Photoelectroactivity of a Hybrid System Constructed by Immobilization of Avidin onto Biotinylated TiO_2 Electrodes

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Immobilization of avidin onto thin film nanocrystalline TiO_2 on ITO was obtained through avidin−biotin binding. In the construction of hybrid systems, dopamine was used as the TiO_2 surface-active ligand, providing a conductive lead to covalently linked biotin. The assembled hybrids were characterized using electrochemical techniques of chronopotentiometry, cyclic voltammetry, and photocurrent action spectroscopy. With each layer of a biomolecule (dopamine, biotin, and avidin) attached onto the TiO_2 film, an increase in overpotential (η) for the oxidation of ferrocyanide was detected by chronopotentiometric measurements. An increase in overpotential of $\Delta\eta \sim 400$ mV was measured for the monolayer of avidin bonded to biotinylated electrodes. The absorption of light by semiconductor nanocrystallites results in charge separation, holes being localized on avidin. The photoinduced charge separation and oxidation of avidin yields to the dissociation of the avidin−biotin complex, promoting changes in the photoelectroactivity of avidin-modified electrodes. The dissociation of this strong noncovalent complex was confirmed by measuring changes in chemiluminescence produced in a reaction of β-galactosidase-labeled avidin and 4-methylumbelliferyl-β-D-galactopyranoside.

Introduction

The design of functionally integrated hybrid systems for artificial photochemical energy conversion employs semiconductor nanoparticles for initial light-induced charge separation while biomolecules are used for subsequent chemical/electrical conversion. Assembling of nanoscopic objects into the arrays that promote collective properties in periodic structures and enable light-induced site-specific redox chemistry presents a major challenge in creating efficient hybrid photoconversion systems.[1] Recently, a hierarchical assembly of semiconductor nanoscale building blocks (CdS/ZnS,[2] TiO_2,[3] or CdTe[4]) have been achieved using proteins as scaffolding media. Taking advantage of the capability of avidin (protein) to strongly bind biotin molecules ($K = 10^{15}$ M^{-1}),[5] we have assembled TiO_2 nanorods in wire-like structures.[3] The ability of enediol ligands such as dopamine (DA) to restore the undercoordinated surface Ti sites of TiO_2 nanocrystallites[6] was exploited for the controlled, site-specific biotinylation of the nanorods.[7] The biotinylated TiO_2 rods were then assembled through avidin into wire-like structures. Using low-temperature electron paramagnetic resonance (EPR) spectroscopy, we have shown that the photoexcitation of wire-like architecture composed of TiO_2−dopamine−biotin−avidin hybrids (TiO_2/DA−BN−Av) results in extended charge separation: the photogenerated electrons localize at TiO_2 rods, while the holes localize at protein.[3] The spatial separation of charges in this hybrid system is a prerequisite for successful charge-transfer induced chemistry on protein. The low-temperature (4 K) EPR experiments also indicated that localization of holes on avidin resulted in the formation of a tyrosine cation radical, an intermediary in redox chemical reactions on attached avidin. The high affinity of the avidin−biotin system is due to multiple hydrogen bonds that involve oxygen from a ureido group of biotin and, among other amino acids, tyrosine 33 of avidin.[8] It has been shown that tyrosine and tryptophan are two amino acids in avidin prone to oxidation, tyrosine having a more negative redox potential, being easier to oxidize.[9,10] Thus, localization of holes on tyrosine 33 would affect the redox potential of avidin and change its chemical/redox properties. The question now arises as to what are the consequences of photoinduced redox chemistry in these hybrid systems. Specifically, we want to address two potential outcomes resulting from accumulation of charges in avidin: the capability to manipulate its binding properties or the possibility of discharging its capacitance. Avidin is a nonconductive protein; the binding of a monolayer film of avidin onto a biotinylated surface results in the increase of surface potential of ~400 mV.[11] It has been shown that when adsorbed on electrode surfaces, avidin

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prevents the transfer of charges.[12] Similarly, streptavidin creates a high tunneling barrier between CdTe nanowires, preventing a current through cross points with the positive bias up to 10 V.[4]

In this paper we are reporting on the photoelectrochemistry of the avidin−biotin complex conjugated onto thin film nanocrystalline TiO_2 electrodes. Combining different electrochemical methods (chronopotentiometry, cyclic voltammetry, and photocurrent action spectroscopy) with the direct observation/determination of bound avidin on biotinylated TiO_2 electrodes, we have addressed the question of photoinduced redox chemistry at room temperature in these hybrid systems. For these studies, we have employed ∼200 nm thick TiO_2 film on indium tin oxide (ITO), and different types of avidin, from native to redox- or enzyme-labeled avidin.

Experimental Section

Materials. Colloidal solutions of TiO_2 particles (diameter 45 Å) were prepared by hydrolysis of $TiCl_4$, as previously described,[13] and used for producing TiO_2 films. ITO was used as transparent back contact for TiO_2 films. Glass slides with a conductive side of ITO ($R_s = 8-12\ \Omega$) from Delta Technologies were cleaned by 30 min of sonication in a series of baths: 1:1 acetone/methanol, detergent, 20% sulfuric acid, 0.2 M NaOH in 1:1 water/methanol, and water. Nanoparticulate films of TiO_2 were prepared by dip-coating method. The clean ITO slides were dipped into 0.12 M TiO_2 solution three times with drying in an oven at 150 °C for 30 min after each deposition. The resulting TiO_2 films were ∼200 nm thick (Supplement 1, Supporting Information). The surface of the electrode was ∼1.0 cm^2. Details on characterization of TiO_2/ITO and dopamine-modified TiO_2/ITO electrodes are published elsewhere.[14]

Redox-labeled avidin with ferrocene (AvFc) was synthesized attaching the *N*-(ferrocenylmethyl)-6-aminocaproic acid (Fc6ac) to avidin. Fc6ac was synthesized according to Padeste et al.[15] and characterized using cyclic voltammetry. The conjugation of Fc6ac to avidin was performed by binding carboxyl groups of ferrocene derivative to amino groups on lysine residues in protein. The activation of a carboxyl group of Fc6ac was performed as follows: 0.5 mL of 50 mM Fc6ac in DMF was mixed with 150 mM of *N*-hydroxy-succinimide (Aldrich) and 22 μL of *N*-(3-dimethylaminopropyl) *N*′-ethyl-carbodiimide hydrochloride (Fluka) and heated at 80 °C for 2 h under a nitrogen atmosphere. The procedure results in the attachment of the succinimide group to the ferrocene derivative. After cooling, 100 μL of the succinimide-activated ferrocene solution was added to 1 mL of 4 mg of avidin in 100 mM phosphate buffer, pH 8.5, in portions of 10 μL in intervals of 1 min. After shaking overnight at room temperature, the solution was separated from precipitates by centrifugation and extensively dialyzed against 50 mM phosphate buffer, pH 7.5. The maximum number of ferrocene derivatives attached to one avidin equals 16, the number of lysine residues.[5,16] The capability of ferrocene-labeled avidin to bind biotin was preserved, as tested by HABA (4-hydroxyazobenzene-2-carboxylic acid) assay. Native avidin and enzyme-labeled avidin, avidin-β-galactosidase conjugate (AvGal), were purchased from Sigma. All other reagents were analytical grade and used as received, without further purification. Milli-Q deionized water (resistivity 18.2 MΩ cm^{-1}) was used for synthesis and in all experiments.

Immobilization of Avidin onto TiO_2/ITO Electrodes. The steps taken in immobilization of avidin onto TiO_2 films by way of avidin−biotin binding are presented in Scheme 1. In the first step, attachment of dopamine to nanoparticulate TiO_2 films was obtained. TiO_2/ITO slides (∼2 cm × 0.5 cm) were dipped in 5 mM dopamine solution for 3 h in an inert atmosphere and afterward washed with 50 mM phosphate buffer, pH 7.5. In the next step, the condensation reaction between the succinimide group on the biotin derivative and the amino group on dopamine was exploited to covalently link biotin to dopamine.[17] The slides were placed in 18 mM biotin *N*-hydroxysuccinimide ester solution in DMF/phosphate buffer solution (1:1) for an additional 6 h, resulting in the formation of biotinylated TiO_2 electrodes (TiO_2/DA-BN). After washing, electrodes were placed in a solution of glycidil isopropyl ether in order to prevent undesirable nonspecific binding of protein. Finally, in the last step, 30 μL of solution of either avidin (Av) or labeled avidin (AvFc and AvGal), 4 mg/mL of protein, was placed on a flat slide and incubated for an hour in the nitrogen box. After incubation, the slides were thoroughly washed with 50 mM phosphate buffer, pH 7.5, by soaking them in 100 mL of buffer solution and leaving for a few hours, the procedure being repeated three times. Due to the comparable sizes of TiO_2 particles in film and avidin on a surface, ∼5 nm for both, the maximum coverage of protein is a monolayer.

Apparatus. All electrochemical measurements were performed with three-component systems containing modified TiO_2/ITO as working, Pt as a counter, and Ag/AgCl as a reference electrode in a single-compartment 10 mL quartz cell. For cyclic voltammetry (CV) and spectroelectrochemical measurements a BAS-100B/W (Bioanalytical Systems) workstation was used. Constant-current chronopotentiometry (CP) measurements were performed using a potentiostat/galvanostat (EG&G Princeton Applied Research, model 273). In all systems a 50 mM phosphate buffer, pH 6.8, as a background electrolyte solution was used. For different experiments/measurements, hydroquinone or ferro/ferricyanide was used as the redox relays. All measurements were performed in deaerated solutions, and measured values were within 10% error.

The white light source was a 300 W xenon arc lamp (ILC). Monochromatic light was provided through a Jobin-Yvon grating monochromator. The power density of the incident light was measured using a calibrated silicon diode detector (Ophir Optronics); a value of 0.05 mW/cm^2 at 500 nm wavelength was measured at the position of electrodes.

Results and Discussion

In constructing hybrid systems on TiO_2 electrodes we used dopamine (DA) as a surface-active ligand and conductive lead between semiconductor particles and biotin. Enediol ligands such as dopamine have a large affinity for undercoordinated Ti surface sites; the OH groups on dopamine form an irreversible bidentate complex with surface titanium atoms.[6,18] Additionally, localized orbitals of surface-attached dopamine are electronically coupled with the delocalized

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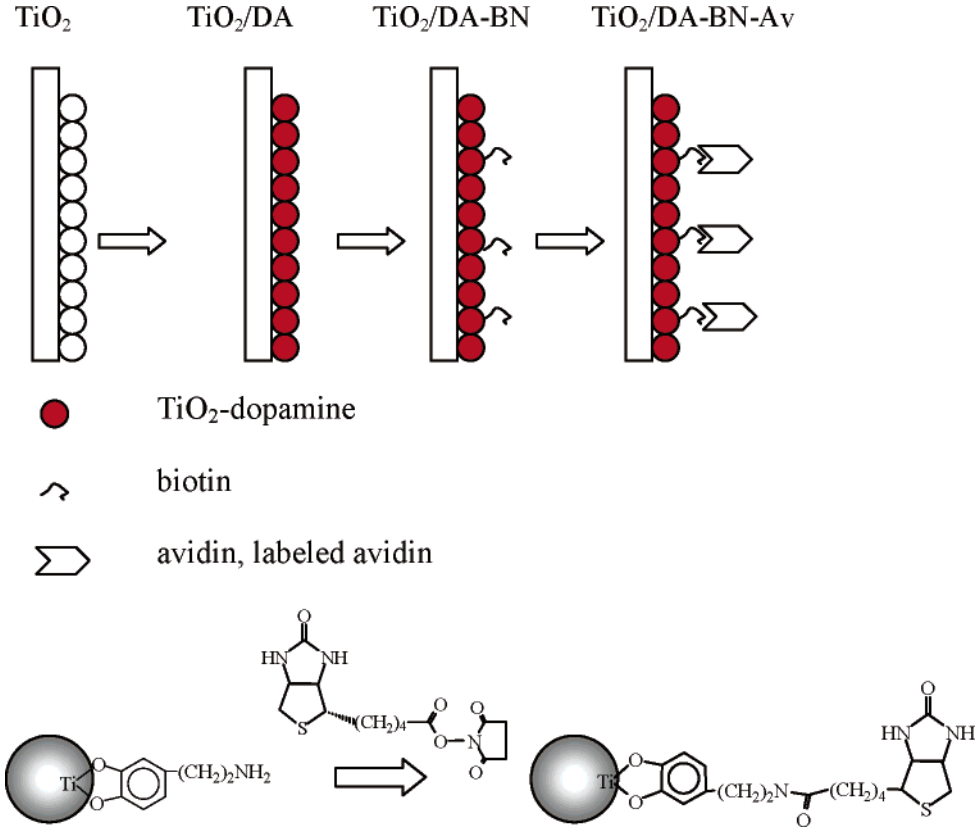
Scheme 1. Salient Presentation of the Steps Carried out in Immobilizing Avidin on Nanocrystalline TiO_2/ITO Electrodes



electron levels from the conduction band of a TiO_2 semiconductor forming a ligand-to-particle charge-transfer complex (TiO_2/DA). This results in the shift of the onset of absorption toward longer wavelengths.[6] By covalently linking the valeric acid chain of biotin to the amino group of dopamine, we have obtained biotinylated electrodes (TiO_2/DA-BN) with exposed ureido oxygen capable of attaching avidin (Scheme 1). The successfulness of making TiO_2−dopamine−biotin−avidin (TiO_2/DA−BN−Av) hybrids was tested, and modified electrodes were characterized by performing electrochemical measurements such as chronopotentiometry (CP), cyclic voltammetry (CV), and photocurrent action spectroscopy.

Chronopotentiometry. As was shown by Willner and coworkers for gold electrodes,[19] chronopotentiometric measurements can be used to demonstrate the attachment of biomolecules onto the surface of the electrode. In chronopotentiometry, and in the presence of a redox couple in solution, constant current is applied between the counter (Pt) and working electrodes (modified ITO/TiO_2), and the potential of the working electrode (measured with respect to the reference electrode, Ag/AgCl) is monitored. The basis of these controlled current experiments is that a redox (electron transfer) reaction must occur at the surface of the working electrode in order to support the applied current. The presence of a nonconductive/insulator layer on the working electrode suppresses the interfacial electron-transfer rate. Thus, an overpotential on the working electrode necessary for the oxidation/reduction of redox couple in solution under constant current provides information on the properties of the electrode surface.[19,20] The relation between overpotential (η) and electrode resistance (R) under constant current (I) is given by eq 1.[19a] The resistance of the modified electrode is thus a sum of the resistance of the unmodified semiconductor (R_{SC}) and variable resistance induced by the attached biomolecule (R_{biomol}).

$$R = \eta/I = R_{SC} + R_{biomol} \quad (1)$$

We have tested stepwise assembly of the layers of biomolecules (dopamine, biotin, and avidin) onto TiO_2 films measuring the chronopotentiometric signals after each step of the electrode modification by applying constant anodic current in a solution of ferro/ferricyanide. Figure 1 presents potential changes upon the application of 10 μA current for 10 s ($E-t$ curves) for three electrodes with a succession of attached biomolecules: dopamine-modified (TiO_2/DA), biotinylated (TiO_2/DA−BN), and avidin-modified (TiO_2/DA−BN−Av). The measurements were obtained in deaerated phosphate buffer solution containing 10 mM $[Fe(CN)_6]^{3-/4-}$ ($E^\circ = 0.315$ V vs Ag/AgCl). The potential required for the oxidation of $[Fe(CN)_6]^{4-}$ at the surface of TiO_2/DA electrode, ~250 mV, was the same as that for bare TiO_2; the TiO_2−dopamine complex did not affect the $E-t$ curve. However, with each additional adjacent biomolecule at the surface of the electrode the overpotential increases, Figure 1. The

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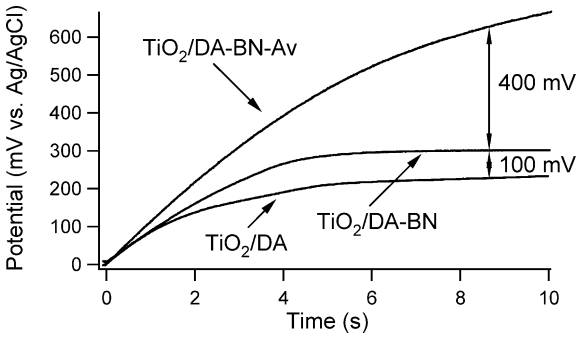

Figure 1. Potential−time ($E-t$) profiles of modified TiO_2 thin film electrodes measured at applied constant anodic current of 10 μA in a deaerated solution containing 10 mM $[Fe(CN)_6]^{3-/4-}$ in 50 mM phosphate buffer, pH 6.8.

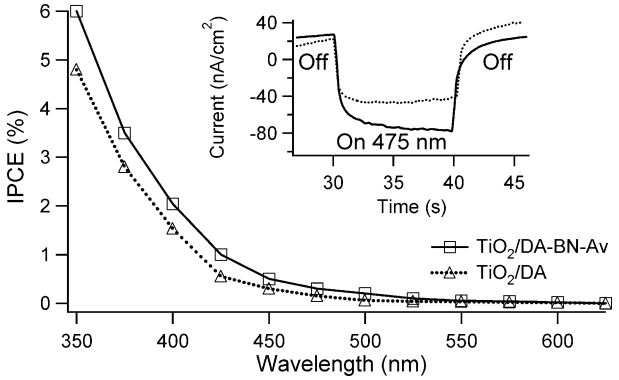

Figure 2. Photocurrent action spectra of avidin-modified (TiO_2/DA−BN−Av) and dopamine-modified (TiO_2/DA) electrodes measured in deaerated 5 mM hydroquinone solution of 50 mM phosphate buffer. Inset: Currents during an illumination cycle with 475 nm light.

conjugation of biotin to dopamine increases the overpotential for $\Delta\eta \sim 100$ mV, while consecutive associations of avidin to a biotinylated electrode yield $\Delta\eta \sim 400$ mV. The value of the increased overpotential for a monolayer of avidin corresponds to the increase in resistance of $\Delta R = 40$ kΩ. The increase in overpotential demonstrates inhibition of the electron-transfer reaction due to the "screening" of the electrode's surface with each layer of biomolecules, revealing successful hierarchical assembly.

Photocurrent Action Spectra. Absorption of light by the TiO_2/DA charge-transfer complex yields the excitation of electrons from the chelating ligand directly into the conduction band of TiO_2 nanocrystallites, resulting in a red shift of the onset of absorption as compared to that of bare TiO_2.[6,14] Thus, measuring the dependence of photocurrent on the wavelength of absorbed light can give information on the preservation and efficiency of the TiO_2/DA charge-transfer complex in avidin-modified electrodes. Figure 2 presents the photocurrent action spectra of TiO_2/DA−BN−Av and TiO_2/DA electrodes measured during light−dark cycles in deaerated 5 mM hydroquinone, 50 mM phosphate buffer solution. The current was acquired as square-shaped signals during the light cycles (in duration of 20−30 s) at different wavelengths, Figure 2, inset. Several repeated measurements of dark−light cycles did not affect the photocurrent signals. The incident photon to current conversion efficiency (IPCE), defined as the number of electrons collected per incident photon, was evaluated from photocurrent (I_{SC}) measurements at different wavelengths (λ), and using eq 2,

$$\text{IPCE\%} = \frac{1240 \times I_{SC}(\text{A/cm}^2)}{\lambda(\text{nm}) \times I_{inc}(\text{W/cm}^2)} \times 100 \qquad (2)$$

where I_{inc} is the incident light power for each wavelength λ. The spectrum of TiO_2/DA−BN−Av electrodes shows the same offset of absorption (~650 nm) as TiO_2/DA electrodes, confirming excitation of electrons from dopamine to the conduction band of TiO_2 particles upon absorption of light.[3,6,14,18] The low values for IPCE compared to literature values for TiO_2 films[21] are due to very thin (~200 nm) low-porosity TiO_2 films used in our experiments. The fast rise of the photocurrent, as shown in the inset of Figure 2, for TiO_2/DA and TiO_2/DA−BN−Av electrodes (detection limit of the instrument is 50 ms) indicates efficient charge separation in these hybrids. Unexpectedly, the higher current was observed for the avidin-modified electrode compared to TiO_2/DA (or TiO_2/DA−BN electrode which exhibits the same photocurrent spectrum as dopamine-modified). Various sources could contribute to this effect. The protein layer can affect photon reflectance, having a different roughness than nanocrystallite film;[22] the space charge layer of TiO_2 electrode could be affected by bound protein, increasing the efficiency for collection of electrons on ITO, and finally, the amino acid(s) of bound avidin could act as traps for photogenerated holes, decreasing electron−hole recombination and increasing the photocurrent. If extended charge separation occurs at room temperature, i.e., if holes localize on avidin, as we have shown with EPR at 4 K,[3] than tyrosine (Tyr) and tryptophan (Trp) are two amino acids most likely to be oxidized in the protein.[23−25]

Cyclic Voltammetry. Applying potentials to semiconductor nanocrystalline electrodes below their conduction band and examining spectroscopic or current response can be used for detection and characterization of surface trapping sites.[11,26] We used CV to examine the possibility of avidin to act as a surface trap for positive charge, which can lead to consequent oxidation of Tyr and Trp. Figure 3 presents voltammograms of avidin-modified electrodes. The low sensitivity was obtained when native avidin was bound to the electrode, TiO_2/DA−BN−Av (Figure 3, black curve). The broad unresolved peak at ~0.75 V indicates the irreversible oxidation of avidin.[10,23] Because the majority of electroactive groups (Tyr and Trp) of avidin are buried inside the macromolecule and are not readily accessible for interaction with the electrode, attaching an electroactive redox marker on protein greatly enhances electrical contact between an electrode and the protein.[15,27−29] Thus, a better sensitivity

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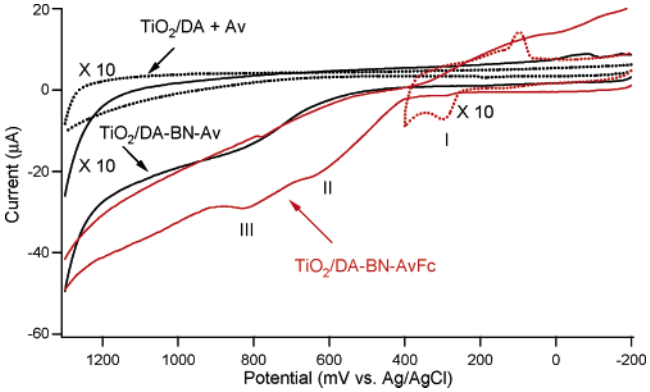

Figure 3. Cyclic voltammograms of avidin-modified electrodes. Black line (TiO_2/DA−BN−Av) corresponds to native avidin, while the red line (TiO_2/DA−BN−AvFc) to ferrocene-labeled avidin. Scan rate 10 mV/s. For comparison a cyclic voltammogram of TiO_2/DA electrode in the presence of 4 mg/mL avidin in solution is also presented (dotted black line). Red dotted line presents enlarged voltammogram for the TiO_2/DA−BN−AvFc electrode measured between −200 and 400 mV.

was achieved using ferrocene conjugated avidin, AvFc. The presence of long-chain ferrocene as a redox relay attached to avidin enables electron hopping, therefore enhancing current response. The two oxidation signals corresponding to Tyr and Trp residues (peaks II and III, Figure 3, red curve) are distinguished.[30] The oxidation potentials, 0.6 V for Tyr and 0.8 for Trp, agree with the values obtained for the avidin−biotin complex adsorbed on the carbon electrode and measured by square-wave voltammetry.[10] In addition, the presence of ferrocene moiety attached to the surface of the electrode via avidin is visible as peaks in both oxidation (peak I) and reduction waves (Figure 3, red dotted line). The observed large difference between oxidation and reduction peaks of ferrocene are indicative of partial coverage of active sites on a microscopic scale.[31] For comparison, a voltammogram for the TiO_2/DA electrode in the presence of 4 mg/mL avidin in solution is also presented in Figure 3 (dotted black curve). Because of the low diffusion coefficient of avidin (macromolecule), no oxidation signals due to Tyr and Trp can be observed, confirming that avidin acts as a trap for positive charges only when it is immobilized on the surface of electrodes.

Photoinduced Changes. As demonstrated by photocurrent measurements, Figure 2, absorption of light by the TiO_2−dopamine charge-transfer complex results in the formation of electrons and holes in the TiO_2/DA−BN−Av hybrid. In the absence of a redox couple in solution that can react with photogenerated charges, and under the open circuit condition, the charges may recombine or may induce chemical reactions within the hybrid. We illuminated modified electrodes with white light for prolonged periods of time (300 W Xe lamp, cut-off filter 355 nm, for 10 min) in order to examine the fate of charge residues formed in the hybrid upon illumination. The electrodes were placed in phosphate buffer solution

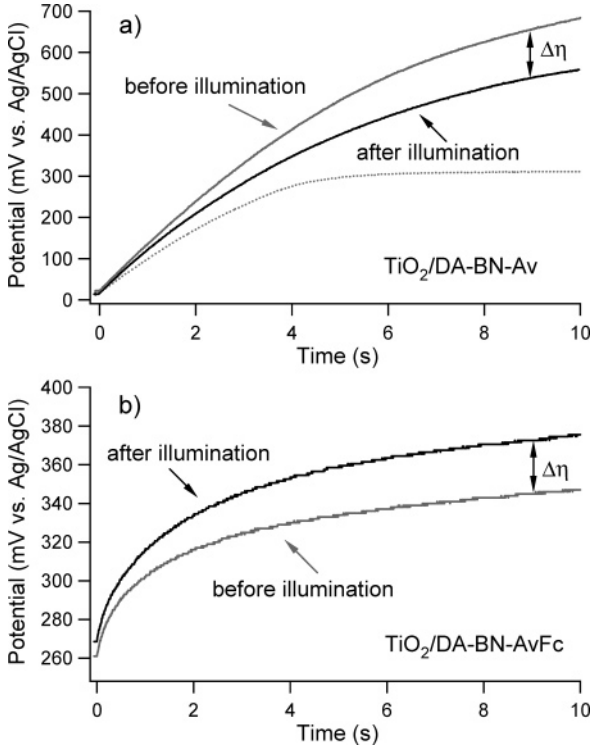

Figure 4. Potential−time profiles of avidin-modified TiO_2 electrodes measured before and after open circuit illumination with white light (350 nm cut-off filter). The 10 μA anodic current was applied for 10 s in a solution containing 10 mM $[Fe(CN)_6]^{3-/4-}$ in 50 mM phosphate buffer. (a) Data for native avidin and (b) for ferrocene-labeled avidin. For comparison, figure contains the chronopotentiometric signal of biotinylated electrode (TiO_2/DA−BN), dotted line.

which was exposed to air, and open circuit conditions were applied during illumination. The oxygen acted as a scavenger of photogenerated electrons, allowing accumulation of holes on avidin. After illumination the electrodes were placed in $[Fe(CN)_6]^{3-/4-}$ phosphate buffer solution and subjected to CP measurements; the conditions for CP measurements were the same as described previously. The $E-t$ curves obtained for illuminated electrodes as compared to the curves obtained for the same electrodes before illumination are shown in Figure 4. Illuminating TiO_2/DA and TiO_2/DA−BN electrodes did not affect their potential−time profiles. However, as can be seen from Figure 4a, in the case of TiO_2/DA−BN−Av the decrease in the overall overpotential/resistance for the oxidation of ferrocyanide toward the value for TiO_2/DA−BN was observed after illumination, indicating that permanent changes occur as a result of photoinduced redox chemistry on avidin. These changes do not suggest discharging; rather they indicate partial removal of avidin from the electrodes as a result of the charging of avidin via oxidation of Tyr and Trp. The same experiments were performed with TiO_2/DA−BN−AvFc electrodes, using ferrocene-labeled avidin instead of native protein, Figure 4b. As can be seen from the $E-t$ curve for this hybrid system before illumination, the attachment of an electroactive moiety at the surface of the electrode results in lowering overpotential for the oxidation of ferrocyanide as compared to the TiO_2/DA−BN electrode, 100 and 300 mV, respectively. When the redox moiety (in the form of AvFc) is removed from the electrode surface to the bulk of the solution, as a result of photoinduced

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charge transfer, the decrease in efficiency of interfacial electron-transfer reactions increases the overall resistance, Figure 4b, black line. Note that the initial potential of TiO_2/DA−BN−AvFc is shifted to the oxidation potential of ferrocene. The partial removal of avidin from electrodes upon illumination results also in the decrease in photocurrent signals (Supplement 2, Supporting Information).

Finally, removal of avidin (in the form of avidin-β-galactosidase, AvGal) from the modified TiO_2 electrode under illumination was established directly detecting chemiluminescence. The reaction of an enzyme β-galactosidase with 4-methylumbelliferyl-β-D-galactopyranoside (MUG) to produce highly fluorescent methylumbelliferone[32] was exploited in the determination not only of the desorption of avidin but also of the bond cleavage: avidin−biotin vs dopamine−biotin bond. Two electrodes modified with AvGal were placed in separate vials and immersed each in 3 mL of 50 mM phosphate buffer; one electrode was subjected to illumination and another was taken as a control. After illumination, electrodes were removed from the vial and 25 μL of 10 mM MUG (in excess of initial concentration of AvGal) was added on each electrode. After incubation for 30 min the fluorescence was observed under UV light, Figure 5. The intensity of luminescence directly depends on the amount of AvGal, confirming the desorption of avidin from the TiO_2/DA−BN−AvGal hybrid upon illumination. In addition, we have tested the solutions where electrodes were immersed for the presence of AvGal. At certain intervals during illumination, portions of solution (300 μL) were taken and mixed with 50 μL of 10 mM MUG. The measured fluorescence spectra ($\lambda_{exc} = 365$ nm) correspond to methylumbelliferone[32] and are presented in Figure 5c. As can be seen from Figure 5c, the increase of AvGal in solution correlates to its dissociation from hybrid. Similar experiments were performed on TiO_2/DA−BN electrodes to examine if the biotin is accessible for binding avidin after illumination. No difference in fluorescence was observed between illuminated and nonilluminated electrodes upon addition of AvGal and subsequent development of chemiluminescence with MUG (Supplement 3, Supporting Information). Although qualitative, these results have verified the dissociation of the avidin−biotin complex as a result of light-induced charge separation in hybrid systems. To dissociate strong noncovalent interaction between (strept)avidin and biotin, different strategies, including high temperatures and treatment with formamide, or modifications of either protein or biotin have been suggested.[33−35] Only recently a chemical/thermodynamic treatment was proposed for the separation of streptavidin and biotin without denaturing protein tetramer, thus enabling reversibility of binding.[36] The effect of light-induced redox chemistry on the structure−function relationship of avidin in hybrid systems was beyond the scope of this work. The studies to determine if the protein's structure is preserved are underway.

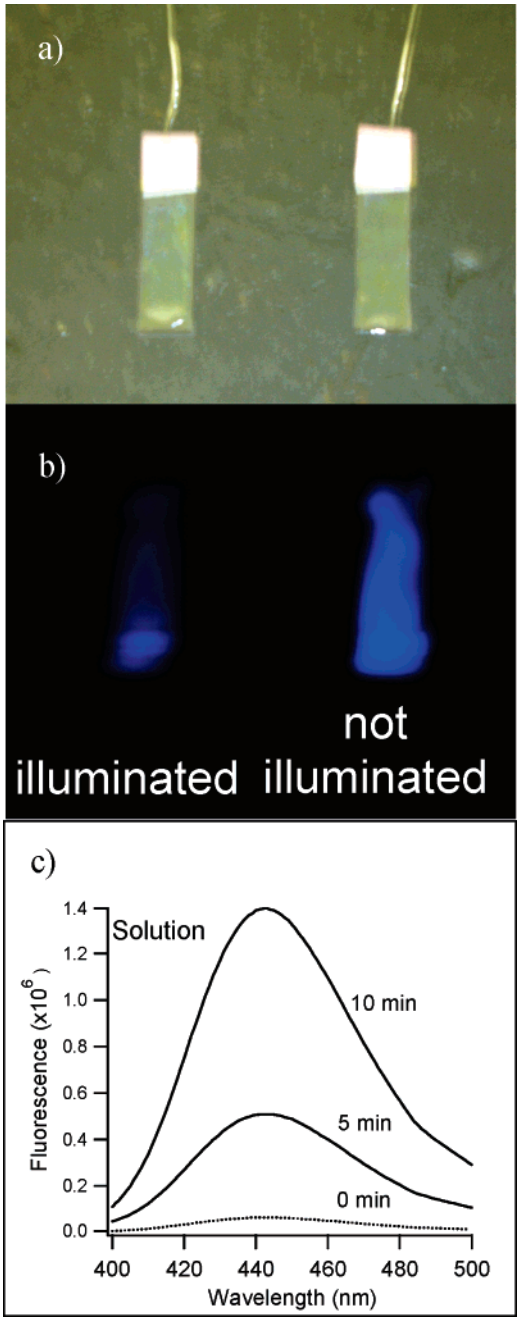

Figure 5. Photographs of modified TiO_2/DA−BN−AvGal electrodes on which 25 μL of 10 mM MUG was added, taken under (a) visible and (b) UV light. The photographs show luminescence due to methylumbelliferone which is a product of chemical reaction between AvGal and MUG. (c) Fluorescence due to the presence of AvGal in a solution in which the electrode was dipped during illumination with white light (cut-off filter 355 nm), showing an increase in fluorescence intensity with the time of illumination.

Conclusion

The possibility to manipulate properties of biomolecules attached onto semiconductor particles opens a new route in the design of solar-based devices. In optimally designed hybrid systems, semiconductor particles, such as dopamine-modified TiO_2, act as light-harvesting material, and photogenerated charges can produce site-specific chemistries in

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biomolecules. We have shown that site-specific oxidation of avidin in TiO_2−dopamine−biotin−avidin hybrids alters the proteins' binding properties, causing dissociation of the avidin−biotin complex, probably due to changes in the chemical/thermodynamic potential of avidin. The changes in avidin's binding properties is a step toward artificial manipulation of enzymatic catalytic reactions, which are characterized by the changes in noncovalent binding.

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Supporting Information Available: Estimation of TiO_2 film thickness from transmittance and morphology of TiO_2 film. This material is available free of charge via the Internet at http://pubs.acs.org.

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