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Short communication

Quantum dots as nano plug-in's for efficient NADH resonance energy routing

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ABSTRACT

The routing of fluorescent signals from NADH to quantum dots (QDs) has been a subject of extensive research for FRET based applications. In the present study, the spectral cross talk of NAD^+/NADH with QDs was used to monitor the reaction of NAD^+-dependent dehydrogenase enzyme. CdTe QD may undergo dipolar interaction with NADH as a result of broad spectral absorption due to multiple excitonic states resulting from quantum confinement effects. Thus, non-radiative energy transfer can take place from NADH to CdTe QD enhancing QDs fluorescence. Energy routing assay of NADH-QD was applied for detection of formaldehyde as a model analyte in the range 1000–0.01 ng/mL by the proposed technique. We observed proportionate quenching of CdTe QD fluorescence by NAD^+ and enhancement in the presence of NADH formed by various concentrations of enzyme (0.028–0.4 U). Hence, it was possible to detect formaldehyde in the range 1000 − 0.01 ng/mL with a limit of detection (LOD) at 0.01 ng/mL and regression coefficient $R^2 = 0.9982$. Therefore, a unique optical sensor was developed for the detection of the formaldehyde in sensitive level based on the above mechanism. This method can be used to follow the activity of NAD^+-dependent enzymes and detection of dehydrogenases in general.

1. Introduction

The routing of fluorescent signals from dehydrogenases, particularly from NADH, to quantum dots (QDs) has been a subject of extensive research for generation of ultra-sensitive biosensors. QDs are colloidal nano-crystalline semiconductors possessing unique spectral properties due to quantum confinement effects (Algar et al., 2010). Properties such as high emission quantum yield, sharp emission spectra, broad absorption spectra, photostability and tunable emission frequencies make them superior tool for QD-based biosensors (Costa-Fernández et al., 2006). In comparison to organic dyes QDs possess distinguishing spectral cross talk and thus can be selectively used for Forster resonance energy transfer (FRET) based applications (Ma and Su, 2011). Their size-tunable and narrow emission spectra can considerably reduce donor spectral leakage into the acceptor channel. At the same time, their broad absorption spectrum at wavelengths to the blue of their emission allows choice of excitation that corresponds to the acceptor absorption minimum, substantially reducing direct excitation (Vinayaka and Thakur, 2011; Sapsford et al., 2006). These unique properties of QDs suggest that the replacement of organic fluorophores in FRET studies with QDs could lead to an experimental setup, which would be ultra-sensitive, economical, and easy to configure, as well as provide the capability to make simultaneous measurements of different macromolecular systems. These features should allow QD-FRET based nano-sensors to generate a very distinct signal efficiently (Ron et al., 2008; Frasco and Nikos, 2009).

Despite progress on FRET phenomenon between NADH and QD interactions, practical applications involving CdTe QD-NAD^+ plugs have been not much envisaged. Little is known about how NAD^+ quenches QD fluorescence as well as enhancement. NAD^+ units have been reported to quench QD fluorescence presumably by electron transfer (ET) mechanism, whereas, NADH formed by enzymatic reaction will act as an energy donor which enhances QD fluorescence (Ronit and Itamar, 2009; Xiangling et al., 2010, Liuqing et al., 2011; Zhenzhen et al., 2011) as depicted in Scheme 1. NAD^+ acts as an electron acceptor and gets reduced to NADH during dehydrogenase reactions. Since NADH formed from enzymatic reaction is a fluorescent compound, CdTe QD can be used as a "plug-in" for NAD^+ co-factor which can possibly route the fluorescent energy from formed NADH and thus can be applied for a wide variety of assay. Plugging of CdTe QD to NAD can offer a great advantage in terms of sensitivity and specificity in dehydrogenase assays. Xiao et al. (2003), proposed gold nanoparticles as plug-ins for routing of electrons from redox enzymes to electrodes which proved to be highly efficient. In addition, gold nanoparticles

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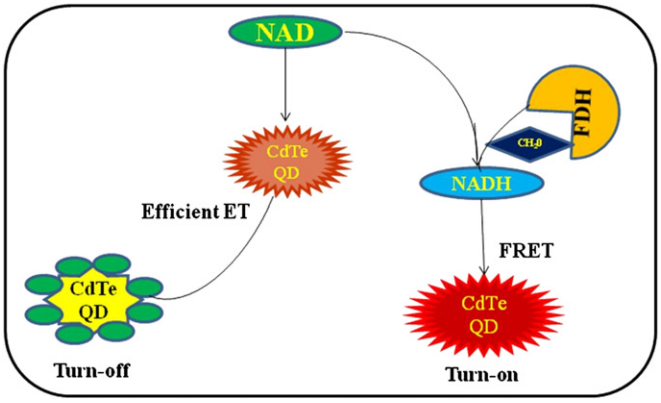

Scheme 1. NAD based "turn off" and NADH based "turn on" of QD fluorescence.

Table 1
Detection of formaldehyde by various reported methods.

State-of-art techniques	Detection limit	Reference
HPLC	0.2 μg/mL	Wu et al. (2003)
GC	0.24–16 μg/mL	Bianchi et al. (2007)
Fluorimetry	3 ng/mL	Helaleh et al. (2001)
Colorimetric methods	0.02–4 μg/mL	OSHA/NIOSHA (1989, 1994) manual
Flow injection systems	0.8 ng/mL	Piyanete et al. (2004)
Cataluminescence	0.1 μg/mL	Kaowen et al. (2006)
Enzymatic methods	10 μg/mL	Korpan et al. (2000)
NAD-QD plug in assay	1 pg/mL	Present studies

were used to develop optical/electrochemical sensors (Shlyahovsky et al., 2005; Zhang et al., 2011). Bahshi et al. (2009) proposed organic dye functionalized QDs for FRET based detection of NADH-dependent dehydrogenases. In contrast, the present work was aimed at investigating possibility of using CdTe QDs as plug-ins to route resonance energy from NADH and monitor NAD^+-dependent formaldehyde dehydrogenase.

Formaldehyde, a colorless flammable gas with a strong and pungent odor, is one of the most significant industrial hazards and air pollutants. Formaldehyde is reported to be carcinogenic and potent neurotoxic agent (IARC Monograph, 2006). Formaldehyde releasers are frequently added to consumable items that may become a reason of growth disorder, blindness, and respiratory diseases of a great number of living organisms at higher accumulated concentrations (IARC Monograph, 2006). Reports on formaldehyde detection on china wines, beers, noodles and many other consumable items have created a need for developing 'non-classical' techniques for rapid and sensitive detection. Classically, formaldehyde is detected by high-performance liquid chromatography (HPLC), colorimetric methods/spectrophotometric methods, gas sensors, nuclear magnetic resonance, etc., whereas potentiometric methods based on immobilized formaldehyde dehydrogenase/alcohol oxidase also been developed (Table 1). In addition chemiluminesce based detection systems are also reported (Akshath et al., 2012). Here in, we have investigated the possibility of using CdTe QDs units as Nano-plugs to route the resonance energy transfer from NADH thus making the assay sensitive and selective.

2. Experimental section

2.1. Materials and Instruments

Cadmium acetate [$Cd(CH_3COO)_2$], MPA, sodium borohydride ($NaBH_4$), tellurium, formaldehyde, formaldehyde dehydrogenase and NAD^+ were procured from Sigma Chemicals, St. Louis, USA. All reagents used were of analytical grade and acquired from standard suppliers. The following instruments were used: UV–Vis spectrophotometer (UV-1601, Shimadzu, Japan) for analysis of spectral changes during synthesis of CdTe QDs, Spectrofluorimeter (RF-5301 PC, Shimadzu, Japan) for photoluminescence measurements.

2.2. Synthesis of CdTe QDs

CdTe QD emitting at 571 nm was synthesized as reported by Li et al. (2007), with modification. In brief, 0.02 M [$Cd(CH_3COO)_2$] was dissolved in 25 mL of double distilled water and mixed with 0.05 M of MPA. The solution was degassed followed by adjusting the pH to 9.2. Sodium hydrogen telluride (NaHTe) was synthesized in a separate reaction by reacting 0.03 M of $NaBH_4$ and 0.01 M of Te in ice-cooled water. The metal dissolved leaving a faint pinkish-colored solution. NaHTe was added drop wise to the above synthesized solution until the solution turned orange. The solution was refluxed at 100 ± 2 °C for 180 min. Both absorption and emission spectra were recorded.

2.3. Enzymatic assay

Formaldehyde dehydrogenase assay was performed using optimized concentration of enzyme, substrate (formaldehyde) and co-factor (NAD^+). Formaldehyde concentration was kept constant at 1 μg/mL during optimization. Assay was performed in the presence of 0.12 U of FDH and 2.5 mM of NAD^+. NADH formation was monitored by recording fluorescence at 450 nm after incubation of reaction mixture for 10 min and at an excitation wavelength of 350 nm.

The effect of NAD^+ on fluorescence quenching of CdTe QD was studied. Various concentrations of NAD^+ (2.5 mM, 5 mM and 10 mM) were prepared in PBS of pH 7.5 and were mixed with CdTe QD separately followed by incubation for 10 min at room temperature. The fluorescence emission of CdTe QD was monitored by exciting CdTe QD at 350 nm both in the presence and the absence of NAD^+.

Experiments were conducted to study possible energy transfer from NADH to CdTe QD during enzymatic reaction. Various concentration of FDH (0.028 U, 0.05 U, 0.1 U, 0.2 U, 0.4 U) were prepared using PBS buffer (pH 7.5). Formaldehyde at 10 μg/mL concentration was used to study the effect of FDH concentration on energy transfer phenomenon. Initially 50 μl of CdTe QD having 0.1 absorption unit at 510 nm was reacted with 2.5 mM of NAD^+. Assay was performed in the presence of FDH of different unit activity in separate experiments in the presence of 2.5 mM of NAD^+ pretreated with CdTe QD. NADH formation was monitored in each instant by recording fluorescence at 450 nm for NADH and at 571 nm for CdTe QD after incubation of reaction mixture for 10 min at an excitation wavelength of 350 nm. Quenching of NADH fluorescence was monitored.

Energy transfer phenomenon between NADH and CdTe QD was applied to detect formaldehyde with optimized FDH and NAD^+ concentrations. Fluorescence spectra for both NADH and CdTe QD were recorded at respective wavelengths as mentioned above. During the assay formaldehyde was varied in the range 1000 ng/mL to 0.01 ng/mL. Standard graph was plotted with CdTe QD fluorescence obtained against respective formaldehyde concentration.

3. Results and discussion

3.1. Synthesis of CdTe QDs

CdTe QD emitting at 571 nm synthesized by aqueous method has shown convincing absorption and fluorescence properties with high quantum efficiency. In general, synthesis of colloidal