Journal of Pharmaceutical and Biomedical Analysis 127 (2016) 9–17

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

The automatic use of capillary isoelectric focusing with whole column imaging detection for carbamazepine binding to human serum albumin

Małgorzata Maciążek-Jurczyk [1], Janusz Pawliszyn *

Department of Chemistry, University of Waterloo, Ontario N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form 1 December 2015
Accepted 10 January 2016
Available online 13 January 2016

Keywords:
cIEF-WCID
Human serum albumin
Fatty acids
Carbamazepine
Isoelectric point

ABSTRACT

The binding of the anticonvulsant drug carbamazepine (CBZ) to human serum albumin, both without (dHSA) and in the presence of fatty acids (HSA) was studied in real time by capillary isoelectric focusing with whole column imaging detection (cIEF-WCID). Reaction mixtures at different CBZ:HSA and CBZ:dHSA molar ratios (0:1/25:1) were prepared in phosphate buffer saline (PBS) solution at a physiological pH (7.4), and incubated for 0–72 h at 37 °C in a water bath. Application of the cIEF-WCID method allowed for observations on the impact of increasing CBZ:serum albumin molar ratios on isoelectric point (pI) shifts, as well as changes in peak area and absorbance, which serve as evidence of structural alterations occurring in the protein in the presence of CBZ. The obtained cIEF-WCID results indicated that the dynamic process of complex formation is not dependent on incubation time. The presented work allowed for recognition of different types of interactions, as well as for the calculation of association constants that demonstrate the stability of the complex. This study was also designed to examine the possible impact of fatty acids (FAs) on protein stability and drug delivery in blood.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The degree to which a drug binds to protein is considered to be a major determinant of drug distribution. Binding plays an important role on drug dynamics, since only the free (unbound) fraction of a given drug can interact with receptors, and consequently induce therapeutic response. The most abundant blood protein, serum albumin (SA), is produced by the liver. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones present in blood, and plays a major role in stabilizing extracellular fluid volume by contributing to the oncotic pressure (known also as colloid osmotic pressure) of plasma [1]. The primary sequence of human serum albumin (HSA) shows that the protein consists of a single polypeptide of 585 amino acids residues containing 17 pairs of disulfide bridges, one free thiol (Cys-34), and a single tryptophan (Trp-214). The protein is composed of three structurally similar, though asymmetrical domains, I–III, each of which is divided into two subdomains, A and B. Albumin has two main drug-binding sites, characterized as Sudlow's site I and Sudlow's site II [2], which bind drugs selectively. Site I, also known as the warfarin binding site, is formed by a pocket in subdomain IIA of HSA [1]. Warfarin is the selective probe drug for this site [3]. Site II is located in subdomain IIIA, and is known as the benzodiazepine binding site. Ibuprofen and diazepam are selective drug probes for site II [1]. It is noteworthy to mention that many different functional groups presented on protein structure participate in drug–protein complex stabilizing processes; these are conducted through van der Waal's force and electrostatic interactions, as well as hydrogen bonds. These interactions can lead to either specific or non-specific interactions between the protein and drug [4]. As the primary ligands for protein are fatty acids (FAs), competition in binding with drugs may occur as a result of direct displacement in the binding site or conformational changes in protein structure induced by the molecules of ligands bound in different subdomains [5]. Each HSA molecule can carry up to seven fatty acid (FA) molecules. Seven common FA binding sites have been identified for medium and long-chain fatty acids (FAs) [6], as well as monosaturated and polyunsaturated FAs [7]. The location of the binding sites of the ligands, as well as the mechanisms of competition between various ligands, can yield valuable information concerning drug design [5]. FA sites 2, 4, and 5 bind FA with high affinity, while sites 1, 3, 6, and 7 exhibit low affin-

* Corresponding author. Fax: +1 519 746 0435.
E-mail address: janusz@uwaterloo.ca (J. Pawliszyn).
[1] On-leave: Department of Physical Pharmacy, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland.

http://dx.doi.org/10.1016/j.jpba.2016.01.023
0731-7085/© 2016 Elsevier B.V. All rights reserved.

ity for FA [8]. The relative arrangements of the three domains are largely changed when fatty acid chains bind to protein. For protein binding, competition between ligands is more important for ligands that have very high binding fractions, as the increase of free fraction can be doubled. While as competitors, FAs do not seem to affect the binding of drugs to a large extent; they can simultaneously occupy several binding sites in the albumin structure, making the drug–protein complex unstable. As such, an evaluation of binding parameters for the drug–albumin complex in all existing binding sites needs to include information about changes in protein affinity toward the drug in the presence of fatty acids [5].

Information on ligand–protein-binding can be provided by the use of various techniques, such as crystallography, fluorescence spectroscopy, nuclear magnetic resonance spectroscopy (NMR), circular dichroism (CD), Fourier transformation infrared spectroscopy (FTIR), ultrafiltration, UV–vis spectrophotometry, calorimetry, mass spectrometry (MS), affinity chromatography (AC) and affinity capillary electrophoresis (ACE) [9–15], and recently, capillary isoelectric focusing with whole column dynamic imaging detection (cIEF-WCID) [16,17]. Capillary isoelectric focusing (cIEF), first reported by Hjerten and Zhu [18], combines high-resolution for biochemical separation gel isoelectric focusing (IEF) with capillary electrophoresis (CE). The cIEF method is used for separation of amphoteric compounds according to different isoelectric points (pIs) [19–22], and can be used for analyses of proteins, biological complexes, and peptides. The isoelectric focusing process can be imaged by a charge-coupled device (CCD) camera equipped in the whole column with the use of UV absorbance or laser-induced fluorescence (LIF). When coupled with dynamic imaging detection, cIEF is referred to as cIEF-whole column imaging detection (cIEF-WCID). The cIEF-WICD coupling is a new, fast, automated, and simple technique that provides high resolution, speed, and high-sensitivity separation of amphoteric biomolecules [17]. The application of WCID provides direct monitoring of diffusion processes in real time. The use of dynamic imaging detection allows for potential applications of this technique in biotechnology, such as monitoring of protein diffusion in real time and estimations of molar masses (g/mol) from diffusion coefficients (D), which is a second primary attribute, besides pI, for protein characterization and analysis. For highly abundant proteins, shifts in their isoelectric point (pI) can impact the function of organs that interact with them. Purtell et al. [23] examined the effects of changes in pI on renal handling of albumin molecules. The authors showed that an increase in pI caused an increase in heterologous albumin secretion, and increased nephron permeability. Indeed, there is a wide body of evidence in the literature that reports on the disease relevance of pI [24–28]. Considering these implications, pI changes can be treated as markers for structural changes in protein.

Today, the discovery of blood-based biomarkers for diagnosis and prognosis of diseases is of extreme importance in the scientific and clinical fields. To this extent, the currently presented work was undertaken with aims to analyze the stability of protein based on its isoelectric point (pI) value by employing the separation technique cIEF-WCID. The presented results are followed by a discussion of the drug interaction capability of the human serum albumin in the absence (dHSA) and presence of fatty acids (HSA), as well as the influence of incubation time on formed complexes. While the interactions between drugs and proteins based on electrophoregram profiles and isoelectric points have been studied and published in the literature [16,29]. for the first time, and to the best of this author's knowledge, the study of the drug–albumin complex is presented based on the binding isotherms and association constants obtained by the cIEF-WCID technique. Carbamazepine (CBZ), an antiepileptic drug, was used in this study as a model drug. To date, the binding of CBZ in the tertiary structure of human serum albumin has been analyzed through the use of volumetric and fluorescence techniques, while alterations in the secondary structure of SA in the presence of CBZ have been shown with the use of circular dichroism and FTIR spectroscopy [30]. In previous studies conducted by our team, in order to analyze the binding of CBZ to human serum albumin and investigate the free fraction of carbamazepine, different techniques were used and compared, such as solid phase microextraction (SPME) with liquid chromatography–ultraviolet detection (LC–UV), as well as the spectroscopic techniques spectrofluorescence (SFM) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR) [31]. The currently presented work is a continuation of the carbamazepine binding study based on another electrophoretic technique, cIEF-WCID, demonstrating its innovative application.

2. Experimental

2.1. Chemicals

Methylcellulose (MC), Carrier ampholyte high resolution (CA, Pharmalytes 3.0–10.0), Tris(hydroxymethyl) aminomethane (Tris, 99.8%), hydrochloric acid (HCl, 37%), carbamazepine (CBZ), albumin from human serum lyophilized powder (HSA), and albumin from human serum lyophilized, fatty acid-free (dHSA), were purchased from Sigma–Aldrich, CA. The pI markers (4.14, 6.14, 8.18) were a gift from Dr. T. Huang (Advanced Electrophoresis Solutions, Ltd., CA). *O*-Phosphoric acid 85% (H_3PO_4) and methanol (CH_3OH) were provided by Fisher Scientific (CA). Sodium hydroxide (NaOH) was purchased from Caledon Laboratories Ltd., CA. Water was purified with an ultrapure water system (Thermo Scientific Barnstead NanoPure system). Acrodisc 25 mm syringe filters were obtained from Pall Life Sciences, USA. 1.5 mL microcentrifuge tubes were obtained from Fisher Scientific (USA). Separation was performed on cartridges with silica capillary tubes (ProteinSimple) with an effective length of 50 mm, 100 μm inside diameter (ID), and 200 μm outside diameter (OD). The multi-use centrifuge device Br4i was provided by the Jouan company. All the chemicals employed in this study were of analytical reagent grade and used without purification.

2.2. Solutions and sample preparation

A 0.01 M Tris(hydroxymethyl)aminomethane–hydrochloric acid (Tris–HCl) solution at pH adjusted to 7.4 as necessary to mimic physiological conditions was prepared by dissolving 0.6267 g of Tris in 42.5 mL of 0.1 M HCl, with the appropriate amount of purified water added to obtain 500 mL total volume. A stock solution of carbamazepine (CBZ, 1.37×10^{-1} M) was prepared in methanol. Working solutions of CBZ at concentrations of 5.5×10^{-6} M–1.37×10^{-3} M were prepared by dilution of stock solution in methanol. The final concentration of methanol was 1% (v/v). Stock solutions of human serum albumin, both in the absence (dHSA) and presence of fatty acids (HSA) (2.2×10^{-4} M) were prepared in Tris–HCl pH 7.4. The CBZ:dHSA and CBZ:HSA molar ratios used for analyses were 0:1–25:1. All reaction mixtures were prepared in triplicate.

2.3. Capillary isoelectric focusing (cIEF)—whole column imaging detection (WCID) system

The cIEF-WCID experiments were recorded on an *i*CE280 analyzer (Convergent Bioscence, Toronto, Canada) and performed at 280 nm. The used separation column was a fluorocarbon internally coated fused-silica capillary supported by a cartridge (ProteinSimple, Toronto, Canada). The whole column ultraviolet (UV) absorption image was captured by a camera, which includes an imaging lens and a charge-coupled device (CCD) sensor. The *i*CE280

Download English Version:

https://daneshyari.com/en/article/1220908

Download Persian Version:

https://daneshyari.com/article/1220908

Daneshyari.com