Graft Polymerization on Gold

Effect of the Helix Macrodipole on Surface-Initiated N-Carboxyanhydride Polymerization on Gold**

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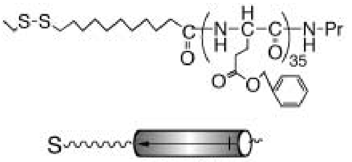
Herein, we describe surface-initiated polymerization of γ-benzyl L-glutamate N-carboxyanhydride (BLG-NCA) in the presence of helical polypeptide (PBLG) rods immobilized on gold surfaces. To our knowledge, it is the first report that the rate of NCA polymerization, initiated with immobilized primary amine, is completely controlled by the direction of macrodipole moment of the adjacent helical rods.

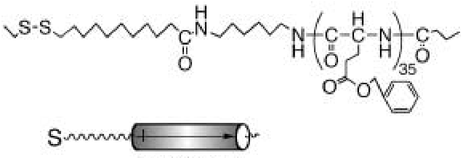
The polymerization of BLG-NCA, initiated with trialkylamines was reported for the first time by Blout and Karlson[1] to produce α-helical polypeptides. Since then, polyglutamates have been the subject of numerous studies.[2]

Growth of polypeptide brushes is an attractive method to modify and control surface properties. The resultant well-confined polypeptide brushes could have applications in the field of waveguides, data storage, and nonlinear optics (NLO) because of their optical activity. Strategies for attaching polypeptide brushes to gold surfaces include the "grafting-onto" method,[3–6] tethering preformed polymer chains from solution onto a surface, and the "grafting-from" method,[7,8] polymerizing from surface-anchored initiators. The latter provides a higher density of polypeptide brushes on a surface because the "grafting-onto" method eventually suffers from serious steric hindrance by neighboring helices and unfavorable dipolar interactions.[5] Whitesell et al. have demonstrated the polymerization of alanine NCAs, initiated by specially designed amionotrithiol-functionalized gold substrates.[7] This initiator molecule exactly meets the spatial requirements of an α helix.

In our study, we employed a mixed monolayer system on gold, composed of an amine-terminated disulfide compound **1** as an initiator and an amine-free disulfide compound **2** as a lateral diluent, so as to satisfy spatial requirements, and examined effects of the presence of a helical PBLG (PBLG-N-SS or PBLG-C-SS), which was incorporated in advance into the mixed monolayers, on the NCA polymerization. The helical polypeptide is known to generate the macrodipole, that is, in this case, the electrostatic potential directed from the N-terminus to the C-terminus.[10,11] Therefore, if the preformed helical PBLG is successfully anchored to the monolayer-covered gold substrates and positioned near the amino group, it would be expected to induce the helix-macrodipole effect on NCA polymerization. To clarify such an effect is important in view of biological processes, such as the folding process of proteins in which many proteins attain their correct conformation only with assistance with molecular chaperones.



PBLG-N-SS



PBLG-C-SS

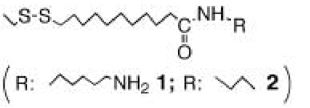


Figure 1 outlines the pathway for the preparation of a ternary self-assembled monolayer (SAM) composed of **1**, **2** and PBLG-N-SS or PBLG-C-SS on gold substrate. The adsorption processes of these compounds onto the gold surface were monitored by a quartz crystal microbalance (QCM). A gold-deposited QCM resonator (AT-cut, 9 MHz) was first placed in pure $CHCl_3$, and the solution of PBLG-N-SS, a typical helix matrix, was then added. The frequency decreased steeply because of adsorption of PBLG-N-SS and stopped changing after 2 h. The total frequency shift (ΔF) can be converted into the mass (Δm) adsorbed on gold surface by Sauerbrey's equation.[12] By using the Δm values, one can

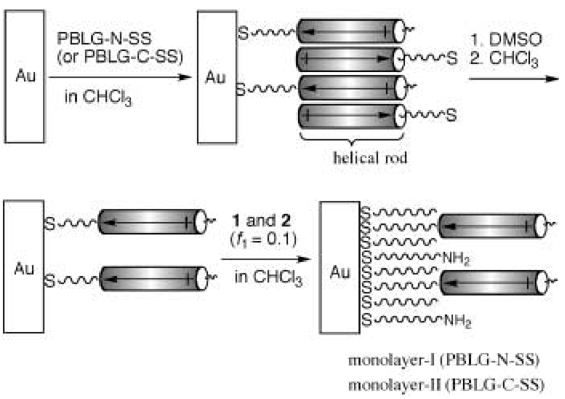


Figure 1. Preparation of the SAM of **1**, **2**, and PBLG-N-SS (or PBLG-C-SS) on gold substrate (the dipoles are reversed with PBLG-C-SS). For full details see text.

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calculate a surface coverage (Γ) of the adsorbents. For PBLG-N-SS adsorption, the Γ value was calculated to be 5.2×10^{-11} mol cm^{-2}. This value strongly suggest the formation of a densely packed α-helix assembly on the gold surface since the theoretical Γ value is in the range 3.2×10^{-11} to 8.3×10^{-11} mol cm^{-2}[13] when a hexagonal packing of helices is assumed. In our previous work, we found that helical PBLG existed mainly in a dimeric aggregate in $CHCl_3$.[5] Therefore, the assembly of PBLG-N-SS on gold thus obtained would be in a double-layered structure consisting of the dimeric aggregate of helices as shown in Figure 1. Such helix-dimer formation must the result of macrodipole interaction between helices which must be in side-by-side, antiparallel orientation. When this gold substrate was first treated with DMSO, which should behave as a helix-braking solvent, and then immersed in $CHCl_3$, the Γ value evaluated from the QCM frequency shift became 2.6×10^{-11} mol cm^{-2}, that is, just half of that before DMSO treatment. This result implies that the double-layered structure would be converted into a single-layered structure, which contains a suitable cavity for growth of a helical PBLG chain (Figure 1). Subsequently, this gold electrode was immersed in the $CHCl_3$ solution of a binary mixture of **1** and **2** until adsorption equilibrium was reached so that the formed cavity would be loaded with the binary mixture containing initiator.

The f_1 value of the mixture composition (f_1:molar fraction of **1** in the mixture) was adjusted to be 0.1, taking into account the lateral distribution of **1**. As a result, the Γ value of **1** was computed to be 2.4×10^{-11} mol cm^{-2} from the total QCM frequency shift. This value is very close to that for PBLG-N-SS in its single-layer form, which implies that about one molecule of **1** would be situated in the gap between PBLG helices as illustrated in Figure 1 (monolayer-I). The monolayer-II containing PBLG-C-SS in stead of PBLG-N-SS was prepared in the same way.

The BLG-NCA polymerization was subsequently carried out at these monolayer surfaces and monitored in situ by measuring QCM frequency shift. Figure 2 shows the frequency shift on addition of a BLG-NCA/$CHCl_3$ solution (2.5×10^{-2} mM) into pure $CHCl_3$, in which the monolayer-covered QCM electrode was immersed. For comparison, the same polymerization experiment was performed for a binary monolayer of **1** and **2** ($f_1 = 0.05$) without PBLG matrix (Figure 2, curve b). It can be seen in every case that the frequency decreases immediately upon addition of BLG-NCA, meaning the progress of polymerization. The rate of polymerization was evaluated from the initial slope of the frequency decay curve (Table 1). The rate of polymerization in the presence of PBLG-N-SS is accelerated by a factor of about 10 compared to that in the absence of PBLG matrix. In contrast, the presence of PBLG-C-SS depressed the BLG-NCA polymerization. These effects of helical PBLG matrices on polymerization must be a result of their macrodipole interaction. In case of PBLG-N-SS, the direction of its helix-macrodipole moment is antiparallel to that of the polymerized PBLG chain, and therefore the growth of PBLG chain by polymerization between PBLG-N-SS matrices would be much more favorable than that without PBLG matrix. Conversely, the presence of PBLG-C-SS, which has a macrodipole moment direction that is parallel to the polymerized chain, led to an energetic disadvantage for the chain growth. Such a macrodipole effect also had a remarkable influence on the degree of polymerization (n) of each polymerized chain (Table 1). The n values were calculated from the total frequency change at 7 h polymerization; they are 95 and 15 for PBLG-N-SS and PBLG-C-SS monolayers, respectively. The value of 95 observed for PBLG-N-SS monolayer is larger than that of PBLG matrix ($n = 35$), which reflects the enhanced polymerization as a result of the helix-macrodipole interaction. On the other hand, the PBLG-C-SS monolayer limits the chain growth to $n = 15$. This chain length of $n = 15$ is consistent with the chain length ($n = 16$),[14] beyond which peptide segments are in general able to commence forming helical conformation and generate a helix-macrodipole moment. The helical PBLG-C-SS matrix is energetically unfavorable for the parallel orientation of the helix macrodipole, and accordingly suppressed any chain growth beyond $n = 15$.

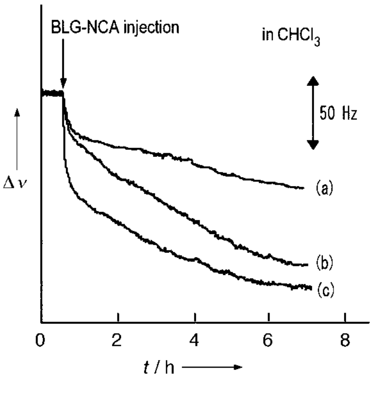

Figure 2. Frequency shifts of gold-coated QCM resonators in $CHCl_3$ modified with the **1**/**2**-mixed monolayer in the presence of PBLG-C-SS (a), in the absence of PBLG matrices (b), or in the presence of PBLG-N-SS (c) on addition of a BLG-NCA/$CHCl_3$ solution.

Table 1: Effects of preformed PBLG matrices on rate and degree of polymerization.

Monolayer	Rate of polymerization $\times10^{11}$ [M s^{-1}]	n[a]
1/**2**-mixed monolayer ($f_1 = 0.1$)	5.5	73
monolayer-I	50	95
monolayer-II	3.9	15

[a] Average degree of polymerization.

To elucidate the orientation of the helical rods thus obtained, FTIR spectra of the C=O stretching region were measured for the monolayer-I before and after NCA polymerization, and shown in Figure 3. In the amide I and amide II regions, both spectra exhibit two bands at 1655 cm^{-1} and 1547 cm^{-1}, which are assigned to the α-helix structure.[15,16] The additional C=O stretching band, arising from the ester groups of the side chain, is observed at 1733 cm^{-1}. Enriquez and Samulski[17] demonstrated that the nature of the order and average orientation of the polypep-

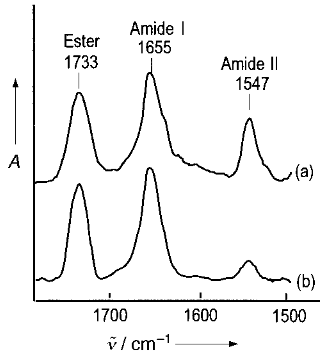

Figure 3. FTIR (raman) spectra of the monolayer-I before (a) and after (b) 7 h polymerization of BLG-NCA.

tide α helix in the films can be inferred from FTIR data. By using the ratio of the integrated intensities of the amide I and amide II regions, the average tilt of the helix axis from the surface normal was evaluated.[18] From the spectral data in Figure 3, the average tilting angle of the helices in the monolayer after NCA-polymerization is calculated to be 27°, which is significantly enhanced in the perpendicular orientation, compared with the 52° tilt found before polymerization. This result suggests that by polymerization of NCA between helical PBLG-N-SS matrices, the monolayer adopts a head-to-tail antiparallel orientation, which is energetically more favorable, and consequently achieves a nearly perpendicular helix orientation.

In conclusion, preformed PBLG helix rods can control the NCA-polymerization on the basis of helix-macrodipole interaction, and eventually allow the successful preparation of densely bound PBLG helical brushes on gold surfaces. Probably the key advantage of this technique is the high grafting density of helix brushes in an antiparallel orientation, which results in monolayers that are uniform at the Å level. We believe that these findings are of importance not only for a new approach to fabrication of polypeptide self-assembled materials but also for a relevant model of chaperone-assisted protein folding.

Experimental Section

Compound **1** was prepared by the reaction of 11-(ethyldithio)undecanoic acid[9] with *n*-(6-aminohexyl) carbamic acid *tert*-butyl ester in the presence of *N'*-(ethylcarbonimidoyl)-*N,N*-dimethyl-1,3-propnaediamine (EDC) and DMAP (DMAP = 4-dimethylaminopyridine) as condensing agent and catalyst, respectively, and then removal of *tert*-butoxycarbonyl group. Compound **2** was prepared by the reaction of 11-(ethyldithio)undecanoic acid with *n*-propylamine in the presence of EDC and DMAP. These products were identified by IR and ^{1}H NMR spectroscopy, and their purity was checked by means of TLC. PBLG-N-SS was prepared by polymerization of BLG-NCA initiated with *n*-propylamine, and then by condensation reaction with 11-(ethyldithio)undecanoic acid in the presence of EDC and DMAP, as reported previously.[5] PBLG-C-SS was prepared by polymerization of BLG-NCA initiated with **1**, and then by treatment with *n*-butanoyl chloride. These PBLG derivatives were characterized by ^{1}H NMR spectroscopy and MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry.

Measurements: Gold-coated AT-cut quartz crystals (USI System Ltd., Japan) with a fundamental resonance frequency of 9 MHz were employed for QCM measurement. Detailed procedures were described previously.[5a,b] FTIR was performed on a Nicolet System 800 spectrometer with a mercury—cadmium–tellurium (MCT) detector. The measurements were carried out with the 1024 scans of interferogram accumulations using a bare gold substrate as a reference for reflection absorption spectroscopy (raman) measurement. The optical path was purged with dry air before and during measurements. A reflection attachment at an incident angle of 80°, together with a polarizer, was used.

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