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Construction of poly(ethylene glycol) tethered chain surface possessing mercapto group at the distal end for protein immobilization

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Abstract: Marcapto-ended PEG tethered chain was constructed on a gold surface by the consecutive treatments with a short mercapto-ended semitelechelic PEG (MeO-PEG-SH; MW=2k), followed by a long mercapto-ended telechelic PEG(SH-PEG-SH; MW=5k). By changing the density of the pre-constructed MeO-PEG-SH(2k) tethered chain, the amount of modified SH-PEG-SH could be controlled, which was monitored by a surface plasmon resonance (SPR) instrument. Under the suitable modification conditions, ideal PEG tethered chain surface possessing the effective amount of the mercapto ends were constructed, avoiding loop formation by the mercapto-ended telechelics. Maleimide-BSA could be immobilized via a Michael type addition reaction on the SH-installed PEG tethered chain surface effectively. The immobilized proteins thus obtained were confirmed to retain a high activity for the specific interaction with antibody or antigen. The surface possessing the mercapto-ended PEG tethered chain thus prepared can be regarded as a promising tool for protein immobilization and protein-protein interaction analyzing surface.
Key words: Poly(ethylene glycol), Surface plasmon resonance, protein immobilization, mercapto group

Introduction

Characterization of protein and protein-protein interaction was a key technology for a proteomics analysis. Protein chips technology has emerged as a promising tool for screening protein-protein interactions and characterizing the levels of proteins expressed in cells[1,2]. Protein chips have also been used as a proteomics screening tool to characterize biochemical processes by identifying novel protein-protein and protein-DNA binding interactions. Particularly, the exploitation of surface plasmon resonance (SPR) analyzer has been widely utilized as a label-free, real-time measurement system for biomolecular inter action[3,4]. The development of surface-based proteomics tools required general and facile methods for the immobilization of protein on the surface by a specific orientation, retaining its functionality[5,6]. Non-specific adsorption of biomolecules such as serum proteins and lipids is one of the other issues to reduce the sensing performance of protein-protein interactions[7,8]. The present demand is to satisfy the specific protein orientation on the surface without denaturation, retaining non-fouling character of the surface[9,10,11,12,13].

So far, numerous types of surface modifications such as self-assembled monolayers (SAMs) of alkanethiolates[14] and hydrophilic polymer brush on the surface have been reporte[15]. These surfaces showed reduced protein adsorption to some extent. For the protein sensing with high sensitivity, however, non-specific adsorptions must be avoided as low as possible.

We have been focusing on preparation of complete non-fouling surface using PEG tethered chain surface. There are two key technologies for our surface modification. One is the use heterotelechelic poly(ethylene glycol) (heteroPEG), which as prepared by our original synthetic method[16]. The other is to construct mixed PEG tethered chain surface, which means that the PEG tethered chain consists of both long (5k) and short (2k) chain length[17,18]. Construction of the mixed PEG surface by long heteroPEG coupled with short semi-telechelic PEG, reduced non-specific adsorption of biomolecules almost completely. In addition, a ligand molecules at the distal end of the tethered PEG chain on the surface could be achieved[19]. However, protein rejection and conjugation of ligand on the mixed heteroPEG tethered chain surface are trade-off relation. Due to the protein repellant character, it is difficult to conjugate biomolecules such as antibodies and membrane proteins on their surface. A convenient and effective protein immobilization on the PEG brushed surface should be required.

In order to construct facile conjugation of proteins on the non-fouling PEG tethered chain surface, we designed mercapto-ended PEG tethered chain surface using telechelic PEG possessing a mercapto group at both end. The idea was to construct short PEG chain (2k) with controlled brush density, followed by the immobilization of the SH-ended telechelics (5k).

In this treatment, mixed PEG chain possessing the mercapto group at the distal end of the long PEG chain was constructed avoiding loop formation. Immobilizations of proteins via a maleimide addition as well as a dithiol conjugation were effectively achieved. Protein-protein interaction of the prepared surface was monitored using SPR sensor.

Experimental Section

Materials

Tetrahydrofuran(THF), triethylamine, *N*-*N*'-dimethylformamide(DMF), sodium hydride(NaH), and methanesulfonyl chloride were purified by conventional methods. Hydroxyl-ended telechelic poly(ethylene glycol)(OH-PEG-OH, M_n = 4600) (Aldrich) and mercapto-ended semitelechelic poly-(ethylene glycol)(MeO-PEG-SH, M_n = 2000)(NOF) were used as received. N-proplyamine(Wako), sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclo-hexane-1-carboxylate(Sulfo-SMCC)(Pierce), Bovine Serum Albumin, (BSA)(Sigma) and all other reagents were used as received. A gold sensor chip (SIA kit Au) for SPR measurements was purchased from Biacore AB. SPR evalutions were carried out on a Biacore 3000 device (Biacore AB).

Synthesis of mercapto-ended telechelic poly-(ethylene glycol)

For the preparation of mercapto-ended telechelics, PEG having methane sulphonyl group at both end (MS-PEG-MS) was prepared from hydroxyl-ended telechelics. The OH-PEG-OH (1.0 mmol) and THF (20 ml) were added to a 100mL round-bottom flask containing an argon atmosphere. After the PEG was dissolved, NaH (20 mmol) and triethylamine (9.0 mmol) were added to the PEG solution. The prepared PEG solution was added to the THF solution of methanesulfonyl chloride (7.0 mmol) at the room temperature with stirring. The mixture was further stirred for over night. The obtained polymer (MS-PEG-MS) was precipitated into an excess amount of diethyl ether and separated by filtration. The precipitate was dried in vacuo and then finally freeze-drying with benzene.

For the preparation of thiol-ended PEG telechelics, the potassuim-*O*-ethyldithiocarbonate (8.8×10^{-4} mol), THF (50 mL) and DMF (3.6 mL) were added to the round-bottom flask under an argon atmosphere and stirring for several minutes. The mixture solution was added to the THF solution of MS-PEG-MS and stirred for several hours. After the reaction, the obtained polymer (DTC-PEG-DTC) was recovered in the same way as described above. In order to convert *O*-ethyldithiocarbonate-end to thiol group, 14 mmol of propylamine was added to THF solution of the obtained DTC-PEG-DTC (2.2×10^{-5} mol) and stirred for several hours. After the reaction the obtained polymer (SH-PEG-SH) was recovered in the same way as described above.

Preparation of mercapto-ended telechelic PEG brush surface

After the bare gold SPR sensor chip was cleaned by piranha solution (H_2SO_4:H_2O_2=3:1) for several minutes. This chip was docked into the SPR instrument. Sodium phosphate buffer (pH 7.4, 50 mM, containing 1 M NaCl) solution of methoxy-PEG-SH (MeO-PEG-SH) (M_n = 2k, 1 mg/mL) was injected at a constant flow rate of 5 μL/min at 37 °C for several minutes by monitoring the amount of immobilized MeO-PEG-SH by the SPR angle shift (0.075-0.1°). To the pre-constructed MeOPEG(2k) tethered chain with the controlled brush density, mercapto-ended PEG telechelics (5k) was modified (1 mg/mL, pH 7.4, 50 mM, containing 1 M NaCl). The mercapto-ended PEG telechelics was obtained just before use by the reaction of 57.5 μL of 1.4 M n-propylamine with 500 μL of DTC-PEG-DTC aqueous solution (1 mg/mL) for 30 min at 37 °C. The obtained SH-PEG-SH was purified by a column separation using NAP-5 column with sephadex G-25 (Amersham Biosciences), 1 mL of SH-PEG-SH fraction was eluted by PBS solution (pH 7.4, 50 mM, containing 1 M NaCl). After the immobilization of SH-PEG-SH, the solution of MeO-PEG-SH was injected again at a constant flow rate of 5 μL/min at 37 °C three times to improve the brush density maximum.

Nonspecific adsorption of protein to PEG modified surface

Adsorption of BSA on the prepared PEGylated surface was measured by SPR. A solution of 10 μM BSA was allowed to follow onto the constructed PEG surface at the a constant flow rate of 5 μL/min for 30 min at 25 °C and then the SPR response shift was measured. As a control, BSA adsorption on a bare gold chip was examined.

Immobilization of Maleimide-Protein to PEG modified surface

For the preparation of maleimide-BSA, 54.4 μL of 1mM sulfo-SMCC solution was added to 500 μL of 10 μM BSA solution and stirred 30 min at 37 °C. The obtained protein solution was purified by using NAP-5 column and 1mL fraction of BSA-maleimide solution was eluted by PBS buffer (pH 7.4, 50 mM, containing 0.15 M NaCl). Immobilization of the BSA-maleimide on the SH-installed PEGylated surface was carried out using the SPR instrument, monitoring the angle shift. A solution of 10 μM BSA-maleimide was allowed to follow onto the constructed PEG surface at a constant flow rate of 1μL/min for 180 min at 25 °C. As a control, 10 μM BSA solution was injected onto the constructed PEG surface at the a constant flow rate of 5 μL/min for 30 min at

25 °C and then the SPR response shift was measured.

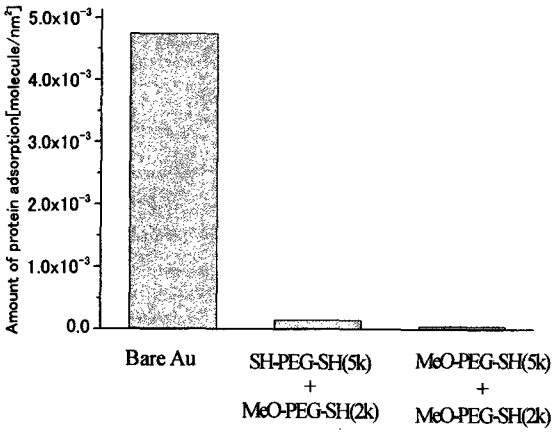

Figure.1 Amount of BSA adsorption via nonspecific adsorption versus various surface.

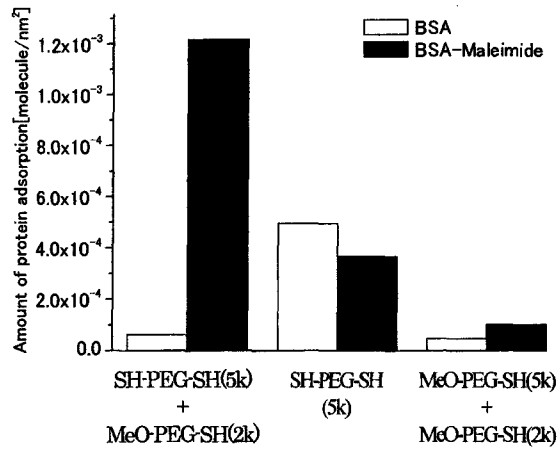

Figure.2 Amount of protein adsorption via specific or nonspecific adsorption versus various PEG surface.

Results and Discussion

The purpose of this study was to construct a PEG tethered chain surface having mercapto group at the distal end of the PEG chain and immobilization of specific proteins at the end of the PEG chain end for high performance detection of protein-protein interaction, retaining the low non-specific interaction of environmental proteins. Our idea was to employ the consecutive treatments of bare gold surface with the short MeO-PEG-SH (MW= 2k), followed by the treatment with long SH-PEG-SH (5k). The pre-immobilized short PEG chain may prevent an loop formation when the surface was modified with long SH-PEG-SH(5k). From the SPR measure ment, the amount of immobilized PEG was monitored. With increasing the amount of the preinstalled short PEG(2k) chain, the amount of immobilized SH-PEG-SH(5k) was decreased. For example, the SPR angle shifts of SH-PEG-SH were 0.14°and 0.09°, when the surfaces were pre-immobilized by the short PEG chain, which angle shifts were 0.05° and 0.1°, respectively. Under the suitable conditions, non-specific adsorption of BSA was assessed as shown in Figure 1. When BSA was contacted with bare gold surface, significant amount of non-specific adsorption took place. On the contrary, the MeO-PEG-SH(2k)/ SH-PEG-SH(5k) mixed PEG chain surface showed almost complete non-fouling surface, which was the same tendency as that of MeO- PEG-SH(2k)/MeO-PEG-SH(5k) mixed surface[19]. This result shows that the PEG brushed layer was effectively constructed by the consecutive treatment of MeO-PEG-SH(2k) followed by the treatment with SH-PEG-SH(5k). From the data obtained in the Figure 1, it is anticipated that the SH-PEG-SH(5k) forms tethered chains on the surface, but not loop formation. Thus, the immobilization of specific proteins can be anticipated via the specific reaction with the SH end group. In order to confirm the specific conjugation reaction of the protein, maleimide- BSA was conjugated via the Michael type addition reaction with mercapto group on the surface. The immobilization data were summarized in Figure 2. On the MeO-PEG-SH(2k)/SH-PEG-SH(5k) mixed surface, very large amount of the maleimide-BSA was immobilized, which was sharp contract to that with BSA. When the PEG surface was constructed with only SH-PEG-SH(5k), the immobilization amount of maleimide-BSA was decreased and almost equal to free BSA, indicating non-specific adsorption of maleimide- BSA on the SH-PEG-SH(5k) surface, but not the covalent conjugation via the Michael reaction with the maleimide. It can be explained by the loop formation on the gold surface by SH- PEG-SH(5k) chains. In the case of MeO-PEG-SH (2k)/MeO-PEG-SH(5k) surface, neither maleimide -BSA nor free BSA showed very low adsorption. On the basis of the obtained results, the mixed MeO-PEG-SH(2k)/SH-PEG-SH(5k) surface showed tethered chain fashion, avoiding loop formation. The mercapto group could be installed at the distal end of the PEG chain and can be utilized for the conjugation of the specific biomolecules via the Michael type addition reaction. Such the SH-installed PEG tethered surface is promising as on of the technique for the construction of specific protein immobilized surface retaining non-fouling character.

Conclusion

In this paper, we described the surface engineering with the mercapto-ended telechelic PEG on the gold surface. Construction of PEG tethered chain on a gold surface by the consecutive treatments with a mercapto-ended semitelechelic PEG (MeO-PEG-SH), followed by a mercapto-ended telechelic PEG (SH-PEG-SH). Under the suitable density of the preconstructed short MeO-PEG-SH(2k), enough amount of long SH-PEG-SH(5k) can be immobilized as a tethered

fashion, but not the loop formation. The mercapto group at the end of the long PEG chain can be utilized for the modification of maleimide-BSA via the Michael type addition reaction.
Thus the protein immobilized surface having high non-fouling character can be easily obtained and it is promising as a new bio-interface for high performance protein recognition.

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