

Surface Grafting of Poly(L-glutamates). 1. Synthesis and Characterization

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The ring-opening polymerization of *N*-carboxyanhydrides (NCA) of γ -benzyl L-glutamate and γ -methyl L-glutamate from (γ -aminopropyl)triethoxysilane (APS) pretreated substrates such as silicon wafers and quartz slides was investigated. FT-IR transmission spectroscopy, circular dichroism measurements, and UV/vis spectroscopy confirmed the pure α -helix conformation of the grafted polypeptide layers. FT-IR spectroscopy also showed that the most important part of the polymer growth took place in the first 5 h of the polymerization. The average orientation of the rather rigid α -helical polypeptides, grown during a short period of time, was more perpendicular with respect to the substrate than the orientation of the polymers grown over a longer reaction time. For concentrations up to 2.0 M, the polymer growth from both NCA monomers showed a pronounced dependence on the monomer concentration. Moreover, it appeared that the higher the monomer concentration, the more perpendicular the average orientation of the helices with respect to the substrate. The thickness of the grafted polypeptide layers up to 400 Å was determined with ellipsometry and small-angle X-ray reflection measurements. The absence of chemical chain termination was demonstrated by additional polymer growth in a renewed polymerization.

Introduction

The surface grafting of polymers in general has been studied intensively during the past decades because of its great importance for improving adhesion in composites,¹ dispersion of pigments,² for biomedical applications of polymers,³ chromatography,⁴ and wettability of fibers.⁵

Among the different methods for attaching polymers to surfaces, surface grafting by initiating a polymerization on the surface through an immobilized initiator has drawn relatively little attention. This method, however, can lead to higher grafting densities than other available techniques. It requires the immobilization of an initiator that will be incorporated in the chain after initiation, a high initiating efficiency, and polymerization conditions under which the polymer chains remain dissolved during the whole polymerization process.

Especially interesting in this respect is the preparation of functional thin films using the surface-grafting process, and particularly the surface grafting of α -helical synthetic polypeptides. In this respect, the use of L-glutamic acid derivatives is quite interesting, and has been studied quite well.^{6–10} The unique features of unidirectional aligned large dipoles of the stable right-handed α -helical polypeptides, as indicated by Hol,¹¹ and experimentally confirmed by Jaworek et al.,¹² in combination with flat silicon or metal substrates that can be used as electrodes, and the possibility of tuning the polymer film properties

by functionalization of the glutamic acid monomer unit, might lead to promising new applications in the fields of optoelectronic devices¹³ and liquid crystal displays.¹⁴

In general, two different methods have been developed to obtain chemically grafted, highly ordered polypeptide films. The first approach, the so-called "grafting-onto" method^{14–21} uses well-characterized preformed polymers. The end groups of the polymer chains react with active, specially designed surface groups. When the grafting density is high enough, the helices stretch themselves away from the surface and take on a more or less perpendicular orientation with respect to the surface.²²

A large disadvantage of this "grafting-to" method is the relatively low surface coverage. Due to steric hindrance

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by neighboring helices and unfavorable dipolar interactions between already grafted and still nongrafted helices, not all grafting sites can be reached and, therefore, holes will be formed in the film. It is also possible that during the grafting process nongrafted helices diffuse into the film in an antiparallel way to cancel the dipole moment. When these films are washed in the proper way, these ungrafted helices will be removed from the film. This will result in a much smaller angle of the remaining grafted helices with respect to the substrate. Another drawback of this system is that the layer thickness is limited by the molecular weight of the used polymers.

To overcome these disadvantages some authors tried the so-called "grafting-from" method.^{12,23–29} This method uses relatively small monomers, which can easily reach the initiator sites on the substrate. So, these polymers actually grow from the substrates. Although the determination of the molecular weight of these polymer chains is much more difficult than for the grafting-onto method, these grafted layers are expected to have much higher grafting densities. Also a broad molecular weight distribution can be expected since not all chains will grow to the same length.

For example, Whitesell^{23,24} published the polymerization of L-alanine and L-phenylalanine *N*-carboxyanhydrides (NCAs), initiated by specially designed aminotrithiol-functionalized gold and ITO substrates. This initiator molecule exactly meets the spatial requirements of an α -helix. They have prepared polypeptide films of about 1000 Å in thickness, with α -helices oriented more or less perpendicular to the substrate.

Simultaneously Oosterling et al.^{25,26} studied the surface grafting, conformation, and orientation of various (co)-polyglutamates and (co)polyaspartates polymerized in solution from (γ -aminopropyl)triethoxysilane (APS) coated Aerosil, glass slides, and silicon wafers.

Heise et al.²⁷ used mixed amine monolayers of long aliphatic trichlorosilanes produced by the SAM technique in order to control the initiator surface concentration and studied the effects of grafting density on the orientation of the helices. The thin polypeptide layers, with a thickness of less than 150 Å, prove how delicate these polymerizations are. Inert reaction conditions, purity of the monomers, and dryness of the solvents require special attention.

Chang and Frank²⁸ studied three different approaches: The grafting-onto reaction between the N-terminus of poly-(γ -benzyl glutamate) (PBLG) molecules with preformed surface chloroformate groups results in low grafting densities with the PBLG molecular axis parallel to the surface. In the second method, the triethoxysilane headgroups of the PBLG molecules initiated by (γ -aminopropyl)triethoxysilane lead to intermolecular cross-linking in the solution prior to the surface condensation reaction, and a film thickness of 50 Å. Chain hindrance effects in the grafting-from method of polymerizing γ -benzyl glutamate *N*-carboxyanhydride from immobilized amine groups lead to rapid chain termination. Therefore, they concluded that the grafting-onto methods are more successful than the grafting-from approach in constructing surface-grafted poly(γ -benzyl glutamate) films.

We published a new method by polymerizing γ -methyl L-glutamate *N*-carboxyanhydride (MLG-NCA) in the melt,²⁹ providing the possibility of a solvent-free polymerization with a very high monomer concentration, leading to a film thickness up to 200 Å.

Chang and Frank³⁰ developed another solvent-free polymerization method. They polymerized NCA monomers with a vapor deposition technique. The layer thickness could easily be tuned by changing the temperature, pressure, and reaction time.

Very homogeneous and therefore pinhole-free polypeptide layers can be obtained using the surface-grafting polymerization of γ -benzyl L-glutamate *N*-carboxyanhydrides in solution from flat substrates such as silicon wafers and quartz slides.¹² The electromechanical properties of these thin surface-grafted polypeptide layers between crossed aluminum electrodes were studied for the first time.

This study reports on the synthetic procedures to make these thin surface-grafted layers. It also gives some new insights into what happens with the orientation of the grafted helices during the polymerization and the ways to alter this orientation by varying the reaction time and monomer concentration. Part 2 of this series³¹ will describe a method to reveal the average angle between the grafted helices within these layers and the substrate by means of FT-IR transmission analysis. Part 3 will focus on the subject of surface-grafted block co-polypeptides.³²

Experimental Section

Materials. All experiments were carried out in a dry N₂ atmosphere. The solvents (Merck p.a.) were dried and distilled before use according to standard procedures. The coupling agent (γ -aminopropyl)triethoxysilane (Acros, 99%) was vacuum distilled before use (55 °C, 0.3 mmHg). γ -Benzyl (Sigma, 99%), γ -methyl L-glutamate (Acros, 99%) and triphosgene (Acros, 99%) were used without further purification.

Various substrates were used depending on the intended characterization technique. Silicon wafers (Topsil Semiconductor Materials A/S, Frederikssund, Denmark, both sides polished, 900 μ m) were used for Fourier transform infrared (FT-IR) transmission spectroscopy, X-ray photoelectron spectroscopy (XPS), and small-angle X-ray reflectometry (SAXR) measurements. Quartz slides (Heraeus) were used for UV/vis and circular dichroism (CD) measurements.

Monomer Synthesis. Benzyl L-glutamate–NCA (BLG-NCA) was synthesized by phosgenation of γ -benzyl L-glutamate using triphosgene in anhydrous tetrahydrofuran (THF) according to the procedure published by Dorman.³³ The BLG-NCA was recrystallized from a warm mixture of THF/*n*-hexane (1/3 (v/v)). MLG-NCA was synthesized in the same way but recrystallized from warm 1,2-dichloroethane.

Substrate Preparation. The water used for cleaning was purified with a reverse-osmosis system (Elgastat spectrum SC 30) and by subsequent filtration through a Milli-Q purification system.

The quartz slides and silicon wafers used as substrates were rinsed with Milli-Q water and ultrasonically cleaned with subsequently warm (30 °C) ethanol (Merck, p.a.) and dichloromethane (Lab-Scan, p.a.). After these precleaning steps, the substrates were treated with a mixture of H₂O₂ (Merck, 30%)/NH₃ (Merck, 25%)/H₂O (1:1:5 (v/v/v)) for 25 min at 60–70 °C, washed several times with Milli-Q water, ultrasonically treated with a mixture of HCl (Merck, 37%)/H₂O (1:6 (v/v)) for 25 min,

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washed with large amounts of Milli-Q water, and again ultrasonically cleaned with methanol (Merck, p.a.), a methanol/toluene (Merck, p.a.) mixture, and toluene for 5 min. After these steps the substrates were blown dry with a strong flow of nitrogen and immediately used in the silanization procedures.

Silanization. After the wafers and slides were cleaned, they were immediately silanized with freshly distilled (γ -aminopropyl)-triethoxysilane. This silanization was performed according to the method published by Haller.³⁴

Polymerizations. The solution polymerizations were performed in anhydrous *N,N*-dimethylformamide (DMF) (Acros, p.a.) at 40 °C in specially designed glassware. The monomer solution (0.5 mol/L) was added to the silanized substrates. After the polymerizations, the films were washed with a mixture of dichloroacetic acid (Acros, p.a.) and chloroform (20/80 (v/v)) for 24 h to remove any nongrafted material and subsequently with chloroform for 1 h. The samples were dried in a vacuum before analysis. Various samples of the same silanized substrates were used for the experiments to study the polymer growth versus reaction time. These silanized samples were subjected to the same reaction mixture and withdrawn at different time increments.

Infrared Measurements. FT-IR transmission measurements were carried out on a Mattson Galaxy 6021 spectrophotometer using silicon wafers as substrates. Each spectrum is an average of 256 scans measured on five different spots per sample at a resolution of 4 cm⁻¹. The spectrum of a clean silicon wafer was used as the reference spectrum. Grazing incidence reflection spectra were recorded with a resolution of 4 cm⁻¹ on a Bruker IFS-88 equipped with a Spectra-Tech fixed-angle (80°) GIR accessory.

Calculation of the peak areas was done with the peak integration option of the Galaxy/FIRST data system software. The amide A (backbone N-H stretching), C=O ester, amide I (backbone carbonyl stretching), and amide II (mainly C-N stretching) peaks were integrated from 3400 to 3200 cm⁻¹, from 1795 to 1695 cm⁻¹, from 1695 to 1575 cm⁻¹ (after subtraction of the phenyl peak area centered at 1609 cm⁻¹ in case of PBLG), and from 1575 to 1500 cm⁻¹ (after subtraction of the phenyl peak area at 1498 cm⁻¹ in case of PBLG and the amide II_{||} peak area³⁵ at 1516 cm⁻¹ in both cases), respectively. Each peak area was integrated 10 times. The deviation from the average value was smaller than 2.5%.

X-ray Photoelectron Spectroscopy. XPS measurements were done on a X-probe 300 of Surface Science Instruments, using monochromatic Al K α radiation with an energy of 1486.6 eV and a takeoff angle of 45°. Charges were compensated and peak locations were corrected on the basis of the C(1s) signal at 285 eV. An overall scan from 0 to 1000 eV (pass energy, 150 eV) is the average of 10 scans measured with an experimental resolution of 1.8 eV. The narrow scans of C(1s), N(1s), and O(1s) (pass energy, 100 eV) are the averages of 25 scans measured at three different spots on each sample with an experimental resolution of 0.4 eV. The atomic percentages were obtained by correcting the data using XPS sensitivity factors given by the instruments manufacturer (C, 1.0000; N, 1.6783; O, 2.4935).

Small-Angle X-ray Reflectometry. SAXR measurements were performed with a Philips PW 1830 generator and a Philips PW 1820 powder diffractometer, Cu K α radiation (λ = 1.542 Å) was used in a $\theta/2\theta$ geometry with a starting angle of 0.8°. The simulations were carried out using a simulation program designed by A. Leuthe which uses a one slab model and two surface roughnesses.³⁶ The polymer film thickness was corrected for a 28 Å SiO₂ layer and a 24 Å APS layer as determined before.

UV/Vis Spectroscopy. UV/vis absorption spectra of the grafted layers on quartz slides were recorded on a SLM-Aminco 3000 diode-array UV/vis spectrophotometer. A blank substrate was used as reference.

Circular Dichroism. CD spectra of the polyglutamate layers on single quartz substrates were obtained with a Lakewood, N.J., AVIV circular dichroism spectrometer, model 62 A DS, by averaging over at least five scans.

Ellipsometry. Ellipsometry was carried out on a custom-made automatic null-ellipsometer at λ = 632.8 Å and an angle of incidence of 70°. All calculations were done with a three layer step-profile model assuming fixed refractive indices of 1.428 for the coupling layer, 1.55 for the PBLG, and 1.50 for the PMLG polymer layer on top of the native SiO₂ layer (this leads to errors of 5–10% in the calculated layer thickness) and using the same thickness for the SiO₂ and APS layers as for the SAXR measurements.

Results and Discussion

Coupling Layer. The presence of the APS coupling layer on the substrate can easily be detected by means of XPS analysis. The existence of nitrogen as indicated by the N(1s) signal at 400 eV resulting from aliphatic free amine groups clearly proves the presence of the APS coupling layer on the silicon wafer. Compared to the overall spectrum of a clean wafer which contains no nitrogen signal at all, also a decrease in signal height for the Si(2s) and Si(2p_{3/2}) peaks at 150 and 99 eV can be seen. The atomic nitrogen concentration is roughly about 15%, which means that there are still some ethoxy groups left.

To determine the surface density of amine groups on the surface, we followed the method developed by Park's group.³⁷ The amine groups of the APS molecules are converted into imines in a reaction with 4-nitrobenzaldehyde. This reaction converts the nonabsorbing amine group into an UV/vis detectable nitrobenzyl substituted imine. From the imine absorbance of 0.0167 at 287 nm, an amine density of approximately 3.2 molecules/100 Å² can be calculated. This is lower than the density of OH groups of 5.0 molecules/100 Å², as determined by Zhuravlev,³⁸ but very close to the values reported by Heiney et al.³⁹ In principle, there are more than enough amine groups available to start the polymerization, since one single PMLG helix requires 155 Å² of space (with a diameter of 14 Å⁴⁰).

SAXR measurements on silanized silicon wafers and quartz slides reveal the layer thickness and surface roughness of the aminosilane layers. The reflection curve of a clean silicon wafer is shown in Figure 1a and that of a clean quartz slide in Figure 1b. These curves can be fitted using a model of one layer on a substrate. The surface roughness of the silicon wafer is 3.5 ± 0.2 Å, and the surface roughness of the quartz slides was 3.9 ± 0.2 Å.

The reflection curves of the silanized substrates are shown in Figure 1c,d, respectively. From the Kiessig fringes a layer thickness of 24 ± 0.3 Å on silicon and 18 ± 0.2 Å on quartz can be deduced. These values agree very well with the thickness of 21 ± 3 Å obtained by means of ellipsometry and 21 ± 4 Å obtained by Kurth and Bein.⁴¹ The thickness of the coupling layer is exceeding the thickness of a monolayer. A monolayer will have a thickness of about 10 Å when the APS molecules are fully stretched.⁴¹ From our measurements it can be concluded that the coupling layer obtained with the method of Haller³⁴ consists of two to three molecular layers resulting in a substrate with very high amine concentration. The surface roughness of the aminosilane films on both substrates did not exceed 6.5 Å. Thus, relatively smooth amino silane coupling layers can be obtained on silicon wafers and quartz slides with Haller's method.

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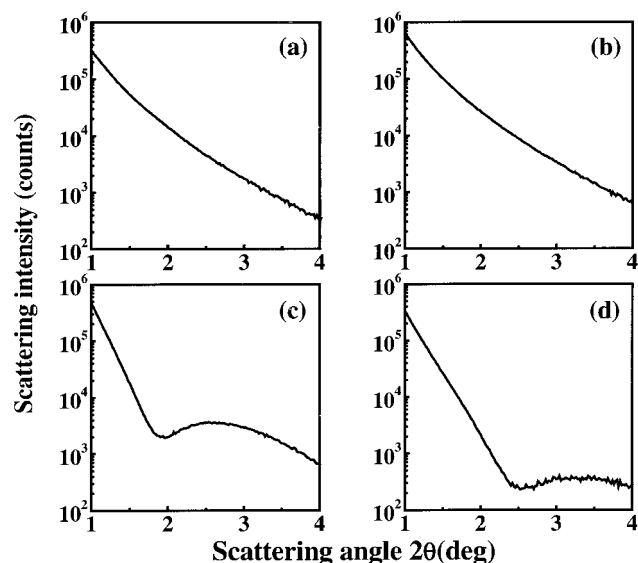
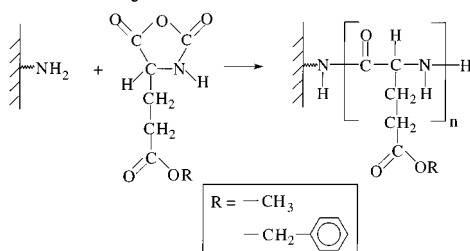


Figure 1. SAXR reflection curves of (a) a clean silicon wafer, (b) a clean quartz slide, (c) an APS silanized silicon wafer, and (d) an APS silanized quartz slide.

Scheme 1. Reaction Scheme of the Surface-Grafting Polymerization Reaction



Monomer Synthesis. To prevent water molecules from initiating the polymerization, which would lead to nongrafted polypeptide material,^{40,42} drying of the solvents is essential. To obtain highly reactive monomers, the procedure of Dorman et al. was followed.³³ Very pure NCA monomers can be synthesized by removal of the hydrogen chloride adduct and a rephosgenation step. After the polymerization, Dorman obtained polypeptides of molecular weights of about 10^6 . The polymerization degrees exceeded by far the reported value of 150 reported in the literature for polymerizations initiated by primary amines.⁴⁰ The synthesized monomers should be stored under dry N_2 atmosphere at $-20^\circ C$.

Polymerizations. The surface-grafting polymerizations from primary amine-coated flat substrates such as quartz slides or silicon wafers were performed with the synthesized NCA's according to Scheme 1. These polymerizations follow the "amine mechanism"⁴⁰ and are very delicate. Compared with Aerosil, flat substrates have much smaller specific surface areas, resulting in a much smaller number of immobilized amine initiator groups. The amine groups are very sensitive to impurities. Water can also cleave an anhydride ring to start the formation of a polymer chain leading to nongrafted polymeric material.^{40,42} These nongrafted polymer chains form aggregates, by growing in an antiparallel way, with the growing helices,⁴² and the completely dissolved state of the growing helices, required for an ideal grafting-from polymerization, has disappeared. Consequently, the polymer growth is blocked. Therefore, ultradry solvents must be used for the monomer

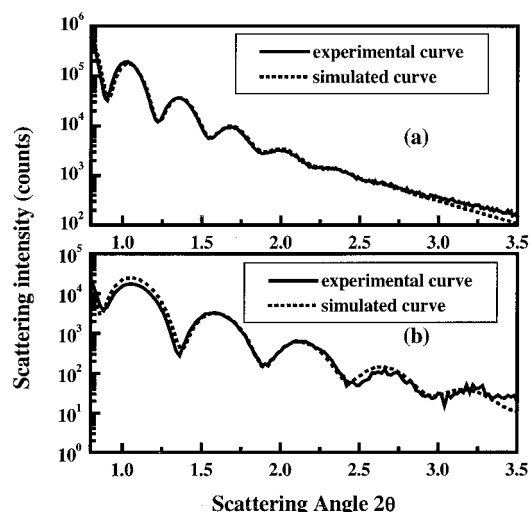


Figure 2. SAXR curves of (a) a grafted PBLG layer of 245 ± 5 Å and (b) a grafted PBLG layer of 140 ± 4 Å on a silicon wafer.

synthesis and also for the polymerizations to minimize the formation of nongrafted material.

DMF was used as a solvent for the polymerization since it is well-known as a nearly nonaggregating solvent for dilute PBLG solutions.^{40,42,43} This solvent, which is highly hygroscopic, was dried on P_2O_5 and distilled three times prior to the polymerization.⁴⁴ A mixture of dichloroacetic acid/chloroform (20/80 (v/v)), which is known to be an effective deaggregant, was used after the polymerizations to remove any nongrafted material.⁴²

General Analysis of the Surface-Grafted Layers.

The XPS spectra of a grafted PBLG and PMLG layers showed no Si(2s) peak at 150 eV and Si(2p_{3/2}) peak at 99 eV due to the thickness of the grafted layers, indicating also that homogeneous films were obtained. The peaks of C(1s) at 285 eV and N(1s) at 400 eV and the elemental composition of the polypeptide films as deduced from the narrow scans also prove that the polymeric layer has been formed. The calculated and experimental data match within 2.5%.

Small-angle X-ray reflection measurements (Figure 2a,b) reveal the film thickness and roughness. From the periodicity of the Kiessig fringes, a film thickness of 245 ± 5 and 140 ± 4 Å, respectively, can be deduced. The variation in thickness between the polypeptide films obtained under the same reaction conditions was about 5%. The layer roughness was determined to be smaller than 20 ± 3 Å. The thickness determined with ellipsometry matches the values obtained with SAXR within 10%.

The measured UV/vis spectra are consistent with results published in the literature for Langmuir–Blodgett (LB) films of the corresponding polypeptides on quartz slides.^{45,46} The shoulder at 206 nm in the UV/vis spectra of both polymeric layers on quartz confirms the α -helix conformation of these polypeptides.⁴⁵ The rather small maximum at 258 nm, observed in the case of the grafted PBLG layer, is caused by the π – π^* transition of the benzene rings of PBLG.⁴⁶

The CD spectra of the grafted PBLG and the grafted PMLG layer on quartz substrates show the characteristics of right-handed α -helices in both cases.⁴⁷ The maximum at 190 nm and a minimum at 208 nm are due to exciton

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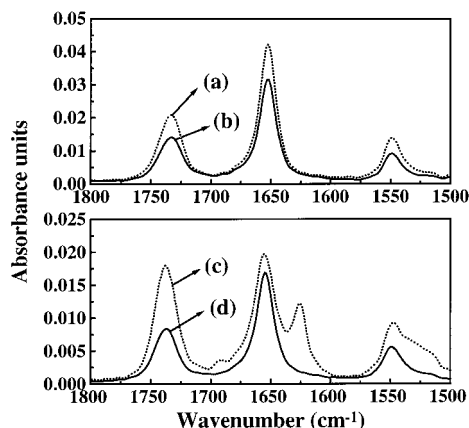


Figure 3. FT-IR transmission spectra of a grafted PBLG layer (a) before and (b) after the washing procedure and PMLG (c) before and (d) after the washing procedure.

splitting of the peptide $\pi-\pi^*$ transition and the minimum at 220 nm to the amide $n-\pi^*$ transition. Rotation of the sample did not change the spectra significantly. This was also observed for the UV/vis spectra and is explained by the homogeneity of the layers and that the helices do not prefer to orient specifically in the x or y direction in-plane of the substrate.

FT-IR Spectroscopy. The FT-IR spectra of surface-grafted PBLG and PMLG layers measured immediately after the polymerization are shown in Figure 3a, c. From the absorptions at 1650 (amide I) and 1546 cm^{-1} (amide II), it can be concluded that only α -helices were formed during the polymerization of BLG-NCA.³⁵ In the case of PMLG, however, a substantial amount of polymeric β -sheet material (amide I at 1630 cm^{-1} and amide II at 1530 cm^{-1}) was observed in addition to the α -helix absorptions (amide I at 1652 cm^{-1} and amide II at 1548 cm^{-1}). The existence of only α -helix material in the case of PBLG formation can be explained by the fact that the α -helix is the most stable conformation for a PBLG molecule. A PMLG α -helix on the other hand changes a lot easier into β -sheet material than a PBLG helix.⁴²

After the polymerization, the nongrafted polymer chains (α -helix material in the case of PBLG and α -helical together with β -sheet material for PMLG) were removed by the washing procedure and layers of pure α -helical polymer chains remained on the substrates in both cases (Figure 3b,d). The formation of these nongrafted polymers is caused by thermal polymerization⁴⁸ and/or initiation by water molecules.

Figure 4 compares the grazing incidence reflection spectra of a surface-grafted PBLG layer after a reaction time of 24 h and a PBLG LB assembly, fabricated following the procedure of Winter and Tredgold.⁴⁹ The orientation of the helices in an Langmuir–Blodgett film is known to be parallel to the substrate.⁵⁰ In grazing incidence reflection measurements only the transition dipole moment components perpendicular to the substrate can be observed. The transition dipole moments of the amide A (A_A , 3290 cm^{-1}) and amide I (A_I , 1652 cm^{-1}) vibrations are oriented in the direction of the helix axis. The amide II $_{\perp}$ band (A_{II} , 1549 cm^{-1}) has a perpendicular orientation with respect to the helix axis. The enlarged absorbances of the

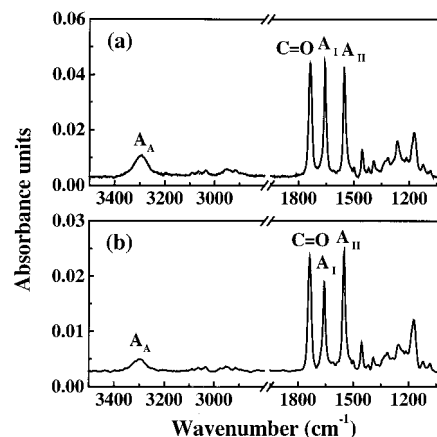


Figure 4. Grazing incidence reflection spectra of (a) a surface-grafted PBLG layer on an aluminum coated substrate¹² and (b) a PBLG LB assembly of 10 monolayers on a gold coated substrate.

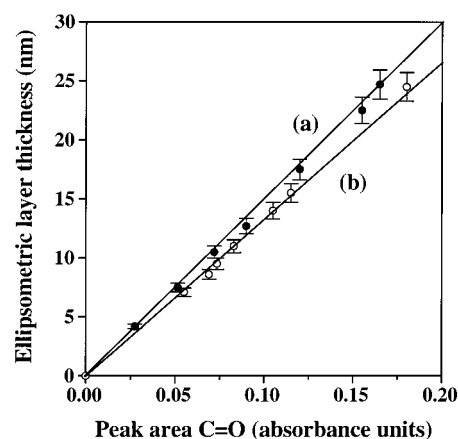


Figure 5. Layer thickness versus the amount of (a) grafted PBLG and (b) grafted PMLG. Error bars indicate an error of 5%.

amide A and amide I peaks and the reduced absorbance of the amide II $_{\perp}$ peak for the surface-grafted PBLG film compared to those of the helices in the LB assembly (Figure 4b) clearly indicate a more perpendicular orientation with respect to the substrate.

Polymer Growth as a Function of Reaction Time. Polymer growth was studied by measuring the area of the $\nu(\text{C}=\text{O})$ absorption peak at 1735 cm^{-1} after different reaction times. This ester side chain carbonyl is randomly oriented because it is not involved in any H-bonding and connected to the helix by two methylene spacers. The area of this C=O stretching vibration can thus be used as a measure for the amount of polymer grown on the substrate. This was confirmed by independent ellipsometric measurements on various samples. A linear relationship was found between the amount of polymer and the ellipsometric layer thickness for both polymers (Figure 5). The larger helix diameter of PBLG of 15.5–26 Å,⁴² depending on the orientation of the side chain phenyl group, compared to 14.0 Å for a PMLG helix, and the almost equal polymer density for both polymers (1.32 g/cm^3 for PBLG and 1.29 g/cm^3 for PMLG⁴²) explain why the same layer thickness is reached with smaller amounts (smaller ester carbonyl peak area) of grafted polymer in the case of PBLG.

To study the polymer growth as a function of time, several surface-grafting polymerizations on silicon wafers were performed in a 0.5 mol/L NCA monomer solution with different time increments (1, 2, 4, 6, and 24 h). The polymer films were characterized by FT-IR transmission

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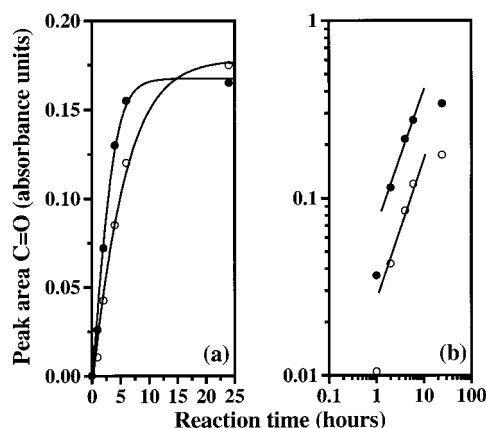


Figure 6. Polymer growth of PBLG (●) and PMLG (○) versus reaction time in (a) linear and (b) logarithmic scale.

spectroscopy, and the results are shown in Figure 6. The growth of PMLG (Figure 6a) is for unknown reasons somewhat slower than that of PBLG, as can be seen by the lower ester carbonyl absorbances in the first few hours. Difference in monomer reactivity in this particular reaction system is the most probable cause. At the end of both polymerizations, however, layers of almost identical thickness were obtained.

The most important part of the polymer growth takes place during the first 5–6 h of the polymerization. During this period, an almost linear dependence of the polymer growth on time is observed. For longer reaction times, this dependency becomes nonlinear and levels off. This effect will be discussed later on.

Peak areas of the three specific amide absorptions (amide A, amide I, and amide II_⊥) of the PBLG and PMLG layers were obtained from FT-IR spectra and are listed in Table 1. The ratio *D* of the peptide amide I and amide II_⊥ peaks can give a good indication about the orientation of the helical chains, because the amide I transition dipole moment has an average orientation parallel to the helix axis and the amide II_⊥ is more or less perpendicular to that. Therefore, they have almost mutually perpendicular transition dipole moments.³⁵ For example, in Langmuir–Blodgett films of polyglutamates, the helices are oriented parallel to the substrate,⁵⁰ and this results in a very high amide I absorption and a very small amide II_⊥ absorption. In short, the higher the *D* value obtained with FT-IR transmission spectroscopy, the smaller the average angle between the helices and the substrate.

The peak areas of the amide A, amide I, and amide II_⊥ were calculated and divided by the area of the ester ν(C=O) to normalize for the amount of polymer. In this way, the peak areas of the analyzed absorbances of the films obtained with various reaction times can be compared with each other. Also the *D* (amide I/amide II_⊥) values are shown. The experimental error in the *D* values is about 5%.

The great change in the *D* value in the beginning of the reaction can be explained by a slow initiation process,⁴⁰ during the first 1–2 h of the grafting process. During this period, new chains will be initiated on the surface, resulting in higher grafting densities and in a more perpendicular orientation due to increasing sterical hindrance between neighboring chains.

After this start, propagation continues, as can be seen by the increase of the ester C=O absorbances (Figure 6a), but the *D* values hardly change over the next 2–4 h. That can be explained by a continuous growth of all helices collectively. The polymerization is first order in time

during this stage of the reaction (see the slope of 1 of the straight line in Figure 6b.)

The *D* values slightly increase at the end of both polymerizations because of the increasing polydispersity of the grafted chains. The extending longer chain parts might bend toward the surface of the film due to van der Waals interactions to minimize surface energy.

A method was evaluated for the determination of the average angle between the grafted helices and the substrate by means of FT-IR transmission spectroscopy. This will be reported in part 2 of this series.³¹ The results of applying this method on the grafted layers in this study are also shown in Table 1 and confirm the rough indication about the helix orientation produced by the evaluation of the *D* values.

The nonlinear polymer growth after a reaction time of about 5 h seems hard to explain. The polymer growth is expected to stay linear, due to the "living" character of the polymerization. The observed nonlinear behavior can be explained by partial chain growth termination. However, we could not find any indication for the chemical termination steps reported in the literature, such as cyclization of chain ends⁴⁰ or nucleophilic attack of the primary amine on the C-2 atom instead of the usual C-5 atom of the anhydride ring, leading to an acid end group.⁴⁰

Termination of the helix growth in this case can be explained by the gradual formation of nongrafted polymeric material. A thick aggregated layer of nongrafted material is formed on top of the surface-grafted helices, as can be seen in FT-IR experiments directly after polymerization and before washing. This might lead to a termination of the growing helices, although there is a substantial amount of monomer left in the solution after 24 h, as was measured with FT-IR transmission spectroscopy as well.

Polymer Growth as a Function of Monomer Concentration. To study the polymer growth dependence on monomer concentration, several surface-grafting polymerizations of BLG-NCA on silicon wafers were performed using different monomer concentrations. A reaction time of 1 h was maintained to avoid the formation of nongrafted material as well as possible. The grafted polymers were characterized by FT-IR transmission spectroscopy. The normalized integrated peak areas of the ester carbonyl absorbances versus monomer concentration are displayed in Figure 7a.

A pronounced effect of the monomer concentration on polymer growth was found within the investigated concentration range for PBLG as well as PMLG. Again the growth of PMLG is somewhat slower than that of PBLG. It is obvious that there is in both cases a more than linear dependence of the polymer growth on monomer concentration.

It is known from literature that the helix growth rate is slow during the beginning of the reaction.⁴⁰ After a certain reaction time, it increases significantly. The exact reason for this phenomenon is still unknown. During the beginning of the surface-grafting polymerization, growth of already grafted helices and initiation of new helices occurs simultaneously. These new ones grow slowly for a certain period. Therefore, the primary amine end groups act in a similar way as the single-center, two-state catalysts known in the olefin insertion polymerizations.⁵¹ This can result in the order in monomer concentration of 1.7–1.8 (Figure 7b).

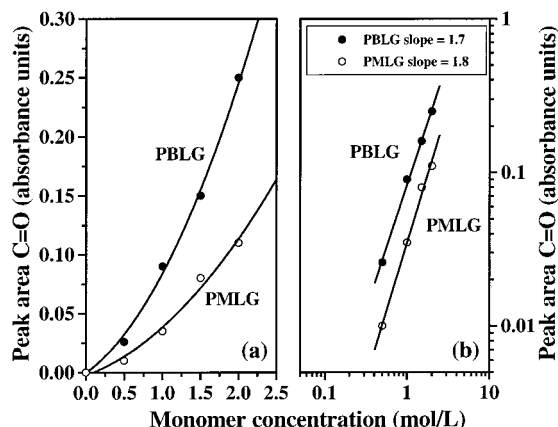
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Table 1. Effects of Reaction Time of the Surface Grafting on Si Wafers, as Measured by FT-IR Transmission Spectroscopy on the normalized Peak Areas of the Studied Amide Absorptions and Therefore on the *D* Values and Tilt Angles of the Surface-Grafted PBLG and PMLG Helices

	normal peak areas (abs. units) for given reaction time (h)				
	1 h	2 h	4 h	6 h	24 h
PBLG					
amide A	2.09	1.67	1.69	1.74	1.82
amide I	2.27	1.81	1.83	1.87	1.96
amide II _{v⊥}	0.56	0.59	0.59	0.58	0.57
<i>D</i> = (amide I/amide II _{v⊥})	4.05	3.07	3.10	3.22	3.44
(90° - <i>θ</i>) ^a	22	42	41	38	32
PMLG					
amide A	2.05	1.64	1.79	1.88	2.02
amide I	2.29	1.94	2.04	2.08	2.11
amide II _{v⊥}	0.52	0.67	0.63	0.59	0.56
<i>D</i> = (amide I/amide II _{v⊥})	4.40	2.90	3.24	3.53	3.77
(90° - <i>θ</i>) ^a	20	44	39	34	30

^a *θ* = angle between the helix axis and the substrate normal.**Table 2. Effects of Monomer Concentration on the Normalized Peak Areas of the Studied Amide Absorptions and Therefore on the *D* Values and Tilt Angles of Surface-Grafted PBLG and PMLG Helices Obtained after a Reaction Time of 1 H**

	normal peak areas (abs. units) for given monomer concentration (mol/L)			
	0.5 mol/L	1.0 mol/L	1.5 mol/L	2.0 mol/L
PBLG				
amide A	2.09	2.05	2.00	1.88
amide I	2.27	2.11	1.82	1.66
amide II _{v⊥}	0.56	0.56	0.56	0.58
<i>D</i> = (amide I/amide II _{v⊥})	4.05	3.76	3.26	3.07
(90° - <i>θ</i>) ^a	22	27	37	42
PMLG				
amide A	2.05	1.85	1.81	1.77
amide I	2.29	2.19	2.00	1.82
amide II _{v⊥}	0.52	0.55	0.61	0.64
<i>D</i> = (amide I/amide II _{v⊥})	4.40	4.00	3.26	2.86
(90° - <i>θ</i>) ^a	20	26	38	45

^a *θ* = angle between the helix axis and the substrate normal.**Figure 7.** Polymer growth of PBLG (●) and PMLG (○) versus monomer concentration in (a) linear and (b) logarithmic scale.

This somewhat strange order in monomer concentration can also be obtained when the rate of initiation (v_i) is different from the propagation rate (v_p). The polymer growth, in such a system, is related to the monomer concentration according to

$$v_p = k_p[M][\text{NH}_2] = k_p[M][\text{NH}_2^*](1 - e^{-k_i[M]t}) \quad (1)$$

Mathematical evaluation of this equation (1) shows that for a specific value of $k_i t$ an order in $[M]$ of about 1.7–1.8 can be deduced.⁵²

The normalized surface areas of the three analyzed amide peaks (amide A, amide I, and amide II_{v⊥}), the *D* values (amide I/amide II_{v⊥}), and the average angles between the helices and the substrates are given in Table

(52) In the first hour of the surface-grafting polymerization the number of growing helices *P* increases. No new helices are initiated after this period. The growing ones are just gaining length. The number of growing chains on $t = 0$: $P = 0$. The number of growing chains on $t = \infty$: $P_\infty = \text{NH}_2^*$, with NH_2^* being the number of primary amine groups that start the growth of a helix eventually. On t (between 0 and 1 h)

$$[\text{NH}_2] = [\text{NH}_2^*] - [P] \quad (\text{A})$$

with $[\text{NH}_2]$ is the concentration of new initiating primary amine groups. Initiation is related to monomer concentration $[M]$ according to

$$v_i = k_i[M][\text{NH}_2] \quad (\text{B})$$

So

$$-d[\text{NH}_2]/dt = k_i[M][\text{NH}_2] \quad (\text{C})$$

Integration over $[\text{NH}_2]$ to $[\text{NH}_2^*]$ and from 0 to t results in

$$[\text{NH}_2] = [\text{NH}_2^*]e^{-k_i[M]t} \quad (\text{D})$$

combined with (A) gives

$$[P] = [\text{NH}_2^*](1 - e^{-k_i[M]t}) \quad (\text{E})$$

Polymer growth is related to the monomer concentration according to

$$v_p = k_p[M][P] \quad (\text{F})$$

combined with (E) gives

$$v_p = k_p[M][\text{NH}_2^*](1 - e^{-k_i[M]t}) \quad (\text{G})$$

So, the growth in film thickness is related to $[M](1 - e^{-k_i[M]t})$. Mathematical evaluation shows that for a specific value of $k_i t$ an order in $[M]$ of about 1.7–1.8 can be deduced.

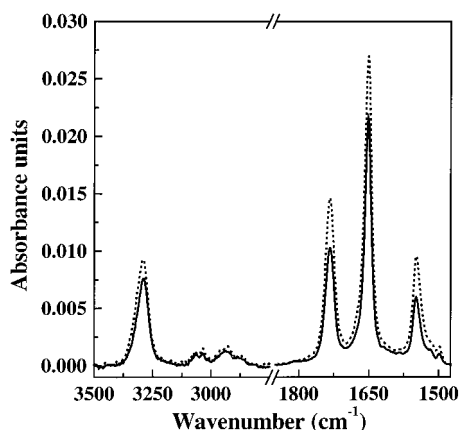


Figure 8. FT-IR transmission spectra of a grafted PBLG layer (solid line) obtained after a reaction time of 24 h, followed by the washing procedure and the obtained PBLG layer after the renewed polymerization (dotted line).

2. From the decreasing D values, we can conclude that the polymer chains are oriented in a more perpendicular way with respect to the substrate at higher monomer concentrations, pointing to higher grafting densities.

An increase in reaction time at higher monomer concentration leads to thick polypeptide layers. Using 1.5 mol/L and a reaction time of 24 h, a layer thickness of about 400 Å for the surface-grafting reaction of MLG-NCA was obtained.

Renewed Polymerization. The absence of chemical chain growth termination is demonstrated further by a renewed polymerization after the washing procedure. After 24 h of polymerization and subsequent washing, a freshly prepared BLG-NCA monomer solution was added. Polymerization was allowed for another 24 h. The increased absorption values in Figure 8 indicate that additional polymer growth occurred. The layer thickness increased from ± 200 Å to about 280 Å. The reactive chain ends that had been enclosed by nongrafted material in the first polymerization period were able to grow again. This explains why the D value changes from 3.51 before the addition of new monomer to 3.11 after the renewed polymerization. This possibility of a renewed polymerization also opens the way for synthesizing surface-grafted block copolypeptides, which will be published in part 3 of this series.³²

Conclusions

The surface-grafting polymerization of BLG-NCA and MLG-NCA in solution can be performed successfully, and the method described in this paper is a step toward a better controllable surface-grafting polymerization. FT-IR, UV/vis, and CD measurements of the grafted polypeptide layers on silicon wafers and quartz slides show that the layers consist of polypeptide material in the pure right-handed α -helix conformation after the proper washing procedure.

Factors that influence the polymer growth and helix orientation, such as reaction time and monomer concentration, were studied by means of FT-IR measurements. The helices formed initially (<1 h) are oriented on average nearly parallel with respect to the substrate. During the next few hours the polymer growth is related to reaction time in a linear way. The helices formed are oriented in a more or less perpendicular orientation.

The monomer concentration plays a very important role. For short reaction times that prevent the formation of nongrafted material, FT-IR, ellipsometry, and SAXR measurements revealed that thicker polymer layers could be obtained with higher monomer concentration, within the same reaction time. The average orientation of the helices is more perpendicular with respect to the substrate when a higher monomer concentration is used.

Nongrafted polymeric material formed in solution aggregates between and on top of the growing helices and causes growth inhibition. After removal of this nongrafted material, the polymerization can be continued again. This opens a way for synthesizing surface-grafted block copolymers.

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Supporting Information Available: Figures showing XPS and UV/vis spectra and a table giving elemental composition of PBLG and PMLG films. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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