of irradiation and a substrate limitation of the catalysis at the end of the process. The rising band at 650 nm, which leads to a color change from orange to green during catalysis, was previously ascribed to the reduction of the tpphz bridging ligand upon irradiation.[33] Furthermore neither in the dark nor in the absence of **RutpphzRhCp*** accumulation of reduced BNA species was observed. Similar changes as in Fig 1 can be found using NAD^+ and $NADP^+$ as substrates under otherwise identical conditions (Fig S2, S3). The fundamental ability of **RutpphzRhCp*** to photocatalytically produce the reduced cofactor hence does not depend on the group attached to the nitrogen but rather on the presence of the *m*-carbamoyl pyridiniumion moiety itself.[11]

Additionally, in contrast to the previously reported heterobinuclear complex $[(tbbpy)_2Ru(tpphz)PdCl_2](PF_6)_2$,[31a] photocatalysis using **RutpphzRhCp*** is not inhibited by the presence of excess chloride ions, proved by an experiment in which BNA^+ was reduced with a similar TOF in absence as well in presence of an 5000 times excess of NaCl (Fig S4).

Furthermore on-off experiments revealed that the catalytically active species is highly reactive with respect to BNA^+ (Fig. 2). After switching off the light source, no further increase in the absorbance at 355 nm was detected, indicating that in presence of a suitable substrate such as BNA^+, no accumulation of the catalytically active species is possible, which would react in the dark with the cofactor analog. Therefore formation of the catalytically active species seems to be the rate determining step of the overall process.

Surprisingly control reactions performed with **Rutpphz** instead of **RutpphzRhCp*** led to similar spectral changes as in Fig 1. By irradiation of a typical catalytic mixture described above, rising bands at around 350 nm in presence of BNA^+, NAD^+ or $NADP^+$ (Fig S5-S7) were observed. Although operating slower than **RutpphzRhCp***, we first believed that the photoreducible tpphz ligand in **Rutpphz** may play a role as hydride mediator to produce the 1,4-reduced product out of the three substrates. To test this hypothesis, we performed a typical catalytic run without adding BNA^+ (see Fig S8). The loss of the two sharp bands at around 365 nm and 385 nm during illumination clearly indicated the photoreduction of the tpphz ligand in presence of TEA as electron donor, as these two bands have been ascribed to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions of the fully aromatic system.[33]

To validate if the tpphz ligand in **Rutpphz** plays a crucial role in transferring reducing equivalents to BNA^+, $[Ru(tbbpy)_3](PF_6)_2$ was tested under similar photocatalytic conditions as described above. As can be seen in Fig 3, a rising band at around 350 nm

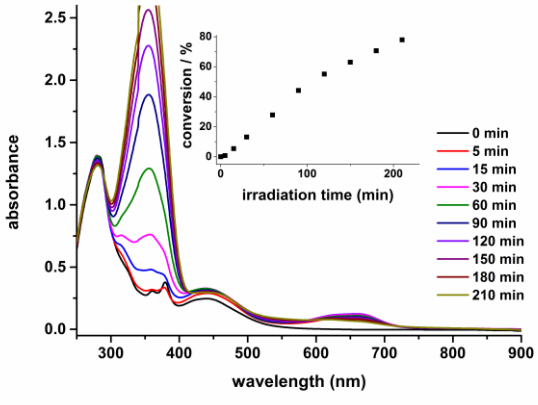


Figure 1. UV-vis spectral changes during irradiation of a catalytic mixture containing **RutpphzRhCp*** (10 µM), BNA^+ (0.5 mM), TEA (0.1 M), NaH_2PO_4 (0.1 M) in degassed MeCN:H_2O = 1:1 (v:v) under an argon atmosphere with blue light (λ = 470 nm, 50 ± 2 mW cm^{-2}).

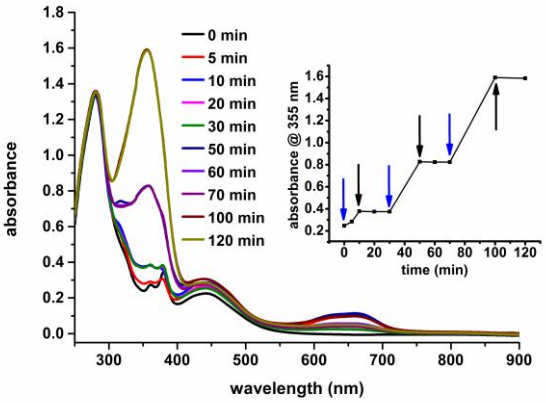


Figure 2. On-off-experiment for the photocatalytic reduction of BNA^+ (c = 0,5 mM) using **RutpphzRhCp*** (c = 10 µM) in a solution of MeCN:H_2O = 1:1 (v:v) containing TEA (c = 0.1 M) and NaH_2PO_4 (c = 0.1 M); UV-vis spectra at different points in time and corresponding changes in the absorbance at 355 nm. The blue arrows indicate that the light source (one blue LED stick, λ = 470 nm, 50 ± 2 mW/cm^{-2}) is switched on, the black arrows that it is switched off.

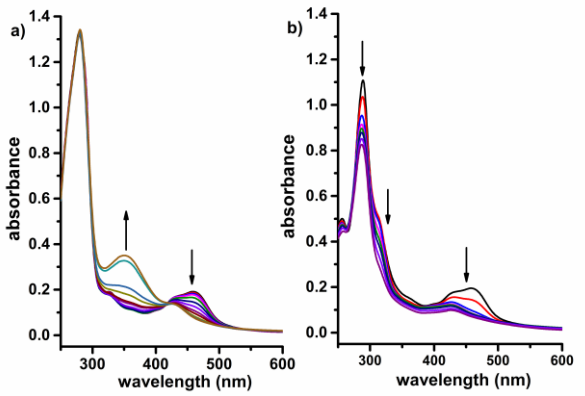


Figure 3. a) UV-vis spectral changes during 4 hours of irradiation of a solution containing $[Ru(tbbpy)_3](PF_6)_2$ (c = 10 μM), BNA^+ (c = 0.5 mM), TEA (c = 0.1 M) and NaH_2PO_4 (c = 0.1 M) in degassed $MeCN:H_2O$ = 1:1 (v:v) under an argon atmosphere with blue light (λ = 470 nm, 50 ± 2 mW cm^{-2}). Diagram b) shows the changes upon irradiation of a solution described above without BNA^+ being present.

occurred in presence of BNA^+ (diagram a)), whereas in its absence (diagram b)) only the photodegradation of $[Ru(tbbpy)_3](PF_6)_2$ was observed, indicated by the lowered absorbance of the MLCT band at 450 nm with increasing irradiation time.

Although the homoleptic complex is able to generate reduced BNA species too, the efficiency of this process compared to **Rutpphz** is lower. Despite the photolability of $[Ru(tbbpy)_3](PF_6)_2$, this result respresents a surprising detail of our studies, since **Rutpphz** would feature due to its low lying tpphz based LUMO a smaller driving force for electron transfer onto a substrate.[31a,34] An attractive explanation for this behavior could be the overcompensation of the lower reduction potential of **Rutpphz** by the formation of a π-π interactions mediated supramolecular aggregate with BNA^+ via the planar tpphz ligand and the aromatic systems of the cofactor analog.

This is demonstrated by concentration dependent ^{1}H-NMR spectroscopy experiments. With an increasing amount of BNA^+ predominantly the tpphz based signals of **Rutpphz** undergo a shift (Fig. S9). This is in line with similar results obtained by the π-π-stacking between pyrene and **Rutpphz**.[35]

On the other hand $[Ru(tbbpy)_3](PF_6)_2$ does not exhibit similarly effective interactions with BNA^+, indicated by constant ^{1}H-NMR spectroscopic shifts of the tbbpy signals upon increasing concentrations of the cofactor analog (Fig. S10). This fortifies the above discussed hypothesis for the superior electron transfers onto BNA^+ using **Rutpphz** via formation of a π-π interactions mediated aggregate.

However, the results obtained from the UV-vis spectroscopic investigations of the catalytic processes using **RutpphzRhCp***, **Rutpphz** and $[Ru(tbbpy)_3](PF_6)_2$ as photoredox active molecules revealed, that the fundamental photocatalytic activity of the three complexes with respect to BNA^+ may simply be related to the photochemistry of ruthenium polypyridyl complexes in presence of a sacrificial electron donor such as the used TEA.

Since it is known that the 1,4-dihydro forms (BNAH, NADH, NADPH) as well as the different possible dimeric structures ($(BNA)_2$, $(NAD)_2$, $(NADP)_2$), obtainable by single electron transfer onto the substrate and successive radical coupling, show similar absorption profiles,[36] discrimination of the photoproducts by this method is not possible.[29] Therefore an enzymatic assay was used to test if the mononuclear complexes **Rutpphz** and $[Ru(tbbpy)_3](PF_6)_2$ as well as **RutpphzRhCp*** were able to produce the biologically relevant two electron 1,4-reduced form of the nicotinamide cofactors, since only this species would be enzymatically consumable.

For this purpose a mixture of **Rutpphz**, $[Ru(tbbpy)_3](PF_6)_2$ or **RutpphzRhCp*** (c = 10 μM), NAD^+ (c = 0.96 mM), sodium pyruvate (c = 10 mM), $MgCl_2$ (c = 8.5 mM), TEOA (triethanolamine, c = 0.2 M) in $MeCN:H_2O$ (1:99, v:v, pH = 8.7) and in presence of lactate dehydrogenase (LDH, 5 units/mL) under an argon atmosphere was irradiated with one LED-stick (λ = 470 nm, 50±2 mW/cm^2) from the bottom side of standard quartz cuvettes that were used as reaction vessels. Based on the fast hydrolysis of the Rh-Cl bond and the determined pK_a value of the rhodium bound aquo ligand in similar systems,[37] the resting state of the photocatalyst might be described best with a RhCp*(OH) moiety at the catalytic center. Although the higher σ donating ability of the hydroxo ligand compared to an aquo ligand might influence the intramolecular electron transfer kinetics, **RutpphzRhCp*** behaves as expected for an efficient catalyst, that produces the biologically consumable reduced cofactor. As can be seen in Fig 4, almost no accumulation of reduced NAD species can be observed in the presence of LDH (diagram a)), indicated by negligible changes of the recorded UV-vis spectra upon irradiation of the sample, whereas in absence of LDH a rise of the 340 nm product absorbance is observable (diagram b)). HPLC analysis after 17 h showed, that in presence of LDH, 3.5 mM of lactate were formed. This correlates to a TON of 350 and an average TOF of 20 h^{-1} with respect to the photocatalyst **RutpphzRhCp*** (Fig S11).

As NAD^+ itself was used in a concentration of 0.96 mM, cofactor recycling was obtained. In the course of irradiation each NAD^+ molecule was reduced on average more than 3.5 times. For the reduction of oxidized nicotinamides, the active catalyst has been proposed to be a Rh-H[11,12] or as previously suggested a reduced Cp* moiety, formed after proton transfer from the rhodium center to a carbon atom of the former aromatic ligand.[38,39]

However, in the absence of LDH no lactate was detected, indicating that the catalytically active species is not able to reduce the keto group of pyruvate in a significant amount itself.[40] Since no stereo information is existing in **RutpphzRhCp*** proximate to the catalytic center, we furthermore would have expected the formation of a racemic lactate mixture.[41]

Similarly no lactate was found after 17 h when the same sample was stored in the dark or **RutpphzRhCp*** was absent, showing that the overall photobiocatalytic process is driven by visible light induced photoredox catalysis of the heterobinuclear complex, providing enzymatically consumable reducing equivalents for LDH.

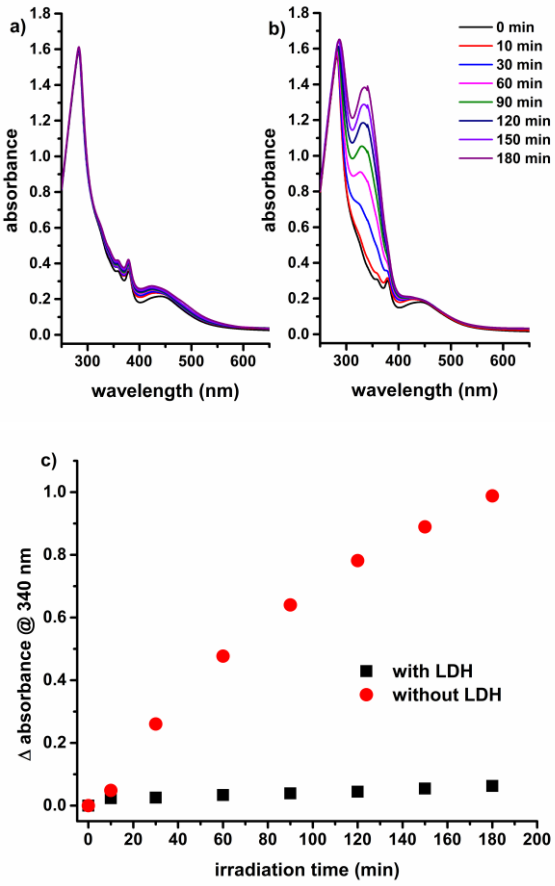


Figure 4. UV-vis spectral changes during the first 3 hours of irradiation of a catalytic mixture using one LED stick (λ = 470 nm, 50 ± 2 mW/cm^{-2}): a) The mixture contains **RutpphzRhCp*** (c = 10 μM), NAD^+ (c = 0.96 mM), sodium pyruvate (c = 10 mM), TEOA (c = 0.2 M), $MgCl_2$ (c = 8.5 mM) and LDH (5 U/ml) in MeCN:H_2O = 1:99 (v:v, pH = 8.7); b) The mixture contains all the above mentioned compounds without LDH; c) Increase in the absorbance at 340 nm with respect to the value before starting the illumination of the catalytic mixtures described above.

The enzymatic test for **Rutpphz** as well as $[Ru(tbbpy)_3](PF_6)_2$ revealed that neither in presence nor in absence of LDH lactate was produced (Fig S12). Consistent with this finding the rise of the 340 nm band was nearly the same for both mononuclear complexes, irrespective of the presence of LDH (Fig S13 and S14). We therefore conclude, that these two complexes generate only biologically inactive reduced NAD species, putatively a mixture of regioisomeric $(NAD)_2$ dimers.[42] Similar findings have already been published, highlighting the reactivity of oxidized nicotinamide cofactors and its analogs towards one electron reduction in presence of suitable chromophores.[29,30] A selective hydride transfer of the photoreduced tpphz ligand in **Rutpphz** to the 4-position of the nicotinamide cofactor can therefore also be excluded. We conclude additionally, that the same dimer formation is true for the experiments described above, using BNA^+ as substrate and the mononuclear complexes **Rutpphz** and $[Ru(tbbpy)_3](PF_6)_2$ as photocatalysts.

In order to strengthen this hypothesis, ^{1}H-NMR experiments using **Rutpphz** or $[Ru(tbbpy)_3](PF_6)_2$ as phocatalysts, TEA as sacrificial electron donor and BNA^+ as terminal electron acceptor showed, that upon irradiation with visible light (λ = 470 nm) the peaks assigned to BNA^+ diminish and new signals appear (Fig S15 and S16). Although this again highlights the ability of ruthenium polypyridyl complexes to photochemically convert BNA^+ under appropriate reaction conditions, comparison with the ^{1}H-NMR signal set of freshly synthesized BNAH were not in accordance with the new peaks emerging under visible light irradiation.

These findings together with the enzymatic tests and the results obtained by following photocatalysis using UV-vis spectroscopy have severe impacts on the molecular design of systems for photocatalytic NADH production and coupled enzymatic transformations using photosensitizers with high reduction potentials such as ruthenium polypyridyl complexes. As **Rutpphz** and $[Ru(tbbpy)_3](PF_6)_2$ are able to photoreduce the oxidized nicotinamide cofactors and its artificial analogs itself, multicomponent systems composed of separated redox chromophores and $[(N,N)Rh(Cp^*)X]^{n+}$ like catalysts have to be designed carefully in order to enable fast electron transfers from the excited/reduced chromophore onto the catalyst, thus avoiding the accumulation of bioinactive dimeric cofactors.

Multimetallic or supramolecular architectures in which the electron transfers are not limited by diffusion due to a well-defined preorganisation of chromophore and catalyst are therefore highly relevant alternatives to multicomponent systems In the latter, by virtue of the independent photochemistry of the long-lived excited/reduced state of the chromophore with nicotinamide cofactors, the need of intermolecular electron transfers would exhibit a severe source of inefficiency inherent to the system.

In order to detect possible side products in the photobiocatalytic processes, we first performed thermal catalysis experiments using **RutpphzRhCp*** as catalyst, $NaHCO_2$ as hydride donor and BNA^+ as hydride acceptor to exclude negative influences of the ligand environment around the rhodium center on the well-known and highly selective hydride transfer of the $[(N,N)Rh(Cp^*)X]^{n+}$ complexes for the generation of the 1,4-reduced product.[10-12] As can be seen in Fig 5, the rising band at 355 nm again indicates the formation of a reduced BNA species. A TON of 13 after 515 min and a TOF of 1.5 h^{-1} could be calculated, which is significantly lower with respect to the photobiocatalytic system described above. This again highlights the potential of light driven cofactor reduction, as here the formation of the active catalyst is much less dependent on the temperature. Moreover, compared to recently published literature,[19] the TOF of our formate driven system is reduced by a factor of 4. As a higher thermal energy content will have a dramatic effect on the overall BNA^+ reduction by facilitating the rate determining step, i.e. generation of the catalytically active Rh species via β-hydride elimination from the metal bound formate,[12] the decreased TOF in our case can be ascribed to the lower applied temperature.

As in Fig 5 the fine structured bands of the fully aromatic tpphz ligand at 350 and 380 nm remain visible until they diminish

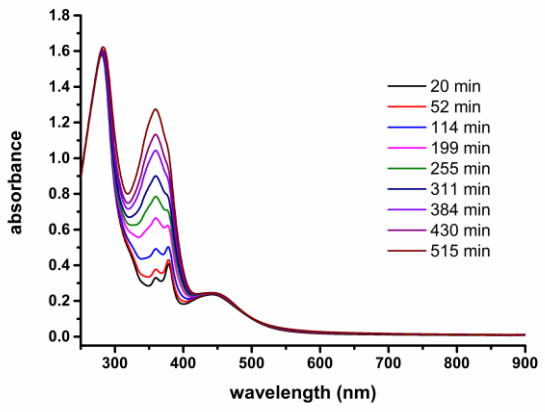


Figure 5. UV-vis spectral changes during the thermal BNAH production by a mixture containing **RutpphzRhCp*** (c = 10 μM), BNA^+ (c = 1 mM), $NaHCO_2$ (c = 50 mM) under argon atmosphere, using degassed solvents in a ratio of MeCN:H2O = 1:9 (v:v) at room temperature.

under the increasing product absorbance, hydride transfer reactions from the initially formed rhodium hydride onto the tpphz bridging ligand with possible concomitant unselective hydride transfers onto BNA^+ are highly unlikely. Control experiments without **RutpphzRhCp*** did not result in the formation of the 355 nm product band. In order to study the product distribution of this thermal process, ^{1}H-NMR experiments under similar conditions were performed (Fig S17). Over time new peaks emerge exactly at these positions, where the independently synthesized 1,4-reduced BNAH exhibits its proton resonances. Therefore the well-known selective hydride transfer property of the Rh(Cp*) catalytic center is preserved in **RutpphzRhCp***.

For a closer look into the product distribution under irradiation using **RutpphzRhCp*** as catalyst, ^{1}H-NMR investigations under catalytic conditions using TEA as electron donor and BNA^+ as terminal electron acceptor were performed. Surprisingly, similar to the experiments performed with the mononuclear complexes **Rutpphz** and $[Ru(tbbpy)_3](PF_6)_2$, no BNAH could be detected (Fig S18). These findings were in strong contrast to the results obtained by performing the enzymatic assays described above, since only the 1,4-dihydro form of NADH can be used by LDH.

A possible explanation for the accumulation of reduced BNA species different to that of the 1,4-dihydro form BNAH in the case of **RutpphzRhCp***, could be the consumption of formed BNAH by an one electron oxidation process involving the photoexcited ruthenium chromophore. BNAH is frequently used as potent sacrificial electron donor in photoredox catalytic systems due to its higher reduction potential than common aliphatic amines.[43] In presence of a base, fast deprotonation of the very acidic formed BNAH radical cation occurs, generating a neutral radical species which then couples with a second BNA radical to yield dimeric BNA species $(BNA)_2$.[44,45]

To elucidate if this fast consumption process of generated BNAH by excited chromophores also occurs for **RutpphzRhCp***, ^{1}H-NMR experiments were performed using the binuclear complex in presence of TEA and BNAH as mutual competing electron donors and BNA^+ as terminal electron/hydride acceptor. As can be seen in Fig 6 (the whole spectrum is shown in Fig S19) the signal, attributed to the two hydrogen atoms located para to the nitrogen atom in the heterocycle of BNAH at 2.97 ppm, decreases with increasing irradiation time relative to the quintett of MeCN-d_2 at 1.94 ppm. This shows that also in the case of **RutpphzRhCp*** consumption of BNAH occurs, generating a mixture of different $(BNA)_2$ dimers.

However, as the enzymatic assay described above proved that the biologically active 1,4-dihydro NADH was successfully generated photochemically using **RutpphzRhCp***, the reduced cofactor was consumed enzymatically faster than by the excited binuclear complex.

These results lead to a second important design parameter of semiartificial photobioctalytic systems using $NAD(P)^+$ as photocatalytically recyclable hydride mediator, which is that the enzymatic transformation has to be significantly faster than the unproductive consumption of the generated NAD(P)H by the excited or photooxidized photosensitizer. This could be achieved on the molecular scale by choosing a quickly operating enzyme for redox biocatalysis and on a macroscopic level by a precise control over the mass flow in the reaction vessel or via the immobilization of multinuclear photocatalysts on semiconductor electrode surfaces. The latter would inhibit the quenching of the photoexcited chromophores by the photocatalytically generated reduced nicotinamide cofactors due to steric reasons as well as by a fast regeneration of the oxidized chromophore with an electron from the valence band of the semiconductor.[46,47] Moreover by immobilizing enzymes on microparticles,[48] this approach would also allow spatial separation of photo- and biocatalyst, which are prone to mutual inhibition.[49,50]

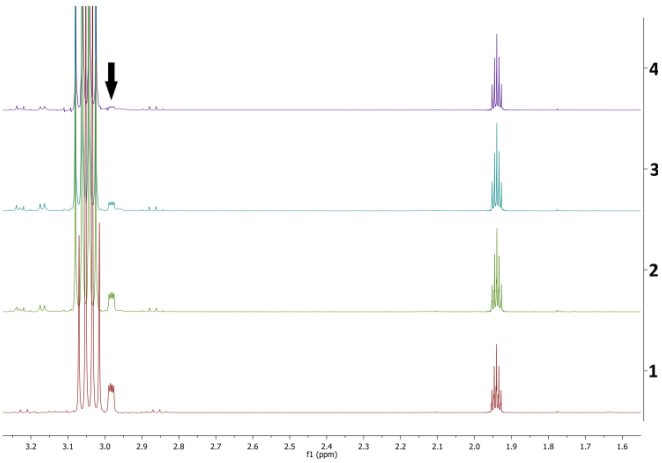
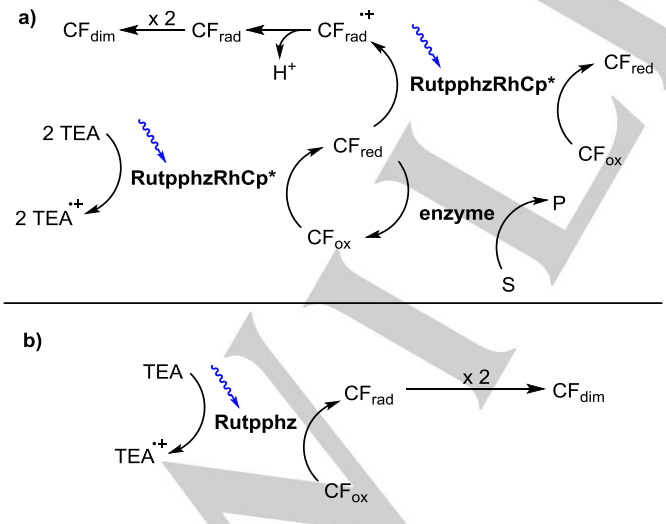


Figure 6. ^{1}H-NMR spectroscopic changes between 1.4 and 3.2 ppm of a solution containing **RutpphzRhCp*** (c = 100 μM), TEA (c = 0.1 M), NaH_2PO_4 (c = 0.1 M), BNA^+ (c = 10 mM) and BNAH (c = 30 mM) under argon atmosphere using a degassed solvent mixture of MeCN-d_3:D_2O = 1:1 (v:v). Spectrum 1) was recorded before, spectrum 2) after 3, spectrum 3) after 22 and spectrum 4) after 42 hours of irradiation with one LED stick (45-52 mW cm^{-2}) at room temperature. The intensive peak at 3.05 ppm is attributed to the methylene group of TEA.

Based on the performed experiments described above, a rational reaction scheme can be drawn. As can be seen in scheme 2, part a), the binuclear photocatalyst **RutpphzRhCp*** is able to use TEA under irradiation with visible light as sacrificial electron donor in order to generate the bioactive reduced cofactor (CF_{red}) from its oxidized analog (CF_{ox}). In presence of an efficiently working enzyme, CF_{red} gets immediately reconverted to CF_{ox} which can then be reused from **RutpphzRhCp*** as hydride acceptor. These coupled processes allow the photocatalytic recycling of nicotinamide cofactors and the use of substochiometric cofactor amounts for enzymatic substrate conversions. In absence of an enzyme, the initially produced CF_{red} gets no longer reconverted to CF_{ox} and by virtue of its function as potent sacrificial electron donor CF_{red} undergoes an one electron oxidation with **RutpphzRhCp***, yielding the radical cationic species, which couples after deprotonation with a second neutral nicotinamide cofactor radical (CF_{rad}) to one of the regioisomeric dimeric species CF_{dim}.[42]

As can be seen in part b) of scheme 2, **Rutpphz** as well as $[Ru(tbbpy)_3](PF_6)_2$ are not able to generate CF_{red} due to the lack of an appropriate catalytic center that guides the stored reducing equivalents selectively to the 4-position of the heterocycle. Therefore the mononuclear compounds only yield CF_{rad} under irradiation, which couple to the enzymatically unusable CF_{dim}.

Although the dimeric $(BNA)_2$ species exhibits an even higher reduction potential than BNAH, previous reports suggest that the $(BNA)_2$ radical cationic species, generated by photooxidation with a ruthenium polypyridyl chromophore, decompose only very slowly.[51] Therefore electron back transfer occurs very efficiently and $(BNA)_2$ represents the final product of photocatalysis as shown in scheme 2.



Scheme 2. Possible reaction pathways under visible light irradiation using **RutpphzRhCp*** (part a)) and **Rutpphz** (part b), same pathway is proposed for $[Ru(tbbpy)_3](PF_6)_2$; CF_{ox} = oxidized nicotinamide cofactor, CF_{red} = reduced nicotinamide cofactor (1,4-dihydro form), CF_{rad} = radical nicotinamide cofactor species, CF_{dim} = dimeric nicotinamide cofactor species, S = substrate for biocatalysis, P = product of the photobiocatalytic process.

Hence semiartificial photobiocatalytic systems have to be designed with great care as the continuous accumulation of CF_{dim} represents an irreversible loss pathway that leads ultimately, due to the ever dwindling CF_{ox} amount, as it is also observable in Fig S19, even in presence of a great excess of a sacrificial electron donor with lower reduction potential than CF_{red}, to an unefficient enzymatically substrate conversion.

A further possible side product during the photobiocatalytic process is H_2, which cannot be detected using standard UV-vis or ^{1}H-NMR spectroscopy. Therefore the photocatalytic hydrogen production under conditions for the initial BNA^+ photoreduction experiments described above was studied via gas chromatography (MeCN:H_2O = 1:1, v:v; c(TEA) =0.1 M, c(NaH_2PO_4) = 0.1 M; c(**RutpphzRhCp***) = 10 μM). After 100 h a TON of only 3 was achieved. In presence of 100 equivalents BNA^+, a delayed onset of hydrogen production as well as a reduced TON of 0.4 was observed. Moreover within the time span of the photobioctalysis experiments described above, no hydrogen was detected at all, indicating that under the applied conditions BNA^+ represents the strongly favored substrate compared to H^+.

Conclusions

In conclusion we presented with **RutpphzRhCp*** for the first time a well-defined binuclear architecture, able to reduce nicotinamide cofactors into the biologically active forms under irradiation with visible light. Herein the rate determining step of the process is the light driven generation of the catalytically active species. Moreover **RutpphzRhCp*** was able to power the LDH catalyzed transformation of pyruvate to lactate in a proof of concept system with a total TON of 350 per photocatalyst, thereby using substochiometric amounts of NAD^+ under implementation of an internal cofactor recycling system.

Furthermore, comparing studies indicated that **RutpphzRhCp*** exhibits under the applied conditions a much higher activity for nicotinamide cofactor reduction than hydrogen evolution, possibly suggesting unequal mechanistic pathways with respect to the formation of the two different products.

First insights into the intertwined photocatalytic reaction pathways using mono- and bimetallic ruthenium polypyridyl complexes in presence of nicotinamide cofactor analogs point towards design criteria for semiartificial photobiocatalytic systems. These arise from the fact that multicomponent systems with separated photo and catalytic centers exhibit a potential source of error by design. Within the elongated lifetime of the highly reactive excited or photoreduced chromophore due to the collision induced electron transfer from the photo- to the $[(N,N)Rh(Cp^*)X]^{n+}$ catalytic center, an one electron reduction of the oxidized nicotinamide cofactors may occur, leading finally to the end of productive photobiocatalysis by continuous accumulation of dimeric species. Hence the combination of oligonuclear photocatalysts with fast operating enzymes could be a promising approach for the future design of efficient photobiocatalytic systems.

Especially in the context of solar fuel production and CO_2 storage,[52,53] photobiocatalysis is intrinsically superior compared to the formate driven cofactor reduction, as in the latter approach, formation of one molecule NADH would always liberate one molecule of CO_2.

Experimental Section

Detailed information regarding the synthesis of the compounds, the sample preparation as well as the execution of the photocatalysis experiments can be found in the electronic supplementary information.

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Keywords: photobiocatalysis • organometallic chemistry • oxidoreductases • rhodium • ruthenium

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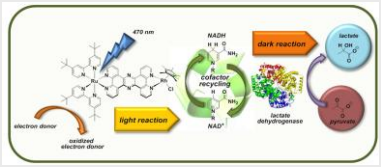
FULL PAPER

A heterobinuclear photocatalyst has been found to drive an enzymatic reduction of pyruvate to lactate by continuous generation of NADH under visible light irradiation.



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Coupling Molecular Photocatalysis to Enzymatic Conversion