Biosensors and Bioelectronics 78 (2016) 213–220

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Detecting trypsin at liquid crystal/aqueous interface by using surface-immobilized bovine serum albumin

Cheng-Hao Chuang [a], Yi-Cheng Lin [b], Wei-Long Chen [b], Yu-Hsuan Chen [b], Yu-Xun Chen [a], Chieh-Ming Chen [a], Hung Wei Shiu [c], Lo-Yueh Chang [c], Chia-Hao Chen [c], Chih-Hsin Chen [b,*]

[a] Department of Physics, Tamkang University, New Taipei City 25137, Taiwan
[b] Department of Chemistry, Tamkang University, New Taipei City 25137, Taiwan
[c] National Synchrotron Radiation Research Center, Hsinchu 30076, Taiwan

ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form
9 November 2015
Accepted 15 November 2015
Available online 17 November 2015

Keywords:
Liquid crystal-based biosensor
Bovine serum albumin
Trypsin
Scanning photoelectron microscopy

ABSTRACT

We report a new mechanism for liquid crystal (LC)-based sensor system for trypsin detection. In this system, bovine serum albumin (BSA) was immobilized on gold grids as the enzymatic substrate. When the BSA-modified grid was filled with LC and immersed in the solution containing trypsin, the peptide bonds of BSA were hydrolyzed and peptide fragments were desorbed from the surface of gold grid, which disrupted the orientation of LC at the vicinity and resulted in a dark-to-bright transition of optical image of LCs. By using this mechanism, the limit of detection (LOD) of trypsin is 10 ng/mL, and it does not respond to thrombin and pepsin. Besides, the cleavage behavior on gold surfaces was directly visualized by the scanning photoelectron microscopy (SPEM), in particular for the chemical composition identification and element-resolved image. The loss of BSA fragments and the enhancement of Au photoelectron signal after trypsin cleavage were corresponding to the proposed mechanism of the LC-based sensor system. Because the signals reported by LC can be simply interpreted through the human naked-eye, it provides a simple method for fast-screening trypsin activity in aqueous solution.

1. Introduction

Trypsin is a digestive enzyme produced in the pancreas. It maintains the function of human digestive system by hydrolyzing the proteins into smaller peptide fragments, which is easier to transport into the small intestine and utilized in cellular metabolism. In addition, it is also responsible for other physiological functions such as immune response and blood coagulation. Loss of trypsin activity can lead to serious conditions such as cystic fibrosis (Noone et al., 2001). Therefore, the development of analytical system which can precisely detect trypsin with high sensitivity is critically needed. To detect trypsin, most of current approaches applied a peptide-immobilized surface as substrate and the specific cleavage events occurred on the surface can be reported by different signals such as fluorescence (Fan et al., 2012; Suzuki et al., 2008), electrochemistry (Abd-Rabboh et al., 2003; Dong et al., 2015) and surface-enhanced Raman scattering (Chen et al., 2013) with high sensitivity. However, these techniques require expensive instrumentation and trained technicians for data processing such that limit their applicability.

Alternatively, liquid crystal (LC)-based sensors have been considered as a cheaper and simpler approach for biological sensing applications. The key advantage of using LC-based system is the pre-labeling of the targets is not required, and the sensing read outs are colorful thus can be identified by naked-eye. By studying the interfacial phenomenon involving the orientation of LC, LC-based sensors have been used to detect various targets of interest such as DNA (Chen and Yang, 2010), proteins (Gupta et al., 1998), bacteria (Sivakumar et al., 2009), viruses (Han et al., 2014), glucose (Kim et al., 2013), organophosphates (Chen and Yang, 2013), bile acids (Bera and Fang, 2013), protons (Bi et al., 2009), and heavy metal ions (Yang et al., 2013). In previous studies, the principles to detect the enzyme at the LC/aqueous interface by using the LC-based sensors can be classified into two types. One of them applied enzymatic substrates which are pre-added in the aqueous phase. The reorientation of LC is caused by the hydrolytic products produced from the specific enzymatic reaction occurred in the aqueous phase. For example, Hu et al. developed the LC-based sensor to monitor urease activity in aqueous solution through the hydrolysis of urea by urease to produce ammonia (Hu and Jang, 2011). The presence of ammonia increases the pH of solution that can be reported by LC-based pH sensor as bright-to-dark transition of its optical signals. Besides, they also developed the LC-based sensor to monitor lipase activity in aqueous solution through the

* Corresponding author.
E-mail address: chc@mail.tku.edu.tw (C.-H. Chen).

http://dx.doi.org/10.1016/j.bios.2015.11.049
0956-5663/

hydrolysis of glyceryl trioleate to produce oleic acids (Hu and Jang, 2012). The presence of oleic acids simultaneously forms a self-assembled monolayer (SAM) at the LC/aqueous interface which blocks the LC from contacting the aqueous phase and leads to bright-to-dark transition of optical images of the LC. However, the results of this system are pH-dependent as it relies on the deprotonation of aliphatic acids to form amphiphilic molecules in order to align the LC at interface. The other type for LC-based enzyme sensor is designed by directly applying a layer of enzymatic substrate at the LC/aqueous interface to block the LC from contacting the aqueous phase. The reorientation of LC is derived from the enzymatic hydrolysis of substrates which allows the LC to contact with aqueous phase. For instance, Hussain et al. applied a monolayer of phospholipids at an LC/aqueous interface to monitor lipase activity (Hussain et al., 2014). In addition, Zhang et al. applied a poly-L-lysine-based polymeric membrane at an LC/aqueous interface to monitor trypsin activity (Zhang and Jang, 2013). In this type of the LC-based sensors, the optical identification is correlated to the ideal immobilization of the monolayer of substrate at the LC/aqueous interface. Because the precise characterization of such monolayer at the interface is challenging, the performance of this type of sensor is not optimal.

Bovine serum albumin (BSA) is a globular protein produced in cows. Because it is chemically stable and does not interfere with biological reactions, it is commonly used as the blocking agent for immunoassays or the stabilizing agent for enzyme storage. Recently, several studies demonstrated that BSA can also act as the enzyme substrate for trypsin detection. For example, Hu et al. applied BSA-stabilized gold nanoclusters as the fluorescence probe to detect trypsin. The addition of trypsin hydrolyzed BSA into amino acids or peptide fragments and therefore lowered the fluorescence intensity of gold nanoclusters, allowing the sensitive detection of trypsin at 0.01 μg/mL (Hu et al., 2012). On the other hand, Yazgan et al. reported that BSA can be used as the substrate to bind with Raman reporter molecules, creating the signal of surface-enhanced Raman scattering (SERS) on solid surface. The enzymatic hydrolysis on BSA lowered the intensity of SERS such that the activity of enzyme was quantified (Yazgan et al., 2010). As a substrate of enzyme, the structure, stability, and orientation of BSA immobilized on solid surfaces have been studied previously. To the best of our knowledge, there is no study focusing on characterizing the interface influenced by the hydrolysis of surface-immobilized BSA. Although an enhanced vibration signal in SERS technology is strongly affected by certain target metals, molecular bonding groups, as well as nanoparticle hybrid substrates (Yazgan et al., 2010), it is not an optimal platform to identify the effect of enzymatic hydrolysis on the chemical nature of complex BSA network immobilized on the surface.

In this study, the LC-based sensor system utilizing a BSA-modified gold grid for trypsin detection is reported for the first time. Previous studies have demonstrated that the orientation of LC at the LC/aqueous interface can be controlled by applying amphiphilic molecules such as surfactants (Brake and Abbott, 2002), polyelectrolytes (Lee et al., 2010) and lipids (Brake et al., 2003) at the interface. We proposed that after BSA is hydrolyzed by trypsin, peptide fragments will be released from the surface of gold grid to the vicinity of LC, leading to the reorientation of LCs as well as a differentiable change in the LC texture. To further investigate the change in the composition of surface-immobilized BSA before and after trypsin hydrolysis, X-ray photoelectron spectroscopy (XPS) was applied to characterize the element-sensitive surface density and chemical bonding of BSA under various conditions. For space-resolved information, we offered a novel imaginable approach with the surface and element sensitivity to characterize the trypsin activity directly by scanning photoelectron microscopy (SPEM) (Chuang et al., 2007).

2. Materials and methods

2.1. Materials

Glass slides were obtained from Fisher Scientific (U.S.A). *N,N*-Dimethyl-*n*-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), sodium cyanoborohydride ($NaBH_3CN$), 50% glutaraldehyde aqueous solution (GA), bovine serum albumin (BSA), fluorescein isothiocyanate isomer I (FITC), trypsin, pepsin and were purchased from Sigma Aldrich (U.S.A). Tris buffer was purchased from J.T. Baker (U.S.A). Liquid crystal 4-cyano-4′-pentylbiphenyl (5CB) was purchased from TCI (Taiwan). 4′-n-decyloxybiphenyl-4-carboxylic acid (DCA) was synthesized following reported procedures (Coco et al., 2000). Water was purified by using a Milli-Q system (Millipore).

2.2. Preparation of DMOAP-coated slides

To clean the surface, glass slides were immersed in a 5% Decon-90 solution (a commercially available detergent) for 2 h, sonicated in deionized water for 15 min, and rinsed thoroughly with deionized water twice. After this, the slides were dried under a stream of nitrogen. The cleaned glass slides were immersed in an aqueous solution containing 0.1% (v/v) DMOAP for 5 min, and then rinsed with copious amounts of deionized water. DMOAP-coated slides were dried under a stream of nitrogen and heated in a 100 °C vacuum oven for 15 min.

2.3. Immobilization of BSA on gold grid

TEM gold grids (75, 100, 200, and 400 mesh, Ted Pella, Inc., U.S.A) were cleaned in methanol, ethanol, and acetone (sonicated in each solvent for 15 min), and then heated overnight at 100 °C to evaporate residual solvent. To immobilize BSA on the surface, the clean grids were immersed in PBS buffer containing BSA (1 mg/mL) for 30 min then rinsed with deionized water. Next, the grids were immersed in an aqueous solution containing BSA (1 mg/mL), glutaraldehyde (5 wt%) and sodium cyanoborohydride (10 mM) under constant shaking for 16 h. After that, the grids were washed thoroughly with deionized water to remove non-specifically adsorbed BSA.

2.4. Preparation of LC samples

The DMOAP-coated glass slides were cut into squares (5 mm × 5 mm) for supporting LC. Then, a BSA-modified grid was placed on the slide, and approximately 0.3 μL of 5CB doped with 0.3 wt% of 4′-n-decyloxybiphenyl-4-carboxylic acid (DCA) was dispensed onto the grid. Excessive LC was removed by using a capillary tube. Finally, the grid containing the LC was immersed in 200 μL of Tris buffer (100 mM, pH = 8.4) containing different concentrations of trypsin. The effect of the pH value of solution on the LC images of the system was shown in Fig. S1. For the stability of the system, we set the pH value of solution at 8.4 because it can maintain the dark LC images for more than 12 h. The optical appearances of these samples were observed by using a polarizing optical microscope (Canon EOS D650, Japan) in the transmission mode. Each image was captured with a digital camera mounted on the microscope with an exposure time of 1/80 sec.

2.5. XPS measurements

To study the surface properties by XPS, the 1000 mesh gold grid (mesh size ~20 μm) was immersed into the 1 mg/mL of BSA and 100 ng/mL of trypsin solution sequentially, controlled by the combined CCD and XYZ positioning system. The BSA/gold sample