Grafting of Polypeptides on Solid Substrates by Initiation of N-Carboxyanhydride Polymerization by Amino-Terminated Self-Assembled Monolayers

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A series of mixed self-assembled monolayers of functionalized (Br(CH2)11SiCl3) and unfunctionalized (CH3(CH2)10SiCl3) alkyltrichlorosilanes of different compositions have been prepared on bulk silicon substrates. By in situ modification of these monolayers the bromo end groups were transformed to amino end groups as shown by X-ray photoelectron spectroscopy measurements. The change of hydrophilicity was monitored by water contact angle measurements, showing the expected decrease of contact angles with increase amino group content on the surface. These substrates were used to initiate N-carboxyanhydrides in dioxane to yield α-helical polypeptides grafted from the surface. The thicknesses of the obtained polymer layers were measured with ellipsometry and X-ray reflectometry. Fourier transform infrared measurements confirm that the grafted polymers are in the α-helical conformation.

Introduction

The tethering of polymers to surfaces has gained increasing interest in recent years. The motivation for this arises not only from fundamental research but also for practical reasons. The combination of the properties of a supporting material and a polymer or rather the fixation of the polymer properties on a substrate is promising for a wide variety of possible applications.5 Due to the large number of polymer systems and substrate materials available, the field of possible applications reaches from covalent tethering of polystyrene1,2 to adhesive tethering of block copolymers.3

The tethering of rigid rodlike synthetic polypeptides like poly(benzyl L-glutamate) (PBLG) to silicon is of special interest, as manifested by several publications.4-8 These rigid rodlike macromolecules differ from polymers with flexible secondary structure resulting in some unique characteristics. For example, PBLG, due to its anisotropic molecular shape arising from its α-helical structure, shows a large dipole moment of 8000 D along the molecular axis, as well as a high hyperpolarization β of 5 × 10−28 esu for Mw = 500,000 g/mol.9 Therefore, it is a promising material for application as a novel orienting layer in liquid crystal displays.8 Furthermore, a nonlinear optical response like second harmonic generation (SHG) can be expected if a monomolecular polylglutamate film has an unidirectional orientation of the polymer backbones.10 An approach to such an unidirectional orientation of the polypeptide helices is covalent attachment of one of the end groups to the substrate with a high grafting density (Figure 1) as first described by Sano et al.5 and named chemical reaction alignment (CRA).

There are two ways to achieve grafting: (1) initiation of the monomer N-carboxyanhydride (NCA) polymerization by the surface (grafting from);6,7 (2) coupling of preformed polymers to the surface (grafting onto).4,5 Regarding the initiation from the surface, one must consider that two mechanisms of NCA polymerization are competing depending on the initiator used. The activated monomer (a.m.) mechanism is initiated by bases and the
amine or protic mechanism initiated by primary amines. Exclusively with the latter the initiator is inserted into the growing polymer chain. Therefore, it is necessary to establish primary amino groups on the surface to graft a polymer from the surface. On the other hand primary amines can also act as a base during initiation leading to nongrafted polymer by a change of mechanism. The greater the steric hindrance of the primary amines sites on the surface, the more the undesired a.m. mechanism is preferred. Taking into account that a polymerization from a surface runs under demanding steric conditions, it is obvious that the initiator layer should have a low surface roughness and a distribution of initiator sites as perfect as possible. The coupling of preformed polymers again requires reactive sites either on the surface or at the polymer terminus which can couple to certain groups of the other component.

The grafting of polyglutamates from a surface has been reported for a case using spherical substrates (Aerosil A200V, carbon black) modified with (aminopropyl)-trimethoxysilane (APS) as initiator on the surface. Although the tethering onto flat substrates presents an even greater challenge because of the lower surface area, resulting in a smaller amount of initiator or reactive sites, it has been carried out by different groups following one of the two ways mentioned above. Enrique et al. for example, used a self-assembling technique of PBLG containing a disulfide moiety attached at its N-terminus onto gold (grafting onto). Coupling of PBLG to silicon modified with (N-(2-aminomethyl)-3-aminopropyl)methyldimethoxysilane was done by Sano et al. with dicyclohexylcarbodiimide. Whitesell et al. first described a “grafting from” procedure for polypeptides on flat surfaces. They used indium–tin oxide modified with APS or a special designed initiator on a gold surface to initiate a NCA polymerization. The latter initiator was designed to fit the surface requirements of the polypeptide helix and therefore reduce steric hindrances. Wieringa and Schouten grafted PBLG and poly(methyl-L-glutamate) from silicon modified with APS or (4-aminobutyl)-dimethyldimethoxysilane using a solution or a melt of the monomers, respectively.

Figure 1. Schematic of an aligned polyglutamate monolayer.

In the present paper we describe our results from polymerization of benzyl-L-glutamate–NCA (Figure 2) using mixed self-assembled monolayers (SAMs) with various concentrations of amino end groups. In contrast to APS, long chain alkyltrichlorosilanes give defined and reproducible monolayers. While thiol-sulfonic acid monolayers on gold due to the exclusive reaction of the thiol group with the gold atoms, silanes form a two-dimensional network at the surface which is tethered to the substrate by some covalent bonds. Nevertheless the monolayers formed by silanes are very robust.

The alkyl chain spacer provides stabilization of the molecular assembly through interchain van der Waals interaction. The order within the monolayer can, therefore, be adjusted to some extent by the length of the alkyl chain or the temperature used for the silanization process. To produce monolayers with active sites, terminal functional groups have to be present in the silanization agent. These terminal groups must not compete with the surface active headgroup for adsorption to the surface or react with it. For this reason amino and trichlorosilyl groups are not compatible. Amino-terminated long alkyl chain trimethoxysilanes are stable but give disordered layers due to the chemical interactions of the amino groups with the substrate. Following Balachander and Sukenik amino-terminated monolayers can be obtained by deposition of bromo-terminated alkyltrichlorosilane and subsequent in situ modification on the surface. In order to reduce steric hindrances for the polymerization reaction, it is favorable to adjust the density of initiating groups at the surface. According to Whitesell et al. this can be done by use of special designed initiator molecules. We choose a more facile way to accomplish this: By preparation of mixed monolayers from functionalized (Br end group) and nonfunctionalized (CH3–end group) undecyltrichlorosilane in different ratios, the amino groups can be diluted at the surface. To the best of our knowledge mixed silane monolayers of this kind have not been previously described. Frank and co-workers reported mixed, amino-terminated SAMs prepared by backfilling (preparation of the SAM in two steps).

**Experimental Section**

**Materials.** Unless otherwise stated all reagents and chemicals were obtained commercially. Dioxane was distilled from sodium. Hexadecane (Alrich) was purified by percolation twice


(22) B. Balachander, N.; Sukenik, C. N. Langmuir 1990, 6, 1621.


through neutral alumina (ICN, 32-63) and used immediately. Triethylamine (Fluka) was distilled from calcium hydride. Acetone (Riedel-de Haen, 99.5%), dichloromethane (Fluka), 2-propanol (Fluka), 1-undecene (Aldrich), undecyl-10-enyl bromide (Lancaster), trichlorosilane (Aldrich), hydrogen hexachloroplatinate(VI) hexahydrate (Johnson Matthey), sodium azide (Aldrich), hydrogen peroxide, sulfuric acid, and triethylamine (Fluka) were used as received.

**Surfactant Synthesis.** 1-Bromo-11-(trichlorosilyl)undecane. Undecyl-10-enyl bromide (12.5 g, 60 mmol) was added to an argon-purged round bottom flask containing approximately 5 mg of hydrogen hexachloroplatinate(VI) hexahydrate in 0.1 mL 2-propanol. A 2 – 3 mL excess of trichlorosilane (10 mL, 150 mmol) was added with stirring. The flask was fitted with a water-cooled condenser, and the mixture was refluxed for 2 h under argon. The excess trichlorosilane was removed by short-path distillation. The residue was purified by Kugelrohr distillation, using a commercially available apparatus (BUCHI GKR-51). The distillation was carried out at 0.01 – 0.02 mbar and ca. 120 – 130 °C.**25** 1H NMR (80 MHz, CDCl3) δ (ppm): 1.0 – 1.65 (m, 18H), 1.70 – 2.0 (m, 2H), 3.3 – 3.5 (t, 2H).

1-Trichlorosilylundecane. 1-Undecene was converted into 1-trichlorosilylundecane by using the above hydrolysis procedure and isolation by Kugelrohr distillation, using a commercially available apparatus (BUCHI GKR-51). The distillation was carried out at 0.01 – 0.02 mbar and ca. 100 °C.**25** 1H NMR (80 MHz, CDCl3) δ (ppm): 0.7 – 1.0 (t, 3H), 1.1 – 1.6 (m, 20H).

**Substrate Preparation.** Silicon wafers (Wacker Chemtronics, both sides polished) with a native oxide layer of 15 – 16 Å thickness were cut to measure 2.5 cm × 6 cm. Substrates were wiped with an acetone-soaked cotton swap, successively sonicated in soap water, rinsed with large amounts of distilled water, and sonicated in dichloromethane. Substrates were then placed in freshly prepared “piranha” solution (70/30 (v/v), concentrated sulfuric acid, 30% hydrogen peroxide) for 1 h to prepare the substrates for further use. Freshly prepared “piranha” solution (70/30 (v/v), concentrated sulfuric acid, 30% hydrogen peroxide) for 1 h at room temperature for 16 h. SAMs were placed in 5% HCl solution for 5 h to complete hydrolysis of the aluminum complexes, rinsed with distilled water, wiped with acetone, and placed in triethylamine for 24 h in order to convert the –NH2 into –NH3+ groups.

**Polymerization.** Benzyl L-glutamate and benzyl L-glutamate–NCA were synthesized according to Block.**28** Amino-terminated SAMs were placed in a 2 – 3% solution of ω-benzyl L-glutamate NCA in dry dioxane under argon at room temperature for 14 days. To remove ungrafted material, the wafers were rinsed with THF in a Soxhlet apparatus for a further 3 days.

**Ellipsometry.** Measurements were carried out with a self-built automatic null ellipsoideter at λ = 632.8 Å and an angle of incidence of 70° using a three-layer step-profile model assuming fixed refractive indices of 1.48 for the SAMs and 1.5 for the polymer layers on top of the native SiO2 layer. This leads to uncertainties of 5 – 10% in the calculated layer thickness.

**Contact Angles.** The advancing and receding contact angles of water on the SAMs and polymer films were measured with a tilting stage method (50°) at room temperature using a Kruß G1 contact angle goniometer mounted on a homemade tilting base. Humidity was controlled to be 100% by keeping the walls of the goniometer filled with water. Receding and advancing angles were measured on three different drops and results were averaged.

**X-ray Reflectometry.** X-ray reflectometry (XR) measurements were performed using Cu Kα radiation (λ = 0.154 nm) from a Rigaku 12 kW rotating anode with a line focus. The reflectometer was designed around Huber goniometers and optical components from BLake Industries, including a grating primary monochromator to a constant wavelength resolution of Δλ/λ = 0.015. Angular divergence was constant at about 2 × 10−4 radians. All measurements, typically lasting several hours, were performed in air at room temperature. Data sets were corrected for background estimated using measurements with the detector offset from the specular condition.

The XR data were analyzed by assuming a reasonable initial model for the scattering length density profile through the film, calculating the reflectivity curve that such a model would give, and then varying model parameters until acceptable agreement between the experimental and calculated model reflectivity was achieved. The model scattering length density profiles were created by first forming a simple model of four distinct layers, PBLG, SAM, SiO2, and silicon. Then the interfaces between layers were broadened by convoluting the sharp interface with a Gaussian function to yield diffuse interfaces characterized by the root mean square roughnesses reported in Table 1. Constraints on appropriate values for the scattering length density and roughness parameters for the Si, SiO2, and SAM are well-known from other work in this research group.**22** Therefore, these parameters can only vary within narrow limits. The primary parameters for “fitting” the data are those associated with the PBLG layer.

**Fourier Transform IR (FT-IR).** FT-IR spectra were recorded on a Mattson Galaxy 6020 spectrometer in transmission mode with a pure silicon wafer as a reference. The sample chamber was purged with dry nitrogen before and during measurements. The resolution was set at 4 cm−1 and 256 scans were recorded.

**X-ray Photoelectron Spectroscopy (XPS).** The XPS measurements were performed with a X-Probe 300 (Surface Science Instruments) instrument using monochromatic Al Kα radiation with an energy of 1486.6 eV. Each sample was measured with an experimental resolution of 1.8 eV and a take-off angle of 45°. Charge correction was calculated from the difference of the observed C(1s) binding energy to the calculated value of 284.8 eV. The N(1s) peak was assigned according to ref 30. The compositions were obtained by fitting the peak area using a Gaussian function after subtraction of the background. The atomic percentages (%) for the SAM were obtained by correcting the data using XPS sensitivity factors given by the instrument manufacturer (C, 1.0000; N, 1.6783; O, 2.4953).

**In Situ Modifications.** Azide-Terminated SAMs. Bromide-terminated SAMs were placed in a supersaturated solution of NaN3 in dry DMF (1.5 g on 100 mL). The solution (together with the undissolved NaN3) was stirred at room temperature. After 24 h the SAMs were rinsed with distilled water and wiped with an acetone-soaked cotton swap.**27**

Amino-Terminated SAMs. The azide-terminated SAMs were placed in lithium aluminum hydride solution (0.2 M in THF). After 24 h the SAMs were soaked in THF for an additional 24 h. SAMs were then placed in 5% HCI solution for 5 h to completely hydrolysis of the aluminum complexes, rinsed with distilled water, wiped with acetone, and placed in triethylamine for 24 h in order to convert the –NH2 into –NH3+ groups.

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difficulties in the original protocol have been corrected.22b, c have been followed by me means of X-ray reflectometry, and surface. Furthermore, the preparation and modification proven that this procedure yields amino groups at the surface according to the method of Balachander and Sukenik22 the bromo end group was displaced by azide. 

The azide was reduced with lithium aluminum hydride for the hydrophilicity of the surface. This is in particular valid for the concentration of functional groups, reflecting the higher contact angles are decreasing with increasing concentration of the contact angles (water) on SAMs.33b,34 Other polar groups are more hydrophilic when they are in the polar environment provided by methyl groups than when the surface of the monolayer is composed largely of other polar groups.35 The nonlinear dependence of the contact angles on the composition of the solution can also be explained because it may be due to a preferential absorption of the bromine-terminated trichlorosilane. Such a preferred absorption in the case of slightly different terminal groups has been found by Whitesides and co-workers for thiol with different terminal groups.35b,36

To verify the in situ modification of the monolayers XPS measurements are under way. Preliminary results of these investigations are shown in Figure 5. A N(1s) peak is found in the XPS spectra of the amino-terminated SAMs which is not present in the Br-terminated SAMs. The atomic ratio N/C for the SAM after in situ conversion increases with increasing amount of Br-terminated silane in the silylation mixture. The nonlinear dependence of the ratio N/C on the composition of the silylation mixture may be an indication of incomplete conversion of the Br end groups into amino groups or a preferred adsorption of the BR-terminated trichlorosilane from the silylation mixture. Curve A in Figure 5 approximates how the atomic ratio N/C would change with the silylation mixture composition if the layer deposited at 20% really had 20% NH2 functionality and complete conversions were achieved for all in situ reactions. The discrepancy between curve A and the measured ratios is quite high and this suggests that the alternative explanation may be the dominant one, although no doubt the in situ conversions do not have perfect yields. It is possible that Br-terminated silanes are preferentially adsorbed from the deposition mixture as has been found by Whitesides and co-workers for thiols on gold.35b,36 In this case, if the yields of the in situ conversions were close to 100%, then the measured atomic ratios should lie above a linear behavior, approximated by curve B in Figure 5, expected for nonpref-

(31) Duevel, R. V.; Corn, R. M. Anal. Chem. 1992, 64, 337. (32) For better clarity only advancing contact angles are shown. Hysteresis is about 8° for all samples.
erential adsorption followed by complete conversion. A combination of both mechanisms is possible and cannot be ruled out at present. More detailed XPS measurements are under way to address this. In any case, the present data clearly indicate that the SAMs do have amino functionality after in situ conversion and that the amount of this functionality increases substantially, though not linearly, with the fraction of Br-terminated silanes in the silylating mixture.

To verify layer thickness, ellipsometry measurements were carried out, giving values of 19 ± 2 Å for all samples. Taking into account that the calculated thickness for a complete SAM layer with fully extended alkyl chains is 17 Å, the measured values are in strong support of a monolayer coverage.

The preliminary results of X-ray reflectometry on the Br-terminated SAMs are in accord with a monolayer coverage, too, but also reflect the aforementioned features of silane SAMs. The fitting of the reflectivity curves requires the assumption of an interfacial layer between the SiO₅ surface and the alkyl chains as first proposed by Tidwell et al. This layer can be ascribed to the two-dimensional polysiloxane network with some adsorbed water. The thickness of this layer is 6.6 ± 1 Å in the case of a 100%Br-terminated SAM. The thickness of the layer of the alkyl chain (with the Br end group) is determined to be 15.6 ± 1 Å, which is in very good agreement with the theoretical value of 15.74 Å. The difference between the thickness of the complete SAM as determined by ellipsometry and from X-ray reflectivity is due to the different sensitivity to the interfacial layer. Concluding, both X-ray reflectivity and ellipsometry reveal a monolayer coverage.

**Polymerizations.** Polymerizations were carried out by immersing the wafers in a solution of BLG-NCA in dioxane. After being withdrawn from the polymerization solution and cleaned, the wafers with amino-terminated SAMs have different contact angles, while the contact angles for wafers with CH₃-terminated SAM remained unchanged (Figure 6). This indicates that the change in the contact angles is due to polymer on the surface. The variation of the contact angles as a function of the amino group concentration on the initiating surface is complex. This may be due to different orientations of the peptide chains (see Figure 6, dotted line). If the chains are lying flat on the surface, they expose the nonpolar side groups to the gas phase. If they are standing upright, they orient the more polar amino end groups to the gas phase. However, a deeper discussion of contact angles is difficult since the exact orientation is unknown so far. On the other hand the data also may be interpreted as scattering around a "constant" value, since the differences are just beyond the experimental error of the contact angle determination itself (see Figure 6, broken line).

The thickness of the polymer layer was determined by ellipsometry and X-ray reflectometry (XR) (see Table 1). Figure 7 shows a typical XR measurement with experimental data and fitted curve. The evaluated scattering density profile of the layer is shown in the insert.

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Almost all thicknesses obtained by XR are slightly higher than the values from ellipsometry, but in general there is a good agreement. The thickness of the polymer layer ranges from 45 Å for the sample with 20% amino groups to a maximum of 65 Å for the wafer with 40% amino groups after polymerization.
aminogroups. If one assumes a perpendicular orientation of the polyglutamates, a thickness of 65 Å corresponds to an average degree of polymerization of 42.\(^\text{38}\) Whether the initiator concentration of 40% at the surface represents an optimal concentration for the graft polymerization must be confirmed by further investigations. However, also the roughness of this sample is lower than for most of the other samples (Table 1). In particular the 20% sample shows not only the lowest thickness but also the highest roughness. This suggests that at least a certain concentration of initiators on the surface is required. Nevertheless, as long as the orientation of the polymers is unknown, an interpretation of the roughness values with respect to the orientation is speculative.

The conformation of the grafted PBLG can be determined by IR spectroscopy from the position of the amide peaks.\(^\text{7}\) Compared to the amine and the ester peaks the position of both amide I and amide II peak is depending on whether the PBLG is in the \(\beta\)-sheet or in the \(\alpha\)-helical conformation. For the \(\alpha\)-helix conformation the amide I peak is located at about 1650 cm\(^{-1}\) and the amide II peak at about 1546 cm\(^{-1}\), whereas for the \(\beta\)-sheet conformation the amide I peak is located at about 1630 and at 1530 cm\(^{-1}\) for the amide II peak, respectively.\(^\text{28}\) As an example the IR spectrum of the sample polymerized on the surface with 100% amino groups in Figure 8 shows only peaks for PBLG in the helical conformation with no detectable amount of \(\beta\)-sheet material.

### Conclusions

Experiments were carried out to clarify whether the grafting of poly(benzyl L-glutamate) from flat surfaces can be improved by adjusting the density of initiator sites at the surface in order to meet the steric requirements of the polymerization reaction. This adjustment was done by simultaneous adsorption of Br- and CH\(_3\)-terminated alkyltrichlorosilanes on silicon wafers resulting in mixed monolayers and subsequent in situ modification of the bromide into amino groups (–NH\(_2\)), which are the initiator sites. The formation of monolayers was confirmed by ellipsometry and X-ray reflection. The modification was done by an established procedure and verified by XPS. The increase in surface polarity encountered with this modification was followed by contact angle measurements. In addition, the gradation of amino group concentration at the surface was qualitatively confirmed by contact angle and XPS measurements. However, so far we have no information about the exact concentration and lateral distribution of the initiator sites.

The results of the polymerization with regard to the thickness and the roughness of the polymer layer varied with the concentration of initiator sites on the surface: A minimum concentration was required to obtain a certain thickness and minimum roughness. Beyond a certain concentration, the polymer layer thickness remained constant while its roughness increased. FT-IR measurements revealed, that all grafted polymers are in the \(\alpha\)-helical conformation. An interpretation of the change of contact angles after polymerization and a deeper discussion of the roughness values will follow after investigation of the polymer orientation.

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