Journal of Chromatography A, 1495 (2017) 31–45

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Affinity chromatographic methodologies based on immobilized voltage dependent anion channel isoform 1 and application in protein-ligand interaction analysis and bioactive compounds screening from traditional medicine

Qian Li, Pan Qiao, Xiu Chen, Jing Wang, Liujiao Bian*, Xiaohui Zheng*

Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Northwest University, Xi'an 710069, China

ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 9 March 2017
Accepted 13 March 2017
Available online 18 March 2017

Keywords:
Membrane protein
Voltage dependent anion channel isoform 1
Protein-ligand interaction
Bioactive compounds screening
Rheum officinale Baill.

ABSTRACT

Voltage dependent anion channel isoform 1 (VDAC-1) serves as an attractive target of anti-cancer drugs by mediating the entry and exit of metabolites between cytoplasm and mitochondria. This work reports on the preparation of a VDAC-1-based bioaffinity chromatographic stationary phase by linking the protein on lecithin modified microspheres. An assay of chromatographic methods including frontal analysis, zonal elution, injection dependent analysis and nonlinear chromatography were utilized to investigate the bindings of ATP, NADH and NADPH to VDAC-1. Electrostatic interactions were found to be main forces during these bindings. The calculated association constants of the three ligands to VDAC-1 showed good agreements between diverse chromatographic methods. Validated application of the stationary phase was performed by screening anti-cancer compounds of *Rheum officinale Baill.* using high performance affinity chromatography coupled with electrospray ionization-quadrupole time of flight mass spectrometry. Chrysophanol, emodin, rhein, aloe-emodin and catechin were identified as the bioactive components of the herb. These compounds targeted VDAC-1 through Thr207 and the N-terminal region of the protein. Taken together, the current stationary phase was possible to become a promising tool for protein-ligand interaction analysis and anti-cancer drug screening from complex matrices.

1. Introduction

Human proteomics reveals that over 30 percent of current proteins are membrane associated [1]. These proteins represent 50–60% targets of marketed drugs owing to their core components in many essential cellular processes, for instances, signal transduction, molecular transportation, transmembrane potential controlling, energy generation and transduction and chemical reactions catalysis [2].

Membrane protein based ligand binding study and new leads identification are of ever-increasing interest for drug discovery. Plenty of modern strategies have been extensively used to achieve these purposes, for instance, high throughput screening (HTS) [3] and fragment-based drug discovery (FBDD) [4]. The two methods of detection often rely on submicromolar interactions and the specific binding of chemical modulators of pharmaceutical targets, and consequently face the challenges for detection of weak bindings. Furthermore, the HTS and FBDD based techniques in combination with nuclear magnetic resonance, X-ray crystallography and surface plasmon resonance are expensive and time-consuming to set up and run.

Bioaffinity chromatography is one of the most commonly used techniques to achieve these purposes [5,6]. It combines the rapid analysis speed of high performance affinity chromatography (HPLC) and the high specificity of a ligand binding to its target protein. This method needs immobilization of target protein onto solid matrix while minimizing the loss of its activity [7]. In this context, many papers have been published on the application of affinity chromatography using immobilized membrane receptors [8–11].

As a classic integral membrane protein, voltage dependent anion channel isoform 1 (VDAC-1) serves as an authentic target of anti-cancer drugs that regulates cell death through mitochondria-mediated apoptosis [12,13]. Using VDAC-1 as a probe, the present work was designed to attach the protein onto lecithin modified

* Corresponding authors.
E-mail addresses: bianliujiao@sohu.com (L. Bian), zhengxh@nwu.edu.cn (X. Zheng).

http://dx.doi.org/10.1016/j.chroma.2017.03.023
0021-9673/

macroporous silica gel to construct a new affinity stationary phase. Feasibility of the stationary phase in real samples was evaluated by analysis the interaction between VDAC-1 and three ligands, plus the screening of anti-cancer biocompounds from *Rheum officinale Baill.*

2. Experimental methods

2.1. Materials and instruments

L-α-Phosphatidylcholine (from egg yolk, lecithin), ATP, NADH and NADPH were acquired from Sigma-Aldrich (St. Louis, MO, USA). Chrysophanol (110796), emodin (110756), rhein (110757), aloe-emodin (110795) and catechin (110877) were from the National Institutes for Food and Drug Control (Beijing, China). Methanesulfonamide was purchased from Tokyo Chemical Industry (TCI) Shanghai (Shanghai, China). Potassium ferricyanide, potassium carbonate, potassium osmate(VI) dihydrate and hydroquinidine 1,4-phthalazinediyl diether ($(DHQ)_2$-PHAL) were obtained from Aladdin Industrial Corporation (Shanghai, China). Nickel-chelated sepharose 6 fast flow pre-packed columns (HisTrap FF, 5 mL) were obtained from GE Healthcare Life Sciences (Uppsala, Sweden). Macroporous silica gel (SPS 300-7, 300 Å pore size, 7.0 μm particle diameter) was purchased from Fuji Silysia Chemical Company (Tokyo, Japan).

Protein purification experiments were performed using an ÄKTA10 system with UNICORN 5.20 software (GE Healthcare). The packing machine was supplied by Dalian Yilite Analytic Instruments Company (ZZXT-A, Dalian, China). FT-IR spectroscopy was performed using Nicolet 5700 Fourier Infrared Spectrometer (Thermo Nicolet Corporation, Madison, WI, USA). The chromatographic system was an Agilent 1100 HPLC apparatus which includes a manual injector fitted with a 20-μL sample loop, a binary solvent manager, a column oven and a diode array detector.

2.2. Preparation of VDAC-1 liposome

Histidine tagged VDAC-1 was prepared from inclusion bodies of a recombinant genetic engineering strain *E.coli* BL21(DE3)pE28b/VDAC-1 according to our previous work [9]. Briefly, the cDNA of human VDAC-1 (GenBank accession number: NM_003374) was cloned into plasmid pET28b through restriction sites of *Nco* I and *Xho* I. Luria-Bertani (LB) medium with kanamycin resistance was utilized to incubate the strain expressing the result gene until an OD_{600} value of 0.4–0.6 was reached. VDAC-1 inclusion body was induced by the addition of 1.0 mmol/L IPTG at 30 °C for extra 6 h incubation. The inclusion body was collected by treating the cells using ultrasonic method in ice-water bath. The target protein in the inclusion was renatured and separated by an on-column method using Ni-sepharose resin. The purity of VDAC-1 was determined as 95% by SDS-PAGE in which the protein presented a single band with the molecular weight of ~35 kDa. The protein was incorporated into liposome according to our previous methods [14], making a protein-liposome ratio of 1:60.

2.3. Immobilization of VDAC-1 and column packing

As illustrated in Scheme 1, the purified VDAC-1 was covalently immobilized onto silica gel surface using phospholipid monolayer that was formed by lecithin.

Step I: Sharpless asymmetric dihydroxylation of lecithin. The reaction was performed to oxidize the two olefins in lecithin to vicinal diols with reference to previous reports [15]. In detail, potassium ferricyanide (9.9 g, 30 mmol), potassium carbonate (4.1 g, 30 mmol), $(DHQ)_2$-PHAL (78 mg, 0.1 mmol), potassium osmate(VI) dihydrate (7.4 mg, 0.2 mmol) and methanesulfonamide (1.9 g, 20 mmol) were added into a 500-mL round bottom flask. Solution of *tert*-butyl alcohol (*t*-BuOH)-water (v:v = 1:1, 200 mL) was utilized to dissolve these reagents by ultrasonic method. An aliquot of 100 mL *t*-BuOH-water (v:v = 1:1) solution containing 0.01 mol/L lecithin was added into the result solution followed by continuous stirring for 24 h at 4 °C. An excess sodium sulfite (15 g, 0.12 mol) was employed for another 1 h reaction that enables accomplishment of the whole reaction. The dihydroxylized lecithin obtained through evaporating method and was identified by infrared spectroscopy.

Step II: Activation of macroporous silica gel. Macroporous silica gel (1.0 g) was aminated by γ-aminopropyl triethoxysilane (3 mL) according to our previous method. The amino density on the gel surface was estimated by HPLC coupled with Schiff base method. The aminopropyl-bonded silica gel (1.0 g) was then mixed with 1.5 g *N,N′*-carbonyldiimidazole (CDI) in 30 mL dry acetonitrile. The reaction remained at room temperature for 2.0 h and the activated macroporous silica gel was obtained.

Step III: Immobilization of VDAC-1. The activated gel (1.0 g) was utilized to initiate next reaction with dihydroxylized lecithin (138 mg, 167 μmol) with duration of 12.0 h. The resultant gel was filtered and rinsed using 50 mL of chloroform, methanol, distilled water and 10 mM Tris-HCl, by sequential. Subsequent immobilization of VDAC-1 was performed by suspending lecithin coated gel (1.0 g) in 10 mL of 2.0% cholate detergent solution containing VDAC-1 proteoliposome-cholate (total protein: 2.0 mg). The reaction was maintained for 2 h under gently stirring condition. The immobilized VDAC-1 was collected by filtration and totally rinsed with Tris-HCl buffer.

The column was prepared by packing the immobilized VDAC-1 into stainless steel column with a size of 50 × 4.6 mm at 4.0×10^7 Pa using slurry assay. The control columns containing lecithin-modified macroporous silica gel or the stationary phase blocked by glycine in prior to the immobilization of VDAC-1 were packed by the same method.

2.4. Protein-ligand interaction experiments

The chromatographic experiments were performed on an Agilent 1100 HPLC apparatus. The mobile phases were 20 mM phosphate-buffer (PB, pH 7.4) for NADH, 100 mM PB (pH 7.4) for ATP and NADPH. The flow rates for the 3 ligands were 0.3, 0.5 and 0.8 mL/min, respectively. The wavelengths were 340 nm for NADPH and 260 nm for ATP and NADH. The temperatures were 10, 20, 30, 37 and 45 °C.

2.4.1. Frontal analysis

In frontal chromatographic studies, mobile phases containing diverse concentrations of ATP (0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0 and 15.0 μmol/L), NADH (0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0, 15.0 and 30.0 μmol/L) and NADPH (0.25, 0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0 μmol/L) were continuously pumped through the column until a series of breakthrough curves were formed. The breakthrough times were derived by differentiation the raw data of breakthrough curves through the software of Origin 8.0.

2.4.2. Zonal elution

Self-competitive studies were performed with the use of identical analyte and competitor. Phosphate buffer saline (PBS, 20 mM) in the presence of ATP (0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0 and 15.0 μmol/L), NADH (0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0, 15.0 and 30.0 μmol/L) and NADPH (0.25, 0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0 μmol/L) served as mobile phases. The injection volumes were 5 μL of 1.2 mmol/L ATP, 2.5 mmol/L NADH and 1.0 mmol/L NADPH. Each injection was estimated in triplicate to record retention times.