Studies of Hybrid Nano-Bio-System: Single-Walled Carbon Nanotubes and Hydrogenase

D. Svedruzic-Chang[*], J.L. Blackburn[**], T.J. McDonald[***], M.J. Heben[†] and P.W. King[§]

National Renewable Energy Laboratory
1617 Cole Blvd., Golden, CO, USA, 80201
[*]drazenka_svedruzic@nrel.gov, [**]jeffrey_blackburn@nrel.gov, [**]timmy.mac@gmail.com
[†]michael_heben@nrel.gov, [§]paul_king@nrel.

ABSTRACT

We have examined changes in single-walled carbon nanotube's (SWNTs) optical signals upon addition of [FeFe] hydrogenases (H_2ase). Evidence was found that stable SWNT/H_2ase charge-transfer complexes self-assemble in solution under conditions of H_2ase catalytic turnover. Raman studies suggest that metallic SWNT can undergo either forward or reverse electron transfer depending on the H_2ase redox state. This amphoteric behavior is due to the electronic band structure of the metallic SWNTs, which leads to both occupied and empty mid-gap electron states at the Fermi level. In contrast, semiconducting SWNT, which had no mid-gap states, can only accept electrons from the reduced H_2ase. In context of hybrid SWNT-H_2ase based devices, metallic SWNTs are more suited for applications as a conductive molecular wire, while semiconducting SWNTs are more suited for use in nanoscale sensors or photovoltaic devices.

Keywords: nano-bio-systems, single-walled carbon nanotubes, hydrogenase, charge-transfer, Raman

1 INTRODUCTION

Molecular hydrogen is promising as the centerpiece of a sustainable, carbon-free energy supply. Unfortunately it is not abundant in nature and current production technology is energy inefficient, expensive, and produces CO_2 when hydrocarbons are utilized as the source of H_2. Various microorganisms are able to produce H_2 through catalytic activity of hydrogenases (H_2ase), a group of metalloproteins [1]. The most common and best studied are [NiFe] and [FeFe] type H_2ase. Their biological roles include disposing of excess electrons produced during certain cellular metabolic activities, catalyzing an exergonic reduction of protons under low-redox potential growth conditions, and use of H_2 as a low-potential "food" source. In the case of photosynthetic microorganisms the source of such electrons is solar-driven water oxidation. As biocatalysts involving only a polypeptide chain and naturally abundant first-row transition metals, H_2ase might potentially be incorporated into solar-to-hydrogen [2] or hydrogen-to-electricity [3] fuel cells, as cheap, renewable and efficient alternative to noble metal catalysts such as platinum.

Although promising, incorporation of biological molecules into nonbiological devices faces many challenges. In the case of redox enzymes the major challenge is to achieve a robust and efficient electronic interaction with non-biological material in a manner that does not compromise enzymatic activity. For H_2ase, an additional obstacle for widespread application is its inherent inactivation by molecular oxygen. Single-walled carbon nanotubes are interesting candidates for use as molecular wires within bio-hybrid systems because of their nanoscale dimensions (comparable to the dimensions of a protein), high surface area, and outstanding electrical conductivity. In addition, SWNT in complex with H_2ase can be potentially applied as a component of nanoscale devices for light harvesting and conversion of solar energy to H_2 either as an active photo-collecting element or for dissociation of bound excitons and charge-transfer from a chromophore to the H_2ase active site.

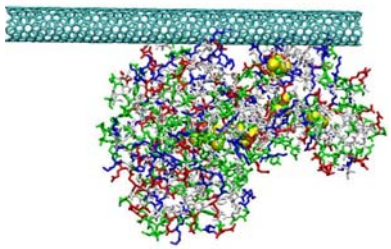

Figure 1. Model of SWNT/H_2ase complex

In our initial studies [4] we have examined changes in SWNT optical signals upon addition of recombinant [FeFe] H_2ase from bacterium *Clostridium acetobutylicum*. We found evidence that novel and stable charge-transfer complexes self-assembles in solution under conditions of H_2ase catalytic turnover (Figure 1). Formation of the complex sensitizes the nanotubes to the proton-to-hydrogen redox half-reaction. Thus, the experimental potential can be altered by changing the pH or H_2 concentration. In the presence of H_2, H_2ase mediates electron injection into the LUMO of semiconducting SWNT (sem-SWNT), which was observed as a quenching of the photoluminescence (PL) signals [4]. By evacuating H_2, PL signals from sem-SWNT were recovered. Here, we will present recent Raman

studies of H_2ase interaction with both semiconducting and metallic (met-) SWNTs, which revealed that SWNT in a complex with H_2ase may undergo either oxidation or reduction, depending on the electronic structure of the SWNT and the oxidation state of the enzyme.

2 RESULTS

2.1 Characterization of SWNT/H_2ase Complex by Raman Spectroscopy

Hydrogenases interaction with H^+/H_2 redox couple can be presented by following chemical equation:

$$[H_2ase]_{red} + 2H^+ \leftrightarrow [H_2ase]_{ox} + H_2 \quad (1)$$

where $[H_2ase]_{red}$ and $[H_2ase]_{ox}$ represent reduced and oxidized redox state of catalytically active H_2ase. To study electronic interaction of H_2ase with SWNT at different potentials we have performed solution-phase Raman spectroscopy of SWNT/H_2ase complex equilibrated with either H_2 atmosphere (H_2ase primarily in reduced form) or with H_2-free atmosphere (H_2ase primarily in oxidized form), while pH was kept neutral. H_2-free samples were prepared by extensive purging with argon.

Raman spectra of SWNT/H_2ase complex equilibrated with either 4 % H_2 in N_2 mixture or Ar were excited at 633 or 532 nm. Figure 2 shows the 633 nm resonance Raman with focus on two distinct regions: G-band (main frame) and radial breathing modes (RBM, inset) of the control SWNT sample (no enzyme, 1) and the same sample treated with the H_2ase (2). Two main peaks dominate the G-band Raman spectrum: sharp G^+ mode at 1592 cm^{-1} corresponds to sem-SWNTs, while the small broad G^- shoulder at 1528 cm^{-1} corresponds to met-SWNTs. The RBM peaks in the 180–220 cm^{-1} range correspond to larger diameter met-SWNTs excited resonantly via the first metallic resonance transition, while peaks from ~280–320 cm^{-1} correspond to smaller diameter sem-SWNTs excited via the second excitonic transition.

It is clear from Figure 2 that addition of $[H_2ase]_{red}$ induces significant attenuation of both G band and RBMs of sem-SWNTs. In the same experimental conditions, PL signals of sem-SWNTs were quenched [4]. Addition of $[H_2ase]_{ox}$ (i.e. extensively purged with Ar) does not effect either G-band or RBM signals of sem-SWNTs. Again, Raman data are consistent with PL data [4], which suggest that charge-transfer interaction occurs between sem-SWNT and $[H_2ase]_{red}$ only. Contrary, met-SWNT RBM peaks are significantly attenuated in both H_2 and Ar atmosphere. At 532 nm excitation, the metallic G^- mode constitutes a larger weighting of the G^- band spectrum in comparison to 633 nm excitation and therefore effects on met-SWNT can be easier analyzed. Addition of H_2ase significantly effect metallic G^- mode changing peak position, width and intensity in comparison to control sample, to an extent determined by the experimental conditions (Figure 3).

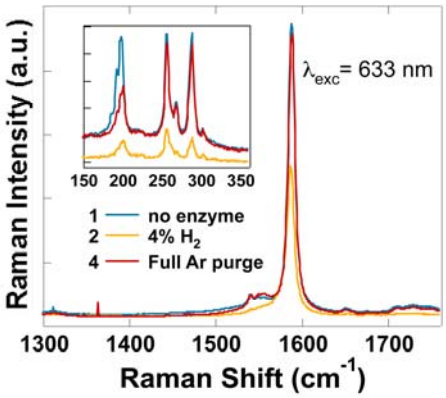

Figure 2: Main frame shows 633 nm excited G band Raman spectra of control SWNT sample (1), SWNT/H_2ase complex under a 4% H_2 atmosphere (2), and SWNT/H_2ase complex extensively purged with Ar (4). Inset shows the RBM spectra of the same samples.

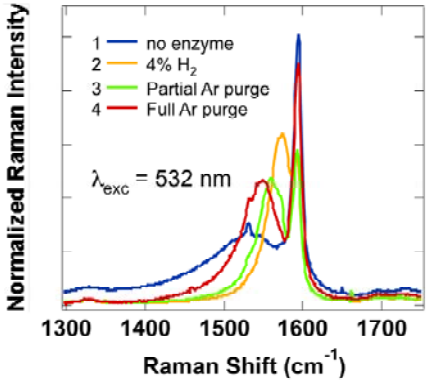

Figure 3: G-band Raman spectrum at 532 nm excitation of control SWNT sample (1), SWNT/H_2ase complex under a 4% (2) and 10^{-5} % (3) H_2 atmosphere, and SWNT/H_2ase complex extensively purged with Ar (4).

2.2 Oxygen Effects on SWNT/H_2ase Complex

In the presence of molecular oxygen, H_2ase is irreversibly inactivated and cannot undergo charge transfer interaction neither with H^+/H_2 couple nor SWNT. PL signals of sem-SWNT, which are completely quenched in the presence of reduced H_2ase, gradually recover when exposed to air to resemble the sem-SWNT signals of enzyme-free sample [4]. In the same fashion, Raman spectra of SWNT/H_2ase sample incubated with O_2 is practically the same as the spectra of the control, enzyme-free SWNT solution (data not shown).

Actual kinetics of H_2ase inactivation could be measured as the rate of decrease in H_2-production activity after exposure to O_2. Without SWNTs added to the solution, $[H_2ase]_{red}$ (i.e. pretreated with 4% H_2) inactivates about 10 times slower than $[H_2ase]_{ox}$ (i.e. pretreated with Ar) (Figure

4). Hence, an insight can be gained about the H_2ase redox state and direction of the charge-transfer in SWNT/H_2ase complex by comparing the rate of H_2ase inactivation in complex with SWNT to the rate of the inactivation of free H_2ase (Figure 4, Table 1).

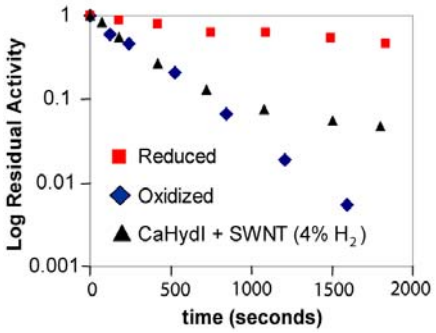

Figure 4: Residual activities of free H_2ase (oxidized and reduced) are compared to the residual activities of H_2ase mixed with SWNT in the reducing conditions (4 % H_2). Activity data are presented in logarithmic scale to better illustrate single versus biexponential functionality.

In addition to enzyme activity, kinetics of O_2 effects could also be measured as the rate of Raman signals recovery. Raman signals from sem- and met-SWNT can be clearly resolved (Figures 2, 3) and therefore the rate of O_2 effect on individual SWNT type can be measured. Here we follow the changes in intensity of RBM peaks at excitation 633 nm upon exposure of SWNT/H_2ase solution to 21 % O_2 for sem- and met-SWNT separately.

Effects of O_2 on activity or RBM signals under either H_2 or Ar atmosphere for sem-SWNT/H_2ase occur with the rate corresponding to the inactivation rate of $[H_2ase]_{ox}$ (i.e. under Ar) free in solution (Figure 4, Table 1). Effects of O_2 on met-SWNT/H_2ase RBM signals under either H_2 or Ar atmosphere follow biphasic kinetics with the two rate constants corresponding to the inactivation rate of reduced and oxidized H_2ase free in solution. This results suggest that met-SWNT in complex with H_2ase in either oxidized or reduced form can undergo charge-transfer. Results of kinetic measurements are summarized in Table 1.

Table 1: Summary of the rate constants obtained by measuring O_2 effects on H_2ase activity or Raman spectra. Changes of the RBM at excitation 633 was measured separately for sem-SWNT (s) and met-SWNT (m).

Sample	Method	$k_1(s^{-1})$	$k_2(s^{-1})$	% k_2
H_2ase, ox (Ar)	activity	0.003	-	0
H_2ase, red (H_2)	activity	-	0.0004	100
SWNT/H_2ase (H_2)	activity	0.001	0.0005	20
SWNT/H_2ase (H_2)	Raman, s	0.005	-	0
SWNT/H_2ase (H_2)	Raman, m	0.003	0.0005	10
SWNT/H_2ase (Ar)	Raman, m	0.005	0.0002	90

3 DISCUSSION

Our recent report showed that [FeFe] hydrogenases spontaneously form stable, catalytic, charge-transfer complexes with SWNTs in solution [4]. Formation of the complex sensitizes the nanotubes to the proton-to-hydrogen redox half-reaction. We followed band-edge emission quenching to study the electron transfer from the catalytically active reduced H_2ase into the LUMO of sem-SWNTs. The rate of the electron transfer process with specific (n,m) sem-SWNTs was depended on the relative redox potential of the SWNT in question and the redox potential of the enzyme, the latter of which could be tuned by the H_2 pressure above the solution.

An appreciable density of both mid-gap carriers and empty states suggest that met-SWNTs may be able to both donate and accept electrons from the H_2ase, essentially catalyzing H_2 production or uptake. However, met-SWNTs are not emissive, due to a finite density of states between the HOMO and LUMO levels and a very low exciton binding energy, meaning our initial PL study could not probe the electronic interactions between met-SWNTs and the H_2ase. Raman spectroscopy is a powerful tool for studying charge transfer reactions of both sem- and met-SWNTs. Resonance enhancement allows for the thorough analysis of vibrations specific to met- or sem- SWNTs, and even specific chiralities, through the careful selection of excitation wavelength. Charge transfer from or to the nanotubes can significantly affect the intensity, position, and shape of SWNT Raman peaks by modifying the resonance conditions and/or altering the electron density between C-C bonds. Raman spectroscopy is therefore an ideal addition to the PL studies of the SWNT/H_2ase complex, providing complimentary information for sem-SWNTs and enabling analysis of the non-emissive met-SWNTs.

Conclusions from PL and Raman data for sem-SWNT are consistent. In both cases sem-SWNT optical signals were effected only in presence of reduced H_2ase. We assumed in equilibrium conditions H_2ase redox potential is equal to the redox potential of H^+/H_2 redox couple, which can be calculated based on Nernst equation. For example, at pH 7, in presence of 4 % H_2, H_2ase potenitial is -0.4 V, which is more reducing than potential of sem-SWNT LUMO (Figure 5). Electron addition to the SWNT LUMO serves to quench PL as well as diminish the resonance Raman conditions for sem-SWNTs, attenuating the intensity of both tangential and radial vibrational modes. By purging the head space with Ar, concentration of H_2 is practically 0, and so as concentration of $[H_2ase]_{red}$ (Equation 1), therefore redox potential is shifted toward more positive and less reducing values (Figure 5) as evidenced by insignificant quenching of the PL and Raman (Figure 1,2) signals of sem-SWNTs. To calculate the limit for H_2ase redox potential in Ar atmosphere, we assumed following conditions: H2ase standard redox potential of -0.4 V [1], concentration of $[H_2ase]_{ox}$ equal to total enzyme

concentration and $[H_2ase]_{red}$ present as a single molecule. Under Ar highest oxidizing potential calculated is +0.8 V, which is significantly more positive than sem-SWNT LUMO and overlaps with empty mid-gap states (Figure 5).

In contrast to sem-SWNTs, met-SWNT electronic band structure is defined by both occupied and empty mid-gap electron states at the Fermi level. Calculated H_2ase potentials: -0.4 and +0.8 V overlap with energy of met-SWNT mid-gap states (Figure 5), allowing for either met-SWNT oxidation or reduction by H_2ase, which explains the effects on met-SWNT Raman signals (Figure 2,3). Because of the symmetry of the G^- electronic coupling, oxidation or reduction of met-SWNT results with G^- peak narrowing and shifting to the blue spectral region. Since both: electron donation or withdrawal leads to similar changes in the G^- mode, the Raman spectrum do not conclusively demonstrate direction of electron transfer between met-SWNT and H_2ase. For further proof, we must carefully analyze the O_2-inactivation kinetics.

Upon exposure to air (i.e. 21% O_2) all [FeFe] hydrogenases undergo inactivation. We have shown here that the inactivation rate depends on the enzyme's redox state (Figure 4). Also, upon exposure of SWNT-H_2ase complexes to O_2, the quenched and/or shifted SWNT Raman modes gradually return to the positions and intensities that correspond to enzyme-free samples. Interestingly, the two rate constants measured for O_2 inactivation of H_2ase are similar to the rate constants measured for the recovery of Raman signals (Table 1). This suggests that time-resolved Raman studies provide a window into the O_2 inactivation kinetics of SWNT-H_2ase complexes. The similar rate constants also suggest that the presence of the SWNT does little to change the O_2-inactivation mechanism.

Raman and PL signals from sem-SWNT effected in the presence of reduced H_2ase recovers in presence of O_2 with the rate corresponding to the rate of O_2-inactivation measured for oxidized H_2ase free in solution. Those results are consistent with our hypothesis that initially reduced H_2ase donates electrons to sem-SWNT LUMO and is in effect oxidized, while initially oxidized H_2ase does not engage in charge-transfer interaction with sem-SWNT and therefore does not change redox state.

The kinetics of recovery of both G-mode (data not presented) and RBM (Table 1) from met-SWNT's in the presence of H_2ase and exposed to O_2 is more complex with two rates being observed. Each rate corresponds to the rate measured for inactivation of oxidized or reduced H_2ase free in solution, again confirming that the observed O_2 effects on Raman signals are coupled to H_2ase inactivation mechanisms. Since both rates are observed, results suggest that H_2ase can be either reduced or oxidized when in complex with met-SWNT, contrary to only oxidized redox state observed in complex with sem-SWNT. For example, initially oxidized H_2ase can accept electrons from occupied mid-gap states of met-SWNT (Figure 5) and therefore show O_2-inactivation kinetic characteristic to reduced H_2ase free in solution.

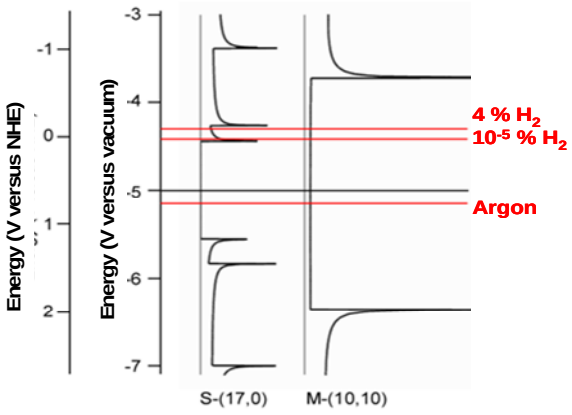

Figure 5: Density of states for a (17,0) sem-SWNT and a (10,10) met-SWNT plotted on an absolute energy scale, versus the normal hydrogen electrode (NHE) and versus vacuum. Red lines show the calculated H_2ase redox potentials

The work presented here suggests that purified met-SWNTs or sem-SWNTs, rather than mixed preparations, would be more suitable in the fabrication of biohybrid devices for specific application, to achieve greater control of the complex electronic properties. Since, met-SWNTs can be easily oxidized or reduced with small perturbations of the system Fermi level, they are more suited for applications that require a conductive molecular wire. For example in energy conversion systems, met-SWNT can serve to electronically couple H_2ase to electrode surface or to a light harvesting species. On the other side, sem-SWNTs are more suited for use in nanoscale sensors or photovoltaic devices, since their optical signals and conductivity are highly dependent on the electronic context.

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