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Electron Donor Solvent Effects Provide Biosensing with Quantum Dots

Baikuntha P. Aryal and David E. Benson*

Department of Chemistry, Wayne State University, Detroit, Michigan 48202

Received September 14, 2006; E-mail: dbenson@chem.wayne.edu

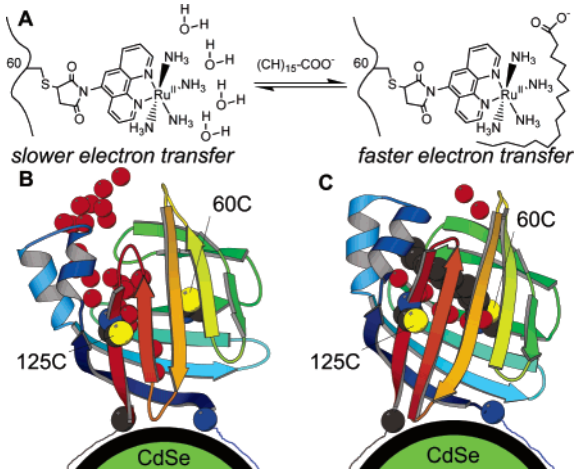
Semiconducting nanoparticles (quantum dots) have emerged as contrast agents for fluorescence microscopy with high quantum yields and photostability.[1] The use of semiconducting nanoparticles to detect small molecule and biomolecular analytes within cells is being pursued.[2] Biosensing of small molecule analytes is an important component of these methods. An electron transfer approach provides small molecule biosensing.[3] This electron transfer method couples ligand-dependent protein motions to electron donor movement relative to the nanoparticle surface to alter the CdSe[3a] and ZnS-coated CdSe (CdSe@ZnS) nanoparticle[3b] emission intensity. Energy transfer also provides a "reagentless" solution to small molecule biosensing,[4] but this approach still relies on organic fluorophores. While biosensors for up to 20 analytes could be detected using structural homologues of maltose binding protein (MBP)[5] from the initial maltose biosensor,[3] additional schemes for coupling ligand−protein binding events with the emission intensity of semiconducting nanoparticles are needed. Ligand-induced changes in the amount of solvent around protein-attached organic fluorophores are routinely used for biosensing.[5] Some electrochemical biosensors have also used changes in solvent occupancy around protein-attached metal complexes; altering the $E°(Ru^{3+/2+})$ of $[Ru(L)(NH_3)_4](PF_6)_2$ (**1**, L = 5-maleimido-1,10-phenanthroline).[6] Here, complex **1** is coupled to intestinal fatty acid binding protein (IFABP) that undergoes a large palmitate-dependent change in binding pocket solvent occupancy (Scheme 1), not conformation. This solvent occupancy effect provides a palmitate-dependent change in CdSe@ZnS nanoparticle emission intensity, which demonstrates that this electron transfer quenching method can be extended to thousands of protein−ligand pairs.

Changes in solvent occupancy have been used to detect fatty acid binding, including palmitate, to IFABP. The 600 Å^3 hydrophobic pocket of IFABP[7] allows a variety of compounds to be covalently attached to the binding site. While IFABP does not possess the typical ligand specificity of most proteins, it serves as a contrasting example to the previously reported MBP system.[3] Similar to IFABP, many proteins have buried ligand binding pockets (10−10000 Å^3) that undergo minimal or no conformation changes but bind ligands selectively.[8] Acrylodan-modified IFABP can detect a variety of fatty acids by emission intensity ratios and lifetimes.[9] Upon palmitate binding, the water occupancy of the ligand binding pocket is decreased,[7] changing the fluorophore emission properties. It is important to note that the polarity and size of the fluorophore attached to the fatty acid binding pocket will influence the fatty acid IFABP affinity.[8] While IFABP-based biosensors are not selective for specific fatty acids, detection of free fatty acids in the blood is needed to detect thrombotic ruptures that lead to heart attacks and strokes. Thus IFABP-based biosensors are significant.

We have generated **1**-modified IFABP fusion proteins attached to CdSe@ZnS nanoparticles. Plasmid DNA that encodes the IFABP fusion protein was generated with a metallothionein domain (COO^- terminal) and a polyhistidine domain (NH_3^+ terminal), termed His_6-IFABP-MT. The polyhistidine domain provides affinity purification, while both domains provide nanoparticle adhesion and minimize IFABP motions on the nanoparticle surface. Two His_6-IFABP-MT constructs were generated with either the V60C or K125C mutation. Cys60 is at the bottom of the fatty acid binding pocket and Cys125 is on the solvent-exposed surface (Scheme 1). Using $CdCl_2$ to reversibly protect the metallothionein Cys residues,[3c] **1** or Bodipy 577/618 maleimide (**2**, Invitrogen) was attached to Cys60/125 through a Michael addition of the Cys60/125 thiolate to the maleimide of **1** or **2**. Incubation of **1**- or **2**-modified His_6-IFABP-MTs with an equimolar (5 nM) amount of mercaptohexadecanoate (MHDA)-capped CdSe@ZnS nanoparticles showed saturated fluorescence responses after 1 h. For **2**-modified His_6-IFABP-MTs, the emission spectra after 1 h indicated energy transfer from the CdSe@ZnS nanoparticle to compound **2** (V60C, 25%; K125C, 23%).[10] The amount of quenching is higher than that observed with **2**-modified N282C MBP,[3c] indicating **1** and **2** are held close to the nanoparticle surface. Comparing the quenching efficiencies of **2**-modified His_6-IFABP-MTs and N282C MBP-MT to Cy3-modified D80C MBP-His_6[4] suggests that **1** and **2** are held around 20 Å from the CdSe@ZnS nanoparticle surface.[10]

Comparison of CdSe@ZnS nanoparticle emission intensity with unmodified and **1**-modified V60C His_6-IFABP-MTs showed decreased emission intensity (Figure 1A, 32% quench), similar to the MBP system (17−44%).[3b] A large emission intensity decrease was observed when **1**-modified K125C His_6-IFABP-MT was attached to CdSe@ZnS nanoparticles (Figure 1B, 58%), suggesting facile electron conduction through the last IFABP β-strand. The improved electronic coupling is consistent with α-helices (MBP) having decreased electron conduction relative to β-sheets.[11] Therefore, the saturated changes in CdSe@ZnS nanoparticle emission intensity

Scheme 1. Model System for Ligand-Induced Solvent Effects in Nanoparticle-Based Biosensing: (A) Palmitate-Altered Solvent Effects on **1**; (B) Apo-IFABP Structure;[8b] (C) Palmitate-Bound IFABP Structure[8a] (Cys, yellow; NH_3^+ terminus, blue; COO terminus, black ball; water, red; palmitate, black)



10.1021/ja066658f CCC: $33.50

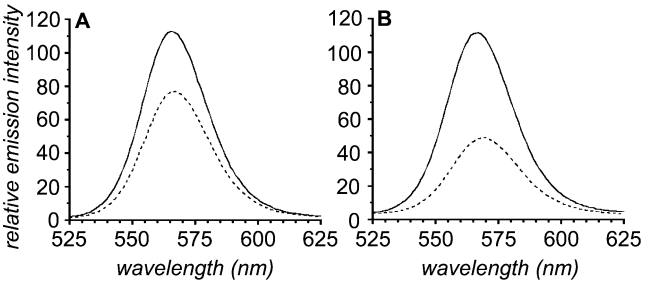


Figure 1. His6-IFABP-MT-mediated changes in CdSe@ZnS nanoparticle emission: **1**-modified proteins (dotted lines); unmodified proteins (solid lines); (A) V60C; (B) K125C. Spectra of **1**-modified proteins alone are overlapped by the *x*-axis.

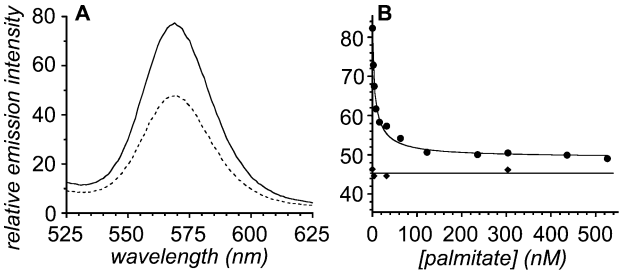


Figure 2. Palmitate-dependent emission intensity in **1**-modified His_6-IFABP-MT-attached CdSe@ZnS nanoparticles. (A) Emission spectra of the V60C protein before (solid line) and after (dashed line) sodium palmitate addition (520 nM). (B) The 565 nm emission intensity versus palmitate concentration: circles, V60C; diamonds, K125C. Lines indicate best fits (K125C, linear; V60C, $K_D = 5$ nM).

upon **1**- or **2**-modified His_6-IFABP-MT addition is consistent with His_6-IFABP-MT binding to the CdSe@ZnS nanoparticle.

Electron transfer quenching provides a method for palmitate-induced solvent effects to modulate CdSe@ZnS nanoparticle emission intensity. Palmitate titrations showed changes in the emission intensity for only **1**-modified V60C His_6-IFABP-MT (Figure 2). A 1.6-fold decrease (32−58% quenching) in CdSe@ZnS nanoparticle emission intensity was observed upon adding 500 nM palmitate. Emission intensity changes were fit to a binding isotherm,[3] which suggested a palmitate association constant of 2×10^8 M^{-1} ($K_D = 5$ nM). The acrylodan-IFABP association constant is 4.2×10^6 M^{-1},[8c] due to the decreased hydrophobic area of acrylodan. The palmitate-mediated effect with **1**-modified V60C His_6-IFABP-MT suggests palmitate excludes water from around **1** and increases the **1**−CdSe@ZnS nanoparticle electron transfer rate. The absolute change in emission intensity reported here is larger than the MBP-based systems,[3b] which are the only unimolecular systems that allow nanoparticle emission intensity to respond to small molecules.

The use of ligand-mediated changes in complex **1** solvation is a significant advance for developing small molecule biosensors with semiconducting nanoparticles. Before this observation, only proteins with large to moderate conformation changes could be considered for biosensor construction.[3,7a] Complex **1** provides this solvent sensitivity through hydrogen-bond-mediated changes in the electron density localized on the Ru^{2+} ion.[6,12] Other substitutionally inert transition metal complexes (d^6) with ammine[12b] or cyano[12c] ligands could also be employed for this strategy. From Marcus theory,[11] there are two potential reasons for this effect: an altered $E°(\mathbf{1}^{3+/2+})$ and/or an altered nuclear reorganization energy for **1**−CdSe@ZnS nanoparticle electron transfer ($\lambda_{\mathbf{1}-CdSe@ZnS}$). The relative contribution of each effect will need to be determined to improve the response of this system.[10] Both the conformationally driven MBP-based system[3] and this solvent exclusion IFABP-based system should be considered as limiting cases. Most protein−ligand binding events are comprised of a combination of binding pocket solvent exclusion[7b] and global conformation change.[7a] The similar responses of the MBP and IFABP-based biosensors suggest that such combinations of ligand-mediated conformation and solvation changes can be utilized for developing new members of this biosensor family. Thus, complex **1** must be within 20−30 Å of the nanoparticle surface to detect a combination of ligand-mediated changes in protein conformation and/or binding pocket solvation. So, this method expands the number of potentially detectable analytes from tens to thousands.

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Supporting Information Available: Sample preparations, emission spectra, and solvent-controlled electron transfer discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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