Biosensors and Bioelectronics 87 (2017) 404–409

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A label-free strategy for measuring the affinity between monoclonal antibody and hapten using microdialysis sampling combined with chemiluminescent detection

Yali Zhou, Pingshi Wang, Jie Xiong, Huan Yue, Yong He, Hui Ouyang, Lin Wang, Zhifeng Fu *

Key Laboratory of Luminescence and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China

ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form
14 August 2016
Accepted 19 August 2016
Available online 20 August 2016

Keywords:
Chemiluminescence
Antibody affinity
Microdialysis
Hapten
Terbutaline
Monoclonal antibody

ABSTRACT

It is of great importance to measure antibody affinity in the course of screening monoclonal antibody (McAb) for immunotherapy, immunoassay and immunological purification. Herein, by using terbutaline mouse McAb as a model, a novel label-free strategy based on on-line microdialysis (MD) sampling combined with flow injection chemiluminescent detection was designed for measuring antibody affinity to hapten in a homogeneous system. After this McAb incubated with its hapten, the unbound hapten was sampled on-line by the MD probe and injected into the chemiluminescent detection system for quantification. The obtained concentrations of the unbound hapten were treated with Scatchard analysis and Klotz analysis to calculate the affinity constant. The MD probe showed a recovery of 26.2% for terbutaline under the chosen conditions. The affinity constants obtained using Scatchard analysis and Klotz analysis were $4.9 \times 10^6\ M^{-1}$ and $4.9 \times 10^6\ M^{-1}$, respectively, showing negligible difference. The obtained affinity constants indicated that the investigated McAb was an antibody with medium affinity. The designed strategy provided a simple, rapid and low-cost approach for direct measurement of antibody affinity. Furthermore, it avoided the decrease of affinity, which was encountered frequently in the conventional approaches based on probe labeling of McAb and protein conjugation of hapten.

1. Introduction

Monoclonal antibody (McAb) is being used widely in various fields such as immunoassay (Tsougeni et al., 2016; Wang et al., 2015; Zhu et al., 2016), immunotherapy (Chari et al., 2014; Ley et al., 2016; Sun et al., 2016) and immunological purification (Fan and Neubert, 2016; Todoroki et al., 2016; Xu et al., 2012) due to its ideal specificity and binding ability. In the course of preparation and screening of McAb, one of the most important work is to evaluate the affinity of McAbs secreted by different hybridoma cell lines (Almanzar et al., 2013; Schallert et al., 2002). It is well known that McAbs with different affinity to antigens/haptens can be utilized in different fields. For example, McAb with high affinity can be adopted in immunotherapy and immunoassay (Chari et al., 2014; Zhu et al., 2016), while McAb with medium affinity is usually chosen in affinity chromatography for immunological purification (Fan and Neubert, 2016; Seiler et al., 1985).

Most of the conventional methods used for measuring antibody affinity are heterogeneous assays based on probe labeling of McAb, which include competitive binding assay (Oyama et al., 2013), thiocyanate elution test (Almanzar et al., 2013) and non-competitive enzyme immunoassay (Beatty et al., 1987; Capiaumont et al., 2000). These methods usually employ enzyme as the signal tracer to label McAb, and then evaluate the antibody affinity using enzyme-linked immunosorbent assay. Obviously, the probe labeling process is time-consuming and labor-intensive (El-Said et al., 2016; Gao et al., 2013). Furthermore, the chemical reaction for probe labeling might result in decrease of antibody affinity due to the steric hindrance or the antibody deactivation (Chang et al., 2013).

In the recent years, some label-free approaches, such as surface plasmon resonance biosensor (Chang et al., 2013; Wang et al., 2011) and quartz crystal microbalance (Deng et al., 2016; Wang and Muthuswamy, 2008), are utilized to measure the affinity between McAb and antigen/hapten. The immunological binding reactions occurring on the sensors surface can change the refractive index or shift the frequency. Thus the signal responses are proportional to the amounts of immunological complex forming on the sensors. For these heterogeneous approaches, the antigens/

* Corresponding author.
E-mail address: fuzf@swu.edu.cn (Z. Fu).

http://dx.doi.org/10.1016/j.bios.2016.08.068
0956-5663/

haptens need to be immobilized onto the sensors surface before measuring the antibody affinity. However, haptens need to be conjugated with carrier proteins to prepare artificial antigens before they are used, since low molecular weight of haptens always results in low immobilization efficiency. To our best knowledge, the chemical structures of many haptens are not suitable for protein conjugation, thus it is always demanded to synthesis their structural analogs with active functional groups. Furthermore, the conjugation reaction sometimes occupies the antigenic determinants or increases the steric hindrance, thus lowers their affinity to McAbs.

Microdialysis (MD) has been exploited extensively as a sampling technique for in vivo analysis and continuous monitoring of small-molecular substances, while macromolecules such as proteins and cells can be excluded. The MD probe consists of inlet and outlet tubings joined together in a cylindrical semipermeable membrane with a defined molecular weight cutoff (MWCO). As the MD probe is perfused with buffer solution (perfusate), small-molecular substances in the external medium diffuse passively through the dialysis membrane into the probe lumen due to the concentration gradient, and then are collected as the exiting dialysate for detection (Gu et al., 2015; Hogerton and Bowser, 2013; Schmerberg and Li, 2013; Zhou et al., 2015). With the aid of a flow-through system, MD is easy to be automated and coupled on-line with some analytical techniques such as capillary electrophoresis (Hogerton and Bowser, 2013), liquid chromatography (Gu et al., 2015; Zhou et al., 2015) and mass spectrometry (Zhou et al., 2015). Therefore, MD has been utilized successfully in many fields, including neurochemistry (Cudjoe et al., 2013; Lin et al., 2014), pharmacokinetics (Carreño et al., 2016; Korf et al., 2010), cell biology (Liu et al., 2014; Olivero et al., 2012) and metabolomics (Vuckovic et al., 2011; Wibom et al., 2010).

Herein, a novel label-free strategy based on MD on-line sampling combined with flow injection (FI)-chemiluminescence (CL) real-time detection has been developed to measure the affinity between monoclonal antibody and hapten, by using terbutaline mouse McAb as a model. In a homogeneous reaction system of McAb and hapten, the unbound hapten was sampled on-line using a MD probe and detected by a FI-CL system. Then the affinity between McAb and hapten was evaluated by Scatchard analysis and Klotz analysis. As described in Table S1 of Supporting information, this proof-of-principle work offers a simple, rapid, low-cost and reliable approach for direct measurement of antibody affinity because neither probe labeling of McAb nor protein conjugation of hapten was required.

2. Experimental

2.1. Reagents and materials

Terbutaline mouse McAb and terbutaline-bovine serum albumin conjugate were obtained from Shandong Lvdu Bio-Sciences & Technology Co., Ltd. (China). Horseradish peroxidase-labeled goat anti-mouse IgG and 3,3′,5,5′-tetramethylbenzidine (TMB) colorimetry kit were provided by Bioss Biotechnology Co., Ltd. (China). Terbutaline sulfate standard was purchased from National Institute for Food and Drug Control (China). Luminol was obtained from Sigma-Aldrich (USA). All other reagents were of analytical grade. Ultrapure water treated using an ELGA PURELAB Classic system (France) was used in the whole investigation. High-affinity polystyrene 96-well microplate was purchased from Greiner Bio-One Biochemical Co., Ltd. (Germany). Vascular MD probe (MD-2310) constructed by a 10-mm polyacrylonitrile hollow fiber membrane (AN69®) with a MWCO of 30 kDa was provided by Bioanalytical Systems Inc. (USA). According to the information provided by the manufacturer, the pore size of the membrane was around 3 nm. The photograph of the whole probe and the scanning electron micrograph showing the tubing structure are shown in Fig. S1.

The MD perfusate and the antibody dilution buffer all were 0.010 M phosphate buffer saline at pH 7.4. The coating buffer for microplate was 0.10 M carbonate buffer saline at pH 9.0. The washing buffer for microplate was 0.010 M phosphate buffer saline at pH 7.4 containing 0.05% Tween-20. SuperBlock® T20 applied as the blocking buffer was provided by Thermo Fisher Scientific Inc. (USA). A 1.0×10^{-5} M luminol solution prepared in 10 mM NaOH was used as CL reagent 1, while a 1.0×10^{-4} M $K_3[Fe(CN)_6]$ solution dissolved in water was used as CL reagent 2.

2.2. FI-CL detection system

FI-CL detection was conducted on an IFFM-E FI-CL analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd., China), which was composed of two peristaltic pumps, an injection valve, a quartz flow cell and a CL detector. The CL reagents and carrier stream were delivered using the peristaltic pumps. Sample loading and injection were conducted on the injection valve. Sample solution was delivered using a syringe pump system (Bioanalytical Systems Inc., USA) that comprised a 1000-μL gastight syringe (MDN-0100), a syringe pump drive (MD-1001) and a hive syringe pump controller (MD-1020). All flow components of the FI-CL system were connected using polytetrafluoroethylene tubings with inner diameter of 0.25 mm. The emitted CL signals were measured by a photomultiplier tube operated at −700 V.

As the injection valve was kept at the loading position, the carrier stream (H_2O), CL reagent 1 and CL reagent 2 were delivered continuously at a rate of 2.2 mL/min until a stable baseline was recorded. Then 30 μL of sample was injected into the carrier stream by switching the valve to the injection position. After 7 s, the sample solution was merged with the CL reagents in the flow cell to produce a CL signal. The CL intensity was used to quantify the amount of terbutaline.

2.3. MD sampling system

The MD sampling system was composed of a MD probe and a syringe pump system described above. The inlet tubing (yellow) and the outlet tubing (green) of the probe were connected to the syringe and the sampling loop of the injection valve, respectively.

To sample terbutaline on-line, the MD probe was immersed into a 1.5-mL Eppendorf tube containing 1.0 mL of sample solution, in which the solution was maintained at 37 °C and constant stirring. The MD perfusate was delivered at a speed of 4.0 μL/min through the dialysis membrane of the probe to sample terbutaline by a concentration gradient. At last the effused dialysate was delivered into the injection valve to conduct FI-CL detection.

2.4. Measurement of antibody affinity using MD sampling combined with FI-CL detection

Terbutaline solutions at the concentrations ranging from 100 to 900 nM were mixed with its mouse McAb solution at a fixed concentration of 700 nM. Then 1.0 mL of the mixture was incubated at 37 °C for 30 min under constant stirring, to reach an immunoreaction equilibrium. After that the MD probe was immersed into the immunoreaction solution to sample unbound terbutaline. In this process 30 μL of dialysate was delivered into the injection valve to detect the concentration of unbound terbutaline in the dialysate. The detected concentration was calibrated with MD probe recovery to obtain the concentration of unbound terbutaline in the immunoreaction system. At last the affinity of terbutaline mouse McAb was evaluated by the