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Highly sensitive nanomechanical assay for the stress transmission of carbon chain

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ABSTRACT

Here, we report the first quantitative experimental study into the molecular basis of the transmission of mechanical signal that originates from biochemical reaction focusing on the length of carbon chain. We designed an experiment by using *n*-alkanethiols with a same carboxyl group and different chain lengths ($n = 1$, 5, 10 and 15) to immobilize a same receptor molecule on the gold surface of a microcantilever, and detected the nanomechanical response of biochemical reaction. The sensitivity of the microcantilever was found to be greatly influenced by the chain length of linker that is between the receptor molecule and the microcantilever surface. The efficiency of stress transmission increases significantly with decreasing length of carbon chain. At the same time, we develop a label-free microcantilever sensor for highly sensitive detection of Glycyrrhizic acid (GL). The detection limit of the microcantilever sensor for GL is found to be as low as 20 pg/mL for the shortest linker ($n = 1$), which is 500 times lower than the longest linker ($n = 15$) and 50 times lower than that of the corresponding icELISA. These findings will provide new insights into the fundamental mechanisms of stress transmission, which may be exploited for biochemical sensor and nanoactuation applications.

1. Introduction

The microcantilever sensor has attracted considerable attention in recent years. Using immobilized receptor molecules, such as nucleic acids, proteins, and lipids, as well as cells and microorganisms, microcantilevers have been applied to a variety of problems to detect molecular interactions [1–8]. Furthermore, microcantilever arrays are extremely sensitive and can detect picomolar amounts of analyte in a complex background [8]. The principle behind the microcantilever sensing mechanism is the transduction of biomolecular interactions into a nanomechanical force. Analytes bind to receptor molecules that are immobilized on the surface of the microcantilever, and this causes changes in surface stress. This, in turn, generates a nanomechanical force that bends the microcantilever. An optical laser, focused on the microcantilever apex, is deflected as the microcantilever bends, allowing for direct measurement of receptor–analyte binding. Microcantilevers have advantages over other sensor techniques, including the ability to measure binding interactions in real time. A unique microcantilever feature is that measurements can be conducted both in air and solution, which may be particularly useful for detecting microbes. Furthermore, in contrast to most comparable technologies, small amounts of receptor and analyte are needed, and molecular labels are not required.

The stress originates from intermolecular interaction in the film of receptor molecules and transmits through the linker between the receptor molecule and microcantilever surface, and then ultimately, arrives at microcantilever surface and causes the bending. For microcantilever sensor based on surface stress effect, how to maximize the stress transmission from the molecular interaction to microcantilever surface is an important way of increasing the detection sensitivity of microcantilever sensor. The linker size between the surface and the receptor may play a key role in the stress transmission. Antibody immobilization on the surface is known as an important factor of the assay sensitivity. Different reagents used in receptor molecule (antibody) immobilization result in differences in the linker molecule sizes. Various reagents suitable for antibody immobilization have been described in literatures for different molecules detections. These reagents include homobifunctional cross-linkers dithiobis

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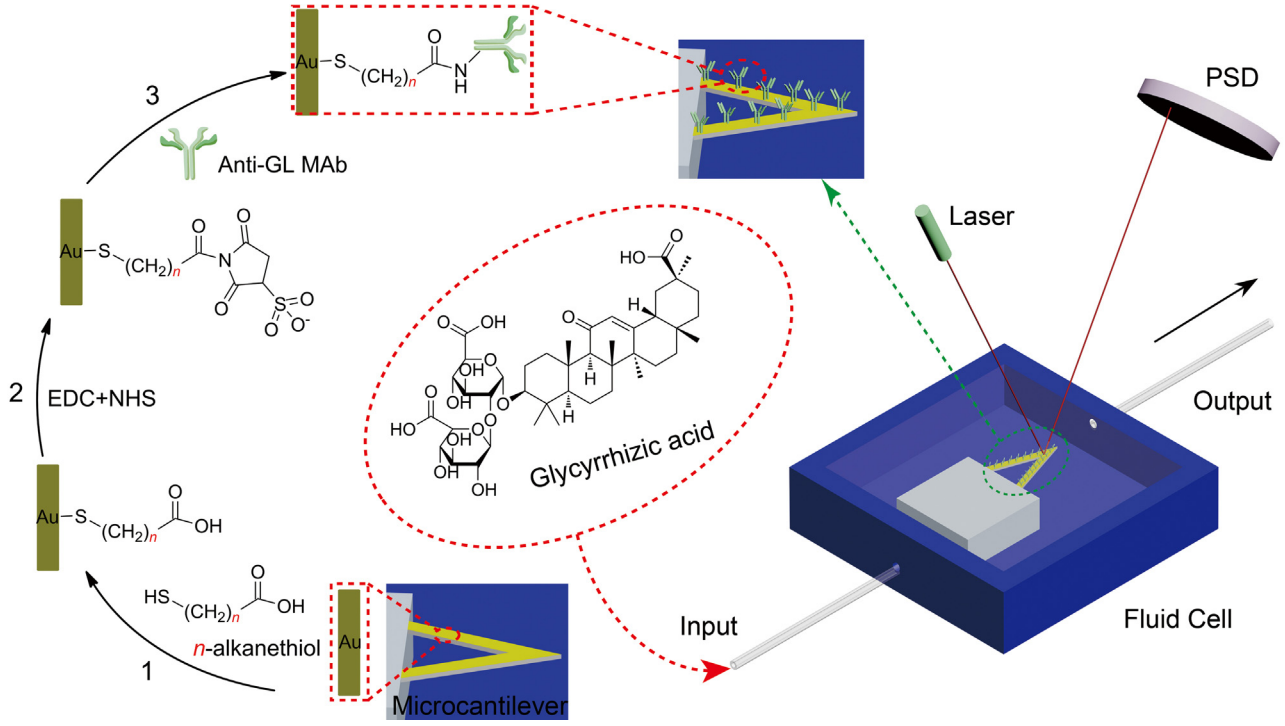

Fig. 1. Schematic of the microcantilever sensor and the antibody immobilization on the gold (Au) surface of microcantilever via self-assembled monolayers (SAMs) of n-alkanethiols ($HS(CH_2)_nCOOH$) with a same carboxyl group and different chain lengths (n = 1, 5, 10 and 15). EDC: N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, NHS: N-hydroxysuccinimide.

(succinimidylundecanoate) (DSU) [9], sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (sulfo-LC-SPDP) [10], N-succinimidyl S-acetyl thioacetate (SATA) [11] and 2-iminothiolane hydrochloride (2-IT) [12–14], and monothiol linkers 3-mercaptopropionic acid [15], 11-mercaptoundecanoic acid (MUA) [16], cysteamine [17] and aminoethanethiol [18]. In these sulfhydrylation reagents, the linker molecules are straight alky chains, and there are 2-carbon chain length between the surface and the antibody for SATA, 3-mercaptopropionic acid, cysteamine and aminoethanethiol; 3-carbon chain length for 2-IT; 9-carbon chain length for sulfo-LC-SPDP; 10-carbon chain length for DSU and MUA. In these different detections (or sulfhydrylation reagents), the microcantilevers show different sensitivities. For straight carbon chain, the carbon chain length may be critical to stress transmission, and ultimately affects the assay sensitivity. However, because of different affinities of antibody used in these detections, it cannot be evaluated how the chain length affects the stress transmission and the sensitivity. A same receptor molecule should be used.

Often, selective receptors are immobilized on the microcantilever surface using alkanethiol linkers, which form self-assembled monolayers (SAMs) on the gold-coated surface of the microcantilever. These thiolated molecules are chosen due to the strong affinity of sulfur head groups with the gold surface of the microcantilever. However, not much information is available on the mechanical properties of SAMs, especially regarding the nature of stress transmission of alkanethiol. Berger et al. showed that, during vapor-phase n-alkanethiol adsorption on microcantilever surface, the difference in surface stress response correlates well with differences in chain length of n-alkanethiol, and longer-chain alkanethiol on the cantilever surfaces results in larger surface stress values [19]. Desikan et al. investigated, in the liquid phase, the effect of chain length on the nanomechanical stress generation on microcantilever surfaces during the adsorption of n-alkanethiols of different chain lengths and showed that adsorption of shorter-chain alkanethiols on the cantilever surfaces results in larger surface stress values [20]. Both Berger and Desikan study the effect of chain length at the step of the alkanethiols adsorption on the microcantilever, due to the binding of sulfur head groups of alkanethiols to the gold surface of the microcantilever. However, there is no report on the effect of chain length of alkanethios when it is used as a linker that links the receptor to the microcantilever surface.

Licorice root is an herbal medicine in China for several thousand years. It is commonly used in Chinese herbal medicines (CHMs) and Chinese proprietary medicines (CPMs). Glycyrrhizic acid (GL) (Fig. 1) is a major active compound and a quality control marker of licorice root. It has anti-viral [21], anti-inflammatory [22], anti-carcinogenesis [23] and anti-hepatitis [24] activities. Its antiviral activity against severe acute respiratory syndrome (SARS)-associated coronavirus has been demonstrated in vitro [25]. GL is also used as food additive and masking agent in pharmaceutical products because of its sweet taste (170 times sweeter than sucrose) [26].

The content of GL in the licorice roots varies considerably with strains, cultivars, growing regions, climatic conditions, and harvest age. The quality of the raw herbs used in the CPMs affects the final therapeutic outcomes and consumer safety. An effective method is needed to screen large numbers of licorice root samples for the quality control. Existing methods for the determination of GL include high-performance liquid chromatography (HPLC) [27], liquid chromatography–ion trap mass spectrometry (LC–ITMS) [28], capillary electrophoresis [29]. However, these methods of detection of herbal ingredients are complex, time consuming, and require costly and bulky instrumentation. Immunoassay based detection techniques are being developed as an alternative to detect these GL in samples. There are reports of enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibody (MAb) against GL [26]. The ELISA reported by Mizutani et al. had a detect range of 20–200 ng/mL. Such assays are highly specific and exhibit the necessary sensitivity and accuracy for the detection of these low