Thin, Highly Crosslinked Polymer Layer Synthesized via Photoinitiated Graft Copolymerization on a Self-Assembled-Monolayer-Coated Gold Surface

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ABSTRACT: A thin, highly crosslinked layer was grafted onto an alkyl thiol self-assembled-monolayer (SAM)-coated gold surface with *N,N'*-methylene bisacrylamide (MBAA), a widely used crosslinker with two polymerizable groups, as the monomer. Surface-initiated photografting copolymerization was achieved through the immobilization of the hydrogen-abstraction photoinitiator benzophenone on the hydrophobic alkyl surface via physical adsorption and subsequent UV irradiation in the presence of an MBAA solution. The growth of the grafted poly-MBAA layers seemed to produce dendritic structures with low surface coverage. At a higher monomer concentration (15 g/L of water), full coverage of the gold surface with a thin layer was obtained and proved by scanning force microscopy and contact-angle measurements. The evaluation of the gold, gold–SAM, and gold–SAM/grafted poly-MBAA layers with a surface plasmon resonance sensor system revealed that the photografted, thin, highly crosslinked polyacrylamide layers had a very low affinity toward the adsorption of protein. Therefore, this provides a very promising approach to tailoring materials for sensors and other applications. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 158–164, 2005

Key words: crosslinking; graft copolymers; proteins; surfaces

INTRODUCTION

Self-assembled monolayers (SAMs) can be formed at the surfaces of plane substrates or particles.[1] This approach has gained increasing scientific attention because it is possible to engineer the surface properties of materials for numerous applications, such as biosensors and biochip arrays. Well-defined monolayers with different exposed chemical functionalities may also serve as models for more complex systems, such as biomaterials in contact with proteins. Usually, a monolayer with a simple chemical composition is not sufficient for those purposes, and so its further modification and functionalization are of great interest. This can be achieved with special functional amphiphilic molecules as components of the SAM[2] or by a surface functionalization of the already formed SAM.[3] Surface-reactive SAMs have also been synthesized for the subsequent immobilization of monolayers of macromolecules. Two variants can be distinguished: grafting-to of macromolecules based on the mutual reactivity of a SAM end group and the macromolecule[4,5] and grafting-from initiated by SAM-conjugated initiator groups, which thus enables radical, ionic, or atom-transfer chain-growth mechanisms.[6–10] Such polymer-grafting reactions offer greatly increased possibilities of producing different surface layer functionalities, including rigidity or flexibility, and thus layer functions. For example, overall properties such as the hydrophilicity are not sufficient to understand or predict the extent and strength of protein binding to surfaces.[3,5,11,12] Thus, the synthesis and characterization of thin, well-defined grafted polymer layers has great relevance for the development of advanced polymer materials.[13]

In contrast to grafting-to, the grafting-from strategy offers much greater flexibility in terms of creating either loose or dense (brush) and crosslinked polymer layers. For example, photoinitiated graft copolymerization from the surfaces of substrate polymers with adsorbed benzophenone (BP)[14] has been established as a very versatile method for the synthesis of various functional materials, including surfaces for protein affinity separation[15] and thin layers of molecularly imprinted polymers (MIPs).[16] Although surface-bound initiators, including SAMs, on glass, silica, and polymers have been explored frequently,[6,7,13] far fewer investigations have been reported for graft copolymerization on thiol-coated gold surfaces.[7–10]

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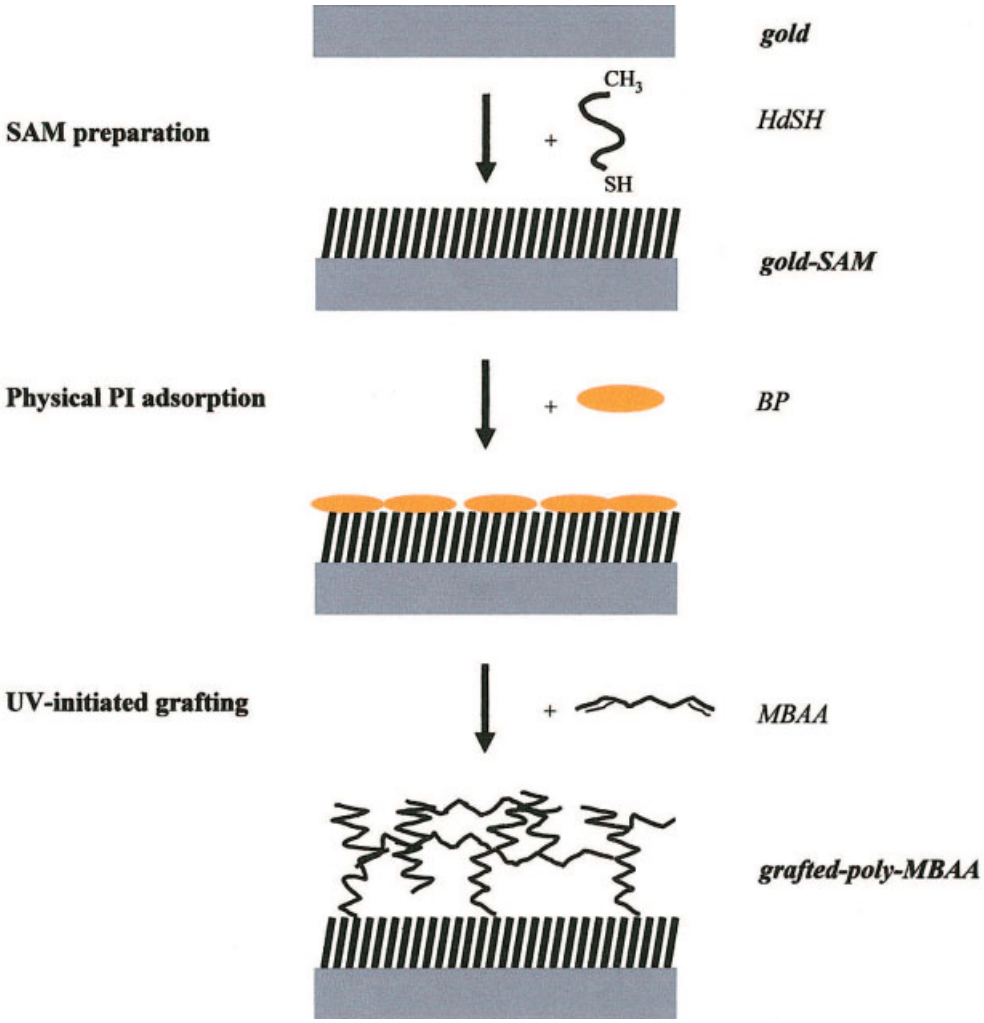

Figure 1 Schematic description of the photoinitiated graft copolymerization of a crosslinker monomer (MBAA) on an alkyl thiol SAM-coated gold surface with an adsorbed hydrogen-abstraction PI (BP). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

However, gold substrates are perfect transducers for various sensors, including surface plasmon resonance (SPR), which has gained great importance for the study of biomolecular interactions.[3,5,17] One concern is the relative thermal and chemical instability of SAMs on gold under polymerization reaction conditions. Furthermore, special syntheses or derivatizations of thiol derivatives have been necessary.[7–10] Therefore, a simple and effective method for immobilizing an initiator on a thiol-coated gold surface along with mild reaction conditions for tailoring the surface structure and properties would have immediate relevance for fundamental and applied research.

This study had several aims. First, the method of photoinitiator (PI) immobilization by physical adsorption on a SAM surface made from simple hydrophobic alkyl thiols was evaluated (see Fig. 1). The feasibility of this approach for the synthesis of an MIP sensor had been indicated, but no surface characterization had been performed, and the origin of the sensor's specificity was not fully clear.[18] Second, for photoinitiated graft copolymerization, reaction mixtures containing only a crosslinker monomer were applied. Such a grafting reaction had not yet been investigated, but mixtures with unusually high contents of crosslinker monomers have outstanding relevance for the synthesis of thin MIP layers.[16,18] Third, the compatibility of the novel grafted polymer layers with SPR were investigated by the evaluation of the binding of a protein. On the basis of the obtained results, the photoinitiated graft copolymerization onto SAM-modified gold with adsorbed BP is a very straightforward and promising approach for the synthesis of tailored functional grafted polymer layers on sensor and other surfaces.

EXPERIMENTAL

Materials

Glass wafers with an effective area of approximately 3 cm^2, which were coated with gold (ca. 50 nm thick)

and were to be used as SPR sensor discs, were obtained from Xantec GmbH (Münster, Germany). *N,N′*-Methylene bisacrylamide (MBAA; pure) was acquired from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). BP (for analysis), 1-hexadecanthiol (HdSH; > 95%), and potassium dichromate were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Chloroform (p.a., 99.98%) was from Fisher Scientific (Loughborough, UK). Acetone (99.5%) and sulfuric acid were obtained from J.T. Baker (Deventer, The Netherlands). Bovine serum albumin (BSA; fraction V powder, fatty acid free, ~99%) was acquired from ICN Biomedicals, Inc. (Costa Mesa, CA). Mucasol and buffer salts were from VWR-Merck (Darmstadt, Germany). Water purified with a Milli-Q system was used for all the experiments.

SAM preparation

First, the gold sensor discs were cleaned by immersion in a solution of the commercial cleaning agent Mucasol (~1 g/L) in water for 15 min, rinsing with water, immersion in a solution of potassium dichromate in concentrated sulfuric acid (70 g/L) for 5 min, rinsing with water, and drying with high-purity nitrogen. Thereafter, the sensor discs were immersed in a solution of HdSH (0.5 mmol/L) in chloroform for at least 24 h. After being washed with chloroform and dried with nitrogen, the materials were ready for the next step.

Photoinitiated graft copolymerization

The UV illumination system UVAPrint (Hoenle AG, Gräfelfing, Germany), equipped with a high-pressure mercury lamp and a special glass filter and providing homogeneous illumination for an area of up to 100 cm^2 with a wavelength 330 to 400 nm (UVA) intensity of approximately 70 mW/cm^2 (measured with a UVA sensor from Hoenle), was used. For the PI coating, the SAM-coated gold substrates were immersed in a solution of BP (1 mmol/L) in acetone for 15 min, and this was followed by drying with nitrogen. Then, the substrates were immediately put into a solution of MBAA in water. After 1 min of residence time, UV irradiation for 15 min followed. Thereafter, the samples were taken out and intensively washed with water.

Contact-angle (CA) measurements

An OCA 15 Plus CA measurement system (Dataphysics GmbH, Filderstadt, Germany) was used. CAs were measured with the sessile drop method. A drop of water was injected from a syringe with a stainless steel needle onto the sample surface; the diameter of the drop was always about 3 mm. At least 10 measurements of drops at least three different locations on the sample were averaged.

Scanning force microscopy (SFM)

A system from Digital Instruments, Inc. (Santa Barbara, CA), was used. All measurements were carried out under ambient conditions with a Nanoscope 3A controller in the noncontact mode. The standard software was used for the quantitative estimation of the surface roughness.

SPR

The biosensor system IBIS I, with a laser with a wavelength of 670 nm, obtained from Xantec, was used. A pH 7.2 saline phosphate buffer (150 mmol/L of NaCl, 9 mmol/L of potassium dihydrogen phosphate, and 30 mmol/L of disodium hydrogen phosphate) was first filtered through a 0.2-μm cellulose acetate membrane (Sartorious AG, Göttingen, Germany) and then was used to prepare a solution of BSA (0.5 g/L). Typical experiments were performed at 20°C and included at least the following steps. After the injection of the buffer solution onto the sensor surface and the stabilization of the baseline, the BSA solution was injected quickly, and the sensor resonance angle was followed for at least 600 s. Then, the buffer solution was injected quickly, and the resonance was followed until a stable value had been reached again. Data were evaluated in terms of the response, that is, the change in the resonance angle due to BSA adsorption, and they were based on an analysis of at least two independently prepared sensors, including the results of two or three measurements on different locations of one sensor. The maximum variation of the sensor response was ±15%.

RESULTS AND DISCUSSION

The applicability of simple physical adsorption of PI to hydrophobic alkyl SAMs on gold (cf. Fig. 1) has been suggested by the high efficiency of this method for the functionalization of polypropylene (PP) from aqueous monomer solutions.[15,16] The main reaction pathway is hydrogen abstraction by photoexcited BP from the (alkyl) surface, and this creates a surface-immobilized starter radical for the (graft) copolymerization.[14] Through the use of the bifunctional hydrophilic monomer MBAA, a grafted polymer with a particular architecture is expected, that is, a thin network layer with a very high crosslinking degree. With photoinitiated graft copolymerization at a constant UV irradiation time, that is, a constant PI conversion, the degree of functionalization (DF) and thus the surface coverage can be adjusted by the monomer concentration.[15] However, with bifunctional MBAA and

TABLE I
Sensor Surface Characterization Data

	CA (water sessile drop, °)	SFM	
		Average roughness (nm)	Mean height difference (nm)
Gold	31.4 ± 1.2		
Gold–SAM (HdSH)	100.3 ± 1.5	0.9	6.7
Grafted poly-MBAA, 6 g/L	79.6 ± 4.6	3.7	20.1
Grafted poly-MBAA, 15 g/L	54.8 ± 2.1	2.6	16.2

PP as the substrate, a significant deviation from the approximately linear dependence, which is typical for monofunctional monomers at low DF values,[15] has been observed: The DF increased almost exponentially with the monomer concentration until a saturation was reached at higher values.[19]

Surface analytical data for the different characteristic stages of the functionalization are presented in Table I and Figure 2. After grafting at an MBAA concentration of 6 g/L, CA was significantly lower than that for the unmodified hydrophobic gold–SAM substrate, but the surface could still be considered hydrophobic, and the variations were rather large. In contrast, at 15 g/L, an even and moderately hydrophilic surface could be obtained reproducibly. For intermediate monomer concentrations, the CAs were between those data, but with large variations. The SFM data provide additional information regarding the surface topology at the different stages (cf. Fig. 2). The gold–SAM substrates were flat and relatively even, and the mean height differences could be attributed to a few linear scratches barely visible on the surfaces of the commercial sensors [cf. Fig. 2(a)]. The surface modification yielded a rather rough structure at a low grafting efficiency, whereas a high monomer concentration yielded a smoother morphology but yet with significantly higher roughness and inhomogeneity than for the gold–SAM substrate (cf. Table I). CA and SFM data, along with the stability of the functionalization under stringent aqueous washing conditions, suggested that a complete coverage of the gold–SAM substrate with covalently grafted poly-MBAA had been achieved at a monomer concentration of 15 g/L.

Another SFM visualization of the samples with low surface coverage revealed a remarkable microscopic pattern of the grafted polymer because a dendritic structure was observed (see Fig. 3). Considering the increase in the mean height difference in comparison with that of the gold–SAM substrate (cf. Table I), we concluded that the polymer thickness in the *z* direction was between 10 and 20 nm. However, the lateral size of the features, with diameters of up to about 100 nm (cf. Fig. 3), that were assigned to grafted poly-MBAA was significantly beyond the dimensions of the monomer building blocks. On the other hand, no larger aggregates, with dimensions in the micrometer range, were seen. This pattern was different from the surface topologies seen for the grafting-from functionalizations of various substrates with many different monofunctional monomers. With low surface coverage, either single polymer coils (mushroom regime) or grafted chain aggregates were observed by SFM; with high surface coverage, more or less well-defined polymer brush structures were achieved.[10,13] With adsorbed BP on spin-coated films of relatively hydrophobic polysulfone, we studied grafting-from syntheses with the monofunctional monomer acrylic acid by SFM, and we found that with increasing UV irradiation time, both the number and size of the grafted polymer spots increased, a fully covered surface was finally yielded.[20] That is, besides the ideally expected increase in the grafting density, a nucleation and growth mechanism was involved. That was explained by a higher probability for starting and growing a grafted chain from the hydrophilic monomer on a hydrophobic substrate in an already hydrophilized surface area (this probability may even be enhanced via initiation by dissolved BP). Hence, the growth of the grafted poly-MBAA seemed to proceed via a pronounced branching of the polymer chains, which was due to the second double bond at each added repeating unit of the polymer chain. High rates for polymer chain growth and branching reactions at the surface resulted in a limitation by monomer diffusion to the surface that could, in analogy to crystallization,[21] cause dendritic growth of the crosslinked polymer. For the very early stage of surface coverage, the position of the dendrite core could be defined by the position of the reacted PI. The core density was proportional to the adsorbed amount of BP on the SAM surface. Finally, the substrate surface was covered by a covalently attached and highly crosslinked polyacrylamide. On the basis of the discussed assumptions for the layer morphology from SFM and the grafting mechanism, the grafted layer thickness should have been between 10 and 20 nm.

SPR is an excellent method for *in situ* and online measurements of biomolecular binding processes on surfaces.[17] If the thickness and composition, that is, refractive index, of the interacting layers are identical,

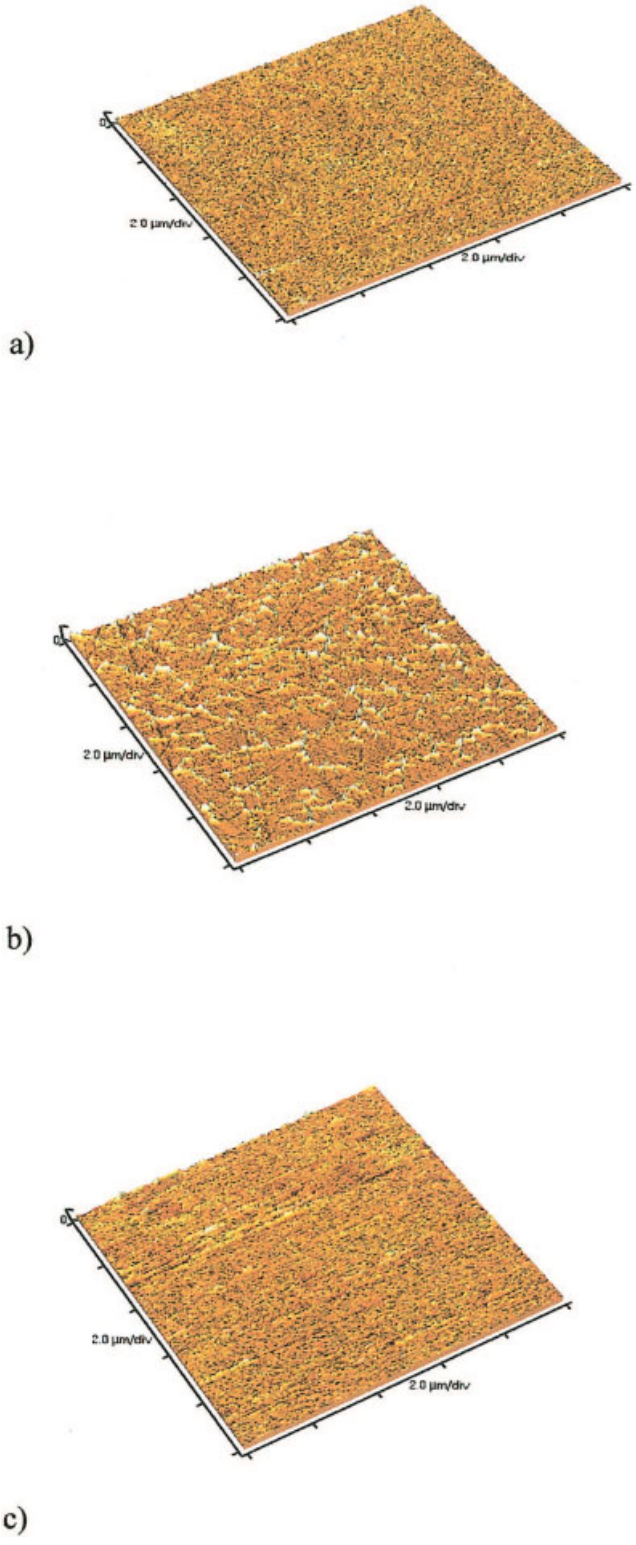

Figure 2 SFM micrographs of different stages of the functionalization of a gold surface (SPR sensor disc, Xantec): (a) gold–SAM (HdSH; *z* axis, 4.0 nm/div); (b) grafted poly-MBAA, 6 g/L (*z* axis, 20 nm/div); and (c) grafted poly-MBAA, 15 g/L (*z* axis, 10 nm/div). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

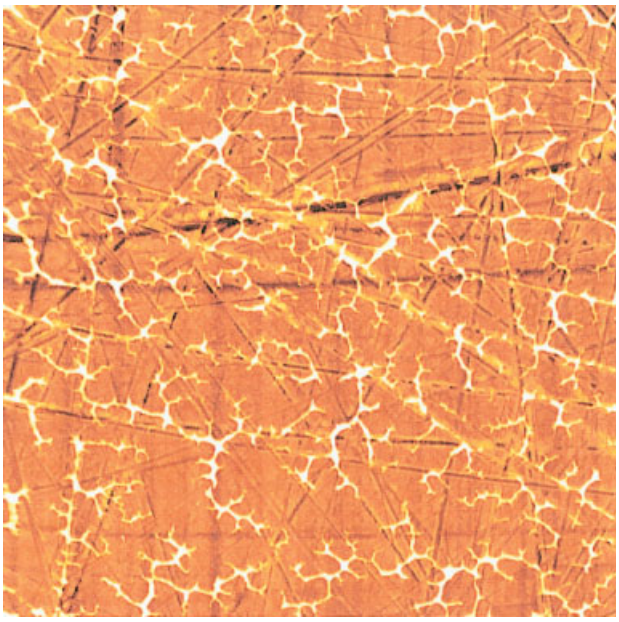

Figure 3 SFM micrograph (SPR sensor disc) of gold–SAM/grafted poly-MBAA (6 g/L, 10 μm $\times$ 10 μm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the SPR responses of different sensors are proportional to the amount bound to/in that layer, that is, to the change in the refractive index in the probe depth.[22] Obviously, the various modification steps changed the layer structure (cf. Fig. 1), but the added layer thickness should not have exceeded approximately 20 nm (as previously discussed). This was far below the SPR probe depth, which under the used conditions (670 nm, aqueous solution) would be about 120 nm (cf. ref. 22). Consequently, the various responses of the sensor to changes in the solution refractive index (e.g., a change from water to a buffer) were, within the range of error, identical for all the samples. Therefore, all data are discussed under the assumption that the sensitivity of the SPR measurements of protein adsorption was the same for all four layers.

Typical results for protein binding to the various modified sensors were measured by SPR with BSA, a serum protein commonly used as a model protein (see Fig. 4). The BSA concentration was selected so that an effective saturation of the surface could be expected, but it was not so high that BSA in the bulk of the solution could significantly interfere with the detection of the BSA adsorption on the surface (cf. refs. 17 and 22–24). A summary of all data revealed the different properties of the various sensor layers with respect to the saturated amount of BSA on the surface and to the percentage of weakly bound BSA that could be washed off from the saturated surface simply with a buffer (see Table II). The differences between the different sensors were striking. For the gold surface,

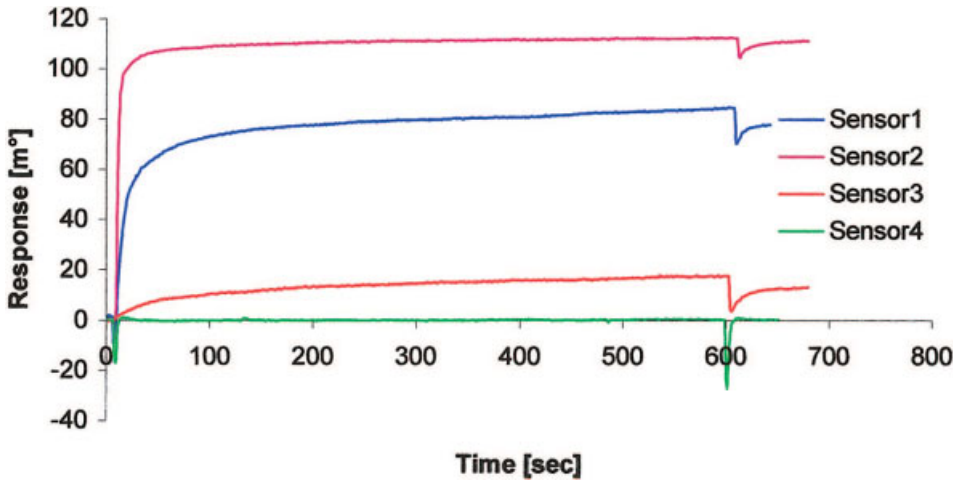

Figure 4 SPR experiments with the following subsequent steps: equilibration with a saline phosphate buffer (pH 7.2), BSA addition (0.5 g/L in a saline phosphate buffer, pH 7.2), and exchange to a saline phosphate buffer (pH 7.2) after 600 s: (a) gold (sensor 1); (b) gold–SAM (sensor 2); (c) grafted poly-MBAA, 6 g/L (sensor 3); and (d) grafted poly-MBAA, 15 g/L (sensor 4). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

fast BSA adsorption was observed, yielding more than 90% of the saturated values in less than 1 min; this was followed by a slower further increase of the total bound amount. Through flushing with a buffer, a small fraction (weakly adsorbed BSA) was washed off. For the gold–SAM surface, the BSA adsorption was even faster, and it led within a few minutes to a stable value that was almost unchanged after the washing with the buffer. In contrast, the BSA adsorption to grafted poly-MBAA with low surface coverage was much slower and led to a significantly lower plateau value; it was also partially reversible. Most remarkably, grafted poly-MBAA with complete surface coverage showed very little BSA adsorption. These very small SPR response values were close to the detection limit, and so the weakly bound fraction could not be determined accurately enough. These interesting properties remained unchanged after repeated exposure to protein solutions or, at least for several weeks, after storage in aqueous buffer solutions. Therefore, the polymer layers were compatible with the SPR method, and the results also proved that the SAM–gold surface was fully covered by grafted poly-MBAA.

BSA is known to adsorb from aqueous solutions to a variety of surfaces into a nearly densely packed monolayer, which results in a surface density of about 250 ng/cm^2.[22] The adsorption of BSA onto gold or gold–SAM had already been measured with SPR,[22–24] and a model-based quantification of obtained SPR response data on a hydrophobic gold–SAM had yielded a value of 170 ng/cm^2.[22] The higher values for gold–SAM in comparison with those for gold, and the much higher percentage of strong BSA binding on gold–SAM than on gold (cf. Table II), could be attributed to the higher hydrophobicity of the surface and to hydrophobic interactions as the main driving force for adsorption (cf. Table I). The reduction of BSA adsorption on incompletely covered grafted poly-MBAA (cf. Fig. 3) was rather large, and the percentage of weakly bound BSA was the highest. This could be explained by a pronounced swelling of poly-MBAA

TABLE II
SPR Responses and Relative Protein Amounts on the Sensor Surfaces from *in situ* Monitoring of BSA Adsorption[a]

	SPR response (mdegree)			
	Plateau value after BSA injection	Plateau value after buffer injection	Total BSA adsorption with respect to gold–SAM (%)	Weakly adsorbed BSA washed off with buffer (%)
Gold	84.4	78.1	75	8
Gold–SAM (HdSH)	112.6	110.8	100	1
Grafted poly-MBAA, 6 g/L	17.6	12.4	16	29
Grafted poly-MBAA, 15 g/L	0.23	0.22	0.2	<5

mdegree, millidegree.
[a] 0.5 g/L in a Saline Phosphate Buffer, pH 7.2.

causing much more effective shielding of the hydrophobic substrate than one would expect from CA and SFM data. Finally, on the basis of these quantifications, the amount of adsorbed BSA on the sensor surface fully covered with thin and highly crosslinked poly-MBAA was less than 0.5 ng/cm^2. These protein-resistant properties were similar to or even better than what was obtained with well-established poly(ethylene oxide) or oligoethylene oxide based coatings prepared with SAMs of amphiphilic thiols with oligoethylene oxide end groups on gold[2,3,23] or via the adsorption of amphiphilic poly(ethylene oxide) triblock copolymers (Pluronics).[24] The first approach requires significant synthetic efforts, whereas for the latter, the instability of the adsorbed layer, that is, the desorption of the surfactant, should be considered.[24]

A recent survey of the structure–property relationships of SAM surfaces that resist the adsorption of proteins indicated that such surfaces should be hydrophilic and neutral, and they should not contain hydrogen-bond donor groups.[3,5] Therefore, it is most remarkable that a surface based on or containing amide groups (—CO—NH—) has such a very low protein adsorption tendency. Further investigations will reveal whether this attractive feature is true for all groups of proteins and if this property can be directly related to the proposed highly crosslinked polymer structure.

CONCLUSIONS

The physical adsorption of a hydrogen-abstraction PI provides a simple and effective method for initiating graft copolymerization on an alkyl thiol monolayer. With a bifunctional acrylamide monomer, a thin, grafted, and highly crosslinked network layer can be prepared that can effectively resist protein adsorption. Moreover, this approach has been demonstrated to be applicable for creating sensors based on gold layers or electrodes. The number of residual double bonds in the layers and possibilities for their further functionalization are under investigation. Syntheses and properties of such thin and highly crosslinked films are of fundamental interest for the further development of receptor layers using immobilized biomolecules or based on fully synthetic MIPs. Therefore, the results of this study may lead to various interesting applications, especially in the life sciences.

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