Effect of Length of Biotin Modified *n*-Alkanethiolates on the Formation of a Self-Assembled Streptavidin-Biotin Monolayer on a Gold Substrate

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ABSTRACT

The molecular recognition between streptavidin and biotin makes the streptavidin-biotin system very useful in a wide range of biotechnological applications. Currently, biotin modified *n*-alkanethiolates have been extensively studied to create a wide variety of self-assembled monolayers (SAMs) on gold surfaces. For the formation of optimum monolayer films, an appropriate biotin-terminated molecule should be selected to minimize nonspecific interactions between streptavidin and the modified surface and to maximize specific binding between them. In the present work, various monolayer films with adsorption of biotin-terminated long-chain *n*-alkanethiolates and of mixtures with 11-mercaptoundecanol (MUOH) from solution onto the gold surfaces were prepared and characterized by optical ellipsometry, contact angle goniometry, X-ray photoelectron spectroscopy (XPS), and scanning tunneling microscopy (STM).

Keywords: SAM, streptavidin, biotin

1 INTRODUCTION

Streptavidin binds biotin with very high and specific affinity ($K_a = 10^{13}$ M^{-1}) and has four binding sites for biotin situated on two opposite sides of the tetrameric protein, which provides us with the possibility of cross-linking between different biotin-containing molecules and adds another dimension to the use of this multi-faceted system [1]. These distinguishing features make a streptavidin-biotin molecular recognition system extremely useful in a wide range of biotechnological applications. To optimize the molecular recognition process of streptavidin-biotin systems, a better understanding of immobilization of streptavidin on a solid surface is necessary.

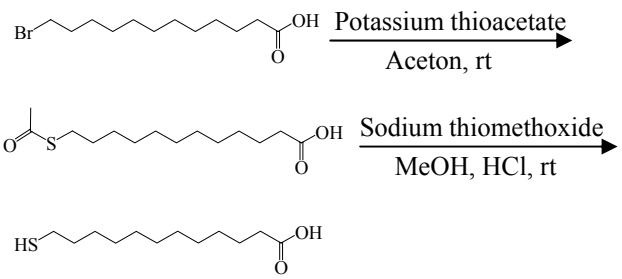
Self-assembled monolayers (SAMs) can provide a reproducible and robust method of fabricating immobilized protein layers. To date, many extensive studies on SAMs of streptavidin on biotin modified gold surfaces have been undertaken [2-7]. The main conclusion of those studies is that the closely packed biotin groups hinder molecular recognition with the biotin-binding pockets of streptavidin. Therefore, addition of hydrophilic spacers and dilution of biotin modified precursor are generally required to reduce the steric hindrance and to increase the flexibility of the biotin modified precursor toward the biotin-binding pockets of streptavidin. Also, it has been reported that biotin-terminated aliphatic thiols and their mixtures of thiols with different chain lengths and/or head groups strongly affect the specific binding between streptavidin and biotin. However, quantitative study on the effect of length of biotin modified *n*-alkanethiolates is very rare. Here, we report experimental results that focus on the length of long-chain *n*-alkanethiolates [$HS(CH_2)_nCOOH$, where n= 10, 11, and 15], in which the terminal functional group (-COOH) can be readily converted to an amide by reaction with an amine, in the streptavidin-biotin molecular recognition system.

2 EXPERIMENTAL SECTION

All chemicals were supplied from Sigma-Aldrich Co. and used as received unless stated otherwise. 16-mercaptohexadecanoic acid (MHA) was recrystallized in hexane before use. 12-mercaptododecanoic acid (MDA) was synthesized in a general and mild condition using KSAc and NaSMe for a synthetic reagent: the first step was a synthesis of thioester from aliphatic bromide and the second step was a conversion of thioester to thiol [8,9]. The synthetic procedure was detailed in scheme 1 [10].

The pure SAMs of MUOH, MUA (11-mercapto undecanoic acid), MDA, MHA were prepared, respectively, by immersing a clean gold substrate into an ethanol solution of the pertinent 1 mM *n*-alkanethiolates overnight (> 16 h) at room temperature. After the formation of pure SAMs, the samples were rinsed in ethanol, followed by ultrasonication in ethanol for 10 min, and then dried under a stream of nitrogen. The mixed SAMs of MUA/MUOH, MDA/MUOH, and MHA/ MUOH were also prepared using the same method as that described for the pure ones. They were prepared as a function of mole fraction of MXA in solution (f_{MXA} = 0.2, 0.4, 0.6, and 0.8, where MXA denotes MUA, MDA, or MHA).

SAMs of biotin modified *n*-alkanethiolates on gold substrates were prepared using 2,2'-(ethylenedioxy)bis-(ethylamine) (DADOO) and (+)-Biotin N-hydroxy succinimide ester (NHS-biotin). This layer-by-layer method is similar to that reported by *Yang* and coworkers [11]. Separately, 10 μM streptavidin solution was prepared using 10 mM phosphate buffered saline (PBS). The substrates of the biotin modified SAMs were incubated in the streptavidin solution for 2 h. The streptavidin-adsorbed substrates were rinsed with PBS and dried with a gentle stream of high-purity nitrogen.



Scheme 1: Synthesis of 12-mercaptododecanoic acid.

Compounds	Au 4f	N 1s	S 2p
Au/MUA	83.38 87.07	-	161.50 162.58
Au/MDA	83.36 87.07	-	161.37 162.63
Au/MHA	83.45 87.14	-	161.59 162.78
Au/MUA-DADOO-Biotin	83.48 87.18	397.60 398.23	161.57 162.70
Au/MDA-DADOO-Biotin	83.48 87.17	397.63 398.27	161.59 162.79
Au/MHA-DADOO-Biotin	83.48 87.17	397.60 398.25	161.60 162.85

Table 1: XPS measurements: peak maxima [eV] of the compounds.

Ellipsometric measurements were performed using an ellipsometer (SE, MG-1000, Korea), equipped with a He-Ne laser (λ = 632.8 nm), at an angle of incidence of 70°. The method for calculating the film thickness was based on a three-phase ambient/film/gold model, in which the film was assumed to be homogeneous and isotropic and was assigned a scalar refractive index of 1.45. Advancing contact angle measurements of water were performed using a sessile drop technique and viewing with a contact angle microscope (Rame-Hart, USA) under ambient conditions. X-Ray photoelectron spectroscopy (XPS: VG-Scientific, ESCALAB 250, USA) was performed to obtain information on the layer composition of the monolayers. An AlKα monochromated X-ray source (h = 1486.6 eV) was used to stimulate photoelectron emission. The spectral envelopes were resolved into Gaussian peaks to fit the spectra, and the gold Au 4f peak was referenced at 84.0 eV. The images of the surfaces of gold substrates were obtained using an STM (Nanosurf easyScan E, Nano-science Instruments, Switzerland) using a Pt/Ir tip at constant current in a variable height mode under ambient conditions.

3 RESULTS AND DISCUSSION

X-Ray photoelectron survey spectra recorded between 0 and 800 eV for the pure SAMs [10]. The S 2p peaks

Compound	d(calc.), nm	d(expt.), nm
Au/MUOH	1.47	1.3 ± 0.1
Au/MUA	1.39	1.2 ± 0.1
Au/MDA	1.47	1.3 ± 0.1
Au/MHA	1.91	1.8 ± 0.1
Au/MUA-DADOO	2.34	1.5 ± 0.3
Au/MDA-DADOO	2.42	2.2 ± 0.2
Au/MHA-DADOO	2.86	2.6 ± 0.2
Au/MUA-DADOO-Biotin	3.12	1.9 ± 0.3
Au/MDA-DADOO-Biotin	3.20	3.0 ± 0.2
Au/MHA-DADOO-Biotin	3.64	3.4 ± 0.3

Table 2: The thickness of SAMs measured by ellipsometry.

indicate that the gold substrates were modified efficiently with MXA, and the N ls peaks also indicate the presence of DADOO and biotin moieties on the surfaces and provide clear evidence that the surface of films was successfully modified with DADOO and biotin moieties (Table 1). Quantitative XPS analysis also provides evidence that the monolayer formed from MDA-DADOO-biotin consists of more closely packed organosulfur compounds than the others. The differences of Au/C signal ratios (the signals are calculated by multiplying intensity and binding energy) that before and after of addition of DADOO and biotin were MUA-DADOO-biotin = 1.07, MDA-DADOO-biotin = 0.01, and MHA-DADOO-biotin =0.52, respectively. The presence of an organic film adsorbed on a gold substrate results in an attenuation of the gold signals. The difference of gold signals indicates that a much thicker organic layer formed by chemisorption of MDA-DADOO-biotin on the gold surface than it did for the others.

The measured thicknesses of MUA, MDA, and MHA are 1.2, 1.3, and 1.8 nm, respectively. The linear change in thickness of the monolayers and similar values of contact angles (18°, 17°, and 18°, respectively) indicate that long-chain MXA [$HS(CH_2)_nCOOH$, $n \geq 10$] were chemisorbed onto the gold surfaces and that ordered and oriented monolayers were formed. The theoretical thickness expected for a monolayer tilted 30° from the normal to the surface was calculated using known bond lengths and bond angles [C-C = 0.155 nm; $\angle$CCC = 110.5°; C-S = 0.181 nm; C-H = 0.11 nm; and C-O (in RCOOH) = 0.136 nm] [12]. The theoretical slope is 0.127 nm/CH_2 unit [13]. The estimated slope is 0.12 which agrees well with the theoretical value. The ellipsometric thicknesses of DADOO-terminated and biotin modified *n*-alkanethiolate monolayers were also measured (Table 2). The thicknesses of the MDA- and MHA-DADOO-biotin SAMs are similar to those of theoretical predictions. This finding indicates that biotin modified *n*-alkanethiolate composed of even numbers of carbon in alkyl chain form ordered and closely packed monolayers. In contrast, there is a large difference between the observed thickness of MUA-DADOO-biotin SAMs and the theoretical thicknesses. When a hydrophilic spacer, DADOO, is attached to the terminal carboxylic acid group of the SAMs, there is considerable difference in the

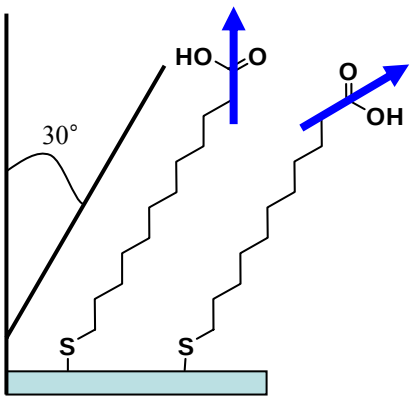


Figure 1: The orientation of head groups in even(a)/odd(b) numbers of carbon in alkyl chains.

degree of change of the film thickness. This difference arises from the orientation of head groups of alkyl chains: the tilt of the head group of the even-numbered alkyl chains is 0° with respect to the surface normal, whereas the tilt of odd-numbered alkyl chains is 60° and, thus, the amide bond that couples the methylene groups to the DADOO moiety is oriented approximately 60° with respect to surface normal (Figure 1) [7,14-16]. The thicknesses of the mixed SAMs of MXA with MUOH as a function of f_{MXA} were also measured by optical ellipsometry. The linear relationship between the ellipsometric thickness and the molar fractions indicates that all of the mixed SAMs are well-organized throughout the entire composition interval.

Figure 2 shows STM images of biotin modified *n*-alkanethiolate monolayers. There exist many islands in the MUA-DADOO-biotin SAMs on gold surfaces, indicating poor surface packing density. In contrast, the images of the MDA-DADOO-biotin and MHA-DADOO-biotin SAMs on gold surfaces show ordered and closely packed features. Those results support the XPS and ellipsometric analysis that the packing density and orientation of the biotin-terminated *n*-alkanethiolates SAMs on gold surfaces are affected by the even/odd numbers of carbon in alkyl chain and DADOO modified *n*-alkanethiolates with even-numbered alkyl chain groups are more efficient at forming closely packed monolayers than are odd ones.

The thicknesses of streptavidin layer adsorbed on pure SAMs using ellipsometric measurements are summarized in Table 3. The nonspecific binding of proteins to hydrophobic surfaces such as those created by methylene groups of alkyl chains is well-documented and, thus, hydrophilic surfaces are often used to reduce nonspecific binding [17]. Table 3 shows that streptavidin is barely adsorbed MUOH SAMs, which indicates that the closely packed hydrophilic head groups of MUOH eliminate nonspecific adsorption of streptavidin to the gold surface. SAMs of MDA-DADOO-biotin and MHA-DADOO-biotin exhibit similar behavior: very little streptavidin binding is observed. This result may be attributed to the close packing

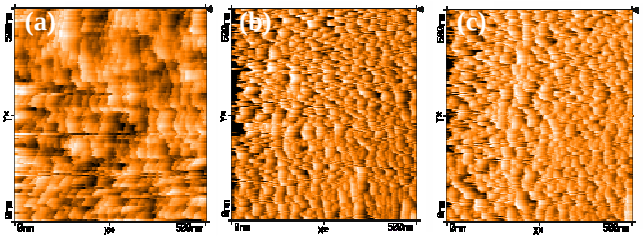
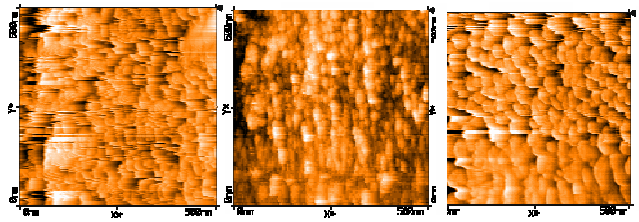


Figure 2: Constant current STM images of monolayers of biotin modified *n*-alkanethiolates; (a) MUA-DADOO-Biotin, (b) MDA-DADOO-Biotin, (c) MHA-DADOO-Biotin.



(a) $f_{MDA} = 1.0$ (b) $f_{MDA} = 0.6$ (c) $f_{MDA} = 0.2$

Figure 3: STM images of streptavidin layers adsorbed on biotin modified SAMs as a function of mole fraction of MDA-DADOO-biotin in solution, f_{MDA}.

Compound	d(expt.), nm
Au/MUOH	0.0 ± 0.1
Au/MUA-DADOO-Biotin	2.8 ± 0.1
Au/MDA-DADOO-Biotin	0.8 ± 0.1
Au/MHA-DADOO-Biotin	0.8 ± 0.2

Table 3: The thickness of streptavidin layer adsorbed on pure SAMs using ellipsometric measurements

of biotin groups that hinder molecular recognition with the biotin-binding pockets of streptavidin [5]. In contrast, very high adsorption of streptavidin to SAMs of MUA-DADOO-biotin was observed. This observation suggests that there is nonspecific binding at the hydrophobic methylene groups, due to exposure of methylene groups of alkyl chains at surface, as well as specific binding with biotin groups. Those results are consistent with the previously discussed obtained data, in which the MUA-DADOO-biotin SAMs are disordered and loosely close-packed. From the results of characterization by contact angle measurements, XPS analysis, ellipsometric measurements, and STM observation, we conclude that MDA-DADOO-biotin monolayers are very well organized and, thus, we believe they are as suitable organosulfur compounds for streptavidin-biotin molecular recognition systems.

The effect of MUOH on the reduction of steric hindrance was also examined by preparing mixed SAMs from a mixture of MDA-DADOO-biotin and MUOH. This mixed SAMs were prepared as a function of mole fraction of MDA-DADOO-biotin in solution (f_{MDA} = 0, 0.2, 0.4, 0.6, 0.8, and 1.0, where f_{MDA} is a mole fraction of MDA-DADOO-biotin in solution).

Figure 3 shows the STM images of the streptavidin layers adsorbed on mixed SAMs of MDA-DADOO-biotin/MUOH. The highest surface density of streptavidin on the mixed SAMs of MDA-DADOO-biotin/MUOH at f_{MDA} = 0.2, which indicates that the maximum binding of biotin with biotin-binding pockets of streptavidin occurred at this mole fraction. In contrast, for SAMs formed from pure MDA-DADOO-biotin, binding between streptavidin and biotin seems to be prevented by the excess biotin present at the surface of the biotin monolayer. The surface density of streptavidin increased as a degree of dilution with MUOH increased.

4 CONCLUSIONS

The effect of the chain length of biotin-functionalized *n*-alkanethiolate SAMs on the molecular recognition of streptavidin-biotin system was investigated. From the experimental data obtained from contact angle goniometry, ellipsometry, XPS, and STM, we reach the following conclusions: (a) The MDA-DADOO-biotin compound is the most suitable in the streptavidin-biotin system, (b) To reduce steric hindrance, it is necessary to add a hydrophilic spacer and a diluting agent with sulfur-based alkyl chains containing hydrophilic head groups, (c) The SAMs formed from biotin-terminated *n*-alkanethiolates having even-numbered alkyl chains were found to be more oriented and closely packed than that of an odd-numbered one. This observation is explained by the fact that the orientation and configuration of the head groups of alkyl chains having even-number of carbon in alkyl chains differ from those of odd ones, (d) Mixed SAMs of MDA-DADOO-biotin and MUOH are desirable for use in streptavidin-biotin molecular recognition systems because both MDA-DADOO-biotin and MUOH form ordered, oriented, and closely packed SAMs whose surfaces are filled with hydrophilic moieties that prevent nonspecific binding.

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