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Chain-length dependent interfacial immunoreaction kinetics on self-assembled monolayers revealed by surface-enhanced infrared absorption spectroscopy

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ABSTRACT

Self-assembled monolayer (SAM) has been extensively applied as ideal interface layer for construction of biosensors. Its chain length and end functional groups determine the physical and chemical properties of the modified surfaces, which will affect the performance of constructed biosensors. Herein, we studied the influence of chain length of n-alkanethiols SAMs on the immunoreaction kinetics employing attenuated total reflection surface-enhanced infrared absorption spectroscopy (ATR-SEIRAS). Antibody (rabbit immunoglobulin) is assembled on carboxyl terminated SAMs \of n-alkanethiols with different chain lengths (n = 3, 6, 11, 16). The whole fabrication steps of the immunoassay can be monitored in situ by the ATR-SEIRAS. From the time-dependent SEIRA spectra, the interfacial immunoreaction kinetics between the immobilized antibody and antigen (goat anti-rabbit immunoglobulin) can be evaluated. We found that the immunoreaction became faster with increasing the chain length of SAMs. This chain length dependent kinetics might be attributed to different orientations of the assembled antibody caused by different packing densities of SAMs. The present research offers a sensing platform to evaluate immunoassay kinetics and provides fundamentals for construction of immunoassay with high performance.

1. Introduction

Immunoassay based on specific recognition of biomolecules plays an indispensable role in accurate and efficient disease diagnostics [1–3]. High performance of the immunoassay requires the proper assembly of active biomolecules on sensing surfaces with the recognition sites exposing to targets in solution [4]. Although the immobilization methods of direct physical adsorption and entrapment have been extensively adopted [5–7], they cannot ensure the proper orientation of the biomolecules, resulting in the decrease of binding activity, selectivity, and sensitivity. SAMs with tunable interfacial properties offer the possibility to manipulate the orientation of biomolecules since this technique is site-specific, thus, biosensors based on SAMs with high sensitivity, selectivity, reproducibility and durability can be constructed [8–11].

When SAMs are used as the sensing interfaces to construct biosensors, the terminal functional groups of SAMs and the chain length will affect the orientation and density of immobilized biomolecules. It has been reported that the direct electrochemistry of cyt c on carboxyl terminated SAMs/Au electrode can be tuned by the surface charges of SAMs in solutions with different pH [12]. In addition, the wettability of SAM interfaces terminated with different functional groups determines the orientation of hemin plane of cyt c, and thus its direct electrochemistry [13]. It has been reported that the hydrophilic SAM favors the hemin plane of cyt c parallel to the surface facilitating direct electrochemistry, while the hydrophobic SAM allows the hemin plane perpendicular to the surface forbidding the direct electron transfer of the assembled protein. On the other hand, the interaction between alkane backbones affects the packing density and stability of the SAMs. It has been reported that the density and stability of SAMs will be improved with the increase of chain length. Thus, performance of biosensor might be affected by the chain length of the

Abbreviations: SAM, self-assembled monolayer; ATR-SEIRAS, attenuated total reflection surface-enhanced infrared absorption spectroscopy; rIgG, rabbit immunoglobulin G; G anti-rIgG, goat anti-rabbit immunoglobulin G; cyt c, cytochrome C; BSA, bovine serum albumin; NHS, N-Hydroxysuccinimide; EDC, N-(3-dimethylaminopropyl)-Nethylcarbodiimide hydrochloride; MPA, 2-Mercaptopropionic acid; MHA, 6-Mercaptohexanoic acid; MUA, 11-Mercaptoundecanoic acid; MDHA, 16-Mercaptohexadecanoic acid (MDHA); AFM, atomic force microscopy; SEM, scanning electron microscopy; hIgG, human immunoglobulin

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SAMs since the structure and organization of a SAM layer significantly affect the subsequent biomolecule modification and bioactivity [11–13].

Up to now, a few off-line methods have been employed to study the influence of chain length in immunoassay. Boujday *et al.* compared the binding efficiency of two proteins on short and long chain amine-terminated SAMs [14]. Chadha's group studied the efficiency of antibody binding on alkanethiol SAMs with different lengths and the further antigen recognition efficiency [15]. Their results exhibited good superiority of long chains in protein binding and immunoassay, which is in accordance with the structural characteristics of long chain SAMs. However, due to the lack of time-dependent information in above researches, studies are mainly focused on quantifying the binding density of protein relating to SAM structure. In reality, the binding kinetics of bio-recognition on different SAMs is another important factor which determines the performance of biosensors. A clear acknowledge of immunoreaction kinetics on top of different SAMs could give us an general guide to select timesaving and efficient biosensor to meet various requirements.

ATR-SEIRAS has recently been proved as an excellent analytical technique for bioanalysis [16–18]. Besides the advantages of conventional IR methods like non-labelling and non-destruction, ATR-SEIRAS exhibits unique superiorities. With the help of ATR configuration and surface coated metal nanostructures, ATR-SEIRAS can successfully restrict the detection region in several tens of nanometers near the interface due to the near field effect and thus suppress the signal from bulk water. In addition, this method supports in situ and real time recording of molecular vibrational signals at the sensing interface at sub-monolayer level. Since IR signals are originated from chemical bond vibration, time-dependent IR spectra enable dynamic analysis for the variation of molecular structure and function during a complex biological event. In recent years, ATR-SEIRAS has been successfully used in bioanalysis including DNA hybridization [19], protein adsorption and conformation changes [12,13,20–22], immunoassay [23,24], artificial membrane system [25–29], and whole cell observation [30,31].

In this paper, we for the first time used ATR-SEIRAS to compare the immunoreaction kinetics on SAMs with different chain lengths (n = 3, 6, 11, 16). This ATR-SEIRAS technique allows monitoring the whole immobilization process and biorecognition process for sensor. The results show that the binding rate between free-state antigen (Goat anti-rIgG) and antibody (rIgG) covalently linked to SAMs increases with the chain length due to the change in orientation of the immobilized proteins. Our research gives a graceful guidance to evaluate immunoassay kinetics and offers a platform to optimize performance of biosensors.

2. Material and methods

2.1. Chemicals and materials

ZnSe semispherical prism (20 mm diameter) was bought from Bosheng Quantum Technology (Changchun, China). BSA, NHS, EDC, MPA, MHA, MUA, and MDHA were purchased from Sigma-Aldrich. RIgG and Goat anti-rIgG were purchased from Southern Biotechnology Associates, Inc., China. Other reagents and chemicals were of analytical grade. All reagents were used as received without further purification. All solutions were prepared with Milli-Q water from a Millipore system.

2.2. Instruments

Infrared spectra were measured with a Nicolet 6700 Fourier transform spectrometer (Thermo Fisher, USA) equipped with a liquid-nitrogen-cooled MCT detector. AFM imaging was performed on an Agilent 5500 AFM/SPM system (Agilent Technologies, USA) using tapping mode under ambient conditions. SEM (FE-SEM, S-4800, Hitachi, Japan) at an accelerating voltage of 5 kV was used to characterize the morphology of the deposited gold films.

2.3. Preparation of surface enhanced substrate

Briefly, gold nanoparticles were deposited on the flat surface of a semispherical ZnSe prism by electroless deposition. Before modification, the surface of ZnSe substrate was polished with aluminum oxide powder of 1 μm in size. Then, the prism plane was immersed in a 10 mM aqueous solution of $HAuCl_4$ at 303 K for 30 s, and the reaction was terminated by adding large amount of water. Finally, the gold nanoparticles coated ZnSe prism surface was cleaned with water and assembled to a home-built poly (trifluorochloroethylene) cell. The morphology and structure of Au nanoparticle film were characterized by SEM and AFM.

2.4. Fabrication of biosensor

The Gold nanoparticles covered ZnSe surface was immersed in 400 μL solution containing 10 mM MPA, 10 mM MHA, 10 mM MUA, or 10 mM MDHA respectively for formation of self-assembled layers through Au-S bond overnight (MPA and MHA in deionized water, and MUA and MDHA in ethanol). After washing with deionized water, the SAMs were activated by 400 μL PBS solution (10 mM, pH=5.8) containing 25 mM EDC/NHS (1:1 M ratio) for 30 min at room temperature and then washed with deionized water. The activated SAMs were then incubated with 200 μL PBS solution (50 mM, pH=7.4) containing plenty of rIgG (20 μg/mL) for 1 h. The SAMs were further washed with deionized water and passivated with 200 μL 1% BSA solution for 10 min. This treatment has been demonstrated to be efficient to eliminate non-specific adsorption of antigen on the SAMs [23].

2.5. IR characterization of sensor fabrication and immunoassay reaction

ATR-SEIRAS spectrum of each fabrication step was recorded by taking the IR spectrum of previous step as reference. For immunorecognition reaction, IR spectra with time of 200 μL PBS solution (50 mM, pH=7.4) containing Goat anti-rIgG (20 μg/mL) were collected with the IR spectrum of 200 μL PBS solution (50 mM, pH=7.4) as reference. IR detection was processed with a homemade ATR accessory and the diameter of the detection cell was 6 mm. Unpolarized IR radiation was totally reflected at the ZnSe prism/solution interface with an incident angle θ = 75° and was detected with a liquid-nitrogen-cooled MCT detector. All the spectra were plotted in absorbance unit relative to a baseline as mentioned. The spectral range was 4000–650 cm^{-1} with a resolution of 4 cm^{-1}.

2.6. IR characterization of free-state MXA molecules

ATR spectrum of each MXA (X=P, H, U, DH) was recorded in solution by taking the IR spectrum of pure solvent (deionized water for MPA and MHA, ethanol for MUA and MDHA). After background recording, solution containing a certain amount of MXA was added to the detection cell. Since the evanescent field locates within several micrometers for normal ATR configuration without gold nanoparticles, the IR spectrum of the free-state MXA molecules was recorded respectively. Other experimental settings were similar to ATR-SEIRAS measurement.

3. Results and discussions

Principle of the biosensor is illustrated in Scheme 1. In the fabrication step, Au nanoparticles film is first prepared on the top of a ZnSe prism via electroless deposition and the morphology of the film