Acta Biomaterialia 9 (2013) 5838–5844

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Electrochemical functionalization of gold and silicon surfaces by a maleimide group as a biosensor for immunological application

Xin Zhang [a], Aleksei Tretjakov [b], Marc Hovestaedt [c,d], Guoguang Sun [e], Vitali Syritski [b], Jekaterina Reut [b], Rudolf Volkmer [c], Karsten Hinrichs [e], Joerg Rappich [a,*]

[a] Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Institut für Silizium-Photovoltaik, Kekuléstr. 5, 12489 Berlin, Germany
[b] Department of Materials Science, Tallinn University of Technology, Ehitajate tee 5, Tallinn 19086, Estonia
[c] Charité Universitätsmedizin Berlin, Institut für Medizinische Immunologie, Abteilung Molekulare Bibliotheken, Hessische Str. 3-4, 10115 Berlin, Germany
[d] Charité Universitätsmedizin Berlin, Institute of Biochemistry 18, Reinickendorfer Str. 61, Haus 10, D-13347 Berlin, Germany
[e] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Department Berlin, Albert-Einstein-Str. 9, 12489 Berlin, Germany

ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 11 September 2012
Accepted 18 October 2012
Available online 29 October 2012

Keywords:
Biosensor
Au and Si surfaces
Maleimidophenyl
Cys-peptide
Antibody

ABSTRACT

In the present study we investigated the preparation of biofunctionalized surfaces using the direct electrochemical grafting of maleimidophenyl molecules with subsequent covalent immobilization of specific peptide to detect target antibody, thereby extending the application of the biosensing systems towards immunodiagnostics. *Para*-maleimidophenyl (*p*-MP) functional groups were electrochemically grafted on gold and silicon surfaces from solutions of the corresponding diazonium salt. A specially synthesized peptide modified with cysteine (Cys-peptide) was then immobilized on the *p*-MP grafted substrates by cross-linking between the maleimide groups and the sulfhydryl group of the cysteine residues. Accordingly, the Cys-peptide worked as an antigen that was able to bind specifically the target antibody (anti-GST antibody), while it was non-sensitive to a negative contrast antibody (i.e. anti-Flag β). The immobilization of both specific and non-specific antibodies on the Cys-peptide-modified surfaces was monitored by infrared spectroscopic ellipsometry, a quartz crystal microbalance integrated in flow injection analysis system and potentiometric response. The results obtained clearly demonstrated that the direct modification of a surface with maleimidophenyl provides a very simple and reliable way of preparing biofunctionalized surfaces suitable for the construction of immunological biosensors.

1. Introduction

In the materials and life sciences there is a great demand for biofunctionalized surfaces, which offer a variety of innovative biotechnological applications in the field of bioelectronics, biosensors and drug delivery. A great number of surface modification techniques have been developed, providing the possibility of specifically attaching biomolecules. The functionalization of metal and glass surfaces with self-assembled monolayers (SAMs) [1,2] is used extensively for the physical and chemical attachment of proteins [3]. SAMs of alkenethiols are widely employed for the modification of gold metal surfaces [4]. One drawback of this method is the instability of the gold–thiolate bond at reductive potentials, which causes the desorption of the layer [5]. Aryl diazonium salts are a possible alternative to SAMs that has considerable promise due to the high stability of the layers produced and the versatility of the chemistry. Nowadays, electrochemical reduction of organic diazonium salt is a well-known technique to graft organic molecules [6–8], especially aromatic rings, onto metal [9–11], carbon [9,12] and semiconductor surfaces [13–15]. The organic layer formed is covalently attached to a substrate, providing a stable functionalized surface suitable for application in different research areas, such as microelectronics [16] and sensors [10,17]. Different functional groups can be introduced to the surface depending on the substituent on the *para*-position of the aryldiazonium salt. For this, electrochemical grafting of 4-carboxyphenyldiazonium [10], 4-nitrophenyldiazonium [18] and 4-aminophenyldiazonium salts [19] have been used. However, for the immobilization of biomolecules on carboxy-, nitro- and amino-derivatized surfaces, further functionalization is needed, including: the activation of carboxyl function through a carbodiimide route to allow subsequent covalent coupling with an amino group of the biomolecule [20,21]; and chemical coupling reactions with a biomolecule-reactive group, such as *N*-hydroxysuccinimide (NHS) or maleimide. Considering that biomolecular coupling through the amino groups of lysine residues in the case of NHS-modified surfaces leads to additional heterogeneities in the population of molecules, the site-specific immobilization of a biomolecule through thiol groups (i.e. cysteines) on maleimide functionalities is the preferred

---

* Corresponding author. Tel.: +49 30806241386.
E-mail address: rappich@helmholtz-berlin.de (J. Rappich).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.10.022

method [22]. Surface modification with maleimide is usually a multistep process that includes the functionalization of the surface with amino groups followed by the attachment of some heterobifunctional crosslinker containing amine-reactive group on the one end and thiol-reactive maleimide on the other [23,24]. Recently, Harper et al. [9] reported the direct grafting of maleimide on Au and glassy carbon electrodes from *para*-maleimidophenyl diazonium tetrafluoroborate (*p*-MPDT). They showed the possibility of immobilizing biomolecules such as ferrocene and the redox-active protein cytochrome *c* on the maleimidophenyl-modified surface for the development of bioelectronic devices, including biofuel cells, biosensors, and DNA and protein microarrays.

In the present study we investigated the possibility of preparing biofunctionalized surfaces using the direct electrochemical grafting of maleimidophenyl molecules with subsequent covalent immobilization of specific peptide to detect a target antibody, thereby extending the application of the biosensing systems towards immunodiagnostics. Fig. 1 presents the method used to construct an immunological biosensor.

The first step is the modification of the electrode surfaces with *p*-MP by the electrochemical reduction of *p*-MPDT (step 1 in Fig. 1). *p*-MP is a very favourable reactive group in the cross-linking process and reacts specifically with sulfhydryl (–HS) groups in aqueous solution at a pH value between 6.5 and 7.5 to form a stable and non-reversible thioether linkage [25]. An HS group could be introduced into a target peptide (antigen) by chemical modification at its N-terminus with cysteine, so that the obtained Cys-peptide could be covalently immobilized on a maleimide-modified surface by cross-linking (as sketched for *p*-MP at step 2 in Fig. 1). We prepared biofunctionalized electrodes by the covalent immobilization of Cys-peptide on *p*-MP modified Au and/or Si substrates and investigated their specificity to interact with the target antibody (anti-GST antibody) (step 3 in Fig. 1), identifying both the specific infrared vibrational signature of amide bonds by infrared spectroscopic ellipsometry (IRSE) [26] and the mass change upon the antibody binding by the quartz crystal microbalance integrated in flow injection analysis (QCM-FIA) technique [27]. IRSE was used to characterize all the steps of the electrode modification process, including the grafting of the maleimide group, Cys-peptide and target antibodies immobilization by their specific vibrational signature. Additionally, the interaction between Cys-peptide and the antibody was monitored by zero current potential (U_Z) measurements between the biofunctionalized electrode (as a working electrode) and the reference electrode during the anti-GST antibody binding. Thus, the biosensing process was displayed in real time via a simple electrochemical response.

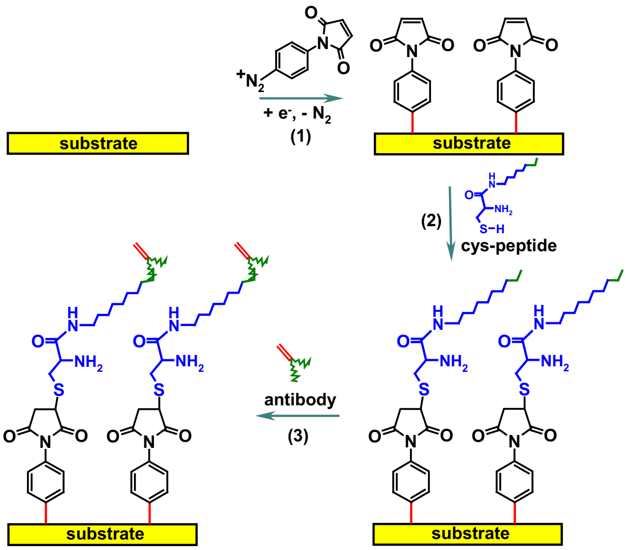

Fig. 1. Scheme of an antibody immobilization onto *p*-MP modified substrate: (1) electrochemical modification by *p*-MP functional groups; (2) coupling of Cys-peptide; and (3) target antibody immobilization.

2. Experimental section

2.1. Substrates

A 200 nm polycrystalline Au film deposited on the glass with a 3 nm Ti coadhesive layer and p-Si(1 1 1) substrates were used as electrodes for the electrochemical grafting process. The Au surfaces were cleaned using fresh piranha solution (97% H_2SO_4:30% H_2O_2 = 1:1) for 20 s, then rinsed copiously with deionized water. p-Si(1 1 1) wafers (0.5–2.5 Ω cm), covered by a 100 nm thick oxide layer and polished on one side, were purchased from the Leibniz Institute for Crystal Growth (IKZ). Before any modification, the SiO_2/Si samples were treated ultrasonically in 2-propanol for 10 min before being washed with deionized water. Then hydrogenated p-Si(1 1 1) surfaces were prepared in three steps. First, the Si samples were immersed in 5% HF for 10 min to remove the oxide layer. Second, the samples were reoxidized for 10 min in freshly prepared piranha solution. Next, the Si samples were rinsed by water and immersed in 40% NH_4F for 6–8 min to obtain a flat and hydrogen-terminated Si surface [15]. Finally, the samples were washed with deionized water and dried by nitrogen. The HF, NH_4F, H_2SO_4 and H_2O_2 solutions used were supplied by Aldrich® with grade Selectipure® (VLSI grade). The water used was purified by a PURELAB® Ultra water system ($\leqslant$18 MΩ cm at 25 °C).

2.2. Chemicals and materials

The *p*-MPDT used in this work was synthesized from *N*-(4-aminophenyl)maleimide (TCI Europa) and identified by nuclear magnetic resonance spectroscopy in CD_3-CN (8.55 dblt, 2H; 8.12 dblt, 2H; 7.07 singlet, 2H). The reagents and solvents employed were used as received, and were as follows: anhydrous acetonitrile (ACN, 99.8%, Sigma–Aldrich) and electrochemical grade tetrabutylammonium tetrafluoroborate (Bu_4NBF_4, $\geqslant$99.0%, Fluka). Phosphate-buffered saline powder (PBS, pH 7.4), and Tris-buffered saline powder (TBS, pH 8.0) were bought from Sigma and dissolved in deionized water to obtained 0.05 M buffer solution.

The specific peptide with the amino acid sequence LAKDLIVPRR was specially synthesized for immobilization of the target antibody – a murine anti-GST IgG (Sigma–Aldrich). The selectivity and sensitivity of the synthesized peptides was verified by complete substitution analysis and affinity determinations with surface plasmon resonance spectroscopy. These binding of these peptides to other antibodies was also tested, including anti-p24 (HIV) CB 4-1 IgG, anti-TGFα-IgG, anti-Flag β antibody and anti-mouse IgG. None of the antibodies showed any interaction with the peptide used. Thus, the LAKDLIVPRR peptide and anti-GST IgG interaction is very specific, and is very well suited as a model reaction for the biosensor study. Anti-Flag ß antibody was taken as the negative contrast. A variant of peptide LAKDLIVPRR was produced then with an N-terminal cysteine (Cys)-linker βAla in milligram amounts by solid-phase synthesis.

2.3. Electrochemical grafting

The *p*-MP layers were electrochemically grafted onto either Au or Si surfaces via the appropriate procedures described below. For the Au surface grafting process, an electrochemical cell accommodating three electrodes, i.e. a gold-coated glass slide as the working