Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 189 (2018) 625–633

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

The effect of glycation on bovine serum albumin conformation and ligand binding properties with regard to gliclazide

Ewa Żurawska-Płaksej [a], Anna Rorbach-Dolata [b], Katarzyna Wiglusz [c], Agnieszka Piwowar [b,*]

[a] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211A, 50-556 Wroclaw, Poland
[b] Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211, 50-556 Wroclaw, Poland
[c] Department of Analytical Chemistry, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211 A, 50-566 Wrocław, Poland

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 11 August 2017
Accepted 31 August 2017
Available online 6 September 2017

Keywords:
Glycated bovine serum albumin
Gliclazide
Drug binding
Circular dichroism
Fluorescence

ABSTRACT

Albumin, the major serum protein, plays a variety of functions, including binding and transporting endogenous and exogenous ligands. Its molecular structure is sensitive to different environmental modifiers, among which glucose is one of the most significant. *In vivo* albumin glycation occurs under physiological conditions, but it is increased in diabetes. Since bovine serum albumin (BSA) may serve as a model protein in *in vitro* experiments, we aimed to investigate the impact of glucose-mediated BSA glycation on the binding capacity towards gliclazide, as well as the ability of this drug to prevent glycation of the BSA molecule. To reflect normo- and hyperglycemia, the conditions of the glycation process were established. Structural changes of albumin after interaction with gliclazide (0–14 μM) were determined using fluorescence quenching and circular dichroism spectroscopy. Moreover, thermodynamic parameters as well as energy transfer parameters were determined. Calculated Stern-Volmer quenching constants, as well as binding constants for the BSA-gliclazide complex, were lower for the glycated form of albumin than for the unmodified protein. The largest, over 2-fold, decrease in values of binding parameters was observed for the sample with 30 mM of glucose, reflecting the poorly controlled diabetic state, which indicates that the degree of glycation had a critical influence on binding with gliclazide. In contrast to significant changes in the tertiary structure of BSA upon binding with gliclazide, only slight changes in the secondary structure were observed, which was reflected by about a 3% decrease of the α-helix content of glycated BSA (regardless of glucose concentration) in comparison to unmodified BSA. The presence of gliclazide during glycation did not affect its progress. The results of this study indicate that glycation significantly changed the binding ability of BSA towards gliclazide and the scale of these changes depended on glucose concentration. It may have a direct impact on the free drug fraction and its pharmacokinetic behavior, including the risk of hypoglycemic episodes or unexpected interactions with other ligands. The use of BSA in examining binding effects upon glycation seems to be good model for preliminary research and may be used to identify a potential drug response in a diabetic state.

1. Introduction

Serum albumin is the most abundant protein in the blood of all vertebrates. It plays many important functions in the organism, serving among others as the main binding and transport protein for endogenous and exogenous ligands [1]. The tertiary structure of albumin is composed of three largely helical domains, which are highly conserved across species. For example, bovine serum albumin (BSA) shows high homology with human serum albumin (HSA), which provides a cost-efficient research strategy [2,3]. The molecular mechanisms of drug binding to albumin are extremely important in the pharmacological aspect, because of determining free drug concentration and its availability for further pharmacokinetic processes. Upon drug binding, conformational changes of the albumin molecule, for example in α-helical content or fluorescence properties of tryptophan (Trp) and tyrosine (Tyr) residues, are observed; these are used to evaluate the interaction between the protein and ligand [4–6]. It is also known that some pathological conditions, such as hyperglycemia, may also lead to structural changes of the albumin molecule, affecting its biological role [7,8]. Increased glucose concentration is the most characteristic symptom of diabetes, a widespread metabolic disease, caused by insulin deficiency or insulin resistance, which is now one of the most important health problems in the world [9]. As a reducing sugar, glucose leads to non-enzymatic glycation of many proteins, including plasma proteins, especially albumin. It is a multistage process, which begins with the formation of the relatively unstable N-substituted glycosylamine (called Schiff base), followed by the reasonably stable aminoketose (called an Amadori

* Corresponding author.
E-mail addresses: ewa.zurawska-plaksej@umed.wroc.pl (E. Żurawska-Płaksej), katarzyna.wiglusz@umed.wroc.pl (K. Wiglusz), agnieszka.piwowar@umed.wroc.pl (A. Piwowar).

http://dx.doi.org/10.1016/j.saa.2017.08.071
1386-1425/

product), which may undergo further rearrangements (such as oxidation, dehydration, cross-linking), yielding pathogenic advanced glycation end products (AGEs) [8]. Consequences of glucose attachment to albumin lead to conformational changes of the native structure of albumin and thereby to loss of its biological function (including binding properties), and also induce signaling cascades transduced by the receptor for AGEs (RAGE) [10,11]. It has been reported that non-enzymatic glycation is intensified in patients with diabetes and may include up to 30% of the total pool of albumin, affecting its physico-chemical properties [12,13].

Gliclazide (GLICL), a second-generation sulphonylurea, is an oral hypoglycemic agent, commonly used in the treatment of non-insulin dependent diabetes (drug structure is presented in Fig. 1). It acts by stimulating β cells of the pancreas to release insulin, and recently the antiglycation effect of this drug on *in vitro* AGE formation was demonstrated [14]. A single oral dose of 40 to 120 mg of gliclazide results in a maximum plasma concentration of 2.2 to 8.0 μg/mL within 2 to 8 h. The protein binding affinity of gliclazide is from 85 to 97%, and this ligand is reported to bind mainly to site II of the HSA molecule, located in subdomain IIIA, with involvement of the aromatic ring of ^{411}Tyr [15–17]. However, Matsuda et al. [18] demonstrated that it also binds to site I (located in subdomain IIA). Due to the high degree of plasma protein binding, gliclazide has a low volume of distribution (13 to 24 L), and thus even small changes in the structure of HSA are capable of producing marked changes in GLICL pharmacokinetics. The overall pharmacokinetics of gliclazide have been thoroughly examined for healthy and diabetic subjects, but there is only one study that has provided some information about the influence of glycation on gliclazide-HSA interactions by employment of high-performance affinity chromatography [18].

To our knowledge, interaction of GLICL with differently glycated BSA has not been investigated so far by fluorescence quenching or circular dichroism (CD) spectroscopy, which are well-established tools to evaluate structural changes in protein molecules and enable calculation of binding parameters. The aim of the present study was to perform a spectroscopic evaluation of the effect of glycation of BSA structure and its implication on the binding properties towards gliclazide in conditions simulating physiological and diabetic conditions. Additionally, we aimed to verify the purported antiglycative properties of gliclazide.

2. Materials and Methods

2.1. BSA Glycation

Bovine serum albumin (Sigma-Aldrich, ≥96%) was used to prepare nonglycated (native) and differently glycated albumin solutions. Firstly, a 1.2 mM stock solution of BSA in 10 mM phosphate buffered saline (PBS), pH 7.4, with addition of 0.1% sodium azide (Sigma-Aldrich) as a preservative, was prepared. Simultaneously, glucose (Sigma-Aldrich, ≥ 99.5%) was dissolved in PBS to obtain 20 and 60 mM stock solutions. Then, the BSA solution was divided into 3 parts, treated with PBS only or 20 or 60 mM glucose solution (v/v 1:1) and incubated at 37 °C for 21 days. The final concentration of BSA was 0.6 mM in each sample and that of glucose was 0, 10 and 30 mM, equivalent to glucose-free (control sample), physiological (gly-10BSA) and diabetic (gly-30BSA) conditions. Next, all samples were dialyzed overnight at 4 °C against PBS (Bionovo, cut-off 14 kDa) with 3 buffer changes. After measuring protein concentration using absorbance of A1% = 6.8 at 280 nm, samples were diluted to a concentration of 4 μM to enable spectroscopic measurements. Next, 0.1 mM stock solution of gliclazide was prepared in PBS by initial dissolving in a small amount of methanol (5% v/v). Gliclazide (Sigma-Aldrich, ≥98%) was added to the albumin solutions in increasing concentrations (in molar ratios of albumin:gliclazide from 1:0 to 1:7) and the mixtures were divided into two parts and incubated at two different temperatures, 27 and 37 °C, for 15 min. Final protein concentration in each sample was 2 μM.

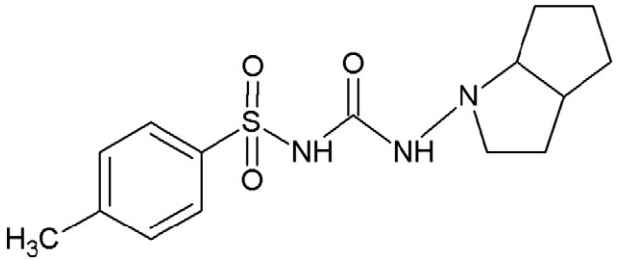

Fig. 1. Structure of gliclazide.

2.2. Antiglycative Properties of GLICL

The above scheme of glycated BSA preparation was repeated with addition of gliclazide (final concentration: 10, 50 and 100 mM) to gly-30BSA solution before 21 days of incubation, followed by exactly the same procedures until obtaining dialyzed samples, which were diluted to a final concentration of 2 μM BSA.

2.3. Spectroscopic Measurements

Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer equipped with a 1 cm quartz cell and excitation wavelengths of 280 nm and 295 nm (taking into consideration the contribution of fluorescence from tyrosine and tryptophan residues, respectively), with the excitation and emission slit widths set at 5 nm. The scanning speed was 200 nm/min. Each spectrum was background corrected by subtracting a spectrum of the phosphate buffer as a blank sample. Moreover, the effectiveness of the glycation process was controlled at an excitation wavelength of 370 nm, characteristic for protein glycation products [11].

Circular dichroism (CD) measurements were recorded on a JASCO J-1500 spectropolarimeter (205–250 nm) with a scanning speed of 200 nm/min and cell length path of 0.1 cm. The final plot was taken as an average of three accumulated plots. Baseline was corrected using phosphate buffer, pH = 7.4.

The UV–vis absorption spectra were recorded on a Perkin Elmer Lambda 20 spectrophotometer against phosphate buffer, pH = 7.4. The measurements were performed in the range 220–450 nm in quartz cuvettes (1 cm × 1 cm × 4 cm).

Warfarin (Sigma-Aldrich, 98%), the specific marker of Sudlow's site I, was used to identify the specific binding sites of GLICL on BSA.

2.4. Data Analysis

2.4.1. Fluorescence Quenching

To analyze the changes in tertiary structure of BSA, gly-10BSA and gly-30BSA after binding with gliclazide, fluorescence studies were conducted. The quantitative analysis of the quenching effect allows also for the assessment of the affinity of GLICL towards non-glycated and glycated BSA. Fluorescence quenching data were determined according to the Stern-Volmer equation [19]:

$$\frac{F_0}{F} = 1 + k_q\tau_0 = 1 + K_{SV}\,Q \qquad (1)$$

where F and F_0 are the fluorescence intensity with and without the quencher (gliclazide), respectively, k_q is the quenching rate constant, τ_0 is the average lifetime of a biomolecule without the quencher ($\tau_0 = 10^{-8}$ s), K_{SV} is the Stern-Volmer quenching constant ($k_q = K_{SV} / \tau_0$) and Q is the concentration of quencher.

Then, the binding parameters were measured for an excitation wavelength of 280 nm (at two different temperatures) and 295 nm