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Control of the Specific Adsorption of Proteins onto Gold Surfaces with Poly(L-lysine) Monolayers

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Monolayers of the polypeptide poly(L-lysine) (PL) are used to control the specific adsorption of proteins onto gold surfaces. A PL monolayer modified with biotin is electrostatically adsorbed onto a vapor-deposited gold film that has been coated with a self-assembled monolayer of the alkanethiol 11-mercaptoundecanoic acid (MUA). The immobilized biotin moieties act as specific adsorption sites for the protein avidin. Adsorption of the biopolymers onto the gold surface is monitored with a combination of surface plasmon resonance (SPR) and fluorescence measurements. By varying the percent biotinylation of the lysine residues on the PL prior to deposition, the surface coverage of avidin can be controlled to create either full or partial monolayers. The thickness of a full monolayer of avidin is 41 Å, as determined by the SPR measurements. At high surface coverages of avidin, an excess of biotin sites is required to overcome steric hindrance. The PL monolayer and any adsorbed avidin can be easily rinsed from the surface with a low or high pH solution. This removal allows for quantitation of the adsorbed molecules by fluorescence measurements in solution rather than on the gold surface. In this manner, fluorescein-labeled PL and avidin are used to determine absolute surface coverages of 4×10^{14} lysine residues cm^{-2} for the PL monolayer and 3×10^{12} avidin molecules cm^{-2} for the full avidin monolayer. SPR imaging experiments are employed to verify that UV photopatterning of the MUA/PL bilayers can be used to spatially direct the adsorption of avidin onto the gold surface. The polylysine attachment methodology will be beneficial in the fabrication of adsorption biosensors.

The fabrication of bioanalytical devices such as adsorption biosensors, enzyme-coated electrodes, and affinity chromatography columns often requires the attachment of protein molecules onto a solid surface.[1-3] In this attachment step, it is frequently necessary to control the specific adsorption of a particular protein and to prevent the nonspecific adsorption of other biological molecules. On metal surfaces, control over the adsorption of proteins and other biomolecules is often accomplished by chemical modification of the surface with self-assembled monolayers.[4-8] In a recent paper, it was established that a monolayer of the polypeptide poly(L-lysine) (PL) could be adsorbed onto gold surfaces via the formation of multiple ammonium-carboxylate ion pairs with a self-assembled monolayer of the alkanethiol 11-mercaptoundecanoic acid (MUA).[9] In this paper, we demonstrate that these PL monolayers offer a simple and attractive method for specifically attaching proteins onto alkanethiol-modified gold surfaces. Proteins can be attached to the surface either by direct cross-linking with the side chain ϵ-amino groups of the PL or by specific interaction with chemical functionalities incorporated into the PL. Since PL is a polypeptide itself, most proteins will not denature upon adsorption onto a PL-coated surface.

As an example of controlling protein immobilization onto gold surfaces with PL monolayers, the specific adsorption of the protein avidin is examined. Avidin (MW = 67 000) is a tetrameric glycoprotein that contains four specific binding sites for the molecule biotin.[10,11] The high affinity of avidin for biotin ($K_{aff} = 10^{15}$ M^{-1}) and the presence of multiple binding sites on the protein has led to its use in numerous biosensor applications.[3,12-19] Avidin and the related protein streptavidin have been adsorbed previously

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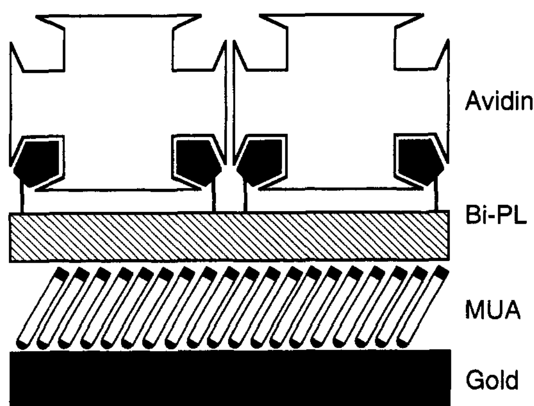

Figure 1. Schematic diagram showing the specific adsorption of the protein avidin onto a gold surface coated with a biotinylated poly(L-lysine) (Bi-PL) monolayer. The Bi-PL is immobilized via ion pair formation with a self-assembled monolayer of 11-mercaptoundecanoic acid (MUA). This diagram is not intended to imply that every avidin is bound to the surface with two biotin moieties.

onto gold surfaces by specific interactions with surface-immobilized biotin[20-24] and by simple nonspecific adsorption.[13]

The approach for the specific adsorption of avidin using PL monolayers is shown schematically in Figure 1. A self-assembled monolayer of MUA is first prepared on the surface of a of vapor-deposited thin gold film.[25-28] A monolayer of PL in which some of the lysine ϵ-amino groups have been modified with biotin functionalities (Bi-PL) is then electrostatically adsorbed onto the surface. Finally, exposure of this surface to an avidin solution results in the specific adsorption of the protein. Similar modification of PL has led to the adsorption of streptavidin on other surfaces.[18] To control the surface coverage of avidin, the biotin moieties are attached to the PL prior to its adsorption. This strategy provides for controlled variation of the percentage of lysine residues that are modified with biotin.

The adsorption of these various biopolymers onto the gold surface is followed with the surface-sensitive optical technique of surface plasmon resonance (SPR).[29-32] In a previous paper, the angle of the SPR reflectivity minimum (denoted as the SPR angle) was monitored in order to follow the adsorption of MUA and PL onto thin (47 nm) vapor-deposited gold films. From the shift in SPR angle upon adsorption, monolayer thicknesses of 17 and 10.5 Å were ascertained for MUA and PL, respectively. Similar measurements of the shift in the SPR angle are used in this paper to monitor the adsorption of avidin onto Bi-PL monolayers. In addition to the SPR angle measurements, SPR imaging[20,33] is used to monitor the specific adsorption of avidin onto photopatterned alkanethiol-coated gold surfaces.[34-36]

To complement the thickness information obtained from the SPR measurements, absolute surface coverage information is obtained from fluorescence measurements of fluorescein-tagged PL and avidin monolayers. The quantitative determination of the fluorescence yield from molecules attached to a metal surface is difficult due to the significant quenching that occurs; therefore, the fluorescently labeled adsorbates are first rinsed from the surface and subsequently quantitated with laser fluorescence measurements of the rinse solution. Removal of the PL (and any protein attached to the PL) from the surface is accomplished by exposure to an aqueous solution with pH values (below 5 or above 12) that disrupt the ammonium–carboxylate ion pairs between the MUA and PL monolayers.[9] The facile removal of specifically adsorbed proteins from the gold surface is an additional benefit of the polylysine attachment strategy.

MATERIALS AND METHODS

Materials. 11-Mercaptoundecanoic acid (MUA) (Aldrich); poly(L-lysine) (PL) av MW = 14 000 (Sigma); sulfosuccinimidyl 6-(biotinamido)hexanoate (NHS-Biotin) (Pierce); D-biotin (Pierce); avidin (Sigma); 4-hydroxyazobenzene-2-carboxylic acid (HABA) (Sigma); 5 (and 6)-carboxyfluorescein, succinimidyl ester (NHS-fluorescein) (Molecular Probes); avidin, fluorescein isothiocyanate labeled (Fl-Av) (Sigma); $NaHCO_3$ (Fluka); and absolute ethanol (Pharmco) were used as received. Millipore filtered water was used for all aqueous solutions and rinsing.

Derivatization of PL. Biotinylated polylysine (Bi-PL) was prepared in solution by the reaction of NHS-biotin with various percentages of the lysine residues. For example, 22% Bi-PL was synthesized by the rapid addition of 1.0 mL of 1.67 mg/mL of NHS-biotin in water (0.003 mmol) to a vortexing solution of 2.5 mL of 1.0 mg/mL of PL in 50 mM $NaHCO_3$ (0.012 mmol of lysine residues). The various other %Bi-PL solutions were prepared in a similar manner. The resulting Bi-PL solutions sometimes were passed through a Sephadex G-25M gel permeation chromatography (GPC) column (Pharmacia); this purification step had no observable effect on the adsorption of avidin. A HABA assay[37] was performed on the GPC column effluent to determine that 90% of the NHS-biotin reacted with the lysine residues. The fluorescein-labeled polylysine (Fl-PL) was prepared analogously to the Bi-PL except that NHS-fluorescein was used with a labeling pecentage of 1.5%, and the Fl-PL product was always purified by GPC.

Surface Preparation and Monolayer Formation. Gold thin films (47 nm) were vapor-deposited onto microscope slide covers (No. 2, 18 × 18 mm^2) that had been silanized with (3-mercaptopropyl)trimethoxysilane as described previously.[38,39] These samples

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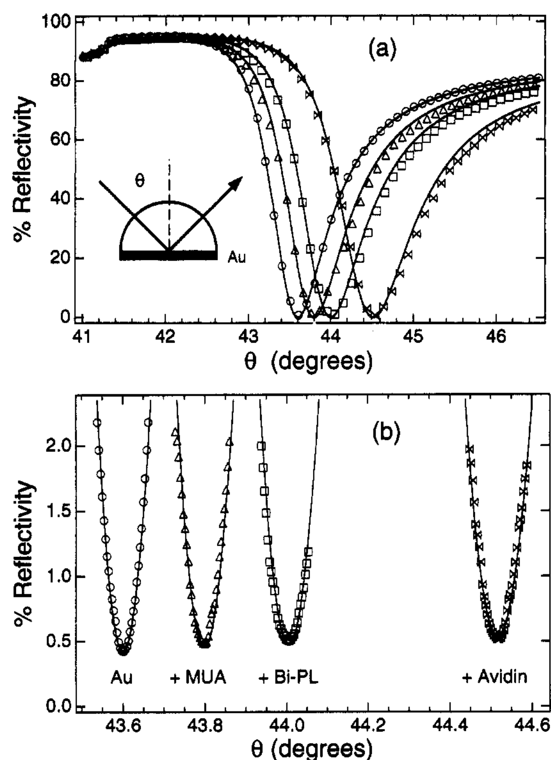

Figure 2. (a) SPR reflectivity curves for a clean gold surface (○), and the same surface after the sequential adsorption of a MUA monolayer (△), a 22% Bi-PL monolayer (□), and a layer of the protein avidin (×). The inset depicts a simplified version of the optical layout for the SPR experiment and defines the angle of incidence, θ. (b) An expanded view of the SPR curves for the same sample as in (a), but with data points taken every 0.004° to precisely determine the reflectivity minimum, referred to as the SPR angle. The shift in the SPR angle upon adsorption is used to determine the thickness of the adsorbed layer via complex Fresnel calculations (solid lines).

were immersed into 1 mM ethanolic MUA solutions for at least 18 h before being rinsed with EtOH and then water. The PL, Bi-PL, or Fl-PL monolayers were adsorbed from 0.1 to 0.2 mg/mL solutions in $NaHCO_3$ buffer (5 mM, pH 8.5) for 30 min, followed by thorough rinsing with water. The PL- or Bi-PL-coated surfaces were exposed for 45 min to 0.03–0.1 mg/mL avidin solutions also in $NaHCO_3$ buffer (5 mM, pH 8.5) and then rinsed with water.

SPR Thickness Measurements. The adsorption of the biopolymers onto the gold surface was followed with the surface-sensitive optical technique of surface plasmon resonance (SPR).[29–32] SPR is sensitive to the thickness and index of refraction of material at the interface between a thin metal film (gold) and a bulk medium (air). The SPR experiment and thickness calculation have been described in detail previously.[9,39] The reflectivity of the 632.8 nm He–Ne laser light was monitored versus the angle of incidence (θ) from a BK7 prism to which a gold-coated slide cover had been brought into optical contact (see inset of Figure 2). A steep drop in the reflectivity was observed near 43.6° for a clean gold sample (Figure 2, circles). The angle of minimum reflectivity, denoted the SPR angle, shifted to higher angles as material adsorbed to the gold surface. This shift in SPR angle was converted to an average monolayer thickness using the material's index of refraction and complex Fresnel calculations.[40]

SPR Imaging Experiments. The SPR imaging experiment had three main differences from the SPR thickness measurements: (i) the laser beam was expanded to ~2 cm diameter to illuminate the entire sample, (ii) the angle of incidence was fixed rather than scanned, and (iii) a video camera served as the detector.[41] Briefly, the prism/sample assembly was held at a fixed angle of incidence, usually near an SPR angle of minimum reflectivity (e.g., 43.6° for a clean Au surface). Any adsorbed material was observed as an increase in the reflectivity at this angle (e.g., for a MUA monolayer, the reflectivity increased to 20%; see Figure 2a). When material was adsorbed onto certain areas of a photopatterned surface but not others, an SPR image was obtained with light and dark regions corresponding to areas of high and low adsorption, respectively. The patterned samples were prepared by UV photopatterning of the alkanethiol monolayer, as described elsewhere.[35]

Fluorescence Measurements. All fluorescence measurements were performed in solution after removal of the fluorescein-labeled monolayers from the gold surface. The Fl-PL (or Fl-Av on Bi-PL) was washed off the MUA-coated surface by immersion into an HCl solution (0.01 M, pH 2) for 1–2 h. Changes in thickness as measured by SPR verified that all material adsorbed to the MUA was removed from the surface by the HCl rinse solution. These HCl solutions were then diluted 20-fold into borate buffer (0.1 M, pH 9), since fluorescein fluoresces much more strongly in basic solution. The fluorescence was excited with 0.25 mW of 488 nm light from an argon ion laser (Lexel model 95) and then collected and finally dispersed with a 0.275 m $f/4$ spectrograph (Acton Research) onto a liquid nitrogen-cooled CCD camera system (Photometrics SDS-9000). The fluorescence at 520 nm of the Fl-PL sample solution was compared to that from various concentrations of Fl-PL standards. For the Fl-Av quantitation, D-biotin was added to the samples and standards because empty biotin sites have been shown to significantly quench the fluorescence of fluorescein.[42]

RESULTS AND DISCUSSION

SPR Thickness Measurements. Surfaces with varying numbers of specific adsorption sites for avidin were prepared by modifying polylysine with different amounts of biotin (0–22% of the lysine residues). These various Bi-PL conjugates were adsorbed electrostatically onto MUA-coated gold surfaces and then exposed to avidin solutions. In this manner, the percent biotinylation of lysine residues needed to produce complete avidin monolayers was determined to be about 20%. The SPR data for the adsorption of monolayers of MUA, 22% Bi-PL, and avidin onto a gold surface are shown in Figure 2. The observed shifts of the SPR angles in Figure 2 are given in Table 1, part A along with the calculated thicknesses for those layers. The calculation of layer thicknesses from the SPR shifts requires the index of refraction of each layer; these values are estimated from the bulk indices of refraction. (The error introduced by this estimation has been shown to be small.[9]) As mentioned in our previous paper, the 17 Å thickness for the MUA monolayer is in excellent agreement with theory and ellipsometric measurements.[26–28] The 22% Bi-PL monolayer was adsorbed onto the MUA monolayer from a 0.1–0.2 mg/mL solution in $NaHCO_3$ buffer (5 mM, pH 8.5). The observed SPR angle shift corresponds to an additional thickness of 17 Å for the 22% Bi-PL monolayer, which is somewhat

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Table 1. SPR Angle Shifts and Calculated Layer Thicknesses

layer		total $\Delta\theta^a$ (deg)	additional $\Delta\theta$ (deg)	index of refraction^b	layer thickness (Å)	surface coverage^c (cm^{-2})
part A	MUA	0.180 ± 0.005	0.180	1.45	17 ± 1	
	22% Bi-PL	0.385	0.205	1.52	17	
	avidin	0.870	0.485	1.45	41	3×10^{12}
part B	MUA	0.180	0.180	1.45	17	
	PL	0.310	0.130	1.52	10.5	4×10^{14} lysines
	avidin	0.310	0.00	1.45	0	

^{a} Total shift in SPR angle from that of the bare gold surface. ^{b} Index of refraction estimated from bulk values. ^{c} Absolute surface coverage determined from fluorescence measurements.

thicker than a monolayer of unmodified PL (10.5 Å). SPR measurements on monolayers of 0.3–15% Bi-PL gave intermediate thicknesses between 10.5 and 17 Å, as expected.

The biotin moieties attached to the surface via the PL acted as specific adsorption sites for avidin. Thus, exposure of the 22% Bi-PL-coated surface to 0.03–0.1 mg/mL avidin in $NaHCO_3$ buffer (5 mM, pH 8.5) produced an additional shift in SPR angle of nearly 0.5° corresponding to an avidin layer thickness of 41 Å. X-ray crystallography and electron microscopy measurements have determined that the size of an avidin molecule is ~40 Å × 55 Å × 55 Å and that two pairs of biotin binding sites are located on opposite sides of the short dimension.[10,11] Other researchers have measured thicknesses of 40–48 Å for full avidin (or streptavidin) monolayers.[13,15,18,21,22] Thus, the monolayer thickness of 41 Å obtained from the SPR results demonstrates that a complete monolayer of avidin has adsorbed onto the 22% Bi-PL-coated surface.

Full monolayers of avidin ~40 Å thick were also obtained for Bi-PL monolayers with as little as 11% biotinylation (see Figure 3). This result agrees with that obtained by Spinke et al., who found that 10% of a biotinthiol mixed with 90% 11-mercaptoundecanol led to the adsorption of a full streptavidin monolayer.[22] Complete 100% biotinthiol monolayers, however, were observed to hinder streptavidin adsorption and yield lower surface coverages.[22,24]

Two control experiments were performed to verify that the avidin was specifically adsorbed onto the biotin sites present on the gold surface. First, SPR measurements determined that no avidin adsorption occurred onto a PL monolayer that had not been biotinylated (Table 1, part B). Second, no avidin adsorption was observed when the Bi-PL-coated surface was exposed to a solution of avidin that had been first saturated with D-biotin. The prevention of nonspecific adsorption of the protein avidin onto the gold surface by the PL (or Bi-PL) monolayers can be ascribed to the fact that at pH 8.5, both PL and avidin (pI = 10) are positively charged. The preferential adsorption of avidin to Bi-PL over PL is investigated further in the SPR imaging experiment described below.

In addition to the full monolayers of avidin obtained from 11–22% Bi-PL monolayers, partial avidin monolayers could be formed by decreasing the amount of biotin on the PL. The effective avidin layer thickness, which is proportional to surface coverage, was determined with SPR measurements. This avidin layer thickness is plotted in Figure 3 versus the %Bi-PL. Notice that partial monolayers of avidin resulted from Bi-PL monolayers with less

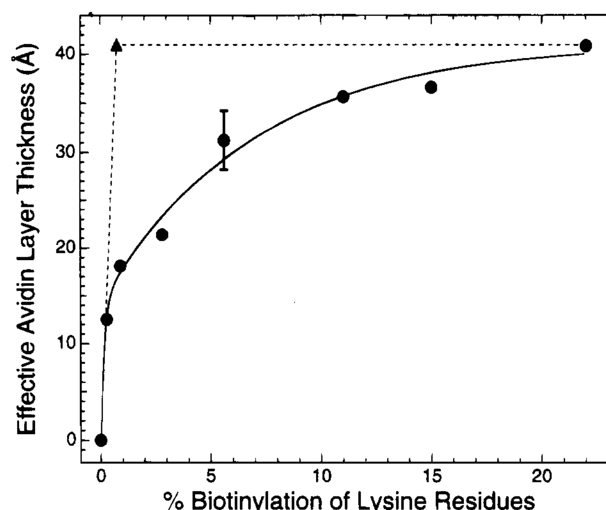

Figure 3. Plot of the effective avidin layer thickness versus the percent biotinylation of the lysine residues of the adsorbed Bi-PL. The experimental data points (circles) were determined by SPR thickness measurements and fit with a double exponential (solid line). The calculated curve (dashed line) was obtained via the fluorescence measurements (see discussion in the text). Comparison of the experimental and calculated curves suggests sterically hindered binding of avidin, which could be overcome by increasing the number of specific adsorption sites (i.e., the %Bi-PL).

than 11% biotinylation. The %Bi-PL was fixed by the stoichiometry of the one-step solution phase reaction between NHS-biotin and the lysine residues of PL *prior* to adsorption of the Bi-PL onto the surface. This method of controlling the spacing of biotin groups on the surface with Bi-PL avoids problems associated with mixed alkanethiol monolayers such as phase segregation[43] and solution composition versus surface composition mismatches.[44,45] This method is also preferable to a surface reaction between NHS-biotin and PL already adsorbed to the gold surface, which was found to offer little control, knowledge, or reproducibility of the biotinylation percentage of the PL. The solution phase reaction prior to adsorption, however, provided precise control over the amount of biotin on the gold surface and the resulting avidin coverage.

As seen in Figure 3, the avidin surface coverage is not a linear function of the %Bi-PL. If all of the biotin moieties in the Bi-PL monolayer were available for binding with avidin, the avidin layer thickness should increase linearly with the %Bi-PL. Once monolayer (saturation) coverage is reached, no further increase in avidin coverage should be observed. The dashed line in Figure 3 depicts this "ideal" behavior. The triangle marks the point of saturation (1:1 ratio of biotin and avidin on the surface), which should be 0.75% Bi-PL as determined from fluorescence measurements (see below). The solid line in Figure 3 is a double exponential fit to the data; several fits were tried, and all gave nearly the same initial slope. This initial slope at low %Bi-PL closely matches the expected slope (dashed line). This agreement indicates that at low surface coverages, an avidin molecule binds to virtually every biotin moiety in the Bi-PL monolayer.

At higher %Bi-PL, however, the surface coverage of avidin no longer increases as quickly as expected. This discrepancy is likely

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due to sterically hindered binding of the avidin; similar steric effects have been observed previously for biotin–lipid-doped Langmuir–Blodgett films on quartz.[14] Once the Bi-PL surface is covered with a significant amount of avidin, avidin molecules from solution have a difficult time finding an adsorption site that is not partially blocked. Nonetheless, at higher %Bi-PL (~20%), a large excess of specific adsorption sites is provided, the steric hindrance problem is overcome, and a complete monolayer of avidin is formed.

Fluorescence Measurements. The absolute surface coverages of both polylysine and avidin were determined by first adsorbing the corresponding fluorescein-labeled protein onto the modified gold surface, and then desorbing it, and subsequently detecting it in solution with fluorescence measurements. Removal of attached proteins from the surface was accomplished by a simple pH change, which disrupted the ion pair interactions between the PL and MUA monolayers. For example, fluorescein-labeled PL (Fl-PL) was adsorbed onto a MUA-coated gold surface, and after the sample was thoroughly rinsed with water, the Fl-PL monolayer was desorbed by immersion into an HCl solution (0.01 M, pH 2). This HCl solution was diluted with 0.1 M borate buffer to raise the pH to 9, and then the fluorescence was measured. The observed fluorescence was compared to that obtained from Fl-PL standards and then used to calculate a value of 4×10^{14} lysine residues cm^{-2} (see Table 1, part B). This surface coverage corresponds to a surface area of 25 $Å^2$/lysine residue, which roughly matches a calculated area of 33 $Å^2$ from a molecular model of PL bound to a surface.[46] This absolute surface coverage result agrees with our previous conclusion (based on the SPR thickness measurements) that the PL molecules cover the MUA-coated gold surface with a complete but single monolayer.[9]

In a second fluorescence experiment, the absolute surface coverage of fluorescein-labeled avidin (Fl-Av) was also determined. After adsorption of Fl-Av onto an 11% Bi-PL monolayer, these monolayers were removed from the surface and the HCl rinse solution was diluted with borate buffer as in the case of the Fl-PL. The fluorescence from the rinse solution was compared with that of Fl-Av standards and yielded a value of 3×10^{12} avidin molecules cm^{-2}. This result relates well to the SPR-determined avidin layer thickness and to the theoretical maximum coverage of 3.3×10^{12} cm^{-2} as calculated from the molecular dimensions of avidin.[10] The easy removal of PL (and proteins specifically adsorbed to the PL) from the gold surface makes these fluorescence measurements simple compared to quantitation of fluorescence on the gold surface itself.

As mentioned above, these fluorescence results were used to calculate the theoretical %Bi-PL monolayer needed to give complete avidin coverage. The number of avidin molecules per square centimeter divided by the number of lysine residues per square centimeter yields the result that a 0.75% Bi-PL monolayer would have one biotin moeity for each avidin molecule of a full avidin monolayer. This outcome is depicted as the triangle in Figure 3, and the slope of a line from the origin to this value (dashed line) matches quite well with the initial slope of the experimental SPR data. The fact that much higher %Bi-PL (~20%) monolayers are required to produce complete coverage of avidin suggests that steric hindrance effects are present in the adsorption of avidin onto Bi-PL, as explained in the previous section.

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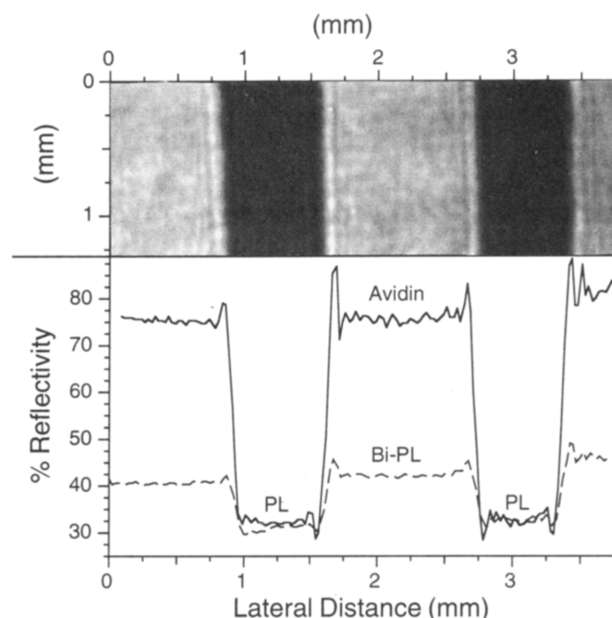

Figure 4. SPR image observed for a photopatterned thin film on a gold surface. The dark stripes correspond to regions of the surface coated with MUA and PL monolayers, whereas the brighter regions are covered with MUA, Bi-PL, and avidin layers. Line profiles of the percent reflectivity were obtained for two different SPR images. The first image (dashed line) was taken using a sample with alternating stripes of MUA/PL and MUA/Bi-PL. The reflectivity in the Bi-PL areas is 10% higher because the Bi-PL monolayer is 6.5 Å thicker than the unmodified PL monolayer. This PL and Bi-PL striped surface was exposed to a solution of avidin, and then another SPR image and corresponding line profile were obtained (solid line and the displayed image). The reflectivity increased in the Bi-PL regions but did not change in the PL regions, thereby demonstrating that avidin only adsorbs onto those areas of the surface having specific adsorption sites (biotin moieties).

SPR Imaging Experiments. In a final demonstration of the utility of PL monolayers for controlling protein adsorption onto gold surfaces, the preferential adsorption of avidin onto Bi-PL versus unmodified PL is demonstrated in the SPR imaging experiment shown in Figure 4. A gold surface was prepared with alternating stripes of 22% Bi-PL and PL by the following procedure: (i) the entire surface was covered with MUA and Bi-PL; (ii) stripes of the MUA/Bi-PL were removed by irradiating the surface with UV light through a mask and then rinsing with ethanol and water;[35] (iii) the sample was immersed into a MUA solution to refill those areas just removed; and (iv) these newly deposited MUA stripes were coated with PL by immersion of the sample into a PL solution. An SPR image was obtained from the resulting surface of MUA/PL and MUA/Bi-PL stripes, and these data are shown as a line profile of the percent reflectivity (dashed line in Figure 4). Notice the 10% difference in the reflectivity between the two regions, which results because the Bi-PL monolayer is 6.5 Å thicker than the unmodified PL layer (see Table 1). After exposure of the surface to avidin (solid line), the reflectivity increased from 40% to 80% in the Bi-PL regions but not at all on the PL covered stripes. The increase in reflectivity for the Bi-PL-coated stripes results from a shift in the SPR angle caused by the adsorption of a 40 Å layer of avidin; conversely, the lack of change in reflectivity for the PL stripes indicates that no avidin adsorption occurred onto those regions. Figure 4 also displays the SPR image of these alternating stripes of MUA/PL (dark regions) and MUA/Bi-PL/avidin (light regions). The SPR

image data clearly demonstrate that the avidin specifically adsorbs to the biotin moieties of the Bi-PL and that unmodified PL prevents the nonspecific adsorption of avidin onto the metal surface.

CONCLUSIONS

Poly(L-lysine) monolayers offer several advantages for attaching proteins or other biomolecules to gold surfaces. Using simple solution phase reactions, a variety of specific adsorption sites (e.g., biotin moieties) can be incorporated into the PL chain. Coating the surface with these derivatized PL monolayers provides for the specific adsorption of a particular biomolecule. As demonstrated in the case of avidin, the PL monolayer allows for control over the surface coverage of the specifically adsorbed protein, and it will also prevent the nonspecific adsorption of proteins with relatively high isoelectric points. A unique advantage of the polylysine attachment scheme is the facile removal of adsorbed proteins by rinsing with either low or high pH solutions. This benefit allows analytical measurements such as fluorescence to be performed more easily in solution rather than on the surface itself. Furthermore, the removal of adsorbed proteins provides the option of regenerating a surface. The ability of PL-coated alkanethiol monolayers to be photopatterned with UV light leads to spatial control over the adsorption of biomolecules onto gold surfaces, and SPR imaging has been demonstrated as an excellent detection method for these patterned surfaces.

Experiments to exploit the advantages of the polylysine attachment strategy in the creation of biosensor surfaces are currently in progress. The avidin monolayers prepared via Bi-PL can be used in biosensor applications by the specific adsorption of either biotinylated probe or target molecules. UV photopatterning of the MUA/PL bilayers allows for the preparation of surfaces with multiple adsorption sites; these surfaces will be beneficial in the production of multielement SPR adsorption biosensors for antibody screening assays and other applications.

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