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Cholesterol recognition system by molecular imprinting on self-assembled monolayer

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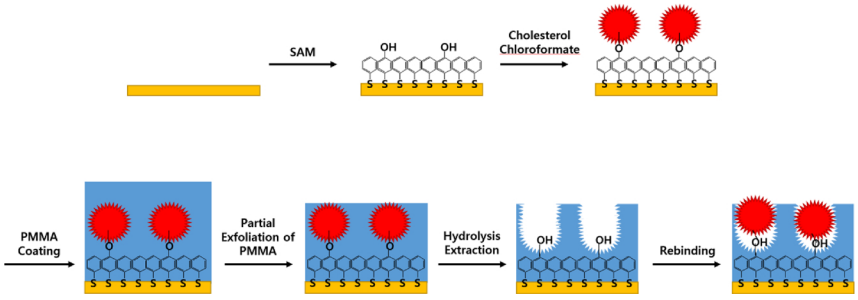
GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the molecular imprinting technique and self-assembled monolayer technique were combined to increase the recognition ability of cholesterol. A self-assembled monolayer with 4-mercaptophenol and benzenethiol was formed on a gold plate, and the reaction of cholesteryl chloroformate with the phenol group in the 4-mercaptophenol was followed. This layer was then coated with poly(methyl methacrylate) (PMMA). In order to expose the cholesterol buried under the coating, the partial upper part of the coated PMMA was then removed by stroking the coated plate in acetone solvent. The molecular imprinted site was prepared by hydrolysis of the carbonate bond and extraction of the hydrolyzed cholesterol. This gold plate was used as a working electrode to test the recognition ability for cholesterol. The result showed that the plate obtained good recognition ability for cholesterol compared with cholic acid. The ratio of 4-mercaptophenol to benzenethiol was a very important factor in the ability to recognize cholesterol. Regulating the thickness of the coated PMMA was also one of the important factors to increase the ability to recognize cholesterol.

1. Introduction

The molecular imprinting technique has attracted considerable attention due to its ability to imitate the biological receptors in a simple way [1–5]. Although the research results produced by the molecular imprinting technique did not show the outstanding ability, many researchers are still studying molecular imprinting because it can be used freely in any circumstance, while biological receptors can only be used in limited circumstances. For example, as one of the important biological receptors, enzymes can be used only in water, in very narrow temperature and pH ranges. However, molecular imprinted analogs can be used in a wide range of organic solvents, and in wide temperature

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and pH ranges [6–10]. Nevertheless, molecular imprinted analogs did not show the same performance as biological materials. Therefore, most of these studies have focused on increasing their performance [11–14].

The most representative method used in the molecular imprinting technique involves the polymerization of the monomer with the target compound to form a very hard three-dimensional network structure containing the target compound. Then, after crushing it into a very fine powder form and extracting the target compounds at the surface of the powder using a solvent, the extracted site serves as a receptor for the target compound. Even though the molecular imprinting technique has been studied for more than 40 years, the practical application is very limited because of two significant disadvantages of this system: the site created by this system is less accurate than the biological receptor and the turnover rate is much slower than the biological receptor.

In the initial studies of the molecular imprinted polymer (MIP), two methods were used: the covalent bonding method and the non-covalent bonding method. In the covalent bonding method, a monomeric compound is bonded to a target compound, followed by polymerization to form a three-dimensional network structure, which is pulverized into a powder form. The functional groups connected to the target compounds are then hydrolyzed and the hydrolyzed target compounds are extracted by solvent. The remaining site can function as a recognition site [15–17]. In contrast, in the non-covalent bonding method, a monomer and a target compound are simply mixed and the polymerization is followed to form a three-dimensional network, which is pulverized into a powder, and a target compound is extracted with a solvent to prepare a recognition site. These two methods have advantages and disadvantages. The merit of the covalent bonding method is that the formed site is more sophisticated than the formed site with the non-covalent bonding method, although the disadvantage of the method is its manufacturing difficulty. However, because this disadvantage is more significant than the merit, the non-covalent bonding method is used in most of the studies reported thus far [1–10].

Since the cholesterol compound causes atherosclerosis, it is one of the important compounds closely related to our health. Therefore, many researchers have attempted to recognize cholesterol using the molecular imprinting technique. However, most of these studies have proceeded with the three-dimensionally crosslinked polymeric compounds using the above described methods [18–23]. Molecular imprinting using a self-assembled monolayer (SAM) is a so-called two-dimensional imprinting method [24–28]. Since molecular imprinting is performed on the surface, the amount of target compound placed in the molecular imprinted site can be detected in real time using an electrochemical signal. It is possible to measure the binding amount immediately after rebinding. We developed a system to detect cholesterol by introducing an imprinting technique on SAM [29]. Subsequently, in order to increase the recognition ability, the hardened surface of SAM was accomplished by using thiol having a very rigid group such as a terphenyl group [30]. In our previous studies, we found that the recognition ability decreases over time because the thiol compounds in SAM moved [29]. In order to solve this problem, we attempted to form a SAM using the compound with the dithiol group [31]. Recently, research has been published in which molecular imprinting was conducted after depositing a thin polymer film on a SAM [32].

In this study, we combined our previous technology with the technology in which the thin polymeric film was used, in order to increase the performance of the molecular imprinted system. We attempted to fabricate a system using the covalent bonding method in order to create a more sophisticated site. We then investigated the effect of the thickness of the thin polymeric film on the ability to detect the target molecule.

2. Experimental

2.1. Materials and instruments

Cholesterol, cholesteryl chloroformate, cholic acid, 4-mercaptophenol, benzenethiol, triethylamine, potassium ferricyanide [$K_3Fe(CN)_6$], tetrahydrofuran (THF), and sodium perchlorate were purchased from Aldrich. Poly (methyl methacrylate) (PMMA) was also purchased from Aldrich with an average molecular weight of 120,000. Cyclic voltammograms were measured using Ivium potentiostat (Ivium Technologys, Netherlands). A gold electrode manufactured in a goldsmith's shop was used as a working electrode. The electrode was fabricated on a round shaped plate and a 2.0 cm Au wire was connected to the electrode. The surface area of the gold plate was 1.0 $cm^2 \times 2$ and its thickness was 0.5 mm. The gold plate was washed with piranha solution before each experiment. This solution was prepared by mixing 20 mL of 30 wt% H_2O_2 and 60 mL of concentrated sulfuric acid solution. In the washing process, the gold plate was placed in a piranha solution for 10 min, washed and then placed in 100 mL of distilled water for 10 min to remove the washing solution, and then washed with distilled water three times. Atomic force microscopy (AFM) was measured using a Dimension Icon from Bruker (Billerica, Massachusetts, USA). Spin coating was carried out using an ACE-200 spin coater from Dong Ah Trade Co. (Seoul, Korea). The thickness of the coated film was measured using a Horiba UVISEL 2 Ellipsometer. (Kyoto, Japan)

2.2. SAM formation on surface of Au plate

220 mg (2.00 mmol) of benzenethiol and 25.2 mg (0.200 mmol) of 4-mercaptophenol were dissolved in 100 mL of ethanol to form a solution. The Au plate prepared as described above was placed in this solution for 12 h to allow these compounds to adhere to the gold surface to form a SAM. The ratio of benzenethiol to 4-mercaptophenol used was 10:1 in the initial experiment. Control of the ratio will be discussed in more detail in the result and discussion section. After forming the SAM, it was soaked in 50 mL of ethanol for 40 min to remove overcoated materials. The gold plate was dried under vacuum for 5 h. All experiments with gold plates were performed with the hanged state, so all reactions or work were performed on both sides of the gold plate.

2.3. Reaction of cholesteryl chloroformate with phenol group on gold surface

The reaction of 4-mercaptophenol with cholesteryl chloroformate proceeded as follows. First, we dissolved 1 mL of triethylamine in 30 mL of dry THF, and the gold plate prepared as described above was placed in the solution. While stirring the solution, the solution of 1.50 g (3.34 mmol) of cholesteryl chloroformate in 15 mL of dry THF was added dropwise. The reaction was finished after stirring for 6 h. After completion of the reaction, the gold plate was washed twice with 30 mL THF.

2.4. Spin coating of PMMA on the above reacted surface

A toluene solution of PMMA was prepared and the above reacted surface of the gold plate was coated with this solution using a spin coater. The concentration of the PMMA solution was 0.5 wt% and spin coating was carried out at 6000 rpm for 1 min. Concentration of PMMA solution and the spin speed of spin coating are discussed in detail in the Results and Discussion section.

2.5. Formation of molecular imprinted site on the coating surface

To create a molecular imprinted site on the coating surface, the overcoated PMMA layer on the cholesterol molecule first needed to be partially exfoliated. In order to remove the exact amount of excessive PMMA coating on the cholesterol molecules and then observe the cholesterol molecules that protrude from the PMMA film, we placed the gold plate into acetone solvent, which is one of the best solvents of PMMA. We then struck the gold plate in acetone. The stroking speed in acetone was 4 s per reciprocating pendulum motion horizontally. This