Analytical Biochemistry 443 (2013) 78–80

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Notes & Tips

Functionalization of quartz crystal microbalances with liposomes containing the *N*-hydroxysuccinimide ester of palmitic acid

Sora Eom, Eunkyeong Yu, Suk-Jung Choi *

Department of Chemistry, Gangneung–Wonju National University, Gangneung, Gangwondo 210-702, Republic of Korea

ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 24 July 2013
Accepted 16 August 2013
Available online 28 August 2013

Keywords:
Biosensor
Quartz crystal microbalance (QCM)
Functionalization
Liposome
N-Hydroxysuccinimide

ABSTRACT

We developed a fast and simple method to functionalize a quartz crystal microbalance (QCM) with liposomes composed of phosphatidylcholine lipid and the *N*-hydroxysuccinimide (NHS) ester of palmitic acid. The liposome was applied directly to a bare gold surface of a QCM to prepare the lipid bilayer presenting NHS groups on the surface. The whole functionalization process was completed within 1 h using stored lipid films. Streptavidin immobilization efficiency of the method was comparable to the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/NHS chemical method, and the activity of the biotinylated antibody immobilized through the streptavidin was stably retained in repeated surface regeneration with the dissociation buffer.

Preparation of *N*-hydroxysuccinimide (NHS)[1] ester is the most common activation chemistry to immobilize amine-containing molecules on quartz crystal microbalance (QCM) or surface plasmon resonance (SPR) biosensor surfaces [1]. In the traditional chemical methods, a self-assembled monolayer (SAM) of ω-mercapto-carboxylic acid is prepared on the surfaces, and the carboxyl groups are converted into NHS esters in situ by consecutive reactions of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS. However, the entire process is laborious and time-consuming. Moreover, the experimental protocols involving chemical reactions are not favorable to many researchers.

Recently, a lipid-based method was developed to introduce functional groups into QCM surfaces without chemical reactions [2,3]. This method took advantage of the capacity of lipids to form a hybrid bilayer membrane (HBM) with a hydrophobic SAM through a spontaneous process. The introduction of biotin or hydrazide groups was achieved by applying liposome-containing biotinylated lipids or dodecanoic hydrazide to the hydrophobic SAM surface. After immobilizing receptor proteins, the QCMs showed sensitivity and stability comparable to the QCM functionalized by the chemical method. Moreover, the biofunctionalized surfaces were highly resistant to the nonspecific adsorption of proteins. One major limitation of the HBM method was that the biosensor surfaces need to be modified by the time-consuming process of SAM preparation prior to the application of the liposome.

In an attempt to develop a simple method to functionalize QCMs with NHS groups, we modified the lipid-based method. At first, *N*-succinimidyl palmitate (NSP) was included in the liposome of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) to present NHS groups on the surfaces. NSP, NHS ester of palmitic acid, was inserted into the lipid layer of DPPC through hydrophobic interactions. Second, the liposome was applied directly to a gold surface of a QCM without SAM preparation to simplify the method. The applied liposome was expected to form a planar lipid bilayer on the gold surface as reported by several authors [4–6]. Third, the liposome was prepared in weak acidic buffer, which may help to reduce the hydrolysis of the NHS ester [7].

The new functionalization method is shown schematically in Fig. 1A. Each lipid film was prepared with 0.2 ml of lipid solution containing 0.44 mg (0.6 μmol) of DPPC (Avanti Polar Lipids, Alabaster, AL, USA) and 0.07 mg (0.2 μmol) of NSP (Sigma–Aldrich, St. Louis, MO, USA) in a glass vial and was used to coat four QCMs (Crystal Sun Life, Tokyo, Japan). For convenience, many lipid films were prepared simultaneously and stored in a freezer until use. The lipid film was hydrated with 480 μl of 0.1 M Mes [2-(N-morpholino)ethanesulfonicacid] buffer (pH 6.0) at 50 to 60 °C for 20 min. The solution was vortexed vigorously for 1 min to prepare the liposome. The QCMs were carefully treated with a minimal volume of piranha solution (7:3 mixture of concentrated H_2SO_4 and

* Corresponding author. Fax: +82 33 640 2264.
E-mail address: sjchoi@gwnu.ac.kr (S.-J. Choi).

[1] *Abbreviations used:* NHS, *N*-hydroxysuccinimide; QCM, quartz crystal microbalance; SPR, surface plasmon resonance; SAM, self-assembled monolayer; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HBM, hybrid bilayer membrane; NSP, *N*-succinimidyl palmitate; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; IgG, immunoglobulin G; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

0003-2697/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2013.08.017

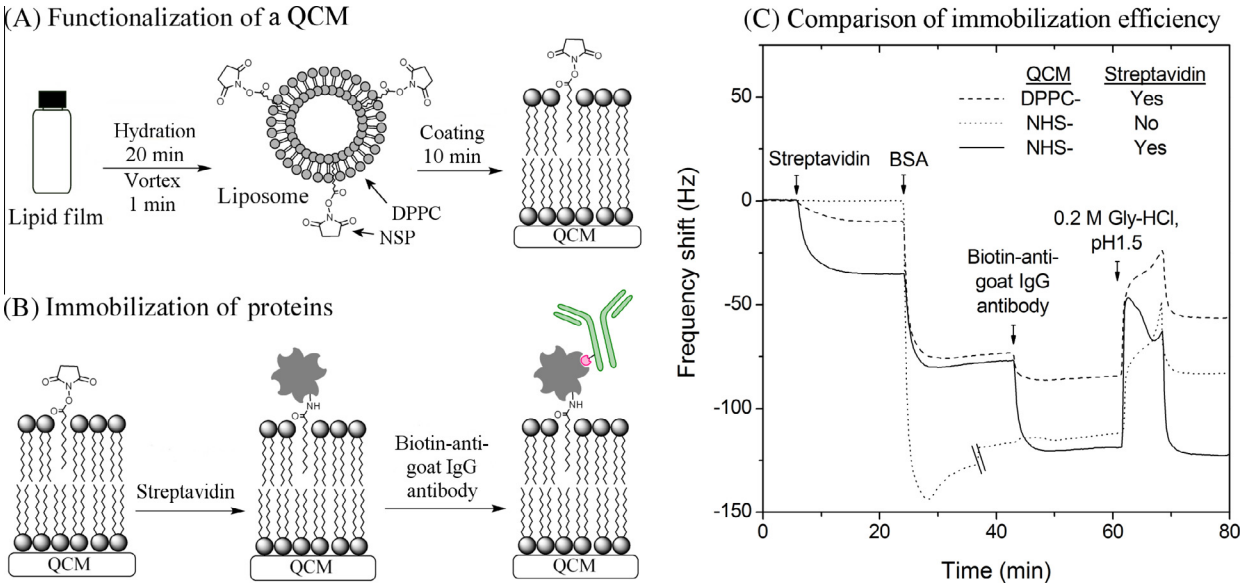

Fig.1. Biofunctionalization of QCMs. (A) Schematic representation of the novel lipid-based functionalization method. (B) Schematic representation of the biofunctionalization of the NHS-functionalized QCM with streptavidin and biotin–anti-goat IgG antibody. (C) Frequency profiles for the biofunctionalization of the DPPC–QCM and the NHS–QCM. The flow rate was 10 μl/min during conjugation of streptavidin and was later adjusted to 20 μl/min.

30% H_2O_2) at 60 °C for 1 min and washed with deionized water and ethanol. The liposome solution (0.1 ml) was added to the cleaned QCM, which was placed in a well cell of an AMO-QC4 QCM biosensor system (Amogreentech, Gimpo, Korea). The cell was incubated at 50 to 60 °C for 10 min to promote lipid layer formation on the electrode surface.

The NHS-functionalized QCM (NHS–QCM) was placed in a flow cell and used for the conjugation of streptavidin to which the biotin–anti-goat immunoglobulin G (IgG) antibody was attached (see scheme in Fig. 1B). A control experiment was simultaneously conducted with a QCM coated with only the DPPC liposome (DPPC–QCM). Throughout the experiments, protein solution or buffer was injected through an injection valve with a 0.1-ml sample loop. When streptavidin (25 μg/ml) was injected into the two types of QCMs, the rate and degree of the frequency shift were much higher in the NHS–QCM compared with the control QCM (Fig. 1C). The result shows that the immobilization of streptavidin on the NHS–QCM was primarily achieved by the NHS groups included in its lipid layer. In contrast, the injection of blocking buffer (10 mg/ml bovine serum albumin [BSA] in phosphate-buffered saline [PBS]) caused a higher frequency shift in the DPPC–QCM (Fig. 1C). More protein adsorption sites were available in the DPPC–QCM because less streptavidin was immobilized. Consequently, the frequency shift after the injection of biotin–anti-goat IgG antibody (30 μg/ml) was higher in the NHS–QCM compared with the DPPC–QCM (Fig. 1C). Binding of biotin–anti-goat IgG antibody was negligible in the second control experiment where blocking buffer was directly injected into the NHS–QCM (Fig. 1C). This result shows that the binding of biotin–anti-goat IgG antibody in the DPPC–QCM was not caused by nonspecific adsorption to BSA. After surface regeneration with dissociation buffer (0.2 M glycine–HCl, pH 1.5), the frequency of the control QCM shifted to a high level, indicating that the dissociation of loosely adsorbed proteins occurred (Fig. 1C). However, the frequency of the NHS–QCM was restored to the same level observed before the injection of the dissociation buffer.

A dose response of the biofunctionalized QCMs was analyzed by sequential injection of samples containing different amounts of goat IgG. The QCMs were regenerated with the dissociation buffer after each injection. As shown in Fig. 2A, the frequency change (ΔF) of the NHS–QCM increased to 32.5 Hz in a dose-dependent manner at a range of 0.16 to 100 μg/ml goat IgG, which was comparable to the results obtained by the EDC/NHS chemical method and the HBM method [2]. However, the DPPC–QCM showed much lower sensitivity compared with the NHS–QCM and was saturated at 20 μg/ml goat IgG. The dose response was negligible in the second control experiment because no biotin–anti-goat IgG antibody was immobilized on the QCM (Fig. 2A). One major concern regarding this novel method was the disintegration of the lipid layer due to the extreme pH conditions of the dissociation buffer. We compared the frequency profiles of four sequential injections of the same goat IgG sample (20 μg/ml), each of which was followed by surface regeneration with the dissociation buffer. The frequency was shifted to approximately −30 Hz by the first injection of the sample but returned to the baseline (0 Hz) after the flow of the dissociation buffer (Fig. 2B, inset). Furthermore, the response of the sensor chip to the same sample was highly reproducible even after repeated surface regenerations (Fig. 2B). These results clearly show that the bound goat IgG was effectively removed by the dissociation buffer; however, the activity of the anti-goat IgG antibody and the integrity of the lipid layer remained intact during the surface regenerations.

This novel functionalization method is fast and simple compared with the EDC/NHS chemical method. The chemical method is usually conducted over 2 days, including the overnight SAM preparation process. Moreover, aqueous EDC or NHS solution is unstable [8,9] and should be freshly prepared before use; however, in the new method, the whole functionalization process was completed within 1 h using the stored lipid film. We used a well-type cell in the lipid-coating step for two reasons. First, the well-type cell can be easily separated from the biosensor system and placed in an incubator for temperature regulation. Second, it was also anticipated that the static condition would be beneficial for the coating process. However, it is possible to conduct the entire process from lipid coating to analysis in a flow cell if the temperature of the flow cell is maintained at 50 to 60 °C because reports have been published regarding the adsorption of the lipid monolayer or bilayer on the QCM or SPR sensor chips encased in flow cells by injecting liposome solution [4,5,10–12].

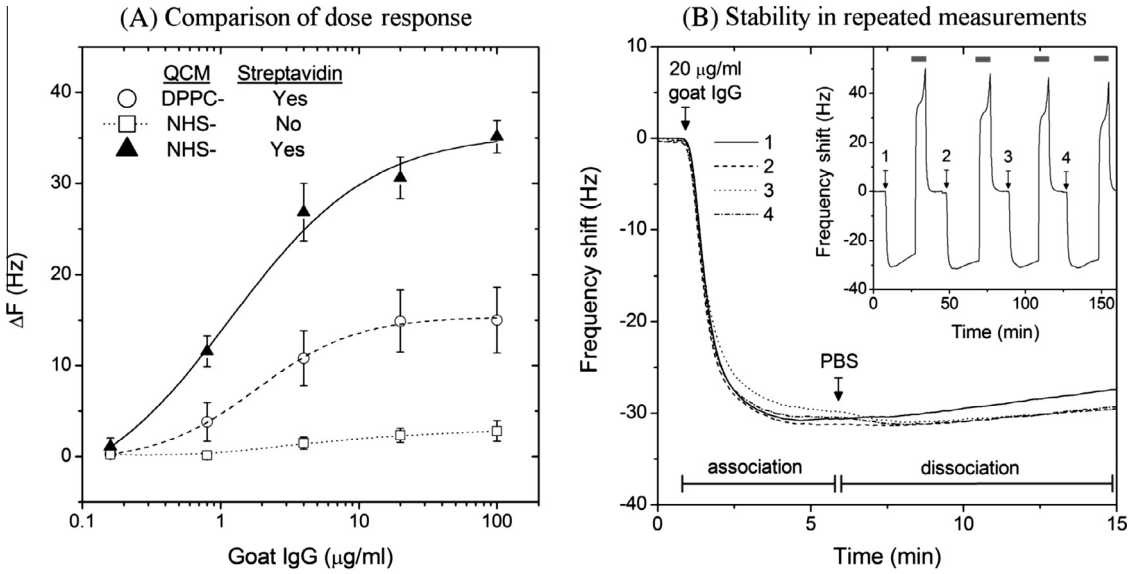

Fig.2. Performance of the biofunctionalized QCMs. (A) Dose response of the biofunctionalized QCMs. Samples containing different amounts of goat IgG were injected into the biofunctionalized DPPC–QCM and the NHS–QCM, and the frequency change (ΔF) was plotted against the goat IgG concentration. The QCMs were regenerated with the dissociation buffer after each injection. Each data point represents the mean from triplicate determinations, and the error bars represent the standard deviation. (B) Stability of the biofunctionalized NHS–QCM. The goat IgG sample (20 μg/ml) was sequentially injected four times into the biofunctionalized NHS–QCM, and the frequency profiles of the four injections were superimposed. The frequency profile of the whole experiment is also shown in the inset. The injection of the goat IgG sample is denoted by arrows. The QCM was regenerated with the dissociation buffer after each injection, and the gray bars represent the periods of flow of the dissociation buffer. Throughout the experiment, the flow rate was 20 μl/min.

Acknowledgments

This research was part of the project titled "Development of Biosensors for Paralytic Shellfish Toxins" funded by the Ministry of Land, Transport, and Maritime Affairs, Korea, and was also supported by the Research Institute of Natural Science of Gangneung–Wonju National University.

References

[1] M.A. Cooper, Sensor surfaces and receptor deposition, in: M.A. Cooper (Ed.), Label-Free Biosensors: Techniques and Applications, Cambridge University Press, New York, 2009, pp. 110–142.
[2] S. Mun, S.J. Choi, Optimization of the hybrid bilayer membrane method for immobilization of avidin on quartz crystal microbalance, Biosens. Bioelectron. 24 (2009) 2522–2527.
[3] S. Mun, S.J. Choi, Direct introduction of a hydrazide group into a quartz crystal microbalance surface with dodecanoic hydrazide embedded in a hybrid bilayer membrane, Anal. Chim. Acta 688 (2011) 70–74.
[4] C.A. Keller, B. Kasemo, Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance, Biophys. J. 75 (1998) 1397–1402.
[5] J.J. Stålgren, P.M. Claesson, T. Wärnheim, Adsorption of liposomes and emulsions studied with a quartz crystal microbalance, Adv. Colloid Interface Sci. 89–90 (2001) 383–394.
[6] E. Reimhult, M. Zäch, F. Höök, B. Kasemo, A multitechnique study of liposome adsorption on Au and lipid bilayer formation on SiO_2, Langmuir 22 (2006) 3313–3319.
[7] P. Cuatrecasas, I. Parikh, Adsorbents for affinity chromatography: use of *N*-hydroxysuccinimide esters of agarose, Biochemistry 11 (1972) 2291–2299.
[8] M.A. Gilles, A.Q. Hudson, C.L. Borders Jr., Stability of water-soluble carbodiimides in aqueous solution, Anal. Biochem. 184 (1990) 244–248.
[9] D. Sehgal, I.K. Vijay, A method for the high efficiency of water-soluble carbodiimide-mediated amidation, Anal. Biochem. 218 (1994) 87–91.
[10] M.A. Cooper, A.C. Try, J. Carroll, D.J. Ellar, D.H. Williams, Surface plasmon resonance analysis at a supported lipid monolayer, Biochim. Biophys. Acta 1373 (1998) 101–111.
[11] M.A. Cooper, D.H. Williams, Kinetic analysis of antibody–antigen interactions at a supported lipid monolayer, Anal. Biochem. 276 (1999) 36–47.
[12] M.P. Beseničar, G. Anderluh, Preparation of lipid membrane surfaces for molecular interaction studies by surface plasmon resonance biosensors, in: N.J. Mol, M.J.E. Fischer (Eds.), Surface Plasmon Resonance: Methods and Protocols (Methods in Molecular Biology, vol. 627), Springer, New York, 2010, pp. 191–200.