The NAD$^+/\text{NADH}$ Redox Couple—Insights from the Perspective of Electrochemical Energy Transformation and Biomimetic Chemistry

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ABSTRACT

The constructions of various photochemical systems and catalysts have become a common theme in the realm of metal-catalyzed energy transformation. The biologically important redox couple $\beta$-nicotinamide adenine dinucleotide (NAD$^+$/NADH) provides a reversible prototype system for the conversion of electrical to chemical energy via the reversible formation of a C–H bond centered on the nicotinamide ring representing an efficient system for numerous biological hydrogen-transfer reactions. In this short review, the first part emphasizes the need to construct operational system for the catalytic transformation of energy from viable sources due to the globally increasing demand in energy consumption. This is followed by a discussion on the redox chemistry of the NAD$^+/\text{NADH}$ reversible redox process centered on the nicotinamide ring as a representative chemical system enabling the efficient transformation of energy. Next, pioneering examples of NAD$^+/\text{NADH}$ mimics providing model systems that can perform non-enzymatic reactions based on the hydrogen (hydride) transfer ability of the model compounds are outlined. And lastly, several examples of ruthenium polypyridyl complexes having NAD$^+/\text{NADH}$ analogous ligands exhibiting excellent photo- and electrochemical properties similar to the NAD$^+/\text{NADH}$ redox couple are given. This is to demonstrate the importance of biomimetic chemistry in realizing novel strategies in the development of catalytic systems that can provide solutions in the alleviation or eradication of the world’s energy problems.

Keywords: NAD$^+/\text{NADH}$ redox couple; redox; ruthenium; biomimetic chemistry

INTRODUCTION

Metal hydrides (LiAlH$_4$, NaH, NaBH$_4$, etc.) are extensively utilized as reducing agents in organic and synthetic chemistry laboratories owing to their propensity towards the catalytic reduction of many classes of substrates. This reactivity arises from the partial negative charge on the hydrogen atom present in the metal-hydrogen bond, shown in Figure 1, enabling the possibility for the metal-hydrogen bond to undergo insertion reactions in various unsaturated compounds and acting as hydride source to effect organic reduction reactions (Fulton and Perhacs, 1998; Brubach et al., 2005; Mitrasinovic, 2003). They produce stable reaction intermediates to obtain the desired product by isolation of the reduced species by either aqueous work-up or by using other suitable solvents like alcohols. One disadvantage, however, is that most of these metal hydrides are very reactive, unstable, and non-renewable.

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In biological reactions, on the other hand, the NAD+/NADH redox couple (Figure 2) in which the oxidized form (NAD⁺) with a pyridinium structure is reversibly converted into the reduced form (NADH) plays a key role in reversible hydride transfer reactions. Chemists have focused much attention on the function of NADH as an organic hydride. Considerable interest has been given in creating model compounds mimicking the activity of the NAD+/NADH redox couple (see references section for a list of preliminary work on this) from the viewpoints of using the electrochemical behavior of the NAD+/NADH redox couple in (a) modeling and studying analogous biological and biochemical phenomena (Elving, 1976; Birrell and Hirst, 2013), (b) electrochemical regeneration step in cyclic redox processes which involves chemical or energy conversion and transformation (Elving, 1976; Bresnahan and Elving, 1981; Scipioni et al., 2010) and, (c) determination of compounds which react enzymatically with NAD⁺ or NADH (Dryhurst, 1977; Vidugiriene et al., 2014).

**Catalytic Energy Transformation.** The invention of the steam engine in the middle of the 18th century marks the advent of increasing energy demands, utilization, and dependence. Burning tremendous amounts of fossil fuels has been common to meet the need for energy in sustaining the processes used in industrial production, manufacturing, and other practices of daily life. Alleviation of problems arising from the issue of global warming mandated the development of artificial photochemical systems. A common theme is an intensive effort towards the photochemical production of carbon monoxide (CO) and formic acid (HCOOH) from carbon dioxide (CO₂). Recent progress along these lines has resulted in advances in our understanding of the interaction of CO₂ molecules with metal complexes, and the factors controlling the efficient storage of solar energy in the form of reduced carbon compounds.
tems designed toward catalytic energy transformation from chemical to electrical energy.

THE NAD\(^+\)/NADH REDOX COUPLE

While the construction of various photochemical systems and catalysts have become a common theme in the realm of metal-catalyzed energy transformation, the biologically important redox couple \(\beta\)-nicotinamide adenine dinucleotide (NAD\(^+\))/1,4,\(\beta\)-dihydronicotinamide adenine dinucleotide (NADH) provides a reversible system in numerous biological hydrogen-transfer reactions. NAD\(^+\) functions as a coenzyme via a proton-coupled electron transfer reaction from a substrate to form NADH. The center for the reversible redox process is the nicotinamide ring. The NAD\(^+\)/NADH redox couple plays important roles as coenzymes for pyridinoproteins (dehydrogenases) in major metabolic pathways such as the electron transport chain.

**Cathodic Reduction of NAD\(^+\).** As summarized in Figure 4, the cathodic reduction of NAD\(^+\) to NADH proceeds through a cycle involving two successive one-electron transfer reactions that are distinctly unique in potential. The first one-electron transfer reaction involves the reduction of a cationic species. This pH independent Faradaic process is accompanied by events like adsorption of NAD\(^+\), free radical NAD\(^\cdot\), and dimeric (NAD\(^-\))\(_2\). The electrochemical pattern for the NAD system is summarized in Figure 5.

The mechanistic pathway for the electrochemical reduction of NAD\(^+\) to the radical NAD\(^\cdot\) is dependent on several factors like the existence of competing adsorption, time-scale of the experiment, the electrode surface and its initial condition, and the background solution defined by its ionic strength and surface activity (Bresnahan and Elving, 1981; Huang and Liu, 2014). Experimentally, it has been suggested that there is a need for a protonation process before any charge transfer occurs since NAD\(^\cdot\) is not reduced in aprotic media (Santhanam and Elving, 1973, Htet and Tennyson, 2016). The measured \(k_d\) for this process, Figure 5, was \(2.7 \times 10^6\) M\(^{-1}\) s\(^{-1}\) from voltammetric analysis (Bresnahan and Elving, 1981). Recently, the reduction of NAD\(^+\) was studied from the viewpoint of its orientation at the solution or electrode interfaces. Studies showed that there is a perpendicular orientation of NAD\(^+\) on a glassy carbon as determined from charge consumed...
on the application of a potential sweep and such is in good agreement with the sequence of chemical reactions (Santhanam and Elving, 1973; Htet and Tennyson, 2016).

Anodic Oxidation of NADH. Shown in Figure 4 are different anodic waves for the oxidation of NADH in various electrodes including glassy carbon (GC), platinum (Pt), and gold (Au). The exact nature of the electrodes affects the response and the interfacial adsorption processes involved in the electrochemical reaction. At the glassy carbon electrode, as an example, the appearance of a pre-wave is due to the weak or negligible adsorption of NADH and strong adsorption of NAD$^+$:

$$\text{NADH} \xrightarrow{+e^-} \text{NAD}^+ \rightarrow \text{NAD}^+_\text{ads}$$ (1)

The oxidation of NADH at any solid electrode involves an apparent single-step two-electron oxidation to enzymatically active NAD$^+$ as shown below:

$$\text{NADH} \rightarrow \text{NAD}^+ + \text{H}^+ + 2e^-$$ (2)

This explains why NADH showed a single diffusion-controlled cyclic voltammetric anodic peak and any reversal in the scan generated a cathodic peak due to the NAD$^+$ at a more negative potential. Summarized in Figure 6 is the reaction pathway for the electrochemical oxidation of NADH. The process involves equilibrium reactions that lead to the establishment of a reaction scheme in the electrochemical oxidation of NADH via an ECE (Electron transfer, Chemical reaction, Electron transfer) mechanism (Bresnahan and Elving, 1981; Huang and Liu, 2014; Ma et al., 2015). The summary of values calculated for the equilibrium constants were summarized on the works of Bresnahan and Elving. The temperature dependence of $k$ at the GC electrode at pH 7.1 is a confirmation of the presence of a chemical limiting step. The magnitudes of $k$ correlate with the extent of electrode coverage by the absorbed NAD$^+$. This supports the idea that NAD$^+$ acts as a mediator with respect to electron and proton transfer during NADH oxidation.

Both reduced and oxidized forms of β-nicotinamide adenine dinucleotide (NADH and NAD$^+$, respectively) are important coenzymes for a large number of dehydrogenase enzymes, catalyzing the removal of a hydrogen from a substrate and the consequential transfer of the hydrogen to an acceptor in a redox active system, and as indespensible components of biomarkers (White, 1982; Ma et al., 2015). Furthermore, given its crucial role in the fields of analytical electrochemistry (Radoi and Compagnone, 2009), bio-electrocatalysis (Limoges et al., 2006; Bardea et al., 1997; Li et al., 2012), and the emerging trends in biofuel cells, the electrochemical evaluation and investigation for the formal potential and redox chemistry of the NAD$^+$/NADH redox couple has attracted considerable attention and interest.

**Figure 6: A Summary of the Reaction Pathway for the Electrochemical Oxidation of NADH.** $U_1^*$ is the oxidation potential of NADH to NAD$^+$; $U_2^0$ is the reversible potential for the NAD$^+$/NAD$^-$ couple; $U_3^0$ is the reversible potential for the NADH$^*$/NADH$^+$ couple; $k$ is the deprotonation rate constant; $k_D$ is a disproportionation rate constant. All potentials are versus S.C.E.

**PIONEERING EXAMPLES OF NAD$^*$ / NADH MIMICS**

Nature provides numerous enzymatic redox transformations that happen rapidly without any realized difficulty in addition to the high stereoselectivity exhibited by these enzyme-mediated reactions. Thus, nature is a source of inspiration towards the development of biomimetic stereoselective redox reagents that can play crucial roles in catalysis, industrial and agrochemistry, as well as in organic synthesis laboratories. NADH mimics, for example, have been extensively studied in biomimetic transformations. Mimics without the actual use of biological enzymes and supramolecular structures such as organic compounds that can perform the biochemical function of NADH and related molecules are highly desirable. For example, some NADH mimics that can perform the stereoselective reduction of benzoylformates to the corresponding mandelates show this reactivity clearly. Chiral dihydronicotinoyl derivatives as NADH mimics can interact with substrates such as benzoylformates in many possible conformations given by the two enantiotopic faces of the α-ketoester and the two diastereotopic faces.
of the dihydropyridine ring, giving four possible approaches as outlined in Figure 7. This reactivity has been studied in the late 1980’s and early 90’s and provides a pioneering example to the synthetic utility of simple NADH mimics.

High stereoselectivity in these biomimetic systems is achievable by realizing possible ways to provide restrictions similar to the enzymatic methods driven by conformational changes within the active site in biologically controlled reaction. With this requirement model systems can be designed with the preferential reactivity while substrate approach to the dihydropyridine moiety is constrained with a fixed orientation. For example, in the stereoselective reduction of benzoylformates to the corresponding mandelates, the ester portion of the substrate can approach directly over the –R* group at the dihydropyridine ring that incorporates sterically demanding or chiral side chains. However, the limitations in the approach pathways (Figure 7, A and B) does not inevitably result in the selective formation of the (R) or (S)-mandelates (Burgess et al., 1991). An approach of the si-face of the substrate to pro-(R) hydrogen in an orientation such as the ester is lying over the R* substituent delivers (R)-mandelate as the product whereas approach of the re-face gives (S)-mandelate, the same is true for pathways C and D shown in Figure 7.

Ohnishi and coworkers (Ohnishi et al., 1975) reported the first NADH model compound capable of stereoselective reduction of a prochiral substrate having a 1,4-dihydropyridine derivative possessing a chiral carboxamide at C-3. This pioneering work also proved the essentiality for the presence of divalent metal ion, which in most instances, is the divalent magnesium added to the reaction mixture in the form of magnesium salts such as magnesium perchlorate. Since Ohnishi and coworkers reported this reduction reaction by a NADH model system, a large number of such biomimetic systems have been developed (for a list of works on benzoylformate reduction by NADH mimics see reference section). Table 1 summarizes some of these model NADH mimics.

Zhao and coworkers reported a more recent development to this transformation in 2007. In their NADH mimic four chiral carbon centers were introduced using (1R,2R)-diaminocyclohexane, Figure 8. This model compound has a C₂-symmetric structure that can take advantage of an efficient bi-directional synthetic preparation methodology (Zhao et al., 2007). Thus, this C₂-symmetric NADH mimics were able to enantioselectively reduce the pyruvate mimic methyl benzoylformate in acetonitrile at room temperature giving (R)-mandelate as the product.

Figure 7: Four Possible Approach Pathways to the Interaction between the Two Enantiotopic Faces of the α-Ketoester and the Two Diastereotopic Faces of the Dihydropyridine Ring. R* at the C-4 position incorporates sterically demanding or chiral side chains.

Figure 8. Enantioselective Reduction of Benzoylformate Using a C₂-symmetric NADH Mimics.

COMPLEXES WITH NAD⁺/NADH ANALOGOUS LIGANDS

Scientists have focused much attention to NADH as an organic hydride donor. Numerous kinetic studies of hydride transfer, reduction of carbonyl compounds in an asymmetric fashion, proton-coupled electron transfer with NADH and its model compounds have been investigated (see reference section for a list of works on this topic). However, all the reactions involving NAD+/NADH model systems present stoichiometric reactions. One example of an electrochemical system behaving in a similar way as the NAD+/NADH redox couple is a model compound reported by our research group (Tomon et al., 2005; Nakajima and Tanaka, 1995), Figure 9. With the goal towards the polypyridyl ruthenium carbonyl complex mediated electro- and photochemical reduction of carbon dioxide, also relying on the ligand based redox reaction as the source of electrons required for the reduction, our group examined the possibility of metallacyclization of Ru(napy-κN)(CO)(bpy)₂(PF₆)₂, where napy and bpy are the 1,8-naphthyridine and bipyridine moieties respectively. Such process can be driven by the ligand based redox reaction on the naphthyridine ring resulting to the enhancement of the nucleophilicity of the non-bonded nitrogen atom triggering an intramolecular attack at the α-carbon of a neighboring ligand in generating a metallacycle.
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The electrochemical reduction of cis-[Ru(napy-κN)(CO)(bpy)₂]²⁺ (Figure 9) at -1.34 V (versus the ferrocene/ferrocenium, Fc/Fc⁺, couple) resulted in the formation of a bond between the non-coordinating nitrogen atom in the naphthyridine ring and the carbonyl carbon followed by the subsequent hydrogenation of the naphthyridine ring. The reduced complex upon re-oxidation at 0.06 V fully regenerated the original complex. This exhibited behavior is in good agreement with the NAD⁺/NADH redox couple.

The reversible formation of C–H bond in the model system showed the versatile properties of the redox couple in using the C–H bond as a way to store energy. Other systems have also been shown to have the ability to store chemical energy and transform this to electrical energy. Tri-nuclear [(bpy)Ru(dpbbpy)]₃⁺·Cl⁻ (bpy = 2,2’-bipyridine, dpb = 2,3-bis(2-pyridyl)benzoquinoline) reported by Molnar and coworkers in 1994, and the tetranuclear [(bpy)₃Ru(dmbbbpy)]₄⁺ (dmbbbpy =2,2’-bis(N-methylbenzimidazole-2-yl)-4,4’-bipyr-
Electrocatalytically, it has been shown that [Ru(bpy)$_2$(pbn)][PF$_6$]$_2$ was able to catalyzed the reduction of acetone to isopropanol with the hydrogenated form of the complex, [Ru(bpy)$_2$(pbnH$_2$)][PF$_6$]$_2$, as the key intermediate. By using pulse and steady-state radiolysis studies the mechanism of hydride donor generation for this complex has been extensively studied. Shown in Figure 11 is the mechanism for the formation of the hydrogenated form of the complex and the generation of the hydride donor (Polyansky et al., 2008).

Another notable ruthenium complex displaying an interesting electro- and photochemical redox behavior is a mononuclear complex [Ru(bbnp)(terpy)][PF$_6$]$_2$ where terpy is 2,2':6',2''-terpyridine and bbnp is 2,6-bis(benzo[b]1,5-naphthyridin-6-yl)-4-tert-butylpyridine (Tannai et al., 2007). The corresponding two- and four-electron reduced complexes of this ruthenium compound are [Ru(bbnpH$_2$)(terpy)][PF$_6$]$_2$ and [Ru(bbnpH$_4$)(terpy)][PF$_6$]$_2$ respectively.

The synthesis of [Ru(bbnp)(terpy)][PF$_6$]$_2$ complex, shown in Figure 12, involved a two-step reaction starting with the coordination of bbnp followed by the coordination of the other ligand terpy. The cyclic voltammetric analysis of this complex showed four reversible redox couples. The redox reactions at $E_{1/2} = +1.48$ V and -1.42 V (all versus S.C.E.) is attributed to the Ru$^0$/Ru$^{II}$ couple and the ligand localized redox process (terpy$^0$/terpy$^-$) couple. The other two remaining redox couples observed at $E_{1/2} = -0.68$ V and -0.88 V resulted from two successive ligand-localized couples: (bbnp, bnp$^+$/bnp$^-$, bnp$^-$) and (bbnp$^-$, bnp$^+$, bnp$^-$). Moreover, the electrochemical oxidation of the four-electron reduced complex fully regenerated the original dehydrogenated complex, Figure 13.

The electrochemical behavior of these inorganic complexes is in good agreement with the NAD-/NADH electrochemistry. This leads to the development of renewable catalysts that can be used for the reduction of organic and inorganic substances. Renewable catalysts for the reduction of alkyl halides, olefins, and ketones in addition to various photo-induced electron transfer and thermal reactions can be realized. Furthermore, this has led to the possibility of using CO$_2$ as a substrate and converts this greenhouse gas to reduced organic compounds like formic acid and methanol that can generate fuel as a renewable and sustainable resource for a clean and green energy-dependent society.

More recently, Kobayashi and coworkers in 2016 reported the photochemical properties of an NAD-/NADH-functionalized ruthenium polypyridyl complex having an NAD+/NADH analogous functionality at the $f$-position of the 1,10-phenanthroline moiety, Figure 14. The NAD form as well as the NADH form showed improved and significant light emission properties as compared with the NAD$^+$ and NADH model complexes.
to those ruthenium polypyridyl complexes mentioned in this review (Ru-pbn complexes). The bpp(benzo[b]pyrido[3,2-f][1,7]-phenanthroline) moiety has been shown to store photoreducing energy via a structural conversion consistent to the NAD+/NADH redox couple.

Electrochemically, the cyclic voltammogram of [Ru(bpp)(bpy)2]2+ exhibited four reversible redox couples at 1.23, −1.28, −1.43, and −1.63 V vs SCE in DMF, Figure 15. The redox couple observed at 1.23 V can be attributed to the Ru(II)/Ru(III) metal-centered redox process. When acetic acid was added as a proton source the reversibility of the redox couple present at −1.28 V was lost with a generation of a new anodic peak at 0.8 V. The remaining two couples occurring at the more negative potential that were unchanged before and after the acid addition can be attributed to the redox processes at the two-bipyridine ligands. The irreversible +0.8 V anodic wave not appearing in the initial anodic potential sweep emerged after the potential sweep was returned at -1.4 V. This demonstrated a large separation between the cathodic and anodic peak potentials of the bpp localized redox process arising from the protonation of the non-coordinated nitrogen atom in the bpp moiety. Furthermore, the NADH form [Ru(bppHH)(bpy)2]2+ has been demonstrated to reduce molecular oxygen while regenerating the original complex [Ru(bpp)(bpy)2]2+ under photoirradiation. By monitoring the NMR spectrum of [Ru(bppHH)(bpy)2]2+ solution, its selective oxidation to [Ru(bpp)(bpy)2]2+ without any trace of degradation products was clearly demonstrated. The production of hydrogen peroxide was also confirmed. Finally, the reasonable mechanism for this transformation was proposed, Figure 16.

The results demonstrated in this ruthenium polypyridyl chemistry bearing a site analogous to the NAD+/NADH redox couple presents a new strategy for the development of novel platforms and molecular design motifs for realizing renewable hydride catalysts towards the grand goal of efficient energy conversion between electrical and chemical energy in creating an energy-secured and sustainable society.

**INSIGHTS AND PERSPECTIVES**

The goal to provide a sustainable society for the next generation has inspired numerous researches in many fields of the sciences. Among these fields, catalysis using transition-metal chemistry has flourished as a useful and attractive strategy in dealing with energy problems that our society is facing now. As with many other systems, nature proves to be the best source of motivation and inspiration. Biomimetic chemistry has advanced greatly because of the potential it offers...
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in delivering solutions to several problems both of the society and difficulties normally encountered in many reactions in the laboratories and in the industry. For example, efficient transformation of electrical to chemical energy has been shown by the NAD+/NADH redox couple via the formation of a chemical bond without any difficulty. By looking into the behaviour of this classical system, researches to mimic its biochemical activity have span over the last few decades. Indeed, nature can provide us with solutions. We simply need to look at the intricacies of nature’s structure and function. In the future, more technological advancement should bring us to encountering nature’s wonders and how we can harvest such wonders to provide real and concrete solutions especially in the world’s increasing energy demand and alleviation of problems arising from global warming.

CONCLUSION

Redox reactions remain the most viable source in providing the chemical motive force essential for all forms of life. The biologically important redox couple, NAD+/NADH provides a reversible prototype system for the conversion of electrical to chemical energy via the reversible formation of a C–H bond centered on the nicotinamide ring. The NAD+/NADH redox couple plays important roles as coenzymes for pyridinoproteins (dehydrogenases) in major metabolic pathways such as the electron transport chain. Biomimetic chemistry of the NAD+/NADH redox couple has provided numerous examples of non-enzymatic reactions mediated by the reactivity analogous to the electrochemical process involved in the redox reactions in the NAD+/NADH redox couple. Moreover, biomimetic ruthenium polypyridyl chemistry has provided excellent model systems that demonstrated functionality similar to the NAD+/NADH redox couple. This has resulted to the preparation of ligand systems and transition metal complexes that can harness the hydride donating ability of NADH for catalytic applications such as reduction reactions of α-keto esters, alcohols and other organic substrates. Moreover, the activation of molecular oxygen via the reduction to hydrogen peroxide was also demonstrated. This should provide novel strategies towards the development of catalytic systems that can give advantageous results in the alleviation or eradication of our energy problems.

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