Biosensors and Bioelectronics 48 (2013) 67–74

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Mechanism and enhancement of the surface stress caused by a small-molecule antigen and antibody binding

Shangquan Wu [a], Tiegui Nan [b], Changguo Xue [a], Teng Cheng [a], Hong Liu [c], Baomin Wang [b,*], Qingchuan Zhang [a,**], Xiaoping Wu [a]

[a] CAS Key Laboratory of Mechanical Behavior and Design of Material, University of Science and Technology of China, Hefei 230027, People's Republic of China
[b] College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, People's Republic of China
[c] Department of Chemical Physics, University of Science and Technology of China, Hefei 230026, People's Republic of China

ARTICLE INFO

Article history:
Received 20 November 2012
Received in revised form
25 March 2013
Accepted 30 March 2013
Available online 17 April 2013

Keywords:
Microcantilever sensors
Nanomechanics
Surface stress
Antibody immobilization
Nanotechnology
Immunoassay

ABSTRACT

Generation of microcantilever bending from biochemical interactions can have wide applications, ranging from high-throughput molecular detection to bioactuation. However, the origin of the biochemically induced surface stress causing the bending is a subject of much scientific debate and interest. Unlike a compressive surface stress caused by biomacromolecule antigen and antibody binding, here we show that a small molecule antigen and antibody binding on the surface gives rise to a tensile stress. We propose that the tensile stress is induced by antibody conformational change which manifests itself as Fab arm motion that exposes the C1q binding site of the antibody due to antigen binding. A microcantilever immunosensor was developed for the detection of Chlorimuron-ethyl (CE). We found that antibodies with oriented immobilization induce a greater resultant surface stress than those with random immobilization. The length of linker between the surface and the antibody plays an important role on the stress transmission. The shorter the length, the greater the surface stress. These mechanism and principles will underpin the design of devices and coatings to significantly lower the small molecule detection limit and may also have an impact on our understanding of antigen and antibody binding.

1. Introduction

Microcantilever sensor techniques, based on surface stress effect, are a new way of developing highly sensitive sensors. Because of high specificity of antigen–antibody binding in an immunoassay, a microcantilever functionalized with antibodies can effectively detect target molecules. Compared with many reports on the detection of biomacromolecules (~100 kDa) (Wu et al., 2001a; Dauksaite et al., 2007; Arntz et al., 2003; Velanki and Ji, 2006; Grogan et al., 2002), there are only a few reports on the detection of small molecules (~300 Da) with microcantilevers functionalized with antibodies (Suri et al., 2008; Tan et al., 2010; Xue et al., 2011; Zhao et al., 2010). In some of these reports, the limit of detection (LOD) can be below nanograms per milliliter. The high sensitivity implies that such microcantilever immunosensors have potential for many applications.

These detection systems use microcantilever bending, which results from a change in surface stress caused by specific interactions between target and probe molecules on one surface of a microcantilever. The origin of such biochemically induced surface stress is a subject of much scientific debate and interest. The biomacromolecule antigen and antibody binding creates a compressive surface stress to bend the microcantilevers toward the opposite surface, and the primary cause of the stress is considered to be the repulsion of target biomacromolecule (Wu et al., 2001a; Ji and Armon, 2010; Backmann et al., 2005). For DNA hybridization, a tensile surface stress was observed and the origin is suggested to be the changes in configurational entropy (Wu et al., 2001b). On the contrary, a compressive surface stress was also observed after DNA hybridization, and the predominantly steric hindrance effect is considered to be the origin (McKendry et al., 2002). The antibiotic–mucopeptide binding, where the mucopeptide probe was a small-molecule, induced a compressive surface stress which is considered to be a product of a local chemical binding factor and a geometrical connecting factor (Ndieyira et al., 2008). However, there is no report about the mechanism of surface stress caused by the binding of small target molecules (~300 Da) and antibodies (e.g., IgG, ~150 kDa).

Antibody immobilization on the surface is known to be an important factor on assay sensitivity. Different antibody immobilization methods on microcantilever surface result in differences in the antibody activity, orientation, and linker structures and sizes between the surface and the antibody. Ultimately, these factors

* Corresponding author. Tel.: +86 10 62731305; fax: +86 10 62732567.
** Corresponding author. Tel.: +86 551 3607613; fax: +86 551 3601248.
E-mail addresses: wbaomin@263.net (B. Wang), zhangqc@ustc.edu.cn (Q. Zhang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.03.086

affect assay sensitivity. Various sulfhydrylation reagents suitable for immobilization of antibodies on gold surface of microcantilever have been described in the literature for the detection of different molecules (Arntz et al., 2003; Velanki and Ji, 2006; Grogan et al., 2002; Suri et al., 2008; Xue et al., 2011; Zhao et al., 2010; Weeks et al., 2003; Raiteri et al., 1999; Alvarez et al., 2003; Velanki et al., 2007). With these reagents, the linkers are straight alkyl chains. And there is a variety of alkyl chain lengths between the surface and the antibody. For microcantilever sensors based on surface stress effects, stress transmission from the antibody to the microcantilever, through a linker, plays an important role. The chain length of linker may be critical to stress transmission, and ultimately affects assay sensitivity.

All of these sulfhydrylation reagents undergo chemical reactions to immobilize the antibody. Such reactions may decrease the antibody activity and cause disorder in the antibody orientation, which may result in loss of binding capability to antigen. One of the best ways to achieve oriented immobilization of antibody molecules is their immobilization on a sublayer consisting of Fc-binding receptors, which specifically bind the Fc part of antibody molecules without a chemical reaction (Hoffman and Oshannessy, 1988; Oh et al., 2004). In our previous studies, a protein A functionalized microcantilever was used to specifically bind the Fc part of the antibody and accurately detected small molecules at a parts-per-trillion level (Tan et al., 2010). However, how antibody activity and orientation affect sensitivity, and how chain length of linker affects stress transmission, which may also affect sensitivity, remain unknown.

In the present study, antibodies against a small-molecule antigen (hapten) were immobilized on a gold (Au) surface of a microcantilever in three different methods: (A) sulfhydrylated antibody using the sulfhydrylation reagent 2-iminothiolane hydrochloride which reacts with the $–NH_2$ group of the antibody to give a thiol (–SH) group for conjugation onto the gold surface (Fig. 1A); (B) sulfhydrylated goat anti-mouse IgG using 2-iminothiolane hydrochloride (Fig. 1B), which can also specifically bind the Fc part of the antibody to achieve oriented immobilization of antibody without a chemical reaction; and (C) self-assembled monolayer (SAM), based on 11-mercaptoundecanoic acid (Fig. 1C, $n=10$). Microcantilevers functionalized with antibodies were used to study the mechanism of bending caused by small-molecule antigen and antibody binding, and the effects of antibody activity and orientation on the sensitivity of the microcantilever immunosensor. Alkanethiols with different alkyl chain lengths (Fig. 1C, $n=1$, 5, 10 and 15, i.e. thioglycolic acid, 6-mercaptohexanoic acid, 11-mercaptoundecanoic acid and 16-mercaptohexadecanoic acid respectively) were used in method C to study the effects of the linker length between the surface and the antibody on assay sensitivity. The surface concentrations of immobilized antibodies were estimated using surface plasmon resonance (SPR). An ELISA was used to evaluate the activity of antibodies immobilized on the gold surface of the microcantilever. A microcantilever immunosensor was developed for the detection of Chlorimuron-ethyl (CE) in buffer and soil sample.

2. Materials and methods

2.1. Reagents and solutions

Chlorimuron-ethyl (CE), chlorsulfuron, goat anti-mouse IgG, 2-iminothiolane hydrochloride, thioglycolic acid, 6-mercaptohexanoic acid, 11-mercaptoundecanoic acid, 16-mercaptohexadecanoic acid, 3,3′,5,5′-tetramethylbenzidine (TMB), bovine serum albumin (BSA), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Goat anti-mouse IgG conjugated with horseradish peroxidase (IgG–HRP) was purchased from Jackson

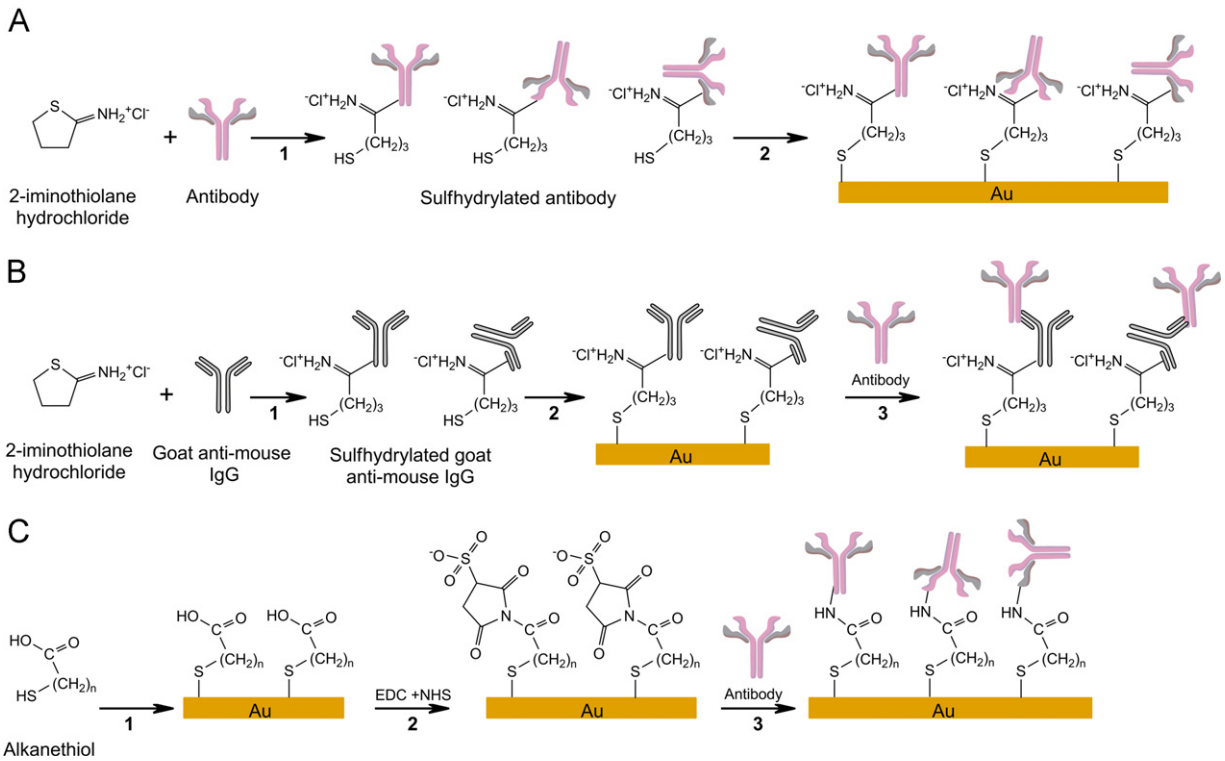

Fig. 1. Principal immobilization schemes of antibodies: (A) antibodies immobilized on the gold (Au) surface of a microcantilever via sulfhydrylated antibody (method A), (B) antibodies immobilized via sulfhydrylated goat anti-mouse IgG (method B), and (C) antibodies immobilized via self-assembled monolayer (SAM) based on carboxyl alkanethiol of alkyl chain lengths $n=1$, 5, 10 and 15. EDC: N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, NHS: N-hydroxysuccinimide.