Journal of Chromatography B, 1021 (2016) 188–196

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Entrapment of alpha$_1$-acid glycoprotein in high-performance affinity columns for drug-protein binding studies

Cong Bi, Abby Jackson, John Vargas-Badilla, Rong Li, Giana Rada, Jeanethe Anguizola, Erika Pfaunmiller, David S. Hage *

Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304, USA

ARTICLE INFO

Article history:
Received 23 September 2015
Accepted 14 November 2015
Available online 27 November 2015

Keywords:
Alpha$_1$-acid glycoprotein
High-performance affinity chromatography
Drug-protein binding
Entrapment
Human serum albumin

ABSTRACT

A slurry-based method was developed for the entrapment of alpha$_1$-acid glycoprotein (AGP) for use in high-performance affinity chromatography to study drug interactions with this serum protein. Entrapment was achieved based on the physical containment of AGP in hydrazide-activated porous silica supports and by using mildly oxidized glycogen as a capping agent. The conditions needed for this process were examined and optimized. When this type of AGP column was used in binding studies, the association equilibrium constant (K_a) measured by frontal analysis at pH 7.4 and 37 °C for carbamazepine with AGP was found to be 1.0 (±0.5) × 10^5 M^{-1}, which agreed with a previously reported value of 1.0 (±0.1) × 10^5 M^{-1}. Binding studies based on zonal elution were conducted for several other drugs with such columns, giving equilibrium constants that were consistent with literature values. An entrapped AGP column was also used in combination with a column containing entrapped HSA in a screening assay format to compare the binding of various drugs to AGP and HSA. These results also agreed with previous data that have been reported in literature for both of these proteins. The same entrapment method could be extended to other proteins and to the investigation of additional types of drug-protein interactions. Potential applications include the rapid quantitative analysis of biological interactions and the high-throughput screening of drug candidates for their binding to a given protein.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The interactions between drugs and serum proteins are known to affect such properties as the transport, excretion and metabolism of drugs in the body [1,2]. One serum protein that is involved in many of these interactions is alpha$_1$-acid glycoprotein (AGP) [3–6]. AGP is a major protein constituent in plasma, with a typical concentration in humans of 0.5–1.0 mg/mL [3]. Human AGP contains a single polypeptide chain of 183 amino acids and has an isoelectric point of 2.8–3.8 [4]. This glycoprotein has a carbohydrate content of 45% and an average molar mass of 41 kDa [4,6]. As a transport protein, AGP is known to bind to many basic and neutral drugs in the blood stream [4,5].

Various techniques have been used to examine the binding of drugs with serum proteins. These techniques have included equilibrium dialysis, ultrafiltration, capillary electrophoresis, and various spectroscopic techniques, including surface plasmon resonance [2,7–11]. High-performance affinity chromatography (HPAC) has also been used in such work [1,12,13]. This method makes use of a biologically-related binding agent (e.g., a serum protein) as a stationary phase in an HPLC column, which can then be utilized to examine binding by this agent to drugs or other targets that are applied to the column [12]. This method has been found to have many advantages as a tool for drug-protein binding studies, including its speed, good precision, ease of automation, compatibility with a variety of detectors, and ability to work with small amounts of a drug or binding agent (e.g., through the use of affinity microcolumns) [1,12,13].

When HPAC is used to study the interactions between a drug and an immobilized protein, one important consideration is the choice of the immobilization method that is used to place the protein in the column [14–16]. Many past reports using HPAC for drug binding studies have employed covalent immobilization (e.g., the Schiff base method) [16–23], as has been used with trans-

* Corresponding author. Fax: +1 402 472 9402.
E-mail address: dhage1@unl.edu (D.S. Hage).

http://dx.doi.org/10.1016/j.jchromb.2015.11.021
1570-0232/© 2015 Elsevier B.V. All rights reserved.

port proteins such as human serum albumin (HSA) and various lipoproteins [16–18,20–23]. AGP has been immobilized for use in chiral separations by employing amine-based crosslinking or coupling through thiol groups [16,24,25]. AGP has also been immobilized for drug binding studies by having the carbohydrate groups on this glycoprotein undergo mild oxidation, followed by coupling of the resulting aldehyde groups with a hydrazide-activated support [16,19,26–28]. Many of these approaches give an immobilized protein that has good correlation with the behavior seen for the same protein in a soluble form; however, this is not always the case [16,17,19,20,23,25]. For instance, covalent immobilization can produce improper orientation, steric hindrance or multi-site attachment for an immobilized binding agent if proper coupling conditions are not selected. These effects, in turn, can lead to a change in actual or apparent activity for an immobilized protein [14,16,19,25,29,30].

Entrapment is an alternative approach for immobilization that can avoid many of these problems. This is a non-covalent method based on the physical containment of a binding agent, as may occur within a porous support or in a cross-linked polymer network [14,30–34]. Sol–gel encapsulation and hydrogel entrapment have been utilized to immobilize proteins and other agents for applications such as protein-based photonic devices, biosensors, chromatographic columns, and enzyme reactors [31–34]. Other entrapment methods have been developed for use with proteoliposomes or red blood cells through the placement of these agents within gels made from dextran or derivatized acrylamide monomers [35,36]. However, many of the materials that are employed in these methods have pressure or flow rate restrictions that make them difficult to use as HPLC supports [30,35,36]. In addition, slow mass transfer effects can be created as a result of such entrapment processes [34].

An alternative entrapment approach has recently been described that can be used with standard HPLC supports, as has been demonstrated in work with human serum albumin (HSA) and modified forms of this protein [14,30]. This method (see Fig. 1) involves the entrapment of a soluble protein on a hydrazide-activated support by using an oxidized form of glycogen as a capping agent [30]. One useful feature of this method is that it can be used directly with HPLC-grade supports such as porous silica. This feature allows this approach to overcome the pressure and flow restrictions that are seen with many sol–gels, hydrogels and low-performance chromatographic supports such as dextran or acrylamide-based gels. The fact that the protein or entrapped agent is held within the pores of the support or at its surface also allows this method to avoid the slow mass transfer properties that are often encountered with other entrapment methods [34]. This method has been shown in preliminary work to be suitable for use with binding agents that span a relatively wide range of sizes (i.e., 5.8–150 kDa) [30]. In addition, this approach has been found to provide good agreement between the binding properties of an entrapped protein like HSA and the soluble form of this protein [14,30].

This report will examine the optimization and extension of this entrapment technique to the immobilization of AGP and the use of such a support in small HPAC columns for the study of drug-protein binding. The conditions needed for the entrapment of AGP will be examined and optimized. The binding behavior of the resulting AGP columns will be evaluated by using various model drugs and both frontal analysis and zonal elution experiments. The combined use of entrapped AGP and HSA columns to screen drug interactions with these serum proteins will also be considered. The results should make it possible to determine how this entrapment technique can be used in future work with other proteins or in applications such as the high-throughput screening of drugs or the rapid characterization of biological interactions.

2. Materials and methods

2.1. Reagents

The AGP (from pooled human serum, 99% pure), periodic acid reagent (H_5IO_6), glycogen (bovine liver), HSA (Cohn fraction V, 99% globulin free, 99% fatty acid free), amitriptyline, carbamazepine, chloramphenicol, chlorpromazine, disopyramide, imipramine, lidocaine, nortriptyline, *S*-propranolol, and quinidine were from Sigma–Aldrich (St. Louis, MO, USA). The Nucleosil silica (7 μm particle diameter, 100 or 300 Å pore size) was obtained from Macherey Nagel (Düren, Germany). Reagents for the micro bicinchoninic (BCA) protein assay were from Pierce (Rockford, IL, USA). All other chemicals were of the purist grades available. All solutions were prepared using water from a Barnstead NANOpure system (Dubuque, IA, USA) or a Milli-Q Advantage A10 purification system (Millipore, Billerica, MA, USA) and were filtered through 0.20 μm GNWP nylon membranes from Millipore.

2.2. Apparatus

The chromatographic system consisted of a series 200 micro pump and absorbance detector from PerkinElmer (Shelton, CT, USA); or a Jasco PU-980i intelligent HPLC isocratic pump (Tokyo, Japan), a Rheodyne Advantage PF ten-port valve (Cotati, CA), and a Jasco UV-975 UV/Vis detector. Injections were carried out by using a six-port Rheodyne Lab Pro valve (Cotati, CA, USA) and a 5 or 20 μL sample loop. The temperature of the columns and mobile phases were controlled by using a Millipore Waters TCM temperature control module, or a PolyScience circulating VWR circulating water bath (Buffalo Grove, IL, USA) and a water jacket from Alltech (Deerfield, IL, USA). The chromatographic data were collected and processed using in-house programs written in LabView 5.1 (National Instruments, Austin, TX, USA). Centrifugation was conducted by using an Eppendorf 5702 RH centrifuge (Hamburg, Germany). The columns were packed using an HPLC column slurry packer from ChromTech (Apple Valley, MN, USA).

2.3. Column preparation

Nucleosil was first converted to diol-bonded silica, followed by conversion of this support into an aldehyde-activated form; this material was then treated with oxalic dihydrazide to form hydrazide-activated silica, as described previously [15]. One factor that was varied was the amount of oxalic dihydrazide that was added during the last step of this reaction. A mole ratio of 5:1 was initially used for the oxalic dihydrazide versus the aldehyde groups on the support (i.e., where the aldehyde content was approximately equal to the original number of diol groups) [15], as utilized in prior reports with this entrapment approach and in the work conducted in Section 3.6 [14,30]. This ratio was later varied from 5:1 to 0.5:1, with a ratio of 1:1 being used in the remainder of this report.

A 17 mg portion of glycogen was added to 4.0 mL of a pH 5.0, 20 mM sodium acetate buffer that also contained 15 mM sodium chloride and 135 mg of the periodic acid reagent [30]. After mixing this solution for 18 h while shaking at room temperature, the oxidized glycogen was purified by using either ultrafiltration or size exclusion chromatography. In the ultrafiltration method, the oxidized glycogen solution was placed in an Amicon Ultra centrifugal filtration device (30 kDa cutoff, Millipore) and washed three times with water, followed by another three times using pH 5.0, 0.10 M potassium phosphate buffer. Each centrifugation step was carried out for 15 min at 20 °C and 4400 rpm. The oxidized glycogen fraction that remained in the filtration device was removed and diluted to 4 mL by adding pH 5.0, 0.10 M potassium phosphate buffer. The method based on size exclusion chromatography was carried out

Download English Version:

https://daneshyari.com/en/article/1211950

Download Persian Version:

https://daneshyari.com/article/1211950

Daneshyari.com