Recognition of lectin with a high signal to noise ratio: carbohydrate-tri(ethylene glycol)-alkanethiol co-adsorbed monolayer†

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Densely packed co-adsorbed ultrathin mono molecular layers of short tri(ethylene glycol)-alkanethiolate (for repelling proteins) and maltoside terminated alkanethiolate (for capturing lectin) provided an extremely high signal to noise ratio surface: the repelling molecules, which had two different functions (highly flexible-hydrophilic arm and rigid packing tail group), worked as "nano barriers" in the recognition monolayer.

There is widespread interest in the formation of nanostructured biological membranes for new applications in sensing and for fundamental studies of interfacial phenomena. The non-specific adsorption of biological species on solid surfaces is a ubiquitous problem as regards to device and biochip design. The prevention of non-specific biomolecule adsorption is linked to a low noise response and the achievement of bio-recognition surfaces with high signal to noise ratios.

The suppression of non-specific adsorption using molecules including oligo(ethlene glycol), and its derivatives, with long alkanethiols has been investigated in terms of layer formation and basic characteristics.[1–7] Protein adsorption has actually been prevented by using polymer layers with oligo(ethylene glycol) terminals.[8] The membrane structure of the oligo(ethylene glycol) polymer is hydrophilic and flexible, but its bulky volume occasionally becomes a problem. It has also been reported that the use of polymer layers with different molecular weights is very effective in suppressing non-specific adsorption.[9,10] From these important reports, we learn that several requirements must be met if we are to realize an excellent biomolecule-repelling characteristic and a good bio-recognition surface; the repellent modifier must have (1) highly flexible and highly hydrophilic parts and (2) structures for forming condensed packing layers. A modifier that provides both of these functions appears to be a contradiction.

Resistance to non specific protein adsorption was increased by increasing the unit number of the terminal oligo(ethylene glycol) group.[4] We confirmed that tri(ethylene glycol) terminated short alkanethiol is a tiny amphoteric modifier that includes a highly flexible-bulky-hydrophilic part and a short alkane backbone to provide dense packing. Tri(ethylene glycol) terminated short alkanethiols (n = 2–8) worked effectively to suppress the non-specific protein adsorption. When we constructed a co-adsorbed mono molecular layer on the substrate using this amphoteric molecule and the protein recognition molecule, target molecules could be captured effectively because the non-specific adsorption on the substrate was greatly suppressed. As a result, the signal to noise ratio was effectively increased.

We synthesized and used tri(ethylene glycol) terminated alkanethiols (EGC_nSH, n = 2, 4, 6, 8, 11, Fig. 1) as protein repelling modifiers. EGC_nSH consisted of a flexible repellent arm and a closely packed tail group as shown in Fig. 1. (1-Mercaptoundec-11-yl)tri(ethylene glycol) ($EGC_{11}SH$) was purchased from SensoPath Technologies and also used in the experiment. 12-Mercaptododecyl β-maltoside (Mal-$C_{12}SH$, Fig. 1) was synthesized and used for the specific recognition of the lectin Concanavalin A (ConA).[11–13] The interface structure and the number of adsorbed Con A molecules were monitored by using surface plasmon resonance (SPR) measurement (Biacore T-100, 2000 and NTT-AT Handy SPR system). The packing ratio of the EGC_nSH monolayers was evaluated from their amount on the substrate by measuring the current, peak area, and peak potential of the electrochemical desorption.[14] Fig. 2(a) shows the specific recognition of ConA on the $MalC_{12}SH$ and EGC_nSH mixed monolayer surfaces ($MalC_{12}SH$: EGC_nSH = 1 : 9 (in surface modification solution); signal response). The real $MalC_{12}SH$ ratio is about 30% by the electrochemical reductive desorption method, although this ratio slightly changes depended on the alkylchain length of EGC_nSH. The non-specific adsorption of ConA on the 100% EGC_nSH monolayers is also shown on the left of the figure (red bars; noise response). ConA recognized the α-1,4-bond of the maltoside part of $MalC_{12}SH$.[11] Large signal responses caused by ConA adsorption were observed on

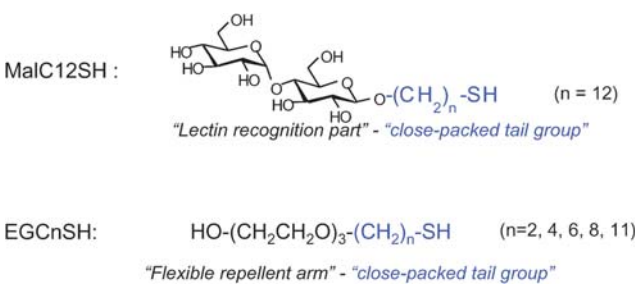

Fig. 1 Capturing ConA molecule ($MalC_{12}SH$) and repelling molecules (EGC_nSH).

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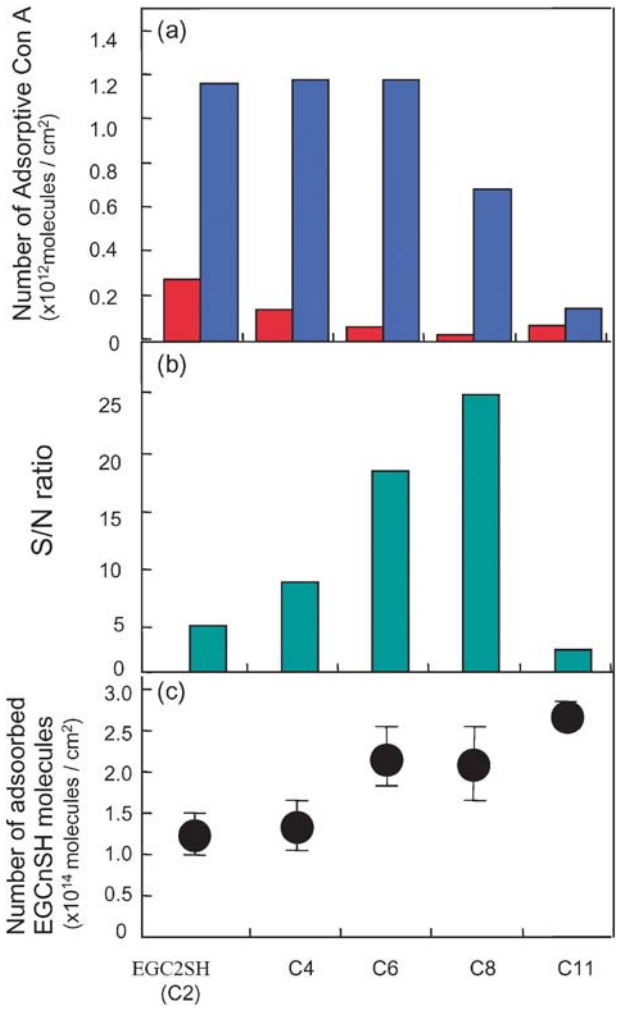

Fig. 2 Relationship between the alkylchain length of EGC_nSH and (a) the number of adsorbed ConA (right (blue) bars: $MalC_{12}SH$ (10%)–EGC_nSH mixed monolayer; left (red) bars: 100% EGC_nSH monolayer), (b) the signal to noise ratio (signal response : noise response; S : N ratio) of ConA adsorption, and (c) the number of adsorbed EGC_nSH molecules evaluated by electrochemical reduction.

each $MalC_{12}SH$–EGC_nSH mixed interface. In particular, when EGC_2SH, EGC_4SH, and EGC_6SH were used as nano barriers, the ConA adsorption signals became higher. When we focused on the results of the ConA non-specific adsorption, the amounts of nonspecific adsorbates decreased gradually as the alkylchain became longer. In fact, EGC_nSH with a longer alkylchain was much more effective in preventing ConA adsorption as shown in Fig. 2(a). The repellant ability as regards to non-specific adsorption reached its maximum value around C6 to C8 of EGC_nSH. With the $EGC_{11}SH$ monolayer, it exhibited a repellant ability similar to that for C6–C8.

The signal to noise (S : N) ratio data is shown in Fig. 2(b) (signal response (S): specific recognition of ConA–maltoside on the $MalC_{12}SH$–EGC_nSH monolayer; noise response (N): non-specific ConA adsorption on the EGC_nSH monolayer). The S : N ratio was improved significantly when using longer EGC_nSH molecules such as EGC_6SH and EGC_8SH. Preventing non-specific molecular adsorption and achieving a high S : N ratio were attributable to a densely packed $MalC_{12}SH$–EGC_nSH monolayer. In particular, the EGC_nSH packing density was the key to improving the ability to prevent adsorption. The number of adsorbed EGC_nSH monolayers on the gold surface was evaluated with the electrochemical reductive desorption method. An alkanethiolate monolayer was desorbed from the gold substrate in an alkaline solution through a one-electron reductive path ($RS\text{-}Au + e^- \rightarrow RS^- + Au$).[14–17] Each EGC_nSH molecule was reduced at around −1.0 V (*vs.* Ag/AgCl(NaCl)) in 0.5 M KOH. As shown in Fig. 2(c), the number of attached EGC_nSH molecules became larger as the alkylchain became longer. The reductive potential was shifted in a negative direction as the alkylchain became longer (EGC_2SH: −0.86 V, $EGC_{11}SH$: −1.08 V (*vs.* Ag/AgCl (3 M NaCl)), indicating that a higher packing density was achieved by lengthening the alkylchain. The position of the reductive peak depends on the length of the alkane chain. The attractive interaction between the alkylchains increases with increasing alkyl chain length and the reductive desorption potential shifts in a negative direction. Moreover, the full width at half-maximum (fwhm) of the reductive desorption peaks indicated the strength of the interaction between the adsorbed molecules. In fact, the fwhm became smaller as the alkylchain of EGC_nSH became longer (EGC_2SH: 46.0 mV, $EGC_{11}SH$: 37.4 mV). The fwhm of the ideal case was around 20 mV for normal alkanethiol ($CH3(CH2)_nSH$; $n > 10$), which formed a rigid self-assembled monolayer.[16] These experimental results supported the idea that (1) longer alkyl-EGC_nSH was adsorbed significantly on the surface and (2) EGC_nSH with longer alkylchains interacted more strongly. In fact, a densely adsorbed EGC_nSH monolayer can prevent non-specific adsorption almost

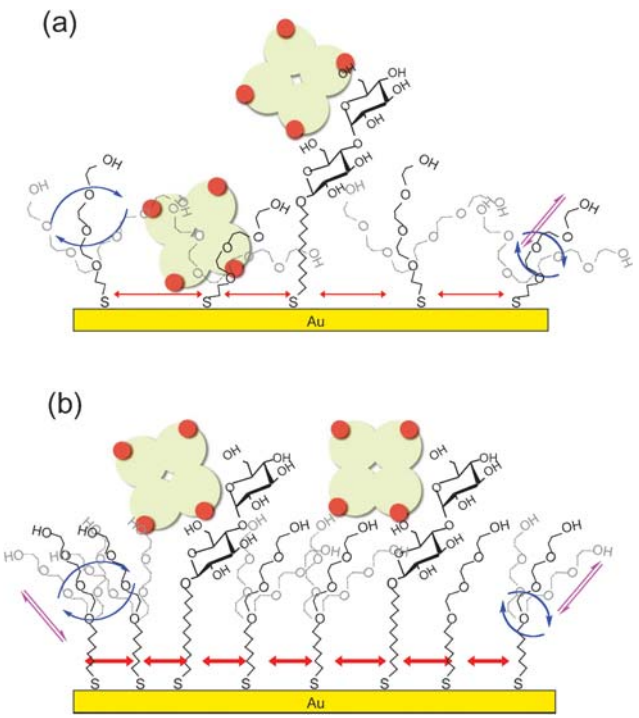

Fig. 3 Models of ConA recognition on $MalC_{12}SH$–EGC_nSH mixed monolayers. (a) $MalC_{12}SH$–EGC_2SH mixed monolayer and (b) $MalC_{12}SH$–EGC_6SH.

Table 1 Repulsion of proteins and peptides on a surface modified with EGC_nSH (n = 2,4,6,8) and polymer (PEG2000) layer (RU is a resonance unit in the Biacore system (1000RU = 0.1 degrees resonance angle shift))

Molecular adsorption/RU		$PEGC_2SH$ (C2)	C4	C6	C8	PEG2000
Proteins	Concanavalin A (MW 104 000, 1 μM)	18.9	9.7	6.3	2.9	11.5
	BSA (MW 66 300, 1 μM)	9.2	5.7	6.5	1.8	7.8
Peptides	Angiotensin (MW 1296.5, 0.1 mg ml^{-1})	7.7	6.4	5.1	1.5	10.0
	Bradykinin (MW 1060.2, 0.1 mg ml^{-1})	6.3	7.0	5.8	1.1	11.4
	RGDS (MW 433.42, 0.1 mg ml^{-1})	9.6	6.9	5.0	0.8	29.1

completely. Thus, the S : N ratio of ConA recognition was improved significantly. The mixed monolayer model is shown in the Fig. 3. The combination of EGC_nSH with a longer alkylchain and a $MalC_{12}SH$ mixed layer was effective with regards to the specific recognition of lectin with a high S : N ratio.

$EGC_{11}SH$ can form a very densely packed self-assembled monolayer because $EGC_{11}SH$ had a longer alkylchain. The non specific adsorption of ConA on the $EGC_{11}SH$ layer was also suppressed effectively (Fig. 2(a)). Unfortunately, with a mixed $MalC_{12}SH$–$EGC_{11}SH$ layer, the amount of ConA adsorption was decreased to about one-sixth that of $MalC_{12}SH$–EGC_2SH, EGC_4SH, and EGC_6SH because the ConA recognition site, α-1,4-bond of maltoside, was hindered in the arms of the $EGC_{11}SH$ molecules. Consequently, the S : N ratio of $MalC_{12}SH$–$EGC_{11}SH$ was unsatisfactory.

These nano scale controlled monolayers (EGC_nSH) completely prevented the non-specific adsorption of biomolecules. In particular, small biomolecules, whose adsorption was difficult to suppress, were prevented from reaching the gold surface. Our molecules, which consist of highly mobile ethylene glycol units and densely packed alkyl-chain units, function as nano barrier molecules. The non specific adsorption of the proteins (ConA, BSA (Bovine Serum Albumin)), and peptides (angiotensin, bradykinin, and RGDS (Arg-Gly-Asp-Ser)) on the each EGC_nSH surface is shown in Table 1. A PEG2000 (mPEG-thiol, M.W. 2000) polymer layer was used for comparison. Our EGC_nSH layers can prevent the non specific adsorption of smaller proteins and peptides. The repelling effect became clearer as the alkylchain became longer, particularly for the tetrapeptide RGDS, which has the smallest MW(433.42).

There have been many reports on protein repellent properties and methods.[8] A polymer PEG layer (PEG2000) is very useful for preventing protein adsorption. ConA and BSA are adsorbed slightly on a PEG2000 surface (ConA: 11.5 RU (1.2 ng cm^{-2}), BSA: 7.8 RU (0.8 ng cm^{-2}); 1RU as 0.1 ng cm^{-2} [14,15]). Mixed PEG polymer layers (both longer and shorter ones) were more effective in preventing non specific adsorption. It has been reported that high density PEG polymer layers formed of mixed poly PEGs provided excellent repellent layers.[9,18] In particular, small molecules (MW < 1000) were repelled from a mixed poly PEG surface.[18] Self-assembled monolayers formed from (semi)-fluorinated organosulfur compounds have also been used to reduce non specific protein adsorption, which was suppressed around 10∼100 RU (1∼10 ng cm^{-2}) for the non-specific adsorption of antibodies.[19] EGC_nSH nano scale monolayers can simply form a rigid repellent monolayer and effectively prevent the adsorption of small molecules. For example, a small peptide such as RGDS was completely repelled (EGC_2SH: 9.6 RU (1.0 ng cm^{-2}), EGC_8SH: 0.8 RU (80 pg cm^{-2})). With a polymer PEG layer, 36 times more RGDS was adsorbed than on the EGC_8SH surface. EGC_nSH nanolayers showed excellent repellent effects for all other small peptides.

The most important result from this work lies in the fact that the nano level short EGC_nSH molecules provided a completely repellent gold surface. Even though the alkanethiol unit (–$(CH_2)_n$–) was short (n = 2–8), EGC_nSH molecules could form a well packed monolayer, and then we could create an inert surface for protein adsorption by using flexible-hydrophilic tri(ethylene glycol) units. EGC_nSH is a small amphoteric molecule and is an excellent tool for forming a ''nano barrier'' on a substrate surface.

Notes and references

1 C. Pale-Grosdemange, E. S. Simon, K. L. Prime and G. M. Whitesides, *J. Am. Chem. Soc*., 1991, **113**, 12.
2 M. Mrksich, G. B. Sigal and G. M. Whitesides, *Langmuir*, 1995, **11**, 4383.
3 L. Deng, M. Mrksich and G. M. Whitesides, *J. Am. Chem. Soc*., 1996, **118**, 5136.
4 K. L. Prime and G. M. Whitesides, *J. Am. Chem. Soc*., 1993, **115**, 10714.
5 S. Herrwerth, W. Eck, S. Reinhardt and M. Grunze, *J. Am. Chem. Soc*., 2003, **125**, 9359.
6 E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama and and G. M. Whitesides, *Langmuir*, 2001, **17**, 5605.
7 B. T. Houseman and M. Mrksich, *Biomaterials*, 2001, **22**, 943.
8 *Hydrophilic Polymers, Performance with environmental acceptability*, ed. J. E. Glass, American Chemistry Society, Washington DC, 1996.
9 K. Uchida, H. Otsuka, M. Kaneko, K. Kataoka and Y. Nagasaki, *Anal. Chem*., 2005, **77**, 1075.
10 T. Satomi, Y. Nagasaki, H. Kobayashi, H. Otsuka and K. Kataoka, *Langmuir*, 2007, **23**, 6698.
11 Y. Sato, T. Murakami, K. Yoshioka and O. Niwa, *Anal. Bioanal. Chem*., 2008, **391**, 2527.
12 D. C. Hone, A. H. Haines and D. A. Russel, *Langmuir*, 2003, **19**, 7141.
13 E. A. Smith, W. D. Thomas, L. L. Kiessling and R. M. Corn, *J. Am. Chem. Soc*., 2003, **125**, 6140.
14 H. O. Finklea, in *Electroanalytical Chemistry*, ed. A. J. Bard and I. Rubinstein, Marcel Dekker, New York, 1996, ch. 2, vol. 19.
15 M. M. Walczak, D. D. Popenoe, R. S. Deihammer, B. D. Lamp, C. Chung and M. D. Porter, *Langmuir*, 1991, **7**, 2687.
16 S. Imabayashi, M. Iida, D. Hobara, Z. Q. Feng, K. Niki and T. Kakiuchi, *J. Electroanal. Chem*., 1997, **428**, 33.
17 E. Stenberg, B. Persson, H. Roos and C. Urbaniczky, *J. Colloid Interface Sci*., 1991, **143**, 513.
18 K. Uchida, Y. Hoshino, A. Tamura, K. Yoshimoto, S. Kojima, K. Yamashita, I. Yamanaka, H. Otsuka, K. Kataoka and Y. Nagasaki, *Biointerphases*, 2007, **2**, 126.
19 E. Klein, P. Kerth and L. Lebeau, *Biomaterials*, 2008, **29**, 204.