Surface Grafting of Poly(L-glutamates). 3. Block Copolymerization

R. H. Wieringa, E. A. Siesling, P. J. Werkman, E. J. Vorenkamp, and A. J. Schouten*

Department of Polymer Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Received December 18, 2000. In Final Form: May 31, 2001

This paper describes for the first time the synthesis of surface-grafted AB-block copolypeptides, consisting of poly(γ-benzyl L-glutamate) (PBLG) as the A-block and poly(γ-methyl L-glutamate) (PMLG) as the B-block. Immobilized primary amine groups of (γ-aminopropyl)triethoxysilane (APS) on silicon wafers initiated the ring-opening polymerization of *N*-carboxyanhydrides of glutamic acid esters (NCAs). After removal of the BLG-NCA monomer solution after a certain reaction time, the amine end groups of the formed PBLG blocks acted as initiators for the second monomers. This method provides the possibility of making layered structures of surface-grafted block copolymers with tuned properties. Ellipsometry and small-angle X-ray reflection (SAXR) measurements revealed the thickness of the polypeptide layers ranging from 45−100 Å of the first block to 140−270 Å for the total block copolypeptides. The chemical composition of the blocks was determined by X-ray photoelectron spectroscopy (XPS). In addition, Fourier transform infrared transmission spectroscopy (FT-IR) revealed that the polypeptide main chains of both blocks consisted of pure α-helices. The average orientation of the helices ranging from 22−42° with respect to the substrate within the first block to 31−35° in the second block could be derived with FT-IR as well.

Introduction

Polymers grafted onto solid supports are of great importance because of the wide range of potential industrial applications in the areas of colloidal stabilization,[1] adhesion,[2] and chromatography.[3] Their behavior is quite different from the behavior of the corresponding free polymer chains in the bulk. For high grafting densities, the anchored polymer chains tend to stretch away from the surface in order to minimize steric interactions. This effect has been part of many studies, both theoretically and experimentally.[4−6]

Ultrathin polymeric layers on flat solid substrates with well-defined thicknesses and properties have enormous potential in the fabrication of electrooptical devices.[7] In this respect, Langmuir−Blodgett (LB) films of polypeptides are of particular interest. Polyglutamate films already proved their applicability in such systems.[8] With the LB technique alternating layers of defined thickness can be made, and through the choice of the ester substituent, the layer properties can easily be tuned.

Until recently, little attention had been paid to the surface grafting of polypeptides, especially polypeptides chemically grafted onto flat solid supports. The synthetic challenge to make surface-grafted polypeptide films can be met by two basic approaches. Preformed polymers that contain reactive or strongly absorbing end groups can be used in the "grafting-onto" method. These end groups will anchor the polymer to the substrate. Polyglutamate homopolymers have been grafted onto substrates by various researchers[9−14] with this approach, but surface-grafted block copolyglutamates have not been reported so far.

In the "grafting-from" method,[15−22] immobilized initiators on substrates are brought in contact with dissolved monomers to obtain covalently bonded polymer chains on the substrate. Primary amines can be used as initiators for the ring-opening polymerization of *N*-carboxyanhydrides of various amino acid esters. As a consequence of the "amine mechanism", the initiator becomes part of the grafted polymer chain,[23] and by stepwise addition of different monomers, block copolypeptides can be synthesized.

Chang and Frank developed the solvent-free vapor deposition polymerization method of NCA monomers on

* To whom all correspondence should be addressed.

(1) Napper, D. H. *Polymeric Stabilization of Colloidal Dispersions*; Academic Press: London, 1983.
(2) Wu, S. *Polymer Interface and Adhesion*; Marcel Dekker, Inc.: New York, 1982.
(3) Ivanov, A. E.; Saburov, V. V.; Zubov, V. P. *Adv. Polym. Sci.* **1992**, *104*, 135.
(4) Milner, S. *Science* **1991**, *251*, 905.
(5) Halperin, A.; Tirrell, M.; Lodge, T. P. *Adv. Polym. Sci.* **1992**, *100*, 31.
(6) Hadziioannou, G.; Patel, S.; Granick, S.; Tirrell, M. *J. Am. Chem. Soc.* **1986**, *108*, 2869.
(7) Roberts, G.; *Langmuir−Blodgett Films*; Plenum Press: New York, 1990.
(8) Hickel, W.; Duda, G.; Jurich, M.; Kröhl, T.; Rochford, K.; Stegeman, G. I.; Swalen, J. D.; Wegner, G.; Knoll, W. *Langmuir* **1990**, *6*, 1403.
(9) Enriquez, E. P.; Gray, K. H.; Guarisco, V. F.; Linton, R. W.; Mar, K. D.; Samulski, E. T. *J. Vac. Sci. Technol. A* **1992**, *10*, 2775.
(10) Worly, C. G.; Linton, R. W.; Samulski, E. T. *Langmuir* **1995**, *11*, 3805.
(11) Machida, S.; Urano, T. I.; Sano, K.; Kawata, Y.; Sunohara, K.; Sasaki, H.; Yoshiki, M.; Mori, Y. *Langmuir* **1995**, *11*, 4838.
(12) Chang, Y. C.; Frank, C. W. *Langmuir* **1996**, *12*, 5824.
(13) Imanishi, Y.; Miura, Y.; Iwamoto, M.; Kimura, S.; Umemura, J. *Proc. Jpn. Acad.* **1999**, *75*, 287.
(14) Niwa, M.; Morikawa, M.; Higashi, N. *Langmuir* **1999**, *15*, 5088.
(15) Chang, Y. C.; Frank, C. W. *Langmuir* **1998**, *14*, 326.
(16) Whitesell, J. K.; Chang, H. K. *Science* **1993**, *261*, 73.
(17) Whitesell, J. K.; Chang, H. K. *Mol. Cryst. Liq. Cryst.* **1994**, *240*, 251.
(18) Oosterling, M. L. C. M.; Willems, E.; Schouten, A. J. *Polymer* **1995**, *36*, 4463.
(19) Oosterling, M. L. C. M.; Willems, E.; Schouten, A. J. *Polymer* **1995**, *36*, 4485.
(20) Heise, A.; Menzel, H.; Yim, H.; Foster, M. D.; Wieringa, R. H.; Schouten, A. J.; Erb, V.; Stamm, M. *Langmuir* **1997**, *13*, 723.
(21) Wieringa, R. H.; Schouten, A. J. *Macromolecules* **1996**, *29*, 3032.
(22) Jaworek, T.; Neher, D.; Wegner, G.; Wieringa, R. H.; Schouten, A. J. *Science* **1998**, *279*, 57.
(23) Kricheldorf, H. R. In *Models of Biopolymers by Ring-Opening Polymerization*; Penczek, S., Ed.; CRC Press: Boca Raton, FL, 1990.

10.1021/la001771j CCC: $20.00
Published on Web 09/18/2001

silicon wafers.[15] The layer thickness can easily be tuned by varying the monomer concentration, reaction time, temperature, and pressure. This technique also has the potential to be applied in the making of surface-grafted block copolypeptides.

When the rigid-rodlike helical polyglutamates are grafted from flat substrates, the helices actually grow away from the surface, forcing the helices into a parallel orientation, which gives rise to a very strong resulting net dipole moment of the combined dipoles of the individual peptide bonds in the main chain.[24] This striking effect in surface-grafted polyglutamate films was experimentally confirmed for the first time by Jaworek et al.[22] who measured the polarization of a very thin layer of parallel aligned helices of poly(γ-benzyl L-glutamate) grafted from Al-coated glass substrates. Such highly ordered materials with a net macroscopic dipole moment can be used for electron-transfer processes in optoelectronic devices.[25]

This paper describes the synthesis of surface-grafted block copolyglutamates with the grafting-from method in solution for the first time. Reaction times for the formation of the first poly(γ-benzyl L-glutamate) (PBLG) block were varied. After the washing and drying procedure the second block can be formed by addition of another monomer solution. The characterization of the block copolymer formation was performed by means of Fourier transform infrared transmission spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), ellipsometry, and small-angle X-ray reflection (SAXR) measurements.

Experimental Section

Materials. The monomer synthesis, substrate preparation, and silanization have been described in detail in part 1 of this series.[26]

Renewed Polymerization. The solution polymerizations were performed in anhydrous *N*,*N*-dimethylformamide (DMF; Acros, p.a.) at 40 °C in specially designed glassware. The monomer solution of γ-benzyl L-glutamate *N*-carboxyanhydride (BLG-NCA, 0.5 mol/L) was added to the silanized substrates. After the polymerizations, the films were washed with a dichloroacetic acid (Acros, p.a.)/chloroform (20/80 (v/v)) mixture for 24 h to remove any nongrafted material and with chloroform for 1 h. After drying in a vacuum, a freshly prepared BLG-NCA monomer solution (0.5 mol/L) was added to the sample for another 24 h to restart the polymerization.

Synthesis of PBLG-PMLG Diblock Copolymer (1). Two silanized substrates were immersed in a BLG-NCA monomer solution (0.5 mol/L in anhydrous DMF) at 40 °C. After 2 h, the monomer solution was removed and the grafted PBLG layers were washed three times with dry DMF in order to remove the remaining BLG-NCA monomer. One substrate was set aside to study the first block formation. The γ-methyl L-glutamate *N*-carboxyanhydride (MLG-NCA) solution (0.5 mol/L) was added to the other substrate and allowed to react for 24 h. The grafted block copolymers were washed in a mixture of dichloroacetic acid/chloroform (1/4 (v/v)) for at least one night to remove all nongrafted material. This was followed by washing in pure chloroform for several hours and drying under vacuum.

Synthesis of PBLG-PMLG Diblock Copolymer (2). The same procedure was followed as for the formation of PBLG-PMLG (**1**), except that the reaction time for the first PBLG block was 1 h.

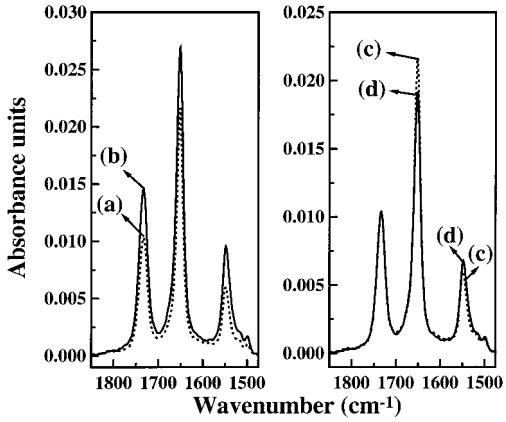


Figure 1. FT-IR transmission spectra of (a) the first PBLG layer, (b) the total PBLG film after renewed polymerization, and (c, d) the "normalized" spectra (scale factor: 0.714×), respectively.

Analysis. The techniques to analyze the block copolypeptide layers are the same as those reported in parts 1[26] and 2[27] of this series.

Results and Discussion

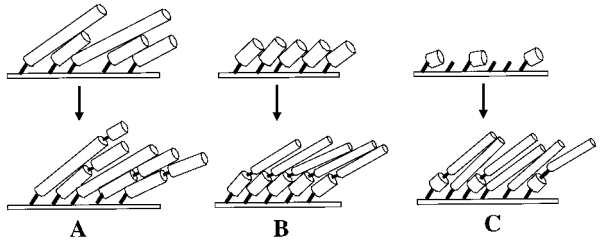
Renewed Polymerization. In part 1 of this series,[26] we already reported that it is possible to restart the growth of surface-grafted helices. This can be done after the washing procedure that is necessary to remove nongrafted material. This nongrafted material that is formed after 5−6 h of polymerization causes helix growth inhibition by interdigitation between and aggregation on top of growing helices.[28] Although the solution still contains a significant amount of monomer even after 24 h of reaction, as has been determined by FT-IR transmission spectroscopy, and the grafted film still has active chain ends, much of the growing polymer then is nongrafted material. This helix growth inhibition caused by nongrafted material seems to be a physical process because we could not find any indication of chemical chain growth inhibition as known from literature. Cyclization of chain ends 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,[23] could not be found.

The first PBLG layer was analyzed with FT-IR transmission spectroscopy. Figure 1a shows the spectrum of this first layer. The amide I and amide $II_{\nu\perp}$ absorption bands at 1652 and 1549 cm^{-1}, respectively, clearly indicate the formation of a PBLG layer consisting of pure α-helical polypeptide.[29] The thickness of this first block is about 205 Å, as determined with ellipsometry.

Ellipsometry revealed a total layer thickness of the grafted film of about 270 Å after renewed polymerization. The increased absorbances of every peak in the FT-IR spectrum as shown in Figure 1b clearly show the additional growth. On the basis of the random orientation of the ester side chain C═O,[30] the area underneath the C═O stretching vibration peak can be used as a measure for the amount of grafted polypeptide and therefore the film thickness. When the areas underneath the ester C═O's of both spectra are normalized, the changes in *D* value,

(24) Hol, W. G.; van Duinen, P. T.; Berendsen, H. J. C. *Nature* **1978**, *273*, 443.
(25) Whitesell, J. K.; Chang, H. K.; Fox, M. A.; Galoppini, E.; Watkins, D. M.; Fox, H.; Hong, B. *Pure Appl. Chem.* **1996**, *68*, 1469.
(26) Wieringa, R. H.; Siesling, A. E.; Geurts, P. F. M.; Vorenkamp, E. J.; Werkman, P. J.; Erb, V.; Stamm, M.; Schouten, A. J. *Langmuir*, companion paper in this issue (part 1).
(27) Wieringa, R. H.; Siesling, E. A.; Angerman, H. J.; Werkman, P. J.; Vorenkamp, E. J.; Schouten, A. J. *Langmuir*, preceding paper in this issue (part 2).
(28) Block, H.; *Poly(γ-Benzyl-L-Glutamate) and Other Glutamic Acid containing Polymers*; Gordon and Breach Publishers: New York, 1983.
(29) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712.
(30) Duda, G.; Schouten, A. J.; Arndt, T.; Lieser, G.; Schmidt, G. F.; Bubeck, C.; Wegner, G. *Thin Solid Films* **1988**, *159*, 221.

Scheme 1. Schematic Representation of (a) the Formation of the Total PBLG Layer after a Renewed Polymerization, (b) the Block Copolymerization of PBLG-PMLG (1), and (c) the Block Copolymerization of PBLG-PMLG (2)



the ratio of the amide I over the amide $II_{\nu\perp}$ absorbances, reveal the helix orientation with respect to the substrate, as described in part 2.[27] The normalized spectra of the first layer and the total polymer film are shown in Figure 1c,d, respectively. The amide I peak decreases significantly, whereas the amide $II_{\nu\perp}$ peak increases after the renewed polymerization. This indicates that still accessible active chain ends are able to grow again, accounting for a more perpendicular helix orientation with respect to the substrate after the renewed polymerization.

Knowing that the maximum helix density is already reached after about 2 h, the initiation from renewedly accessible initiator sites on the substrate, leading to newly formed helices with the same diameter, is doubtful after a reaction time of 24 h for the first PBLG layer, but it cannot be ruled out completely. This should, of course, increase the average angle between the PBLG helices in the first layer and the substrate.

Evaluation of the *D* values can be performed by calculation of the ratio between the amide I and amide $II_{\nu\perp}$ absorbances. This ratio decreases slightly from 3.51 to 3.11, indicating a more perpendicular helix orientation with respect to the substrate. This means an increase from 30° to about 41° for the average angles between the helices and the substrate using the method described in part 2 of this series.[27] As explained above, this decrease can be explained mainly by a renewed growth of all the chain ends (Scheme 1A) that were enclosed by nongrafted polymeric material.

Diblock Copolymer Formation. *Synthesis of PBLG-PMLG Diblock Copolymer (***1***).* The absence of chemical chain growth termination and consequently the presence of still active chain ends of the helices opens the way for synthesizing surface-grafted block copolypeptides, as schematically shown in Scheme 2. The first PBLG block was formed by polymerizing for only 2 h. At this stage in the polymerization process, as described in part 1,[26] propagation of the polymerization occurs by a collective growth of all the helices. This is supported by the nearly constant *D* value over the next few hours (3.07−3.10), indicating that due to sterical limitations the maximum grafting density of PBLG helices has been reached, so that no new PBLG helices are formed anymore. Because of such short reaction times, there is almost no formation of nongrafted material in the solution that can cause termination of the helix growth by aggregation.[28] So, rinsing with DMF should be sufficient to extract the remaining BLG-NCA monomers and low molecular weight nongrafted PBLG material from the surface-grafted first PBLG block. Helix growth will mainly occur on the active chain ends of the former layer in this way (Scheme 1B).

*Synthesis of PBLG-PMLG Diblock Copolymer (***2***).* As the results in part 1[26] have shown, the helices are lying relatively flat on the substrate in the first hour of the

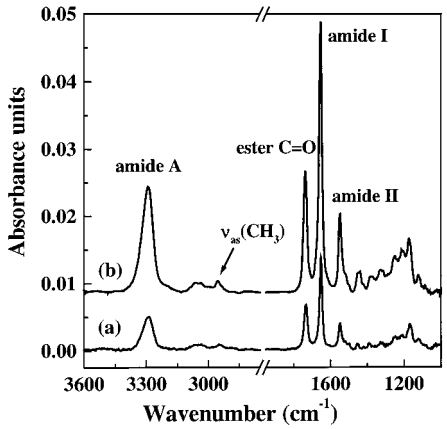
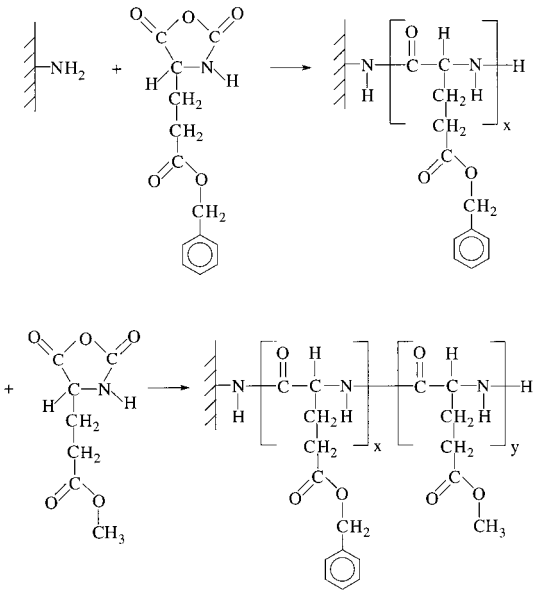


Figure 2. FT-IR transmission spectra of (a**)** the first PBLG block and **(**b**)** the PBLG-PMLG (**1**) diblock copolymer. Spectrum b is *y*-shifted by 0.009 absorbance units.

Scheme 2. Reaction Scheme of the Surface-Grafting Block Copolymerization



polymerization after the work-up procedure. Not all available amine initiator groups have already started to grow helices, due to a slow initiation step.[23] At this stage of the polymerization, there should be sufficient space between the already growing helices to grow helices with a smaller diameter (Scheme 1C).

FT-IR Measurements. *PBLG-PMLG (***1***).* The first PBLG layer of PBLG-PMLG diblock copolymer (**1**) with a thickness of about 100 Å, as determined by ellipsometry, consists of pure α-helical material as indicated by the peak positions of the amide I and amide $II_{\nu\perp}$ absorption bands at 1650 and 1549 cm^{-1}, respectively (Figure 2a). Application of the method from part 2[27] to study the average helix orientation revealed an average angle of the helix main axis of 42° with respect to the substrate for the PBLG helices of the first block.

The diblock copolypeptide, **1** with a total thickness of about 270 Å was characterized by FT-IR measurements as shown in Figure 2b. The absorbances of all bands are much higher than in the case of the first block, indicating that additional polymer growth has occurred. The $\nu_{as}(CH_3)$ at 2954 cm^{-1} clearly shows that the second block consists of PMLG. The C═O stretch absorption peak of the ester side group of the diblock copolypeptide shifted 3 cm^{-1} from 1734 cm^{-1} typically for grafted PBLG toward 1737 cm^{-1},

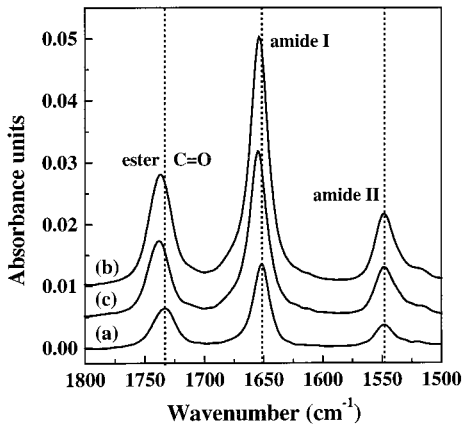


Figure 3. Wavelength shifts of the ester C═O and amide I absorption bands of (a) a first PBLG block, (b) PBLG-PMLG (**1**), and (c) the second PMLG block (b − a). Spectra b and c are *y*-shifted by 0.01 and 0.005 absorbance units, respectively.

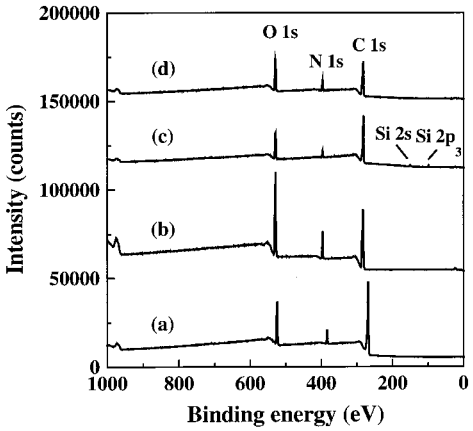


Figure 4. XPS spectra of (a) a first PBLG block, ∼100 Å thick, (b) the PBLG-PMLG (**1**) diblock copolymer (PMLG second block of ∼170 Å thickness), (c) a first PBLG block, ∼45 Å thick, and (d) a PBLG-PMLG (**2**) diblock copolymer (PMLG block of ∼95 Å thickness). Spectra b−d are *y*-shifted for clarity.

as a weighted average between typical PBLG and PMLG values, as shown in Figure 3. Also the amide I absorption shifted from 1650 to 1653 cm^{-1} in the same trend. The main chains of the PMLG block (see Figure 3c), obtained by subtracting the spectrum of the PBLG block (Figure 3a) from that of the diblock copolymer (Figure 3b), are in the α-helix conformation (amide I, 1654 cm^{-1}; amide $II_{\nu\perp}$, 1549 cm^{-1}).

Due to the smaller helix diameter of a PMLG helix of 14 Å compared to 15.5−26 Å for PBLG,[28] depending on the orientation of the ester side group, a smaller angle between the helices of the PMLG block and the substrate has to be expected. From the spectrum of the PMLG block (Figure 3c) an average angle of the PMLG helices with respect to the substrate of 31° was evaluated, what is indeed smaller than the angle of 42° as evaluated for the PBLG helices of the first block. This indicates that the amine chain ends of the PBLG block, indeed, initiate the formation of the PMLG block on top of the first, as schematically shown in Scheme 1B.

*PBLG-PMLG (***2***).* Stopping the surface-grafting polymerization of the first PBLG block after 1 h results in a layer thickness of about 45 Å as measured by ellipsometry. Again FT-IR, not shown here because the spectra are similar to those depicted in Figure 2, shows the presence of pure α-helical material. An average angle of 22° with respect to the substrate was determined.

FT-IR spectroscopy on the diblock co-polypeptide **2** with an overall thickness of 140 Å showed again that additional growth had occurred, as indicated by the higher intensities of all the absorption bands. The $\nu_{as}(CH_3)$ at 2954 cm^{-1}, together with the wavenumber shifts of the C═O ester and amide I bands to the weighted average value of typical PBLG and PMLG values, proves the formation of the PMLG helices on top of the PBLG helices of the first layer. In this case, the possibility that PMLG helices grew between already grafted PBLG helices cannot be ruled out completely.

From the spectrum of the PMLG layer, obtained through subtraction of the spectrum of the PBLG layer from that of the total diblock copolypeptide, similar to the procedure depicted in Figure 3, an average angle between the PMLG helices and the substrate was determined. Instead of a decreasing tilt angle with respect to the substrate for the second block as in the case of **1**, the angle of the helices here has increased from 22° for the PBLG block to 35° for the PMLG block. This can be explained by the earlier postulated growth of PMLG helices between and on top of already grown PBLG helices, as shown in Scheme 1C, leading to a higher grafting density of helices and leading to a larger average angle of the helices with respect to the substrate.[5]

Table 1. Element Composition of the Grafted Blocks As Derived from XPS Measurements

		calc	exp
PBLG first blocks	C %	75.0	74.9
	N %	6.3	6.4
	O %	18.7	18.7
PBLG-PMLG (1)	C %	60.0	60.4
	N %	10.0	9.8
	O %	30.0	29.8
PBLG-PMLG (2)	C %	60.0	66.6
	N %	10.0	8.4
	O %	30.0	25.0

If there is indeed growth of PMLG helices between the already grafted PBLG ones, this will, of course, affect the orientation of the helices within this first PBLG layer. This effect is now neglected in the subtraction step. Taking this effect into account lowers the average helix orientation of the PMLG helices in the second layer with respect to the substrate by a maximum of 8°. This does not change the general idea that the PMLG helices, in this case, grow on top of and between the already grafted PBLG helices.

We did several surface-grafting block copolymer reactions. The helix orientation varies within a deviation of about 3° using the same monomer batches. Deviation in layer thickness stays within 2.5−7.5%. The variation in helix tilt angles and layer thicknesses is slightly higher when the results obtained with different monomer batches are compared (see also part 2[27]).

XPS Measurements. *PBLG-MLG (***1***).* Because of the small XPS penetration depth of only 50−100 Å,[31] the substrate silicon peaks, Si(2s) and $Si(2p_3)$ at 150 and 99 eV, respectively, are not present in the overall spectrum of the sample of the first 100 Å thick PBLG film (see Figure 4a). The chemical composition closely matches that of pure PBLG, as shown in Table 1.

Additional growth of PMLG helices on top of the first PBLG block results in a PBLG-PMLG diblock copolymer, **1**. Ellipsometry revealed a second block length of about 170 Å. XPS showed the chemical composition to be pure PMLG, as derived from Figure 4b and shown in Table 1. This indicates that a homogeneous PMLG layer is formed on top of the PBLG layer.

(31) Stamm, M. *Adv. Polym. Sci.* **1992**, *100*, 357.

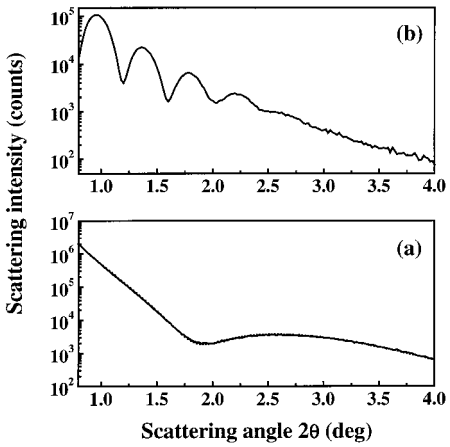


Figure 5. SAXR curves of (a**)** the APS initiator layer of ~24 Å thickness and **(**b**)** the surface-grafted PBLG-PMLG (**2**) film of ~140 Å total thickness.

XPS revealed a chemical composition of nearly theoretical PBLG values for the 45 Å thick PBLG layer of **2**, as shown in Table 1. The silicon substrate peaks Si(2s) at 150 eV and Si($2p_3$) at 99 eV are still present (Figure 4c) due to the low film thickness of this PBLG layer.

As discussed in part 1,[26] the grafting density does not reach its maximum within an hour. In this case, it is thus possible that PMLG helices grow between the already grafted PBLG helices. As expected, XPS analysis of the block copolypeptide **2** (Figure 4d) reveals an elemental composition corresponding to a mixture of pure PMLG and the underlying PBLG block (see Table 1), because of the thickness of the PMLG second block of about 95 Å as determined by ellipsometry. The disappearance of the silicon substrate peaks Si($2p_3$) at 99 eV Si(2s) at 150 eV is the result of the total thickness of the diblock copolypeptide of about 140 Å, which exceeds the limiting depth of analysis of the XPS technique. So, in this case, additional growth of PMLG results in the formation of a surface-grafted layer containing **2** and PMLG homopolypeptide.

SAXR Measurements. PBLG-PMLG surface-grafted from a silicon wafer was also studied with SAXR-measurements. The SAXR curve, shown in Figure 5b, is a superpositioning of the underlying APS coupling layer of about 24 Å (Figure 5a) and the total polypeptide film thickness of about 140 Å as obtained from the Kiessig fringes. The thickness obtained with SAXR measurements and ellipsometry match very well in this case. The deviation between both techniques is normally smaller than 10%. The surface roughness of less than 10 Å proves that rather smooth block copolypeptide layers can be produced.

Conclusions

Very smooth films of block copolypeptides can be synthesized on flat substrates by surface-grafting of *N*-carboxyanhydrides of L-glutamic acid esters from silicon wafers. These wafers have been pretreated with a primary amine-functionalized silanization agent. Due to the absence of chemical chain termination, it is possible to synthesize a second polypeptide block initiated by the still active primary amine chain ends of the first block. This results in an AB-diblock copolypeptide.

Variation of reaction time and monomer concentration are tools for tuning the length of the blocks. The polymerization of the first block (PBLG) can be stopped at a stage of maximum grafting density (2 h). When the diameter of the helices of the second block (PMLG) is smaller, the average angle between the helices in the second block grown on top of the helices of the first layer and the substrate will be smaller.

When the reaction time of the first block (PBLG) is short (1 h), there is even sufficient space for the smaller helices of the second block (PMLG) to grow not only on top of but also between already grafted PBLG helices. This leads to a more perpendicular average helix orientation in the first layer with respect to the substrate.

The method described in this paper can lead to layered surface-grafted films of defined thickness with tuned properties.

Acknowledgment. This research was supported by The Netherlands Foundation for Chemical Research (SON) with financial aid of The Netherlands Organization for Scientific Research (NWO).

Supporting Information Available: Figures showing FT-IR transmission spectra and wavelength shifts of the first PBLG block and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

LA001771J