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Cross-Linking-Induced Permanently Perpendicular Helix Orientation in Surface-Grafted Polyglutamate Films

Jeroen Luijten, Daan Y. Groeneveld, Gerard W. Nijboer, Eltjo J. Vorenkamp, and Arend J. Schouten*

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Using a chemical cross-linking procedure, surface-grafted polyglutamate films with a permanently perpendicular helix orientation were prepared. A surface-grafted α-helical polyglutamate film containing polymerizable side groups was synthesized by ring-opening terpolymerization of 50 mol% γ-methyl-L-glutamate N-carboxyanhydride (NCA), 30% γ-stearyl-L-glutamate NCA and 20% γ-4-vinylbenzyl-L-glutamate NCA initiated from a silicon substrate functionalized with primary amino groups. The average tilt angle of the end-grafted helices in this film is approximately 66°, indicating a nearly parallel helix orientation with respect to the substrate surface. After swelling of the grafted terpolyglutamate film in stearyl methacrylate and subsequent radical cross-linking, the average helix tilt angle decreases to about 11°, indicating an almost perpendicular helix orientation. The film thickness increases accordingly from 151 Å before to approximately 390 Å after cross-linking. Extensive solvent treatment of the cross-linking film shows that the perpendicular helix orientation is permanent.

Introduction

In surface-grafted polyglutamate films prepared by “grafting from” and “grafting to” techniques, the end-grafted α-helices are almost always oriented nearly parallel to the substrate surface.1−10 For many technological applications, however, a permanently perpendicular helix orientation would be highly beneficial because of the enhanced degree of molecular organization in the film and maximization of the trans-film macrodipole moment.2,11,12

Several techniques to manipulate the helix orientation in end-grafted polypeptide films have been reported. One of these methods is based on poling of the macrodipole moments present in polyglutamates13−15 by means of an electric field applied over a grafted film5 or during self-assembly of end-functionalized polyglutamates on a substrate.16 In principle, it should even be possible to switch the helix orientation between parallel (electric field off) and perpendicular (electric field on). A drawback of this approach is that the perpendicular helix orientation can only be maintained with a permanently applied electric field to keep the helices in their perpendicular state.

Wang et al.17 reported a procedure to induce perpendicular helix orientation in surface-grafted poly(γ-methyl-L-glutamate) (PBLG) films by means of a technique called “solvent-quenching”. First, the grafted film is swollen in a suitable organic solvent such as chloroform to bring the end-grafted helices into a perpendicular state. Next, the swollen film is rapidly “precipitated” in a nonsolvent such as acetone. The resulting perpendicular helix orientation is completely stable in the solid state. Unfortunately, this method only seems to work with relatively thick grafted films (on the order of 1000 Å),18 and the perpendicular alignment is lost upon contact with a solvent.

Wieringa et al.19 demonstrated that swelling of a surface-grafted poly(γ-methyl-L-glutamate-co-γ-stearyl-L-glutamate) (PMSLG 70/30) film in hexadecane results in a perpendicular helix orientation. In this case, hexadecane (a low molecular weight oleophilic molecule) is “dissolved” in the interhelical amorphous alkyl (stearyl) regions19 and the helices are forced into a perpendicular state. Fourier transform infrared (FT-IR) analysis showed that the average helix tilt angle θ (the angle between the surface normal and the helix main axis) decreased from 46° before to 15° after swelling. Unfortunately, removal of hexadecane restores the original, more parallel helix orientation.

In order to achieve the perpendicular helix orientation in the above-mentioned system permanent, the perpendicular alignment obtained after swelling should be “frozen” so the helices cannot return to the parallel state. This could be achieved by permanent incorporation of the solvent/swelling agent and/or physically connecting the helices to each other by means of interhelical cross-linking. This approach requires a surface-grafted polyglutamate film containing polymerizable side groups and a

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(13) Martin, P. G.; Stupp, S. I. Polymer 1987, 28, 897.
(14) Block, H.; Shaw, C. P. Polymer 1992, 33, 2459.
(18) The “solvent-quenching” procedure reported in ref 17 was tested with a surface-grafted PBLG film (thickness ∼150 Å) on a silicon substrate prepared as described in ref 8. FT-IR analysis did not show a perpendicular helix orientation in the film.
polymerizable solvent (swelling agent). In terms of chemistry, the following requirements for the polymerizable side group can be formulated: (1) the reactive group should be relatively stable in order to prevent premature polymerization during L-glutamic acid γ-ester synthesis, N-carboxyanhydride (NCA) monomer preparation, and surface-grafting polymerization; (2) the NCA monomer must be crystalline enough to allow purification by means of recrystallization; (3) in order to achieve a good helix grafting density, the reactive side group should not be too bulky; (4) the reactive group must be able to copolymerize with the polymerizable solvent.

In literature, various polyglutamates with polymerizable side groups have been described. Polylactides with (meth)acrylate-based side groups\(^{20}\) are highly reactive and easily lead to premature polymerization during the various synthetic steps. Also, the chain length of the ester side group (C\(_3\)–C\(_6\)) complicates the purification of the NCA monomer due to poor crystallinity.\(^{21}\) Polylactides containing allyl ester side groups\(^{22}\) are relatively easy to synthesize, but, due to the stability of the allyl radical, the cross-linking efficiency would be very low.\(^{23}\) An epoxy group is considerably more reactive, but can only be prepared by epoxidation of the corresponding allyl-functionalized polyglutamate.\(^{24}\) Finally, polymerizable side groups based on cinnamic acid derivatives\(^{25}\) are relatively bulky, which could limit the grafting density of the surface-grafted films.

Suitable reactivity, good crystallinity and limited side group bulkiness might be achieved with an L-glutamic acid γ-ester derivative containing a styrene-like polymerizable side group (γ-4-vinylbenzyl-L-glutamate (VBLG), see Figure 1). The benzylidyne character of the ester side group should facilitate crystallization of the NCA monomer (similar to γ-benzyl-L-glutamate NCA (BLG-NCA)), enabling the preparation of ultrapure monomer necessary for surface-grafting polymerization. Stearyl methacrylate (SMA) can be used as a polymerizable swelling agent. It contains a long alkyl chain for swelling of the grafted film (similar to hexadecane), and (meth)acrylates generally copolymerize well with styrene-like monomers.\(^{25}\)

This study describes a procedure to fabricate surface-grafted polyglutamate films with a permanently perpendicular helix orientation. For this purpose, a silicon-grafted terpolyglutamate film with pendant unsaturated groups was synthesized (see Scheme 1). Using the previously described surface-grafted film with pendant unsaturated groups was synthesized (see Figure 1. γ-4-Vinylbenzyl-L-glutamate.

Figure 1. γ-4-Vinylbenzyl-L-glutamate.

<table>
<thead>
<tr>
<th>Scheme 1. Synthesis of L-Glutamic Acid γ-Ester NCAs and Preparation of a Surface-Grafted Terpolyyglutamate Film with Pendant Unsaturated Groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

30% in order to maintain the solubility of the end-grafted chains in the polymerizable solvent SMA. A part of the γ-methyl-L-glutamate (MLG) content was replaced by VBLG (20%). This amount was chosen arbitrarily and corresponds roughly to one polymerizable side group per helix turn. The grafted terpolymer film was swollen in SMA and cross-linked by radical polymerization. The helix orientation in the grafted film before and after cross-linking was investigated by means of FT-IR spectroscopy.

**Experimental Section**

**Materials and Procedures.** L-Glutamic acid (Aldrich, 99%), 1-octadecanol (Janssen Chimica, 95%), 3-aminopropyltriethoxysilane (Acros, 99%), copper(II) acetate monohydrate (Merck), N,N,N,N′-tetramethyleneguanidine (Merck, > 98%), N,N-dimethylformamide (DMF; Acros, 99%), ethylenediaminetetraacetate (EDTA) dihydrate (Fluka, ≥ 97%), 4-vinylbenzyl chloride (Aldrich, 90%), SMA (TCI-EP ≤ 95%), triphosgene (Acros, 99%), DMF (Acros, extra dry, water < 30 ppm), chloroform (Acros, extra dry, water < 50 ppm) and 1,1,1,3,3,3-hexamethyldisilazane (Acros, 98%) were all used as received. Perkadox 16S radical initiator (di(4-tert-butylycyclohexyl) peroxycarbonat) was a kind gift from Akzo-Nobel and was also used without further purification. MLG (Sigma) was recrystallized from ethanol (70%) before use. Acetonitrile was distilled from CaH\(_2\), THF was freshly distilled from sodium/benzophenone, and n-hexane was freshly distilled from P\(_2\)O\(_5\). Water used for substrate cleaning and Langmuir–Blodgett (LB) experiments was purified by a reverse-osmosis system (Eligastat Spectrum SC 30) and by subsequent filtration through a Milli-Q purification system. All other solvents and reactants were reagent grade and used as received. Glassware used for substrate aminosilanization, NCA synthesis, and surface-grafting polymerizations was flame dried before use. All reactions were performed under an atmosphere of dry nitrogen.

**Measurements.**\(^{1}\)H NMR spectra were recorded on a Varian VXR-300 (300 MHz) spectrometer. The residual \(^{1}\)H atoms in the deuterated solvent were used as the internal standard. Single-reflection
attenuated total reflection (ATR) spectra were recorded on a Bruker IFS 88 spectrometer using a Golden Gate Single Reflection Diamond ATR accessory (Graseby Specac). Each spectrum is an average of 50 scans measured at a resolution of 4 cm⁻¹. FT-IR transmission spectra of surface-grafted polyglutamate films on silicon substrates were measured on a Matson Galaxy 6021 FT-IR spectrometer. Each spectrum is an average of 500 scans measured at a resolution of 4 cm⁻¹. A clean silicon substrate was used as a reference, and all polymer films were measured at three different positions. The polyglutamate peak areas were calculated using the peak integration function of the OPUS 4.0 system software (Bruker). Curve-fitting of the C=O ester, amide I, and amide II peaks was carried out as described by Wieringa et al. The C=O ester, amide I, and amide II peaks were integrated from 1780 to 1605–1600, and 1590–1520 cm⁻¹, respectively. Ellipsometric measurements were performed on a Nanofilm EP3 ellipsometer (λ = 532 nm) at an angle of incidence of 72.0°. Calculations were carried out using a three-layer model, consisting of a native SiO₂ layer (d = 28 Å, n = 1.462), a 3-aminopropyltriethoxysilane (APS) initiator layer (n = 1.428), and a polyglutamate layer (estimated n = 1.5). In addition, an estimated refractive index of 1.47 for poly(stearyl methacrylate) (PSMA) was used. The measurements were carried out at different spots on the sample.

**Synthesis of SLG (1).** The following procedure is based on the method described by Wasserman et al. An alternative reaction solvent and a modified purification procedure were employed in order to make the overall synthesis more convenient. A suspension of 5.0 g (34.0 mmol) of L-glutamic acid and 36.8 g (136 mmol) of triethylamine in 100 mL of t-amyl alcohol was heated to 60 °C. After the dropwise addition of 4.5 mL of concentrated H₂SO₄, the reaction mixture was stirred for 1.5 h at 60 °C. Afterward, the clear solution was allowed to cool to room temperature, and 8 mL of triethylamine was added dropwise. The resulting white suspension was poured into ether (1 L) and the precipitate was collected by filtration. After drying, the product was purified by recrystallization from a water/isopropanol 1:1 (v/v) mixture. Yield: 7.62 g (56%) of a white solid. ¹H NMR (300 MHz, CDCl₃/trifluoroacetic acid (TFA)): δ = 7.74 (s, br, NH⁺), 4.15 (t, 1H, α-CH), 4.03 (t, 2H, OCH₂), 2.66 (t, 2H, γ-CH₂), 2.33–2.10 (m, 2H, β-CH₂), 1.57 (t, 2H, OCH₂CH₃), 1.23 (s, 30H, OCH₂CH₃–(CH₂)₃), 0.82 (t, 3H, CH₃). IR (ATR, cm⁻¹): 2930 (v(CH₃)), 2849 (v(CH₂), 1728 (v=O ester), 1579 (v(COOH)).

**Synthesis of VBLG (2).** This compound was prepared by alkylation of the t-glutamic acid copper(II) complex with 4-vinylbenzyl chloride. Prior to use, the substrates were sonicated in ethanol and dichloromethane. After drying, the product was purified by recrystallization from a mixture of water and isopropanol 1:1 (v/v) at 70 °C to remove any remaining t-glutamic acid and DTA disodium salt. Finally, the γ-ester was purified by recrystallization from hot water. Yield: 14.6 g (28%) of 72.0 g of the C=O ester, amide I, and amide II peaks was carried out as described by Wieringa et al. The C=O ester, amide I, and amide II peaks were integrated from 1780 to 1605–1600, and 1590–1520 cm⁻¹, respectively. Ellipsometric measurements were performed on a Nanofilm EP3 ellipsometer (λ = 532 nm) at an angle of incidence of 72.0°. Calculations were carried out using a three-layer model, consisting of a native SiO₂ layer (d = 28 Å, n = 1.462), a 3-aminopropyltriethoxysilane (APS) initiator layer (n = 1.428), and a polyglutamate layer (estimated n = 1.5). In addition, an estimated refractive index of 1.47 for poly(stearyl methacrylate) (PSMA) was used. The measurements were carried out at different spots on the sample.

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with toluene, baked at 120 °C for 30 min (under vacuum), and stored under dry nitrogen atmosphere.

**Surface-Grafting Terpolymerization.** Surface-grafting polymerization was performed in a dry chloroform/DMF 5:1 (v/v) mixture at 40 °C in specially designed glassware. The 1.5 M monomer solution (containing 50 molar% MLG-NCA, 30% SLG-NCA and 20% VBGL-NCA) was added to the aminosilane substrate using a syringe fitted with a filter (Sartorius Minisart SRP15 0.45 μm). After a polymerization time of 24 h, the substrate was washed with a chloroform/dichloroacetic acid 9:1 (v/v) mixture for 24 h (at room temperature) to remove the non-grafted material. Finally, the substrate was thoroughly rinsed with chloroform, dried under vacuum at room temperature, and stored under nitrogen atmosphere.

**Swelling in SMA and Cross-Linking.** The surface-grafted poly-(γ-methyl-l-glutamate-ter-γ-stearyl-l-glutamate-ter-γ-4-vinylbenzyl-l-glutamate) 50/30/20 (PMSVBLG 50/30/20) film was swollen in a solution of Perkadox 16S radical initiator (400 mg, 1.00 mmol) in SMA (3.00 g, 8.86 mmol) at 40 °C for 48 h. Next, the temperature was raised to 80 °C, and the system was allowed to polymerize for 24 h. Afterward, the substrate was washed with warm chloroform for 5 days in a Soxhlet apparatus and dried under vacuum at room temperature.

**Isolation of PSMA.** The Soxhlet extraction solution was used to isolate PSMA. The polymer was purified by precipitation in methanol, and the obtained product was dried under vacuum at 50 °C for 2 days to give a white, slightly waxy material.

**Substrate Hydrophobization for LB Film Preparation.** Silicone substrates were cleaned following the previously described procedure. After rinsing with Milli-Q water, the substrates were sonicated in methanol, methanol/chloroform 1:1 (v/v), and chloroform, respectively, and immersed in a 1,1,1,3,3,3-hexamethyldisilazane/chloroform 1:4 (v/v) mixture at 50 °C for 1 h. Finally, the substrates were rinsed with chloroform and dried in a stream of nitrogen.

**Preparation of LB Film.** Non-grafted PMSVBLG 50/30/20 that was formed as a side product during the surface-grafting polymerization was purified by precipitation in methanol and dried under vacuum at 50 °C for 2 days. The terpolymer was dissolved in chloroform (Uvasol quality, Merck) at a concentration of 0.15 mg/mL, and the solution was spread on a computer-controlled Lauda film balance, using Milli-Q water as the subphase. Assemblies of 28 monolayers (T = 20.1 °C, Π = 20 mN/m; γ-transfer: 15 mm/min downstroke, 10 mm/min upstroke) were deposited onto hydrophobized silicon substrates.

## Results and Discussion

**Ester and NCA Synthesis.** SLG was prepared by direct esterification of l-glutamic acid with stearyl alcohol using concentrated sulfuric acid as a catalyst and a tertiary alcohol as the reaction solvent. The synthetic procedure described in this study is a modification of the method reported by Wasserman. First of all, the reaction solvent tert-butyl alcohol was replaced with tert-amyl alcohol because the latter does not have to be melted before use. Second, after the esterification step, the reaction mixture was poured directly into diethyl ether. Only the vinyl double bond remains intact during the esterification and the subsequent purification steps.

The l-glutamic acid γ-ester NCA monomers were prepared by phosgenation with triphosgene in THF at 50 °C. The total reaction time should be limited to 3 h, otherwise discoloration can occur, especially in the case of MLG-NCA. In order to remove the hydrogen chloride l-glutamate adduct (salt), the crude NCAs have to be treated with acetonitrile, which is a nonsolvent for the adduct salt. In general, four to seven washing steps with a sufficient amount of acetonitrile are necessary to obtain a salt-free NCA. After several subsequent recrystallizations from THF/n-hexane, a sufficiently pure monomer is obtained, so a rephosgenation step is not necessary. The NCA of VBGL exhibits excellent solubility in THF, chloroform, acetonitrile and DMF. Although this monomer crystallizes slower from THF/n-hexane than BLG-NCA, it does occur without problems. Again, 1H NMR analysis shows an intact vinyl double bond in the purified monomer.

**Substrate Cleaning and Aminosilanization.** Silicon substrates were functionalized with primary amino groups by aminosilanization with APS. This procedure resulted in an initiator layer with a thickness of 9 ± 2 Å, as determined by ellipsometry. In general, an APS layer with a thickness on the order of 6–11 Å is considered to be a monolayer. 34–36 Using the quantification method described by Moon et al.,33 an amino group density of approximately 2.4 per 100 Å² was calculated. Wierenga et al. 8 reported an aminosilanization procedure based on the adsorption of APS from the gas phase with refluxing toluene as the carrier and obtained an initiator layer with a thickness of 21 ± 3 Å and an amino group density of 3.2 per 100 Å². Although the number of amino groups is lower in the present case, there are still more than enough initiator groups available for ring-opening polymerization of NCAs. Surface-grafting of polyglutamates typically results in grafting densities on the order of 3–4 helices per 1000 Å, so actually only 1–1.5 out of 10 amino groups on the surface is used for grafting polymerization. 3 All in all, the substrate cleaning and aminosilanization procedure described here is simple and fast and results in the formation of an initiator monolayer with an amino group density that is sufficient for surface-grafting polymerization of NCAs.

**Surface-Grafting Polymerization.** A silicon-grafted PMSVBLG film was successfully prepared by adding a 1.5 M solution of the corresponding NCA monomers in a molar ratio of 50/30/20 in a 5:1 (v/v) chloroform/DMF mixture to an APS-functionalized silicon substrate. Ellipsometric measurements indicated a layer thickness of 151 ± 2 Å per substrate side. If the grafting polymerization is carried out in DMF, precipitation occurs within several hours, and a very thin (<50 Å) and inhomogeneous film is obtained. The use of chloroform significantly improves the overall solubility of the terpolyglutamate because it is a much better solvent for the stearyl side chains than DMF. A small amount of DMF in the reaction mixture minimizes the helix aggregation during the grafting polymerization. 21,37

Figure 2 shows the transmission FT-IR spectrum of the silicon-grafted PMSVBLG 50/30/20 film. The corresponding IR band positions are summarized in Table 1. For comparison, the band positions for surface-grafted PMSLG 70/30, 9 poly(γ-methyl-l-glutamate) (PMLG), 3 and PBLG films on silicon substrates are also included in the table.

From the band positions listed in Table 1 it can be concluded that the end-grafted terpolymer chains have a 100% right-handed α-helical conformation. 38 Compared to PMSLG 70/30, there are some small shifts in the amide bands, indicating a slightly different polymer composition. Interestingly, the amide II band of the

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I/amide II peak area ratio
In short, an LB film of the (non-grafted) terpolyglutamate was determined as described in the Supporting Information. This will be discussed later.

The average helix tilt angle in the grafted terpolyglutamate film was calculated using the following relation:

\[
\sin(90 - \theta) = \frac{h}{L}
\]

where \(\theta\) is the average helix tilt angle, \(h\) is the layer thickness, and \(L\) is the helix length. On the basis of a layer thickness of 151 Å (per substrate side) and a tilt angle of 66°, a helix length of 371 Å can be calculated. The helical rise per residue is 1.5 Å, so \(P_\alpha\) is 371/1.5 = 247. Since the exact terpolymer composition is unknown, it is not possible to calculate an exact molecular weight. A theoretical terpolyglutamate composition (based on the monomer feed) of 50% methyl groups (\(M_\alpha = 143.14\) g/mol), 30% stearyl groups (\(M_\alpha = 281.59\) g/mol), and 20% 4-vinylbenzyl groups (\(M_\alpha = 245.27\) g/mol) results in an \(M_\alpha\) of approximately 58,000 g/mol. Taking into account that the actual stearyl content is less than 30%, the final \(M_\alpha\) will be lower.

Swelling and Cross-Linking. In general, the immobilized \(\alpha\)-helices in a surface-grafted polyglutamate film will have the tendency to adopt a more perpendicular orientation in a good solvent due to the swelling effect. This was demonstrated by both Wang et al.17 (PBLG in chloroform) and Wieringa et al.9 (PMSLG 70/30 in hexadecane). Here, SMA is used as a polymerizable solvent. In a first attempt, SMA was interdiffused in the surface-grafted PMSVBLG 50/30/20 film in order to obtain a perpendicular helix orientation. Next, a small amount of benzoyl peroxide (BPO) radical initiator was added to the system, and the temperature was raised to start the cross-linking. This resulted in a very thick and hard layer of PSMA on the substrate, which hardly dissolved in chloroform.

The number average degree of polymerization (\(P_\alpha\)) of the end-grafted terpolymer can be estimated from the layer thickness and the average helix tilt angle using the following relation:

\[
\sin(90 - \theta) = \frac{h}{L}
\]

where \(\theta\) is the average helix tilt angle, \(h\) is the layer thickness, and \(L\) is the helix length. On the basis of a layer thickness of 151 Å (per substrate side) and a tilt angle of 66°, a helix length of 371 Å can be calculated. The helical rise per residue is 1.5 Å, so \(P_\alpha\) is 371/1.5 = 247. Since the exact terpolymer composition is unknown, it is not possible to calculate an exact molecular weight. A theoretical terpolyglutamate composition (based on the monomer feed) of 50% methyl groups (\(M_\alpha = 143.14\) g/mol), 30% stearyl groups (\(M_\alpha = 281.59\) g/mol), and 20% 4-vinylbenzyl groups (\(M_\alpha = 245.27\) g/mol) results in an \(M_\alpha\) of approximately 58,000 g/mol. Taking into account that the actual stearyl content is less than 30%, the final \(M_\alpha\) will be lower.

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Table 1. Experimentally Obtained Transmission FT-IR Band Positions for the Silicon-Grafted PMSVBLG 50/30/20 Film and Various Other Silicon-Grafted Polyglutamate Films

<table>
<thead>
<tr>
<th>polyglutamate</th>
<th>C=O ester</th>
<th>amide I</th>
<th>amide II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSVBLG 50/30/20</td>
<td>3294</td>
<td>1737</td>
<td>1654</td>
</tr>
<tr>
<td>PMSLG 70/30</td>
<td>3292</td>
<td>1737</td>
<td>1655</td>
</tr>
<tr>
<td>PMLG⁵</td>
<td>3292</td>
<td>1737</td>
<td>1655</td>
</tr>
<tr>
<td>PBLG⁶</td>
<td>3291</td>
<td>1734</td>
<td>1652</td>
</tr>
</tbody>
</table>

Notes: Reference 9, Reference 8.

Table 2. Overview of the C=O Ester and Amide Peak Areas in the Unscaled Transmission FT-IR Spectra of the Silicon-Grafted PMSVBLG 50/30/20 Film and the PMSVBLG 50/30/20 LB Film, the Corresponding Ratio \(D\) for Both Films, and the Calculated Average Helix Tilt Angle in the Grafted Film

<table>
<thead>
<tr>
<th>polyglutamate film</th>
<th>C=O ester</th>
<th>amide I</th>
<th>amide II</th>
<th>(D) ((A_{II}/A_{I}))</th>
<th>(\theta) (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>surface-grafted</td>
<td>0.0876</td>
<td>0.165</td>
<td>0.0315</td>
<td>5.24</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>LB</td>
<td>0.0995</td>
<td>0.201</td>
<td>0.0311</td>
<td>6.46</td>
<td>90</td>
</tr>
</tbody>
</table>

Notes: Calculated according to the procedure described in the Supporting Information.
the ratio $D$ summarizes the corresponding $C$ before and after swelling and cross-linking are shown. Table 3 FT-IR spectra of the silicon-grafted PMSVBLG 50/30/20 film degradation of the grafted film. In Figure 4 the transmission be removed relatively easily by washing with chloroform without dissolved in SMA before interdiffusion. This resulted in a cross-linking. For a better comparison of the band positions, the original spectrum of PSMA was scaled down (54.5×). The inset shows the unscaled transmission FT-IR spectrum of PSMA.

to a perpendicular helix orientation. In addition, the FT-IR band positions of the amide A, I, and II peaks do not change, which is indicative of a complete preservation of the $\alpha$-helical structure of the surface-grafted polypeptide chains. Also, the $C=O$ ester peak increases considerably due to the incorporation of PSMA. The ratio $D$ decreases from 5.24 to 1.92 after swelling and cross-linking, which corresponds to a change in the average helix tilt angle from 66° to 11°. So, the helix orientation changes from almost parallel to nearly perpendicular.

A similar swelling and cross-linking experiment with a surface-grafted PMSLG 70/30 film did not result in a permanently perpendicular helix orientation after Soxhlet extraction with chloroform (see Table 4). This is indirect proof of the presence of polymerizable vinylbenzyl side groups in the surface-grafted terpolyglutamate film. If PSMA is not covalently incorporated into the grafted film, it will be washed out completely, and the perpendicular helix orientation due to swelling will be lost.

In Figure 5 the $C=O$ ester peaks in the transmission FT-IR spectra of PSMA (-----) and the silicon-grafted PMSVBLG 50/30/20 film before (---) and after (•••••) swelling and cross-linking. For a better comparison of the band positions, the original spectrum of PSMA was scaled down (54.5×). The inset shows the unscaled transmission FT-IR spectrum of PSMA.

of the cross-linked film with warm chloroform, FT-IR analysis showed severe degradation of the film. Similar results were obtained when 2,2'-azo-bis-isobutyronitril (AIBN) was used to initiate the polymerization. A possible explanation for these results might be the poor solubility of both BPO and AIBN in SMA. This leads to an inhomogeneous distribution of the radical initiator in the system, which can result in the (local) formation of very high molecular weight PSMA. During the Soxhlet extraction with chloroform, the osmotic pressure in the grafted film can become so high that the polyglutamate chains (partially) break, resulting in degradation of the film.

In order to realize a more homogeneous polymerization, a different radical initiator was used, and the swelling procedure was slightly modified. First of all, BPO was replaced with Perkadox 16S (di(4-tert-butylcyclohexyl) peroxydicarbonate), which is more soluble in SMA. Second, the radical initiator was dissolved in SMA before interdiffusion. This resulted in a cross-linked film with a PSMA layer on top of the substrate that could be removed relatively easily by washing with chloroform without degradation of the grafted film. In Figure 4 the transmission FT-IR spectra of the silicon-grafted PMSVBLG 50/30/20 film before and after swelling and cross-linking are shown. Table 3 summarizes the corresponding $C=O$ ester and amide peak areas, the ratio $D$ for both films, and the calculated average helix tilt angles.

Figure 4 clearly shows the change in all three amide peaks after swelling and cross-linking. Both the amide A and the amide I peaks decrease, whereas the amide II peak increases. This is characteristic for the transition from a parallel helix orientation

Table 3. Overview of the $C=O$ Ester and Amide Peak Areas and Band Positions in the Transmission FT-IR Spectra of the Silicon-Grafted PMSVBLG 50/30/20 Film before and after Swelling and Cross-Linking, the Corresponding Ratios $D$, and the Calculated Average Helix Tilt Angles in Both Films

<table>
<thead>
<tr>
<th>Si–PMSVBLG 50/30/20 film</th>
<th>$C=O$ ester</th>
<th>amide I</th>
<th>amide II</th>
<th>$D$</th>
<th>$\theta$ (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>before peak area wavenumber</td>
<td>0.0876</td>
<td>0.165</td>
<td>0.0315</td>
<td>5.24</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>after peak area wavenumber</td>
<td>0.205</td>
<td>0.0895</td>
<td>0.0467</td>
<td>1.92</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

$^a$ Calculated according to the procedure described in the Supporting Information.
Figure 6. Schematic representation of the relation between the thickness (h) and the average helix tilt angle (ı) in a surface-grafted polyglutamate film with a nearly parallel helix orientation (A) and an almost perpendicular helix orientation (B). L represents the length of the end-grafted helix.

Table 5. Calculation of the C=O Ester Peak Area of PSMA and the Corresponding Fractions of Terpolyglutamate and PSMA in the Surface-Grafted Film after Swelling and Cross-Linking

<table>
<thead>
<tr>
<th>total C=O ester peak area</th>
<th>C=O ester terpolyglutamate</th>
<th>C=O ester PSMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.205</td>
<td>0.0876</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>42.7%</td>
<td>57.3%</td>
</tr>
</tbody>
</table>

and cross-linking should be identical. In other words, the C=O ester peak area of PSMA is the difference between the total C=O ester peak area and the C=O ester peak area before swelling and cross-linking (see Table 3), as summarized in Table 5. From these peak areas it is possible to estimate the composition of the cross-linked film. Interestingly, the film consists of 42.7% terpolyglutamate and 57.3% PSMA, indicating the incorporation of a considerable amount of PSMA into the grafted film.

When the helix orientation in the surface-grafted terpolyglutamate film changes from parallel to perpendicular, the thickness of the film should increase accordingly (see Figure 6).

Wang et al. reported a surface-grafted PBLG film in which the average helix tilt angle decreased from 49° to 3° after solvent quenching. Their ellipsometric measurements showed that the refractive index of the cast film decreased from 1.560 in the parallel state to 1.268 in the perpendicular state. This considerable change in the refractive index is due to the changing density of the film. The total volume of the film increases due to the perpendicular helix orientation, whereas the total amount of polymer remains the same. So, the density of the cast film will decrease considerably and so will the refractive index. In the present case, not only does the volume of the film increase, but also the amount of material on the substrate due to the incorporation of PSMA. The ratio of the C=O ester peak areas before and after cross-linking (see Table 3) gives a rough indication of the increase in the amount of polymer. Assuming a completely homogeneous system, the amount of material on the substrate increases with a factor of 0.205/0.0876 = 2.34. The volume V of the grafted film (per substrate side) can be estimated from the relation

\[ V = A \cdot h \]  

where A is the area of the substrate and h is the layer thickness. The increase in volume after swelling and cross-linking can be defined as the ratio of the volume after (V₂) and before (V₁) swelling and cross-linking:

\[ \frac{V₂}{V₁} = \frac{A \cdot h₂}{A \cdot h₁} = \frac{h₂}{h₁} \]  

Using eq 1, the following relation for the film thickness after swelling and cross-linking can be derived:

\[ h₂ = h₁ \frac{\sin(90° - ı₂)}{\sin(90° - ı₁)} \]

The helix tilt angle decreases from 66° (ı₁) to 11° (ı₂), and the layer thickness of the cast film before swelling and cross-linking (h₁) is 151 Å, so the theoretical layer thickness of the cast film with perpendicular helix orientation obtained after cross-linking (h₂) is 364 Å. In other words, if the film thickness increases from 151 Å to 364 Å, the volume of the film increases with a factor of 364/151 = 2.41. So, the increase in volume due to the perpendicular helix orientation is, to a certain extent, compensated by an increase in the amount of polymer on the substrate. This implies that the difference in the density of the cast film (and therefore also the refractive index) before and after swelling and cross-linking is relatively small. Assuming a homogeneous film consisting of 42.7% terpolyglutamate (with n = 1.5) and 57.3% PSMA (with n = 1.47), an average refractive index of 1.48 can be calculated. On the basis of this value, the thickness of the cross-linked film was determined to be 390 ± 14 Å, which is in fairly good agreement with the theoretical value of 364 Å.

Conclusions

Using the novel l-glutamic acid γ-ester derivative VBLG, a surface-grafted polyglutamate film containing polymerizable side groups could be prepared. Terpolymerization of 50 molar% MLG-NCA, 20% VBLG-NCA, and 20% SLG-NCA initiated from a silicon substrate functionalized with primary amino groups resulted in an end-grafted terpolyglutamate film in which the polypeptide chains have a stable right-handed α-helical conformation and an intact pendant unsaturation available for polymerization. FT-IR analysis showed that the average helix tilt angle in the cast film is approximately 66°, so the end-grafted helices are oriented to the substrate surface in a parallel fashion. After swelling of the cast terpolyglutamate film in the polymerizable solvent SMA and cross-linking of the system by radical polymerization, a nearly perpendicular helix orientation was obtained, as indicated by an average helix tilt angle of approximately 11°. In accordance with the decreasing tilt angle, the film thickness increased from 151 Å to approximately 390 Å, as determined by ellipsometry. The obtained perpendicular helix orientation was permanent and could not be changed by solvent treatment.

Acknowledgment. The authors thank Dr. Marijn Devillers (Radboud University of Nijmegen) for performing ellipsometric measurements.

Supporting Information Available: Equations for the calculation of the average helix tilt angle in surface-grafted polyglutamate films. This material is available free of charge via the Internet at http://pubs.acs.org.

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