JOURNAL OF
Materials
Communication
CHEMISTRY

Spontaneous formation of single- and double-layered polypeptide assemblies based upon a helix–macrodipole interaction

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Received 9th October 1998, Accepted 26th October 1998

A novel gold-adsorbable poly(γ-benzyl-L-glutamate) has been prepared, and its adsorption processes onto gold substrates from $CHCl_3$ solutions have been examined by means of a quartz crystal microbalance technique. The aggregation state of the resultant polypeptide assemblies was found to be controllable on the basis of the helix–helix macrodipole interaction.

To model the higher-ordered structures of biopolymers such as proteins by using purely synthetic polyelectrolytes, we have devised a strategy in which polyelectrolytes such as poly(L-glutamic acid) are aligned on two-dimensional media.[1] Au-deposited plates have been employed as two-dimensional solid substrates since well-organized monolayers can be prepared on Au by spontaneous adsorption of organic thiols and disulfides.[2,3]

Recently, there has been considerable interest in constructing self-assembled polypeptides on solid substrates. Such structures are expected to be tethered to the surface while having a controlled orientation and molecular conformation. Enriquez and coauthors coupled a disulfide moiety to a poly(γ-benzyl-L-glutamate) (PBLG) molecule, so that PBLG could directly react with a gold substrate.[4] Whitesell *et al.* have shown that an anisotropic, α-helical film of poly(alanine) or poly(phenylalanine) can be formed on gold by graft-polymerization of peptide monomers (*N*-carboxy anhydrides of amino acids, NCAs) initiated with surface-attached species.[5] Here we describe for the first time reversible formation of single- and double-layered α-helical polypeptide (PBLG) assemblies on gold-deposited substrates from nonaqueous solutions based upon helix–macrodipole interaction.[6–9]

A novel gold-adsorbable PBLG (**1**)[†] consists of a PBLG segment (number-average degree of polymerization, $n = 29$) and two long alkyl chains whose ends are modified with disulfide bonds.

The secondary structure of **1** in solutions was first examined by means of ^{1}H NMR spectroscopy since it has been shown that the proton signals of α-CH and α-NH of the peptide main chain shift depending on secondary structures such as α-helix and random coil.[10] In $CHCl_3$, **1** was found to take an α-helix conformation (100%), while in DMSO-d_6 the helix content decreased to 63% with increasing random coil content. This effect of DMSO on the PBLG conformation is consistent with that observed for poly(β-benzyl-L-aspartate).[11] The adsorption processes of **1** in $CHCl_3$ onto gold substrates were subsequently monitored by using a quartz crystal microbalance (QCM). A gold-coated QCM resonator (AT-cut, 9 MHz) was placed in pure $CHCl_3$, and a **1** solution (10 mmol dm^{-3} in $CHCl_3$, 1 ml) was then added at 20 °C (Fig. 1a). The frequency decreased steeply with addition and became unchanged after 2 h at a frequency shift (ΔF) of -520 ± 10 Hz. The ΔF value is proportional to the mass (Δm, ng) of **1** adsorbed on the gold surface.[‡] By using the value of mass, a mean occupied area of **1** molecule on the gold surface can be evaluated to be 0.42 ± 0.01 (nm^2 molecule^{-1}) (Table 1). An FT-IR RAS spectrum (Nicolet System 800) of the adsorbed film of **1** prepared in the same way on a gold-coated glass plate showed that the PBLG segment took α-helical conformation with a high helix content of 94% even in such a self-adsorbed assembly. Since the cross-sectional area of α-helical PBLG segment has been known to be in the range of 0.8–5.3 nm^2, depending on the configuration of its side chain,[12] the evaluated molecular area (0.42 ± 0.01 nm^2 molecule^{-1}) is about half of the minimum cross-section of PBLG helical rod, suggesting that **1** would adsorb on the gold surface in a double-layered structure and not in a monolayer. In order to reveal the aggregation state of **1** in solution, vapor pressure osmometry (Knauer) was carried out for $CHCl_3$ and DMSO solutions of **1**. The molecular weights were evaluated to be 11000 and 6300 in $CHCl_3$ and DMSO, respectively. This result strongly suggests that the **1** molecule exists mainly in a dimeric aggregate in $CHCl_3$ and as monomers in DMSO since the calculated molecular weight

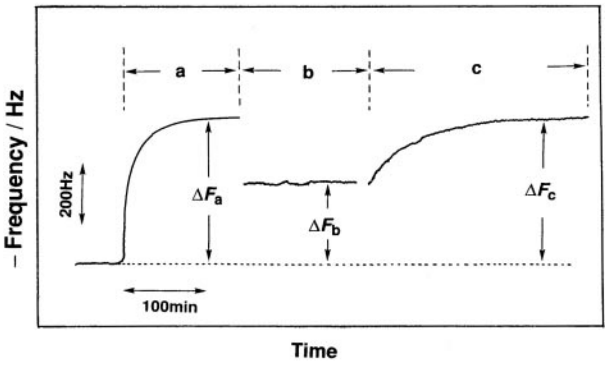

Fig. 1 Time course of QCM frequency shifts at 20 °C when a **1** solution (10 mmol dm^{-3} in $CHCl_3$, 1 ml) was added (a), treated with DMSO (b), and then a **1** solution (10 mmol dm^{-3} in $CHCl_3$, 1 ml) was again added (c). Detailed procedures are described in the text.

Table 1 Total frequency changes due to several treatments of QCM electrodes

Treatment^a	ΔF^b/Hz	Δm^c/ng	Occupied area^d/nm^2 molecule^{-1}
a	-520 ± 10	557 ± 10	0.42 ± 0.01
b	-270 ± 20	289 ± 20	0.80 ± 0.05
c	-490 ± 20	525 ± 20	0.44 ± 0.02

^{a}Symbols a, b, and c correspond to those in Fig. 1. ^{b}Total frequency changes (ΔF_a, ΔF_b, and ΔF_c) caused in the a, b, and c regions in Fig. 1. The ΔF values are the average of four repeated runs. ^{c}The Δm values were calculated by the following equation: $\Delta m = -1.07 \times \Delta F$. ^{d}Occupied area $= AMN_A/\Delta m$: A, area of Au electrode; M, molecular weight; N_A, Avogadro's number.

of **1** is 7100. If the proposed, double-layered model (Fig. 2) can be assumed to be stabilized on the basis of macrodipole interactions of head-to-tail molecular pairs working among helix rods, the double-layered structure should be transformed to the monolayer structure by weakening the helix–macrodipole interaction. The QCM resonator covered with **1** was thus immersed in DMSO for 1 h, to cause a conformational change of the PBLG segments from the helix to the random coil. In fact, an FT-IR RAS spectrum for the film treated with DMSO (data not shown) showed an obvious decrease in the helix content from 94 to 62%, compared with the spectrum before treatment. After 1 h immersion in DMSO, the QCM resonator was washed carefully with $CHCl_3$ several times to remove DMSO and the frequency change was then measured in $CHCl_3$ at 20 °C. This procedure was repeated until the frequency became unchanged (Fig. 1b). The frequency was found to increase at $\Delta F = -270 \pm 20$ Hz, meaning that about half of the **1** molecules adsorbed were removed by the DMSO treatment, probably because the conformational change from α-helix to random coil induced weakening of the helix–macrodipole interaction. In part c of Fig. 1, a **1** solution (10 mmol dm^{-3} in $CHCl_3$, 1 ml) was again added, and interestingly, the frequency was found to revert slowly to the original value, indicating formation of a double-layered structure due to adsorption of helical **1** molecules through helix–helix interactions.

In conclusion, we have demonstrated in this study that single- and double-layered helix polypeptide assemblies can be spontaneously produced on gold substrates from organic solvents on the basis of a helix–macrodipole interaction which is readily controlled by the polypeptide conformation.

Notes and references

†The preparation procedure of **1** is as follows. First, poly(γ-benzyl-L-glutamate) (PBLG) was prepared by polymerization of γ-benzyl-L-glutamate-*N*-carboxylic anhydride initiated with *n*-propylamine. Subsequently, esterification of ω-(ethyldithio)-undecanoic acid[11] with diethanolamine was carried out in the presence of toluene-*p*-sulfonic acid and then treated with triethanolamine, and subsequent reaction with succinic acid anhydride. Finally, the carboxylic acid group of the compound thus obtained was reacted with the residual amino group at one chain-end of the PBLG obtained above in the presence of DCC as a condensing agent.

‡The QCM, which was used in this study, was 0.20 cm^2 in an effective surface area followed Sauerbrey's equation[13] that is expressed as $-\Delta F = 0.93 \times \Delta m$.

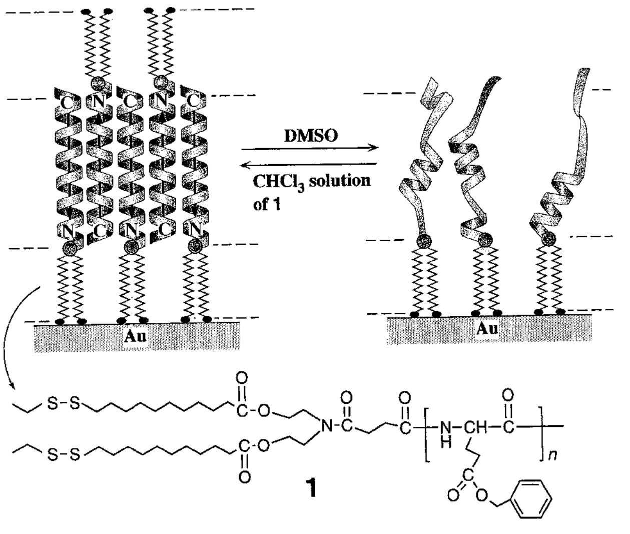

Fig. 2 Schematic illustration of the single- and double-layered polypeptide assemblies on a gold substrate due to a helix–macrodipole interaction, and chemical structure of **1**.

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