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Non-toxic hydride energy source for biochemical and industrial venues: ORP and NAD^+ reduction analyses

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Abstract

A recently described novel compound, silica hydride, was used to investigate potential alternative hydrogen energy sources for use in industry, pharmacology and biochemistry. Acting as an anionic hydride, the silica hydride does not react violently with water and produces stable oxidation-reduction potential readings of greater than −860 mV for extended periods providing capacity as an alternative for current transfer, hydrogen production and fuel cell applications in industrial arenas. In a biological venue, the silica hydride definitively reduces the pyridine NAD^+ to the NADH form, indicating potential for use as a biochemical fuel cell. Subsequent analyses of the compound indicate no induced cytotoxicity as a result of the silica hydride and the increased NADH in a cell.

Keywords: Silica; Hydride; NAD; Antioxidant; Microcluster; ORP

1. Introduction

The use of hydrides as an alternative energy source in fuel cells has been well documented in research [1–4]. Metal saline hydrides, such as LiH, NaH, CaH_2, Na_2AlH_6, and others have demonstrated hydrogen storage capacity of up to 6% weight [5–7]. The release of the stored hydrogen may be regulated by the reaction with water vapor or controlled volume liquid water, although many saline hydrides react violently and without control upon direct contact with a stoichiometric amount of water [8]. Other well established methods of hydrogen energy production may involve vapor-solid hydrolysis or electrolysis methods [9]. The limited resources of elements, such as lithium, and the cost prohibitive nature also factor into the overall efficacy of current fuel cell and energy reserve mechanisms [10]. Hydride energy resources are not only commodable in industry, but may be increasingly useful in the biological arena as biochemical hydrogen "fuel cells". The problem with most current hydrogen energy sources is the induced toxicity and harmful effects resultant from biological exposure to compounds such as LiH, $NaBH_4$, $NaCNBH_3$, etc [11]. Of the few biologically friendly hydrides, such as alkyldiphenyltin hydride, the reduction potential and overall ability to provide useful energy is limited [12].

To aid in the evaluation of hydride energy sources, ORP is one indicator of the energy reserves available for use. This is particularly true of liquid environments. Another indicator is through the evaluation of pH responses to the solution. The problem exists, however, that the ORP may be biased by the pH and *vice versa*. To accommodate for this, Clark reported the idea of computing the absolute reducing potential of a compound by using a variation of the Nernst equation that measures ORP taking into account hydrogen ion activity [13]. The equation developed (1) related hydrogen pressure and reduction potential in units of rH.

$$E_h = 1.23 - \frac{RT}{F}\mathrm{pH} - \frac{RT}{4F}\ln\frac{1}{P_o}, \quad (1)$$

where E_h is the measured oxidation-reduction potential, F is the Faraday constant, R is the universal gas constant and T is absolute temperature. The 1.23 references that the measured potential under one atmosphere pressure of oxygen is 1.23 V

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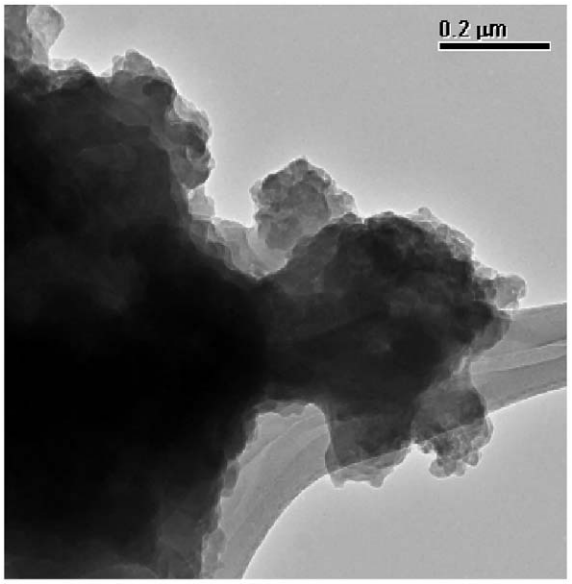

Fig. 1. TEM image of silica hydride clusters. Silica hydride's organosiliceous nano-cages with interstitially embedded hydride anions provide a favorable arena for radical and enzyme reduction without cytotoxic effects.

greater than in a solution of the same pH. rH is defined explicitly as the negative logarithm of the oxygen pressure, P_o(2):

$$rH = -\log P_o. \quad (2)$$

The use of rH gives a hydrogen proton-unbiased look at the absolute reducing potential of a compound, eliminating the effects of pH in the ORP measurement. It is a true indication of a compounds reduction potential capacity. The shifts in rH can be used to quantify the reducing ability and energy reserves of the compound as well as illustrating a qualitative comparison between different reagents. Recently, a novel organosiliceous hydride compound with biochemical properties was described [14]. The compound, silica hydride (Fig. 1), is a sub-micrometer, caged-silicate structure with interstitially embedded hydrogen anions, which acts as a strong reducing agent, while not violently reacting with an aqueous environment. Iterations in synthesis methods have produced up to a 17% hydride content. Low concentrations in aqueous solution provide ORP readings approaching −850 mV, and maintain stability for extended periods for upwards of months at neutral pH and interpolated data suggest multiple years stability at slightly alkaline pH [15]. The silica hydride has additionally been shown not to induce a toxic environment biologically and to efficiently reduce free radicals in vitro [16].

The release of hydrogen may be controlled through reacting the solution with other compounds or through an acid-catalyzed reaction. Acid-catalysis of silica hydride requires only a weak acid, such as citric or malic. ORP and rH are excellent tools for theoretical calculations and industrial tests, and are a solid hypothetical for biochemical testing. However, they do not guarantee the ability to provide energy in the biological arena.

In biological systems, pyridine nucleotides play important roles as coenzymes in oxidation-reduction reactions in the body. Of the biological nucleotides, nicotinamide adenine dinucleotide (NAD^+/NADH) is the most common redox system [17–19]. The reduced coenzyme form, NADH, is critical to the intracellular energy production of adenosine triphosphate (ATP) within the biological system (Fig. 2). Preserved energy is created in the body when NADH is oxidized to NAD^+. The products of the oxidation include water and ATP [20]. The more NADH that is available in the cell, the more demand for cellular energy may be fulfilled. The needed NADH in the body may be synthesized via a niacin complex or through amino acid amination of ribonucleotides [21]. Oxidized NAD^+ generally plays a coenzymatic role in its own reduction, although some compounds are able to reduce the NAD^+ as a substrate [22–24]. The ability of a compound to reduce NAD^+ *in vitro* is a great indicator to evaluate a biochemical hydrogen energy source when used in conjunction with ORP and rH.

The reduction of NAD^+ to NADH has numerous implications in glycolysis and the electron transport chain for the subsequent production of ATP in the cell. Conventional theory illustrates the transfer of two electrons and a proton by related, yet distinctly separated steps. Current theorists claim the direct transfer of a hydride anion mechanistically to reduce the nicotinimide [25–27]. The one-step mechanism for the direct electron reduction of the nicotinamide pyridine is illustrated in Fig. 3.

The hydride anion can be viewed not necessarily as a $1s^2$ orbital configuration, but rather a $1s1s'$ configuration, where the s' orbital maintains an analogous open, *s*-type shell, orthogonal to the 1*s* orbital [28]. Each hydride is gyroscopically resonance-stabilized through the interactions of the electrons between shells. Looking at the hydride ion in this light offers insight to support the theories of a direct transfer of a resonance stabilized hydride ion in solution. Early studies on the hydridoborate anions support the notion of the stability of the anions, particularly in a neutral or alkaline environment [29]. Additional nuclear magnetic resonance (NMR) studies of carbonium ions concur with the idea of a direct hydride transfer [30–32].

The reduction of NAD^+ has an energetically favorable standard reduction potential (E_0) of −0.315 V [33,34]. If the reduction were looked at as a function of a two electron transfer with a proton, the half-redox reaction corresponding to the half equation for the oxidation from water to O_2, H^+ and e^- is $E_0 = 0.815$. The overall reaction then corresponds to $\Delta E_0 = 1.130$ V, which is not particularly energetically advantageous [35]. The direct gain of a hydride anion would perhaps be energetically more favorable to the electron affinity of the NAD^+ or other biological

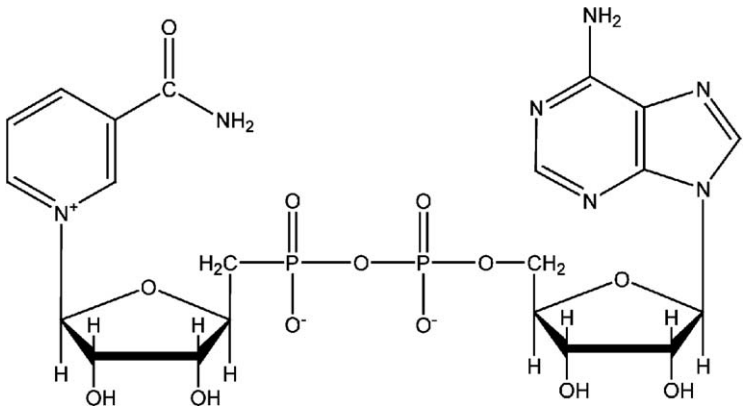

Fig. 2. The structure of the oxidized nicotinamide adenosine dinucleotide molecule. The coenzymatic redox-couple of NAD^+/NADH are major constituents of biochemical reactions and an important indicator of a compound's biological reduction capacity.

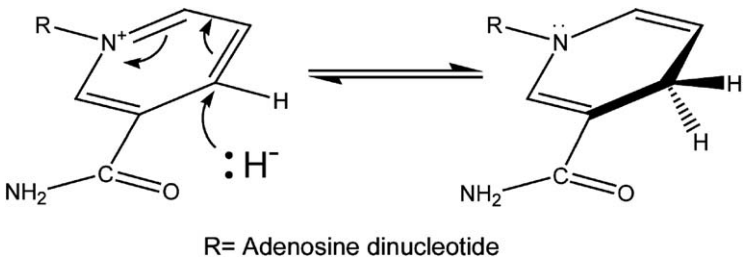

Fig. 3. The mechanism of the direct hydride transfer by the $1s1s'$ electron of the hydrogen anion to the NAD^+ molecule. In vitro data of NAD^+/silica hydride reactions have illustrated the long term maintenance of the reversible, reduced product, NADH.

molecule. Additional studies on the electrochemical behavior of the redox patterns of NAD^+ confirm the indication an overall potential of between 1.1 and 1.8 V needed to reduce to NADH [27]. With these particularly high potentials, one of two circumstances should exist for the reduction of the nucleotide-catalysis of the reaction to reduce the energy needed to promote the spontaneity of the reaction or a reducing agent of great enough strength and efficiency to efficably reduce the compound.

Reduced NADH has illustrated significant biochemical implications including aiding in amelioration of the symptoms of Parkinson's disease [36], increasing norepinephrine production, relating to the treatment of depression [37], beneficially affecting Alzheimer's disease [38], cellular aging and DNA repair [39], and increased athletic energy and performance [40,41].

This work represents a detailed analysis of the novel silica hydride as a hydride energy source for both industrial and biochemical venues. Analysis by spectrophotometry, nuclear magnetic resonance spectroscopy and ORP/rH investigation gain insight into the potential uses of the compound.

2. Materials and methods

Buffers, calibration standards and reagents were of reagent grade and purchased from Spectrum Chemical (Los Angeles, California), with the exception of the silica hydride solution which was produced in-house as previously described [14].

2.1. ORP analysis

An IQ Scientific IQ-400 ORP meter with a platinum solid-state electrode was calibrated with both pH 4 and pH 7 hydroquinone standard solutions. A measure of 30.0 mg of silica hydride was suspended in 250.0 ml of deionized H_2O in an Erlenmeyer flask and magnetically stirred while data were acquired. Data were collected at 30 s intervals for 4 h and 3 replicates were performed. Additional pH and temperature data were collected simultaneously by an ISFET combination electrode.

The experimentation was repeated using a 50 mM Tris-HCl buffer, pH 7.8, using the fore mentioned methodology.

2.2. Spectrophotometric analysis

A standard addition plot was made from adding 100 μl aliquots of a 0.1 mg/ml solution of silica hydride to 0.5 ml of a 60 μM solution of NAD^+ in 50 mM Tris-HCl buffer, pH 7.8 and tested for linearity at 340 nm in a V-580 spectrophotometer (Jasco, Inc., Easton, MD) in a 1 cm quartz cuvette and zeroed relative to a 60 μM NAD^+ standard in buffer solution. Secondly, 500 μl of a 60 μM solution of NAD^+ (Na salt) in 50 mM Tris-HCl buffer was added to a 1 cm quartz cuvette and zeroed. Five hundred microliters of a solution of 200 μg/ml silica hydride solution was pipetted into the cuvette and the absorbance at 340 nm was monitored for 4 h. The experiment was performed in triplicate. An additional assay was performed in triplicate with the same experimental parameters although in non-buffered, deionized water.

2.3. ^1H-nuclear magnetic resonance spectroscopy

Five hundred microliters of a 60 μM solution of NAD^+ (Na salt) in a 50 mM Tris-HCl buffer made from analytical D_2O was placed in an NMR sample tube. The sample was placed in an ARX-300 MHz NMR (Bruker, Germany), shimmed with a deuterium lock and 20 scans taken. An additional 500 μl of a solution of 200 μg/ml silica hydride solution was added to the sample tube and subsequent 20 scan analyses were taken in 30 min increments for 4 h.

3. Results

3.1. ORP analysis

Plotting the average ORP values in mV as a function of time produced the data illustrated in Fig. 4. ORP values changed at an initial rate of −322.6 mV/min and had a maximum redox potential of −862.3 mV occurring at 23 min into the assay. The final data value taken at 4 h was −859.7 mV. The buffered replicates varied less than 2% to the non-buffered replicates.

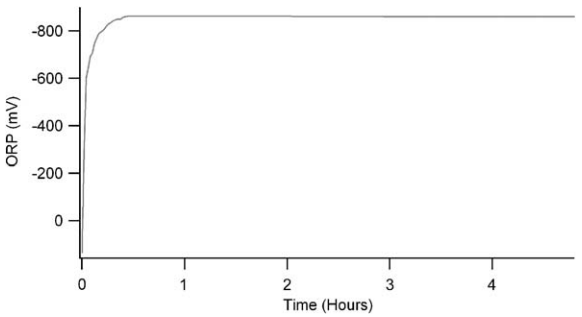

Fig. 4. Redox potential values over time of a 120 μg/ml solution of silica hydride in distilled water. An initial rate of −322.6 mV/min with a maximum redox potential of −862.3 mV maintained stability over the 4 h analysis.

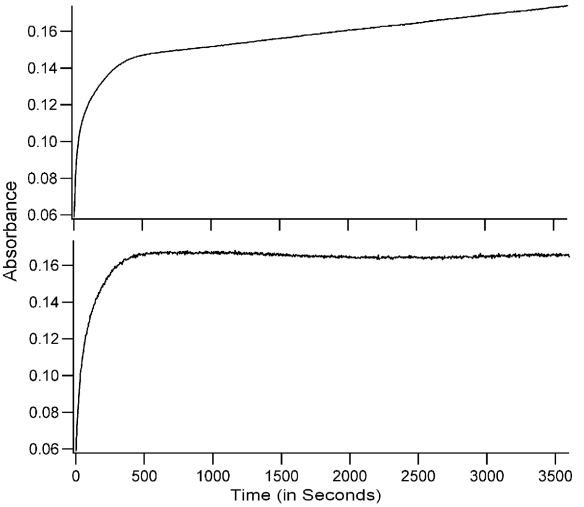

Fig. 5. Three hundred and forty nanometers absorbance spectra for the reduction of (A) non-buffered and (B) Tris-HCl buffered aqueous 60 μM NAD^+ solutions by 200 μg/ml silica hydride.

3.2. rH/pH

Calculation of P_0 for the reaction with an ORP reading of −862.3 and a pH of 8.4 results in value of 6.46×10^{-6} with a calculated rH value of −5.19, which is off the standard scale that ranges from rH = 0 (for H_2) to rH = 42 (for O_2), indicating a highly reduced environment.

3.3. Spectrophotometric analysis

UV-Vis spectraphotometric assays resulted in the reduction of the NAD^+ to NADH in the presence of the silica hydride. The standard addition plot for the addition of the 100 μl aliquots produced a linear relationship between the concentration of silica hydride and the amount of NAD^+ reduced, measured as a function of the absorbance at 340 nm. Sequential analyses of buffered and non-buffered NAD^+ solutions monitored over time indicate the maintenance of the reduced NADH for extended periods. The TRIS-HCl buffered assay with silica hydride reduced the 60 μM NAD^+ (0.14 absorbance units) consistently for 4 h. The unbuffered assay reduced the 60 μM NAD^+ starting at 0.16 absorbance units and slowly increasing to 0.20 absorbance units after 4 h. Fig. 5 illustrates both spectraphotometric assays of the NAD^+ reduction.

3.4. NMR

^1H NMR produced results similar to that previously described [31,32]. Monitoring the evolution and stability of

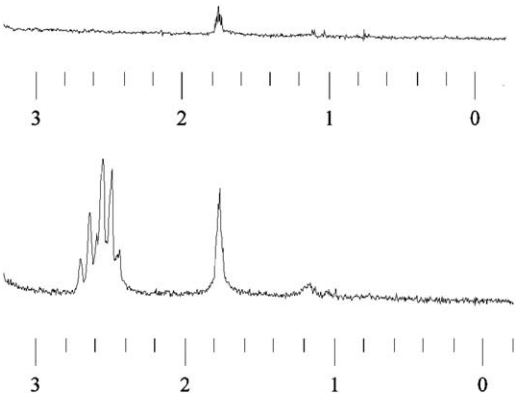

Fig. 6. ^{1}H-NMR of a 60 μM NAD^{+} solution in Tris-HCL buffered D2O (A) before and (B) after treatment with 200 μg/ml silica hydride. The evolution and maintenance of the 5 peak multiplet at δ2.5 ppm is indicative of reduced nicotinamide.

the multiplet centered at δ2.5 ppm from TMS is indicative of the presence and abundance of reduced NADH (Fig. 6). Zero and first-order phase corrections were made to the Fourier transformed FID data and baseline corrected with two points. Integrations of the peak areas centered at 2.5 ppm were followed and produced consistent values at 30 min intervals for the 4 h analysis.

4. Discussion

This analysis of the silica hydride demonstrates the compound as a significant reducing agent and potential energy source. The utilization of this energy is shown through the exceptionally high negative ORP readings and pragmatic reduction potential calculated by rH. The stability of the low ORP in solution for extended periods offers a vast opportunity for the compound to be used as an alternative energy source. Subsequent experimentation on the low ORP solution gives rise to potential uses as means to regulate or transfer current, produce hydrogen gas or use as a reduction agent.

From the biological standpoint, the silica hydride compound has considerable additional possibilities, as a reducing agent for free radicals in the body [16] and as an internal, cellular fuel cell. The reduction of the pyridine NAD^{+} to NADH offers a definitive look at the true reducing capacity in vitro for the silica hydride. The amount of NAD^{+} reduced is consistent, as measured through NMR and 340 nm spectrophotometry, and illustrates a significant reducing capacity. In the body, 6 units of adenosine triphosphate (ATP) are produced per 2 units of NAD^{+} reduced. Theoretically, incorporating this reducing agent into a cellular environment could produce more cellular ATP as a function of increased NADH/NAD^{+} ratios. Previous tests have analyzed cellular cytotoxicity of silica hydride/NAD^{+}-treated cells through fluorescent viability probes consisting of ethidium homodimer-1 and calcein AM using Chinese hamster ovary (CHO) cells [42]. The results indicate no induced cellular toxicity from the silica hydride or the resultant increased NADH concentration. Combinatorially, the low ORP, reduced rH, and ability to consistently reduce NAD^{+} provide a solid basis for further investigation into this hydride family as alternate hydrogen energy mechanisms in both biological and industrial arenas.

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