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Specific detection of avidin–biotin binding using liquid crystal droplets

Mashooq Khan, Soo-Young Park*

School of Applied Chemical Engineering, Department of Polymer Science and Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea

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ABSTRACT

Poly(acrylicacid-b-4-cynobiphenyl-4′-undecylacrylate) (PAA-b-LCP)-functionalized 4-cyano-4′-pentylbiphenyl (5CB) droplets were made by using microfluidic technique. The PAA chains on the 5CB droplets, were biotinylated, and used to specifically detect avidin–biotin binding at the 5CB/aqueous interface. The avidin–biotin binding was characterized by the configurational change (from radial to bipolar) of the 5CB droplets, as observed through a polarized optical microscope. The maximum biotinylation was obtained by injecting a >100 μg/mL biotin aqueous solution, which enabled a limit of detection of 0.5 μg/mL avidin. This droplet biosensor could specifically detect avidin against other proteins such as bovine serum albumin, lysozyme, hemoglobin, and chymotrypsinogen solutions. Avidin detection with $5CB_{PAA\text{-}biotin}$ droplets having high sensitivity, specificity, and stability demonstrates new applications of the functionalized liquid crystal droplets that can detect specific proteins or other analytes through a ligand/receptor model.

1. Introduction

Liquid crystal (LC) droplets are considered a promising platform for sensing applications. LC droplets exhibit a large surface-area-to-volume ratio, which facilitates the reorientation of the LCs at their interfaces. In addition, LC droplets possess several director configurations, which enable them to provide in-depth quantitative information of analytes [1]. LC droplets dispersed in an aqueous solution have recently emerged as simple probes to detect the adsorption and interaction of biological species such as proteins [1,2], lipids [3], endotoxin [4], glucose [5], and urea [6] at the LC/aqueous interface. It is known that the director configurations of the LC droplets, such as radial, preradial, axial, and bipolar, reflect the balance between the elasticity and the surface anchoring of the LCs inside the droplets [7]. The adsorption and interaction of biological species at the LC/aqueous interface may disrupt this balance, inducing a configurational transition of the LC inside the droplets, which can be observed through a polarized optical microscope (POM) [8,9].

Recently, the surface of the aqueous-dispersed LC droplets functionalized by the adsorption of polyelectrolytes (PEs) at the LC/aqueous interface has been utilized in nonspecific biosensors [2,10–14]. The dissociation of the electrolyte groups from PEs induces a charged state, which creates an electric field and changes the configuration of the LC droplet [6]. Several PEs were tested for nonspecific protein detection with LC droplets. For example, poly(acrylicacid-b-4-cyanobiphenyl-4′-oxyundecylacrylate) (PAA-b-LCP) functionalized 4-cyano-4′-pentylbiphenyl (5CB) (a nematic LC at room temperature) droplets made with microfluidics have been used as biosensors for nonspecific protein detection [13]. Another approach for protein detection was the functionalization of LC droplets with surfactants. For example, the pH- and temperature-responsive 5CB droplets functionalized with poly(N-isopropylacrylamide-b-4-cyanobiphenyl-4′-undecaylacrylate) (PNIPAM-b-LCP) and sodium dodecyl sulfate (SDS) have been used for protein detection between the low critical solution temperature and the isoelectric points of tested proteins [15]. However, the specific detection of analytes using 5CB droplets as biosensors through a receptor/ligand model is more highly desirable than nonspecific detection.

Detection of the ligand/receptor binding is the foundation for screening of a specific analyte. Current methods used to detect the ligand/receptor binding generally require an analytical lab apparatus or a bulk assay that involves species labeled with latex beads, enzymes, radioactive isotopes, or fluorophores [16]. The biotin–avidin binding is a well-known receptor/ligand model that is used to amplify and transduce receptor-mediated binding. Avidin is an egg white protein that binds the vitamin biotin with highly

* Corresponding author. Tel.: +82 539505630.
E-mail address: psy@knu.ac.kr (S.-Y. Park).

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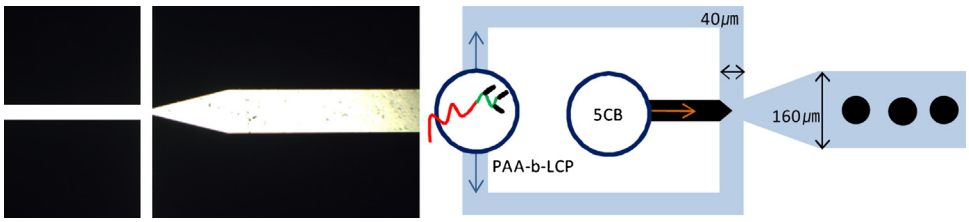

Fig. 1. An optical image (left) and a schematic (right) of the microfluidics channel with dimensions.

specific affinity. The non-covalent avidin–biotin binding provides a general bridge for many diverse applications with an affinity constant of 10^{15} L/mol [17,18], one of the highest affinity constants reported (~10^3 to 10^6 times greater than that for the interaction of other ligands with their specific antibodies) [18–21]. This affinity ensures that once the avidin–biotin complex is formed, it will not be disturbed by a change in pH, the presence of chaotropes, or assay protocols such as multiple washes. Due to the formation of this highly stable complex, the biotin–avidin interaction has become very useful in a wide variety of bioanalytical applications such as affinity, chromatography, and biosensors [17,21,22].

In this study, PAA-b-LCP-functionalized 5CB droplets were generated by a microfluidic technique to demonstrate the selectivity of the LC droplet biosensor by employing the specific receptor on the LC droplet. The PAA chains coated on the 5CB droplets were strongly bound to the LCP block and were biotinylated ($5CB_{PAA\text{-}biotin}$) with covalent coupling. The droplets were tested for the specific detection of avidin at the 5CB/aqueous interface. The specific avidin–biotin complex exhibited a radial-to-bipolar (R-B) orientation change of the 5CB droplets, as observed through a POM. The R-B orientation change of the $5CB_{PAA\text{-}biotin}$ droplets in response to avidin–biotin binding may establish a new platform for the specific detection of proteins and other analytes.

2. Materials and methods

2.1. Materials

5CB (TCI Japan), poly(dimethylsiloxane) (PDMS) kit (Sylgard 184; Dow Corning, USA)containing the prepolymer and a cross-linker, biotin hydrazide, avidin, rhodamine 6G, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide (NHS), bovine serum albumin (BSA), hemoglobin (Hb), chymotrypsinogen (ChTg), and lysozyme (Lyz) were bought from Sigma–Aldrich. Milli-Q water (resistivity higher than 18.2 MU cm) was used in all experiments. Micro-slide glasses (S9213, Matsunami, Japan, 76 mm × 52 mm × 1.3 mm) were cleaned using a hot piranha solution (H_2O_2 (35%): H_2SO_4 (98%) = 1:1 (v/v)) for 30 min, rinsed with water, and dried with nitrogen gas. CAUTION: Piranha solution is extremely corrosive and must be handled carefully. PAA-b-LCP was prepared using the same method reported previously [23]. The molecular weight was PAA(15k)-b-LCP(7k) with a M_w/M_n of 1.19; the number in parenthesis represents the number average molecular weight (M_n), which was calculated from the gel permeation chromatography (GPC) data of poly(tert-butyl acrylate) (PtBA)-b-LCP using 100% conversion.

2.2. Device fabrication

The flow-focusing devices were fabricated by the same method as reported [5]. Briefly, the PDMS was prepared by mixing the prepolymer and the cross-linker thoroughly at the supplier's recommended ratio of 10:1 (w/w). The PDMS was degassed for 40 min in a desiccator to remove the remaining air bubbles. The final mixture was poured on a silicon wafer mold and cured inside an oven at 65 °C for 4 h before removing from the silicon wafer. This patterned piece of PDMS was bonded to a pre-cleaned micro-slide glass using a 46-s oxygen plasma treatment (Femto Science Inc., Korea). The width of the inlet channels, width and length of the orifice, and width and height of the outlet channel were 110, 40, 40, 160, and 40 μm, respectively, and the depth throughout the channel was 100 μm. Fig. 1 shows an optical image and a schematic of the microfluidics channel with dimensions. The channel walls and chip assembly were made hydrophilic by an oxygen plasma treatment. The channel was filled with water until the chip was used.

2.3. $5CB_{PAA}$ droplet formation and its biotinylation

Formation of LC droplets was carried out using the same method previously reported by Khan et al. [24]. Briefly, the liquids were supplied to a microfluidic device via a flexible plastic tubing (Norton, USA, I.D. 0.51 mm, O.D. 1.52 mm) attached to precision syringes (SGE Analytical Science, Australia) operated using digitally controlled syringe pumps (KD Scientific, KDS 100 Series, USA). The flow rates through the microfluidic channels were controlled using two independent syringe pumps. The continuous and dispersed phases were an aqueous PAA-b-LCP (2 mg/mL) solution and 5CB, respectively. The continuous phase was pumped into the two side inlet channels, and the dispersed phase was delivered to the middle channel. Both phases met at the junction, and droplet formation took place when the fluids crossed the neck of the channel. The typical flow rates used for droplet formation were 0.01 and 0.2 mL/h for the dispersed and continuous phases, respectively. The $5CB_{PAA}$ droplets were acquired in a reservoir, and the PAA chains were activated with 0.4 M EDC·HCl and 0.1 M NHS for 1 h. The activated $5CB_{PAA}$ droplets were kept in a biotin hydrazide solution for 12 h at room temperature to obtain biotin-functionalized 5CB droplets ($5CB_{PAA\text{-}biotin}$ droplets). The tested concentrations of the biotin solution (C_bs) were 5, 7.5, 10, 50, 75, and 100 μg/mL.

2.4. Labeling of avidin

The labeling of avidin was carried out following a method reported by Khan et al. with a slight modification [6]. Briefly, avidin was dissolved in phosphate buffered saline (PBS) buffer (pH = 7) in a reaction vial to obtain a 100 μg/mL solution into which the chemical coupling agents, EDC·HCl and NHS, were added and kept for 1 h at 4 °C to activate the carboxyl group in avidin. Subsequently, 1 mg of rhodamine 6G was added and stirred for 12 h at room temperature. A saturated aqueous ammonium sulfate solution (0.5 g/mL) was added dropwise to the mixture. The labeled avidin ($avidin_{rhodamine\ 6G}$) was precipitated and centrifuged twice at 5000 rpm for 20 min. The supernatant was then removed by filtration, and the filtered $avidin_{rhoddamine\ 6G}$ powder was dried under vacuum.