Formation of Biofunctional Thin Films on Gold Electrodes by Electrodeposition of Poly(acrylamide-*co*-tyrosineamide)

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ABSTRACT: A new method was developed for modifying gold electrode surfaces through electrochemically triggered adsorption of acrylamide copolymers containing a low percentage of tyrosineamide side chains. The amount of copolymer adsorbed, as monitored by surface plasmon resonance (SPR), was proportional to the percent of tyrosineamide side chains in the polymer over the range 0−3 mol %. The modified gold surfaces were hydrophilic and resisted nonspecific adsorption of green fluorescent protein (GFP). Incorporation of nickel-binding nitrilotriacetic acid (NTA) side chains into the tyrosineamide copolymers allowed specific immobilization of His_6-tagged GFP. The Ni(II)-dependent GFP binding was measured by SPR and verified by fluorescence microscopy. The method may find utility as a means to electrically address the immobilization of unique ligands in biosensors or other diagnostic devices based on arrayed ligands.

Introduction

Controlling molecular interactions at the interface between synthetic materials and biomacromolecules, especially proteins, remains a significant obstacle during the development of medical or diagnostic devices that come into contact with biological fluids. The first challenge is to prevent nonspecific surface adsorption and denaturation of proteins, the initial step on the path to a foreign body response leading ultimately to encapsulation and device failure.[1] The second challenge, in some cases, is to promote specific molecular adhesion on top of the low-binding interface to add biofunctionality to the surface.[2,3] The low-binding surface preserves the structure and function of immobilized macromolecules and prevents nonspecific background binding.

Numerous approaches have been developed to modify solid−liquid interfaces by chemical or physical grafting of polymers to surfaces. A convenient means to modify the surface of conducting materials is through the electropolymerization of thin polymeric films from solutions of monomers.[4,5] Interest in this approach has been driven by several factors: polymerization is limited to the surface of the electrode, the film thicknesses can be readily controlled, films are uniform and reproducible, a wide range of suitable monomers are available, and electrodes of any scale and with complex geometries can be modified. Although electropolymerized films have been applied predominantly in electronic devices, there are examples of biological functionalities being added to electrode surfaces by electropolymerization. For example, redox enzymes have been entrapped near electrode surfaces in amperometric biosensors by electropolymerization of films under mild conditions that preserve enzyme function.[5,6] Electropolymerized films also have been explored as a means to add biorecognition capability to electrode surfaces for specific immobilization of proteins.[7]

Phenol and phenolic derivatives, including the amino acid tyrosine, are a category of electropolymerizable monomers that has been extensively investigated.[8−10] Application of a moderate (∼0.6 V vs Ag/AgCl) oxidizing potential to an aqueous solution of phenol results in the formation of a thin (50−100 nm), hydrophobic, nonconducting film on the electrode surface.[11,12] The mechanism is not completely understood, but the films adhere strongly to gold, platinum, and platinum oxide electrode surfaces.[9,13] We have exploited the affinity of electrochemically oxidized tyrosine for metal surfaces to develop a novel method of biofunctional film formation on electrodes. The method is based on the voltage-dependent deposition of preformed acrylamide copolymers containing a low percentage of tyrosineamide side chains. The cotyrosineamide films adhered strongly to gold, were hydrophilic due to the polyacrylamide backbone, and resisted nonspecific adsorption of green fluorescent protein (GFP). Ligands for specific immobilization of biomacromolecules onto the low-binding surface can be copolymerized into the acrylamide polymers. To demonstrate, nitrilotriacetic acid (NTA) side chains incorporated into the copolymer allowed specific immobilization of His-tagged GFP.

Experimental Section

Materials. Tyrosineamide and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO). The remaining chemicals and solvents were purchased from Acros (Geel, Belgium) and used as received unless otherwise noted.

Monomer Synthesis. The methacrylated NTA group, 2-(methacrylamidobutyl)nitrilotriacetic acid (MABNTA), was synthesized according to published procedures.[14] The tyrosineamide monomer (*N*-methacryloyltyrosineamide) was synthesized by dissolving *N*-hydroxysuccinimide (2.31 g, 20 mmol) in 60 mL of THF, and the pH was adjusted to ∼8 with *N*,*N*′-diisopropylethylamine (DIPEA). This solution was cooled to between −5 and 0 °C, and while stirring, distilled methacryloyl chloride (1.9 mL, 19 mmol) was added dropwise. After addition, the mixture was stirred at room temperature for 1 h. The reaction was again cooled on ice, and tyrosineamide (3.6 g, 20 mmol) in DMF was added dropwise. The reaction was stirred overnight at room temperature. The solvents were removed by rotary evaporation. Approximately 80 mL of water was added to the powder and extracted with 4 × 80 mL of ethyl acetate. The ethyl acetate fractions were combined, and the solvent was removed by rotary evaporation. 50 mL of water was added to the residue, which was filtered and washed with ∼500 mL of water. The filtrate was lyophilized and analyzed by HPLC.

Copolymer Synthesis. Poly(acrylamide-*co*-tyrosineamide) was synthesized by free radical copolymerization of acrylamide with 0

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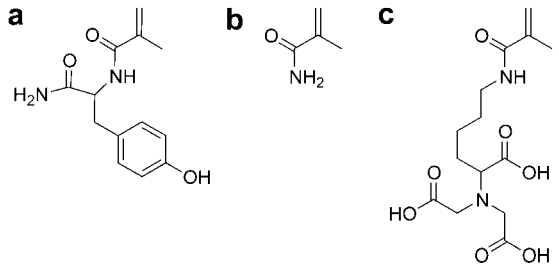

Figure 1. Copolymers were synthesized with various ratios of (a) *N*-methacryloyltyrosineamide, (b) acrylamide, and (c) 2-(methacrylamidobutyl)nitrilotriacetic acid (MABNTA).

to 6 mol % *N*-methacryloyltyrosineamide at 50 °C in methanol (90 wt %) with 2,2′-azobis(isobutyronitrile) (AIBN, 0.5 wt %) as the initiator under nitrogen for 24 h. The copolymer was dialyzed against water for 2 days before lyophilization. The poly(acrylamide-*co*-tyrosineamide-*co*-MABNTA) copolymers were synthesized by the same procedure using 3% tyrosineamide and 3% MABNTA. The structures of the monomers are shown in Figure 1.

Cyclic Voltammetry and Impedance Measurements. A three-electrode cell with a 0.3 mm Pt wire counter electrode and a 0.2 mm Ag/AgCl reference electrode was used for all electrochemical experiments. Planar Au electrodes (1 × 1 cm square) were created by sputtering Au on Mylar using a TMV Super Series SS-40C-1V multicathode sputtering system. Ti, used as the seed layer, was deposited at 90 W for 5 min followed by deposition of Au at 90 W for 4 min at 20 mTorr in Ar gas. A Pine Instrument Co. (Grove City, PN) RDE4 potentiostat driven by a BNC-2090 terminal block from National Instruments (Austin, TX) was used in CV experiments. The planar Au electrodes were placed in the background solution of 0.1 M NaCl, and the current was scanned from −0.9 to +0.9 V at 100 mV/s. The electrodes were placed in a 0.2 wt % polymer solution of the 6 mol % tyrosineamide copolymer in 0.1 M NaCl, and the current was measured from −0.9 to +0.9 V at 100 mV/s. Contact angles were measured by pipetting 20 μL of 18 Mohm water onto the same spot of the electrode before and after electrodeposition of the copolymer.

Disk electrodes used for impedance measurements were prepared similar to an already published procedure.[15] A 1 cm piece of 0.1 mm 99.99% Au wire (Simga-Aldrich, St. Louis, MO) was electrically contacted to a W rod using Ag conductive adhesion paste (Alfa Aesar, Ward Hill, MA). This was placed in an oven at 150 °C for 5 min to dry the Ag paste. The Au wire was inserted into a 10 cm length Prism glass capillary (1.65 mm o.d., 0.75 mm i.d., softening point 700 °C, Dagan Corp., Minneapolis, MN), leaving ∼2 mm space between the tip of the Au wire and the end of the glass capillary. This end was placed in a H_2/O_2 flame, and the glass was melted around the Au wire. A microscope was used to confirm the Au wire was completely sealed by the melted glass and that no bubbles had formed around it. After the Au had been sealed in the glass, the W wire was secured to the glass capillary with an epoxy (Loctite, Henkel, Dusseldorf, Germany). The electrodes were again placed in an oven at 150 °C for 5 min to harden the epoxy. The melted capillary end containing the sealed Au wire was polished flat with successively finer grit sandpaper (180-, 400-, 800-, and 1200-grit Carbimet, Buehler, Lake Bluff, IL) and last with aluminum oxide powder (300 nm, Alfa Aesar, Ward Hill, MA) on a wetted felt pad. A Gamry Instruments femtostat and Gamry Framework Version 4.10 software (Warminster, PA) were used for impedance measurements with the disk electrodes. The impedance was measured before surface modification in 0.1 M NaCl. The electrode was allowed to adsorb overnight in a 0.2 wt % solution of a 3 mol % tyrosineamide copolymer solution in 0.1 M NaCl. A 0.6 V potential was applied for 5 min to the electrode in the polymer solution using chronocoulometry, after which the impedance was remeasured in 0.1 M NaCl.

Surface Plasmon Resonance. SPR was measured with the Spreeta SPR3 integrated three-channel sensor module model TSPR1K23 (Nomadics, Stillwater, OK) using v. 20.97 of the Spreeta5 Multiple Channel Spreeta program. The sensors were washed in 0.1 M NaOH with 1% Triton X-100, rinsed with deionized water, and dried with flowing nitrogen gas prior to use according to the manufacturer's instructions. They were calibrated in water at a refractive index of 1.3330. To apply a voltage to the SPR sensor surface for copolymer electrodeposition experiments, a 0.1 mm 99.99% gold wire was attached to the edge of the sensor with tape. The gold surface of the sensor served as the working electrode in the three-electrode configuration diagrammed in Figure 2.

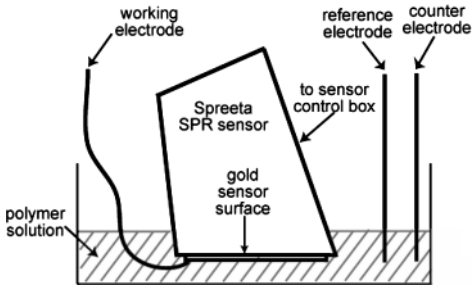

Figure 2. Schematic of the three-electrode configuration used to monitor copolymer passive adsorption and electrochemical deposition by SPR. The gold SPR sensor surface functioned as the working electrode.

To monitor the adsorption of copolymers onto gold, calibrated Spreeta sensor modules were placed in 0.2 wt % solutions of the 0−3 mol % tyrosineamide copolymers (Figure 2). After ∼2 h of copolymer adsorption, either the sensor was returned to water or a 0.6 V potential was applied for 5 min before returning the sensor to water. After polymer deposition by either passive adsorption or electrochemical deposition, the sensors were washed in 0.1 M NaOH with 1% Triton X-100 for 10 min.

Protein Binding. A recombinant His_6-peptide tagged green fluorescent protein (GFP-H_6) was used as a model protein for studies of protein adsorption to the gold SPR sensor surface. GFP-H_6 binding to copolymer modified and unmodified gold surfaces was monitored by SPR. Nonspecific adsorption of GFP-H_6 to unmodified and copolymer modified sensors was monitored by transferring SPR sensors equilibrated in phosphate buffered saline (PBS) pH 7.4 to solutions of 0.05 mg/mL GFP-H_6 in PBS. After 20 min the sensors were washed three times with PBS and then monitored until a steady SPR baseline (in refractive index, RI, units) was achieved. Cotyrosineamide-modified sensors were prepared by electrodepositing a 3 mol % tyrosineamide copolymer onto the surface as described above.

Specific immobilization of GFP-H_6 was tested with a sensor modified by electrodeposition of polyacrylamide-*co*-tyrosineamide-*co*-MABNTA. The RI was monitored as the sensor was first placed into a 0.05 mg/mL solution of GFP-H_6 in PBS (pH 7.4) to test for nonspecific adsorption. The sensor was washed and equilibrated with PBS and then metalated by incubation in a solution of 10 mM nickel acetate. After washing away excess nickel acetate with PBS, the sensor was again placed in the GFP-H_6 solution. To further demonstrate that the GFP-H_6 binding was specifically through His_6−Ni(II) complexes, the sensor was washed twice with 100 mM EDTA in PBS to sequester Ni(II) and reequilibrated in PBS.

Specific immobilization of GFP-H_6 was verified using fluorescent microscopy. Planar gold electrodes (1 cm square) were dipped halfway into a solution of polyacrylamide-*co*-tyrosineamide-*co*-MABNTA, and a 0.6 V potential was applied. After copolymer electrodeposition, the electrodes were washed with water several times. For specific immobilization the copolymer modified electrodes were soaked in 10 mM nickel acetate solution in water for 10 min followed by washing. Metalated and unmetalated electrodes were placed in a 0.05 mg/mL GFP-H_6 solution for 20 min and then washed three times with PBS. The electrodes were examined by fluorescence microscopy. The images were digitized, and the average pixel intensity was determined using ImageJ image processing software. To confirm specificity of binding, the elec-

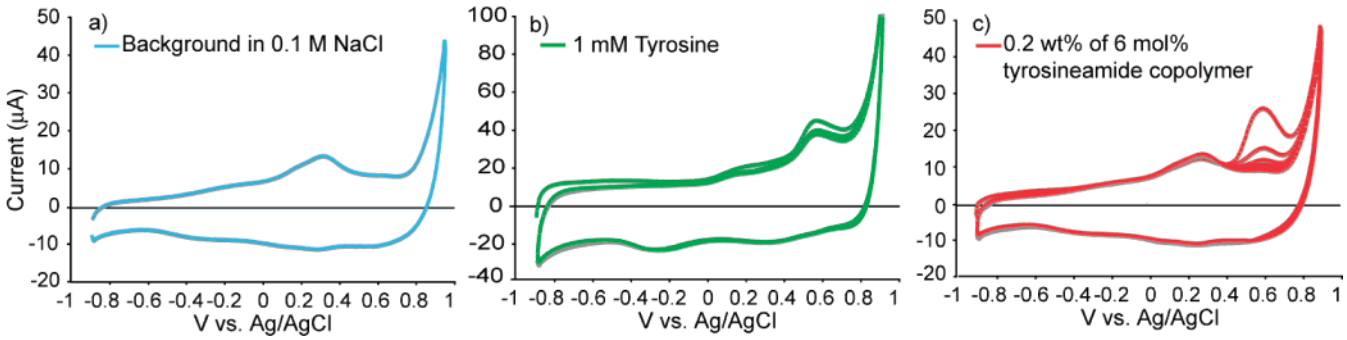

Figure 3. Cyclic voltammetry of tyrosine and poly(acrylamide-*co*-tyrosineamide) in 0.1 M NaCl. Scan rate was 100 mV/s.

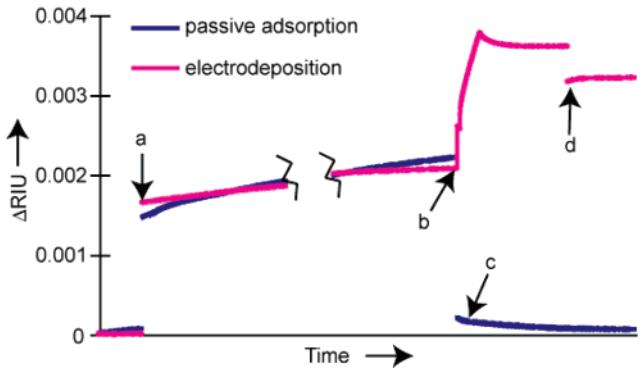

Figure 4. Change in refractive index (ΔRIU) of passively adsorbed vs electrodeposited copolymer containing 3 mol % tyrosineamide side chains. (a) Transfer of SPR sensor into cotyrosineamide polymer solution. (b) Application of 0.6 V potential for 5 min. (c) Sensor with passively adsorbed copolymer was washed in 0.1 M NaOH and 1% Triton X for 10 min and reequilibrated in water. (d) Sensor with electrodeposited copolymer was washed with 0.1 M NaOH and 1% Triton X-100 for 10 min and reequilibrated in water.

trodes with Ni(II)-bound GFP-H_6 were washed three times with 100 mM EDTA and reexamined by fluorescence microscopy.

Results

Cyclic Voltammetry. Cyclic voltammetry (CV) with gold electrodes in solutions of 0.2 wt % copolymer containing 6.0 mol % tyrosineamide side chains resulted in irreversible oxidation peaks at ~0.6 V vs Ag/AgCl (Figure 3c). The peaks occurred at a similar voltage as oxidation peaks observed during cyclic voltammetry of free tyrosine (Figure 3b). In contrast to free tyrosine, the magnitude of the oxidation peak of the tyrosineamide copolymer was diminished in each subsequent scan cycle. Contact angles measured before and after CV of the tyrosineamide copolymer solution demonstrated that the initially hydrophobic gold surface became hydrophilic (data not shown). The impedance of a 0.1 mm gold disk electrode measured at 1 kHz before and after CV in the tyrosineamide copolymer solution increased from 112 to 177 kohm. Together, these observations suggested that a hydrophilic surface layer of the tyrosineamide copolymer was electrochemically deposited on the electrodes that passivated the gold surface to further tyrosineamide oxidation.

Adsorption and Electrodeposition of Tyrosineamide Copolymers. When a Spreeta SPR sensor module was placed in a 0.2 wt % solution of a 3 mol % tyrosineamide copolymer (Figure 4, arrow a), the refractive index increased as the copolymer passively adsorbed onto the sensor surface. After 2 h of adsorption (arrow b) the refractive index returned to near baseline when the sensor was washed with 0.1 M NaOH and 1% Triton X-100 (blue line, arrow c). When a 0.6 V potential was applied for 5 min (pink line, arrow b), the refractive index increased dramatically. Washing with 0.1 M NaOH and 1% Triton X-100 (arrow d) removed a comparatively small amount of the copolymer, in contrast to the passively adsorbed copolymer. The stability of the electrodeposited tyrosineamide copolymer layer under harsh washing conditions suggested a robust attachment to the gold sensor surface.

Additional SPR experiments demonstrated a correlation between the amount of tyrosineamide in the copolymer with the mass of adsorbed copolymer over the range of 0−3 mol % tyrosineamide, demonstrating that the tyrosineamide side chains are responsible for adsorption (Figure 5A). In each case the passively adsorbed copolymer could be almost entirely removed by washing the sensor surface with 0.1 M NaOH and 1% Triton X-100. Likewise, an increasing mol % of tyrosineamide side chains led to increasing amounts of electrodeposited copolymer resistant to harsh washing conditions (Figure 5B).

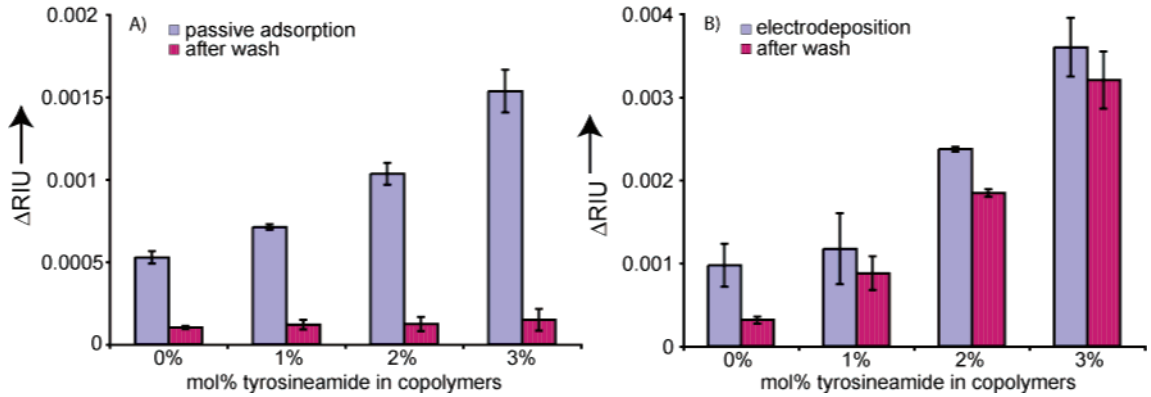

Figure 5. Copolymer adsorption vs mol % tyrosineamide side chains. (A) Passive adsorption before (blue bars) and after (purple bars) washing with 0.1 M NaOH and 1% Triton X-100. (B) Electrodeposition before and after washing. Error bars represent the average ±SD of at least three experiments.

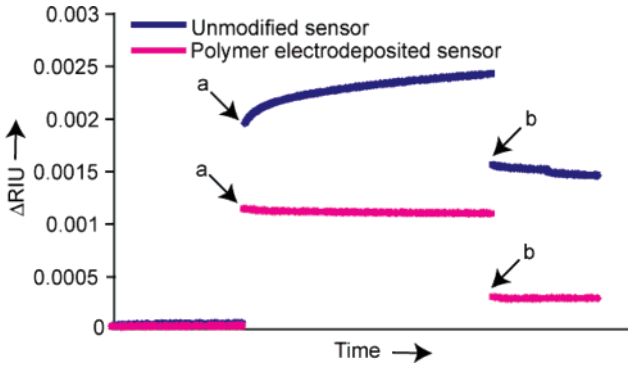

Figure 6. Nonspecific GFP binding to an unmodified (blue) and a sensor modified by electrodeposition of 3 mol % tyrosineamide copolymer (pink). (a) Sensor in 0.05 mg/mL GFP-H_6 solution. (b) Sensor reequilibrated in PBS (pH 7.4).

Nonspecific Protein Binding. A refractive index baseline was established by equilibrating a clean, unmodified SPR sensor in PBS (pH 7.4) before transfer to a 0.05 mg/mL solution of GFP-H_6 in PBS (Figure 6, blue line, arrow a). After 2 h the sensor was returned to PBS (arrow b). The refractive index remained substantially above the baseline value, indicating that GFP-H_6 had bound nonspecifically to the hydrophobic gold surface. In contrast, the initial refractive index change was much lower when a sensor precoated with a 3 mol % tyrosineamide copolymer layer by electrodeposition was incubated in 0.05 mg/mL GFP-H_6 (Figure 6, pink line, arrow a). When reequilibrated in PBS buffer (arrow b), the final change in refractive index from the baseline was 0.0002, about 1/8th the final refractive index change of the unmodified sensor.

Specific Protein Binding. Sensors were modified by electrodeposition of acrylamide copolymers containing 3 mol % tyrosineamide and 3 mol % (2-methacrylamidobutyl)nitrilotriacetic acid (MABNTA) side chains. When incubated with 0.05 mg/mL GFP-H_6 (Figure 7A, arrow a), the refractive index increased initially but returned to near baseline when reequilibrated with PBS buffer (arrow b). Following metalation of the NTA-containing sensor surface with Ni(II), the refractive index increased dramatically when incubated with 0.05 mg/mL GFP-H_6 (arrow c). When washed and reequilibrated with PBS buffer, the refractive index remained elevated (arrow d). The difference in RIU between arrow d and arrow b represents specifically bound GFP-H_6. To demonstrate that GFP-H_6 binding was specifically through metal coordination bonds between [NTA−Ni(II)] and the His_6 tag, the sensor surface was washed with 0.1 M EDTA to sequester the Ni(II) and disrupt metal coordination bonds. The refractive index returned to near baseline as expected (arrow e). The specific binding was confirmed by quantifying the fluorescence of GFP-H_6 immobilized on a gold-sputtered electrode coated with cotyrosineamide-*co*-MABNTA acrylamide (Figure 7B). After metalating the electrode surface with Ni(II), 4−5-fold more GFP was bound than without Ni(II). Furthermore, the bound GFP was nearly entirely removed by chelating the Ni(II) with EDTA.

Discussion

Regarding the adhesive mechanism, we first consider the electropolymerizaton of phenol since similar mechanisms may account for the strong adhesion of the electrodeposited cotyrosineamide polymers described here. First, phenol reportedly adsorbs to and completely displaces water from platinum surfaces.[16] The subsequent electropolymerization of adsorbed phenol is a complex process.[8,9,17] The reaction pathway begins with the formation of phenoxy radicals adsorbed to the electrode surface. The phenoxy radicals form dimers through ortho and para coupling, which are in turn oxidized into radicals that couple into oligomers and higher molecular weight insoluble polymers. Reaction of the phenoxy radicals and polymer radicals with surface oxide species creates hydroquinones, catechols, and benzoquinones that can undergo further dimerization and cross-linking reactions to result in impermeable and strongly adherent films. Robust adhesion of the films may be due to the increase in molecular weight and insolubility of the polyphenoxides. Additionally, the presence of catechols and quinones that are known to adsorb strongly to gold, platinum, and metal oxide surfaces[18,19] may contribute significantly to adhesion.

Likewise, initial copolymer adsorption was likely due to interaction of the phenolic side chains with the gold surface as indicated by the increased passive adsorption with increasing tyrosineamide mol % (Figure 5A). Application of a 0.6 V potential may generate tyrosineamide free radicals that react with O_2 to form strongly adherent dihydroxyphenylalanine (DOPA).[20−22] The tyrosineamide free radicals may also homocouple into ditryrosine cross-links increasing the molecular weight of the adsorbed copolymers. Beyond dimerization, it seems unlikely that extensive oligomerization occurs as is the

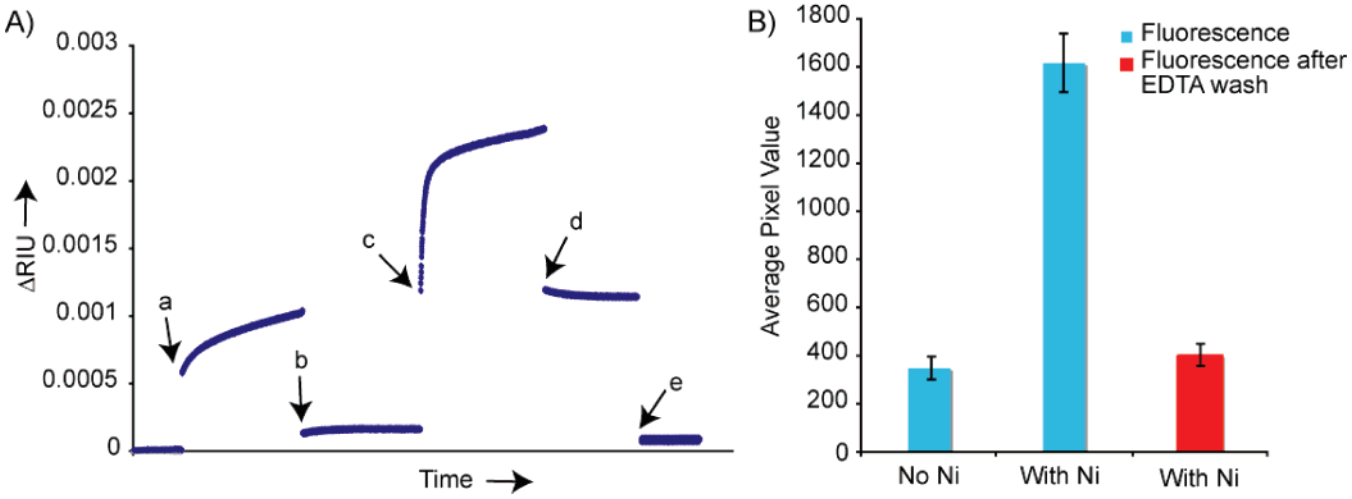

Figure 7. (A) Specific binding of GFP-H_6 to modified sensor. (a) Sensor placed in 0.05 mg/mL GFP-H_6 without Ni(II). (b) Sensor reequilibrated in PBS. (c) Sensor in 0.05 mg/mL GFP-H_6 after metalation with Ni(II). (d) Sensor reequilibrated in PBS. (e) Sensor washed with 0.1 M EDTA and reequilibrated in PBS. (B) Fluorescence of GFP-H_6 bound to gold electrodes. Error bars represent the average ±SD of at least three experiments.

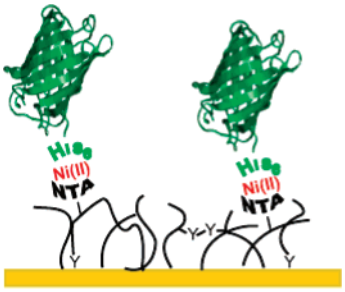

Figure 8. Schematic of GFP-H_6 protein binding to NTA−Ni(II) complexes incorporated into a poly(acrylamide-*co*-tyrosineamide-*co*-MABNTA) film electrodeposited on a gold surface.

case for phenol electropolymerization because of the relatively low concentration of the tyrosineamide side chains.

The adsorbed cotyrosineamide films were hydrophilic due to the high polyacrylamide content in contrast to hydrophobic films formed by electropolymerization of phenolic compounds.[11] The hydrophilic films suppressed nonspecific binding of GFP to 1/8th the level of unmodified gold. This is consistent with other reports of low protein binding by polyacrylamide.[23,24] Further improvements in the nonfouling characteristics may be possible through additional optimization of the process to increase the copolymer surface density. Incorporation of metal-chelating NTA side chains into preformed copolymers demonstrated that ligands for specific interactions can be conveniently added to the low protein binding films. It should be possible to copolymerize a wide variety of moieties, including peptide ligands, into the preformed tyrosineamide copolymers to functionalize gold surfaces. Electrodeposition of hydrophilic cotyrosineamide polymers may find its greatest utility as a means to engineer specific molecular adhesion on low protein binding surfaces during the development of medical diagnostics based on protein−ligand and protein−protein interactions. The nonfouling interface would preserve the structure and function of specifically immobilized proteins, limit nonspecific background, and thereby increase the accuracy, sensitivity, and potential feature density of the device. The electrodeposition method might be particularly useful for diagnostic devices and biosensors based on microarrayed ligands. Because adsorption of the cotyrosineamide polymers is triggered by a localized potential, it may be possible to selectively modify selected electrodes in an array of electrodes as a means to fabricate ligand microarrays. Tyrosineamide copolymers copolymerized with unique ligands could be sequentially introduced while applying potentials only to the electrode or pattern of electrodes to be sequentially surface modified.

Conclusions

The electrochemically triggered deposition of preformed copolymers through tyrosineamide sidechains appears to provide a robust method for modifying gold electrodes and sensor surfaces. Gold electrodes of any size, shape, and geometric arrangement can be modified in aqueous solution under physiological conditions. The polyacrylamide matrix creates a hydrophilic, protein-resistant surface which may be useful in engineering the interface of metallic materials that come into contact with biological fluids.

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References and Notes

(1) Polikov, V. S.; Tresco, P. A.; Reichart, W. M. *J. Neurosci. Methods* **2005**, *148*, 1−18.
(2) Cui, X.; Lee, V. A.; Raphael, Y.; Wiler, J. A.; Hetke, J. F.; Anderson, D. J.; Martin, D. C. *J. Biomed. Mater. Res.* **2001**, *56*, 261−272.
(3) Ignatius, M. J.; Sawhney, N.; Gupta, A.; Thibadeau, B. M.; Monteiro, O. R.; Brown, I. G. *J. Biomed. Mater. Res.* **1998**, *40*, 264−274.
(4) Shirakawa, H.; MacDiarmid, A.; Heeger, A. *Chem. Commun.* **2003**, *1*, 1−4.
(5) Yuqing, M.; Jianrong, C.; Xiaohua, W. *Trends Biotechnol.* **2004**, *22*, 227−231.
(6) Ahuja, T.; Mir, I. A.; Kumar, D.; Rajesh *Biomaterials* **2007**, *28*, 791−805.
(7) Haddour, N.; Cosnier, S.; Gondran, C. *J. Am. Chem. Soc.* **2005**, *127*, 5752−5753.
(8) Gattrell, M.; Kirk, D. W. *J. Electrochem. Soc.* **1993**, *140*, 1534−1540.
(9) Ferreira, M.; Varela, H.; Torresi, R. M.; Tremiliosi-Filho, G. *Electrochim. Acta* **2006**, *52*, 434−442.
(10) Long, D. D.; Marx, K. A.; Zhou, T. *J. Electroanal. Chem.* **2001**, *501*, 107−113.
(11) Bartlett, P. N.; Cooper, J. N. *J. Electroanal. Chem.* **1992**, *362*, 1.
(12) Bruno, F.; Pham, M. C.; Dubois, J. E. *Electrochim. Acta* **1977**, 22.
(13) Stickney, J. L.; Soriaga, M. P.; Hubbard, A. T.; Anderson, S. E. *J. Electroanal. Chem. Interfacial Electrochem.* **1981**, *125*, 73.
(14) Tang, A.; Wang, C.; Stewart, R. J.; Kopecek, J. *J. Controlled Release* **2001**, *72*, 57−70.
(15) Zhang, B.; Zhang, Y.; White, H. S. *Anal. Chem.* **2004**, *76*, 6229−6238.
(16) Wieckowski, A.; Szklarczyk, M.; Sobkowski, J. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *113*, 79.
(17) Gattrell, M.; Kirk, D. W. *J. Electrochem. Soc.* **1993**, *140*, 903−911.
(18) Soriaga, M. P.; Hubbard, A. T. *J. Am. Chem. Soc.* **1982**, *104*, 2735−2742.
(19) Stern, D. A.; Salaita, G. N.; Lu, F.; McCargar, J. W.; Batina, N.; Frank, D. G.; Laguren-Davidson, L.; Lin, C.-H.; Walton, N.; Gui, J. Y.; Hubbard, A. T. *Langmuir* **1988**, *4*, 711−722.
(20) Lee, H.; Scherer, N. F.; Messersmith, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12999−13003.
(21) Yu, M.; Hwang, J.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 5825−5826.
(22) Waite, J. H.; Jensen, R. A.; Morse, D. E. *Biochemistry* **1992**, *31*, 5733−5738.
(23) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605−5620.
(24) Senaratne, W.; Andruzzi, L.; Ober, C. K. *Biomacromolecules* **2005**, *6*, 2427−2448.

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