Colloids and Surfaces B: Biointerfaces 83 (2011) 58–60

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Visual observation of avidin–biotin affinity by fluorescent G4.5 poly(amidoamine) dendrimer

Govindachetty Saravanan[a], Kenji Daigo[b], Toyoko Imae[c,d,*], Takao Hamakubo[b]

[a] Graduate School of Science and Technology, Keio University, Hiyoshi, Yokohama 223-8522, Japan
[b] Department of Molecular Biology and Medicine, RCAST, The University of Tokyo, Komaba, Meguro, Tokyo 153-8904, Japan
[c] Keio Advanced Research Centers, Keio University, Hiyoshi, Yokohama 223-8522, Japan
[d] Graduate Institute of Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Taipei 10607, Taiwan

ARTICLE INFO

Article history:
Received 30 August 2010
Received in revised form 22 October 2010
Accepted 23 October 2010
Available online 2 November 2010

Keywords:
Avidin–biotin affinity
Fluorescence microscopy
Poly(amidoamine) dendrimer
Fluorescent dendrimer

ABSTRACT

An air-treated G4.5 poly(amidoamine) (PAMAM) dendrimer displayed the enhanced fluorescence enough to be utilized as a fluorescence marker to visualize avidin–biotin affinity: On a fluorescence microscopic image, the avidin labeled by a fluorescent G4.5 PAMAM dendrimer was observed to be selectively bound on the biotin pattern that was prepared by amide-bonding of biotin on a carboxylic acid-terminated self-assembled monolayer and in turn by UV-irradiation with a photomask on the monolayer.

1. Introduction

It has been found that the family of N-branched dendrimers showed the fluorescence [1,2]. Recently, this luminescent property of these dendrimers is taking attention and it was anticipated that these dendrimers can be used as a fluorescence marker on several potential applications. For instance, the fluorescence of these dendrimers and dendrons was used to visualize the cotton fibrils, hydrogels, porous silica, etc. [3–5]. These dendritic fluorescence markers can also be expected to label the biomaterials owing to bio-compatible, nonimmunogenic, and water-soluble characters of N-branched dendrimers [6], which allow us easily to monitor the properties of biomaterials and their assemblies, e.g., molecular recognition [7].

The well-known avidin–biotin system was selected as a model to visualize its affinity, because the association constant (K_a) of various biotin molecules towards avidin is in the high order such as 10^{13}–$10^{15}\,M^{-1}$ [8]. A few studies were performed to characterize the avidin–biotin affinity by using fluorescent dyes (sulforhodamine, fluorescein) on a fluorescence microscopy with plasmon spectroscopy, atomic force microscopy, and cyclic voltammetry [8,9]. However, the rapid and facile methods are still to be developed to recognize the avidin–biotin affinity. Therefore, it is expected that the affinity of avidin on the biotin can be visualized easily by labeling of fluorescent N-branched dendrimers as a fluorescence marker on avidin molecules.

In this preliminary report, we visualize the avidin–biotin affinity by using the enhanced emission of G4.5 poly(amidoamine) (PAMAM) dendrimer as a fluorescence marker. An air-treated dendrimer was labeled on an avidin molecule through the formation of amide chemical bond. The affinity of dendrimer-labeled avidin on the biotin surface was determined by means of a simple fluorescence microscopy.

2. Experimental

G4.5 PAMAM dendrimer, avidin and amine-PEO_2-biotin were purchased from Aldrich, Invitrogen, and Pierce chemicals, respectively. 11-Mercaptoundecanoic acid (11-MUA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were purchased from Aldrich, Dojindo, and Wako chemicals, respectively. Avidin and amine-PEO_2-biotin were used as received. Doubly distilled and deionized water (18 MΩ cm) were used throughout this work. A phosphate-buffered saline solution was prepared from 10 mM phosphate, 2.7 mM KCl, 138 mM NaCl, and 0.05% (v/v) Tween 20 (PBS, pH 7.2).

* Corresponding author at: Graduate Institute of Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Taipei 10607, Taiwan. Tel.: +886 2 2730 3627; fax: +886 2 2730 3627.
E-mail address: imae@mail.ntust.edu.tw (T. Imae).

0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.10.043

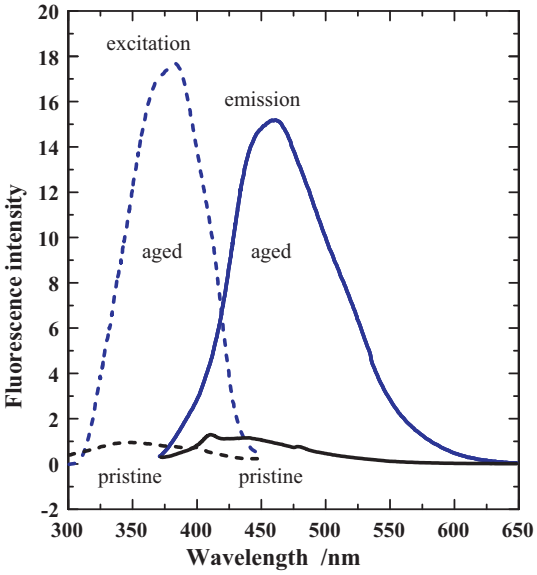


Fig. 1. Emission (solid line) and excitation (dotted line) fluorescence spectra of pristine (black color) and aged (blue color) 0.5 mM G4.5 PAMAM dendrimer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

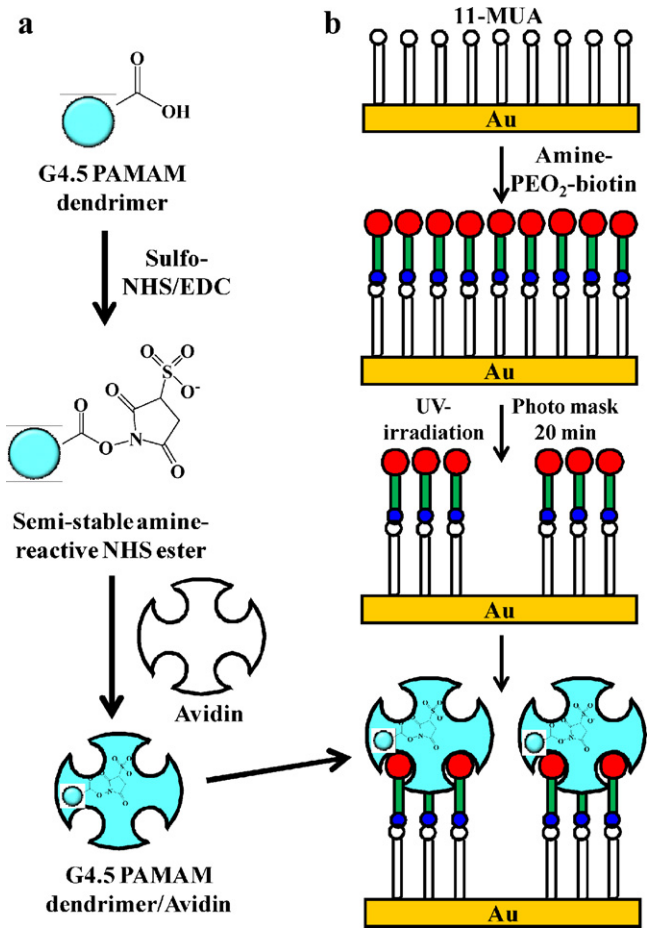
3. Results and discussion

Prior to the label of G4.5 PAMAM dendrimer on avidin molecule, an air-purged solution of G4.5 PAMAM dendrimer was kept for eight days under an air atmosphere in order to enhance its fluorescence property [2,5]. Fig. 1 shows the comparison of emission and excitation fluorescence spectra for air-treated and pristine 0.5 mM G4.5 PAMAM dendrimers in an aqueous solution. The pristine G4.5 PAMAM dendrimer showed a weak emission band at 460 nm with an excitation wavelength at 360 nm which increased steeply after air-treated. Similarly, a weak excitation band observed at 380 nm with an emission wavelength at 462 nm for the pristine dendrimer also increased remarkably by the air-treatment.

This air-treated G4.5 PAMAM dendrimer was labeled on an avidin molecule through amide chemical bond, as shown in Scheme 1a. Briefly, the peripheral carboxyl groups of fluorescent G4.5 PAMAM dendrimer (100 μl, 5 wt%) were converted into active esters (semi-stable amine-reactive NHS ester groups) by reacting for 15 min with sulfo-NHS/EDC in a PBS saline buffer solution. The activated G4.5 PAMAM dendrimer was separated from unreacted sulfo-NHS and EDC by using a PBS buffer in desalting NAP-10 column. The activated fluorescent G4.5 PAMAM dendrimer was further reacted with avidin (10 mg/ml in a PBS buffer) for 2 h at room temperature. Then, 20 μl of a 10% glutamate solution was added to terminate the labeling reaction.

Amine-PEO_2-biotin patterns on a gold coated glass substrate (Nihon Laser Electronic Co., Ltd. 20 mm × 13 mm × 0.7 mm) were prepared by producing the self-assembled monolayer (SAM) and adopting the photolithography in order to visualize the avidin–biotin affinity. Prior to the patterning of amine-PEO_2-biotin on a gold coated substrate, the gold substrates were cleaned carefully by ultrasonication for 5 min each in a piranha solution, water, acetone, and ethanol, followed by drying at oven.

As shown in the Scheme 1b, first, the self-assembled monolayer of 11-MUA was prepared onto the well-cleaned hydrophobic gold substrates (contact angle, $\theta = 76 \pm 2^\circ$) by immersing the substrate for 16 h in an 11-MUA solution, and thus the gold substrate became hydrophilic, having carboxylic acid groups on its surface which reduces the contact angle of pure water to be $40 \pm 2^\circ$. Amine-PEO_2-biotin was immobilized on the SAM of 11-MUA using an NHS/EDC



Scheme 1. (a) Procedure for labeling of a fluorescent G4.5 PAMAM dendrimer on an avidin molecule. The light-blue color on avidin represents fluorescent characteristics adjected by the fluorescent G4.5 PAMAM dendrimer. (b) A scheme for examination of affinity of biotin surface with fluorescent G4.5 PAMAM dendrimer-labeled avidin. The biotin patterns were prepared by irradiation of UV light for 20 min with photo mask. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

solution by the covalent (amide) bond. For details, the carboxylic acid-terminated gold substrate was immersed into a freshly prepared aqueous solution of NHS/EDC (2 mM each in a PBS buffer) to convert carboxyl group into active ester group [10]. After rinsed by a PBS buffer and water, the activated ester substrate was reacted with an amine-PEO_2-biotin solution (2 wt% in a PBS buffer) at room temperature, so that the amine terminal group of biotin formed the amide covalent linkage with carboxyl group of 11-MUA. Then the final concentration of biotin was adjusted about 20 mM.

The biotin-modified gold substrate was patterned further by photolithography: The substrate was irradiated for 20 min by UV light through a photomask in the presence of oxygen so as to completely remove biotin/11-MUA in the selected area [11]. The UV irradiation was carried out at a pressure 1.5×10^2 Pa with a proximate gap of <10 nm between the substrate and the photomask. Then the biotin-patterned substrate was immersed for 24 h in a PBS solution of fluorescent G4.5 PAMAM dendrimer-labeled avidin (100 μg/ml). The modified substrate was washed with a PBS buffer and water, dried under vacuum, and then examined by an optical (Halogen lamp, LHS-H100P-1, 12 V, 100 W) and a fluorescence (super high pressure mercury lamp, Model C-SHG1, 100 W, Nikon-Eclipse TE 2000-U) microscope. For imaging by a fluorescence microscope, the patterned substrates were irradiated at excitation wavelength at 365 nm using DM 400 and RA 400 filters.

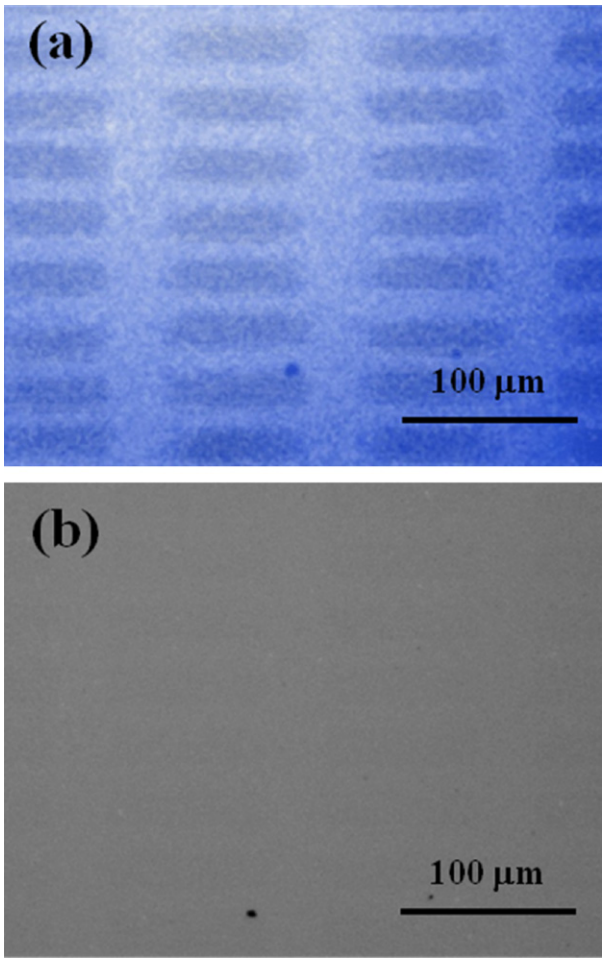


Fig. 2. Images of fluorescence (a) and optical (b) microscope of G4.5 PAMAM dendrimer-labeled avidin on biotin patterns.

The image of fluorescence microscope shows clearly distinct bright-and-dark regions, as shown in Fig. 2a. The bright frame region is indicative of fluorescent G4.5 PAMAM dendrimer-labeled avidin on the biotin pattern. This indicates that fluorescent dendrimer-labeled avidin molecules were bound selectively on the amine-PEO_2-biotin surface by the affinity, displaying the strong fluorescence frame patterns. However, no such affinity was observed in the UV light-irradiated regions, that is, rectangular closed regions, due to the absence of biotin, and therefore there is no fluorescence in these regions. In other words, the well-defined dark regions suggest that the biotin moieties were selectively removed during the UV light irradiation, which leaves the patterned biotin surfaces on the gold substrate. Similar patterns are slightly defined in the image on an optical microscope of G4.5 PAMAM dendrimer-labeled avidin/biotin patterns. However, it was not as clear as an image on a fluorescence microscope, as seen in Fig. 2b. Therefore, it can be mentioned that the observation by a fluorescence microscope is the highly excellent technology to detect the avidin–biotin affinity.

The most attractive feature of the present simple technique is the visualization of the avidin–biotin affinity by using fluorescence of PAMAM dendrimer as a marker. The pattern of G4.5 PAMAM dendrimer-labeled avidin on the biotin substrate fabricated through this technique is extremely reproducible, suggesting that the family of fluorescent PAMAM dendrimers can be available to label on biomaterials. This allows us to monitor the properties of the targeted systems. The G4.5 PAMAM dendrimer-labeled avidin can be valid as a sensing element to recognize the affinity with sensing surfaces, e.g., biotin surfaces. Kim et al. [9] have prepared the regenerable affinity-sensing surface consisting of avidin and biotin molecules and have examined by cyclic voltammetry. In the present study, we visually detected the avidin–biotin affinity on a fluorescence microscope by using fluorescent G4.5 PAMAM dendrimers. Especially, labeling of a biocompatible fluorescent probe, such as PAMAM dendrimer, enable us the visual adaptation to bioscience such as the separation of biomolecules, the determination of biomolecular interactions and affinity biosensing.

4. Conclusions

In conclusion, the affinity between avidin and amine-PEO_2-biotin was visualized by using air-treated G4.5 PAMAM dendrimer as a fluorescence marker. The air-treated G4.5 PAMAM dendrimer showed the enhanced fluorescence, different from the pristine one. Fluorescent G4.5 PAMAM labeled-avidin molecules were selectively bound on the patterned biotin surface, which displays strong fluorescent pattern. Air-treated PAMAM dendrimers with unique luminescent property can be labeled to biomaterials, as a fluorescence marker that gives the information about the properties of biomaterials, such as interactions, assembling, and so on. This method of visual observation should potentiate to further development of biosensors and biochips.

References

[1] D.M. Watkins, Y. Sayed-Sweet, J.W. Kilmash, N.J. Turro, D.A. Tomalia, Langmuir 13 (1997) 3136–3141;
M.H. Kleinman, J.H. Flory, D.A. Tomalia, N.J. Turro, J. Phys. Chem. B 104 (2000) 11472–11479;
W. Chen, D.A. Tomalia, J.L. Thomas, Macromolecules 33 (2000) 9169–9172;
P.M.R. Paulo, R. Gronheid, F.C. De Schryver, S.M.B. Costa, Macromolecules 36 (2003) 9135–9144;
J. Zhang, J.T. Petty, R.M. Dickson, J. Am. Chem. Soc. 125 (2003) 7780–7781.
[2] I.W. Lee, Y. Bae, A.J. Bard, J. Am. Chem. Soc. 126 (2004) 8358–8359;
D. Wang, T. Imae, J. Am. Chem. Soc. 126 (2004) 13204–13205.
[3] D. Onoshima, T. Imae, Soft Matter. 2 (2006) 141–148.
[4] D. Wang, T. Imae, M. Miki, J. Colloid Interface Sci. 306 (2007) 222–227.
[5] C.-C. Chu, T. Imae, Macromol. Rapid Commun. 30 (2009) 89–93.
[6] A.K.J. Patri, U.J. Majoros, J.J. Baker, Curr. Opin. Chem. Biol. 6 (2002) 466–471.
[7] W. Ong, M. Gomez-Kaifer, A.E. Kaifer, Chem. Commun. 15 (2004) 1677–1683.
[8] M. Hoffmann, W. Muller, H. Ringsdorf, A.M. Rourke, E. Rump, P.A. Suci, Thin Solid Films 210/211 (1992) 780–783;
W. Muller, H. Ringsdorf, E. Rump, G. Wildburg, X. Zhang, L. Angermaier, W. Knoll, M. Liley, J. Spinke, Science 262 (1993) 1706–1708;
E.-L. Florin, V.T. Moy, H.E. Gaub, Science 264 (1994) 415–417.
[9] H.C. Yoon, M.-Y. Hong, H.-S. Kim, Langmuir 17 (2001) 1234–1239;
M.-Y. Hong, H.C. Yoon, M.-Y. Hong, H.-S. Kim, Langmuir 19 (2003) 416–421;
E. Kim, K. Kim, H. Yang, Y.T. Kim, J. Kwak, Anal. Chem. 75 (2003) 5665–5672.
[10] T. Yamazaki, T. Imae, J. Nanosci. Nanotech. 5 (2005) 1066–1071.
[11] The oxidation of methyl groups of ODS on Si substrates by UV light was strongly depended on the proximate gap between the substrate and photomask, and the pressure of oxygen.