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Effects of non-enzymatic glycation in human serum albumin. Spectroscopic analysis

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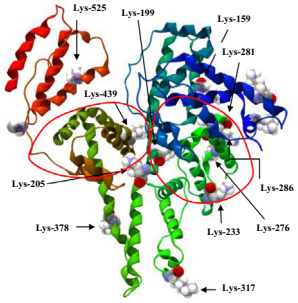
GRAPHICAL ABSTRACT



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ABSTRACT

Human serum albumin (HSA), transporting protein, is exposed during its life to numerous factors that cause its functions become impaired. One of the basic factors – glycation of HSA – occurs in diabetes and may affect HSA-drug binding. Accumulation of advanced glycation end-products (AGEs) leads to diseases e.g. diabetic and non-diabetic cardiovascular diseases, Alzheimer disease, renal disfunction and in normal aging.

The aim of the present work was to estimate how non-enzymatic glycation of human serum albumin altered its tertiary structure using fluorescence technique. We compared glycated human serum albumin by glucose ($gHSA_{GLC}$) with HSA glycated by fructose ($gHSA_{FRC}$). We focused on presenting the differences between $gHSA_{FRC}$ and nonglycated (HSA) albumin used acrylamide (Ac), potassium iodide (KI) and 2-(p -toluidino)naphthalene-6-sulfonic acid (TNS). Changes of the microenvironment around the tryptophan residue (Trp-214) of non-glycated and glycated proteins was investigated by the red-edge excitation shift method. Effect of glycation on ligand binding was examined by the binding of phenylbutazone (PHB) and ketoprofen (KP), which a primary high affinity binding site in serum albumin is subdomain IIA and IIIA, respectively.

At an excitation and an emission wavelength of λ_{ex} 335 nm and λ_{em} 420 nm, respectively the increase of fluorescence intensity and the blue-shift of maximum fluorescence was observed. It indicates that the glycation products decreases the polarity microenvironment around the fluorophores. Analysis of red-edge excitation shift method showed that the red-shift for $gHSA_{FRC}$ is higher than for HSA. Non-enzymatic glycation also caused, that the Trp residue of $gHSA_{FRC}$ becomes less accessible for the negatively charged quencher (I^-), K_{SV} value is smaller for $gHSA_{FRC}$ than for HSA. TNS fluorescent measurement demonstrated the decrease of hydrophobicity in the glycated albumin. K_{SV} constants for gHSA-PHB systems are higher than for the unmodified serum albumin, while K_{SV} values for gHSA-KP

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systems are only slightly lower than that obtained for HSA-KP. The affinity of PHB to the glycated HSA is stronger than to the non-glycated in the first class binding sites within subdomain IIA, in the vicinity of Trp-214. Ketoprofen bound to unmodified human serum albumin stronger than for glycated albumin and one class of binding sites is observed (Scatchard linear plots).

Introduction

Tertiary structure of HSA is composed of three homologous, predominantly helical domains: I, II and III, each divided into two subdomains A and B [1,2]. According to the Sudlow's nomenclature, two principal regions of ligand binding sites in the albumin structure are located in hydrophobic cavities in subdomains IIA (Sudlow site I) and IIIA (Sudlow site II) [3,4]. Sudlows site I, also known as the warfarin–azapropazone site, binds anti-inflammatory drugs such as azapropazone, phenylbutazone, and salicylate. Sudlows site II, or the indole benzodiazepine site, binds aromatic carboxylic acids and profens such as ibuprofen and ketoprofen. Thus, the binding of particular drug molecules to a serum albumin may change considerably binding abilities of the HSA with other molecules. On the other hand, the impact of pathological conditions, such as hyperglycemia can significantly alter the binding of the drugs. There are several reports suggesting that the glycation of HSA that occurs during diabetes may affect its drug-binding properties *in vivo* [5–7]. In the circulation, the HSA becomes non-enzymatically glycated by reducing sugars, and the reference range of a healthy person vary between 1% and 10% [8,9], and this proportion may increase from 2- to 3-fold in the case of diabetes mellitus [10]. As a result, glycated albumin has been proposed as a useful marker for short-term control (2–4 weeks) of diabetic patients. The level of glycated albumin might also be of some value as the indicator of the degree of hyperglycemia in diabetics [11]. The first effect of high reducing sugars concentration in biological fluids and tissues is an important multistep chemical process known as non-enzymatic glycation "*Maillard reaction*". The initial stage of glycation is a spontaneous condensation reaction between reducing sugars and a free amine group such as the ε-amino group of lysine, arginine or N-terminal amino group of protein. In the case of glucose, the reaction initially leads to the formation of acid-labile Schiff bases, which undergo rearrangement to early glycation Amadori-adducts such as fructosamine. If the right environment is provided, these relatively stable Amadori products can undergo further modifications by oxidation and polymerization to form a class of heterogeneous compounds collectively referred to as advanced glycation endproducts (AGEs) [12]. In the case of fructose, the rearrangement of the Schiff base is termed the Heyns rearrangement. Heyns compounds have been detected in liver extracts and in human ocular lens proteins [13]. The extent of modification that occurs on a given protein depends on the amount of time the protein is incubated with a reducing sugar [14], the type of reducing sugar that is present [15], and the environment (i.e. location in the body) in which the Maillard reaction is occurring [16].

AGE-related complications in the body can be classified into two different areas. In one case, the interaction of AGEs with the receptor protein for AGEs (RAGE) is responsible for a wide range of inflammatory responses which eventually lead to tissue damage [17,18]. The second case involves direct modification of proteins by AGEs, leading to a loss of protein function [19] or tissue damage that results from protein crosslinking [20,21]. The non-enzymatic glycation process has been suggested to occur both *in vitro* and *in vivo* and has been demonstrated to play a key role in the long-term complications of diabetes [22], and this alters the binding of physiological and pharmacological ligands [9].

The aim of the present work was to estimate how non-enzymatic glycation of human serum albumin altered its structure using fluorescence methods. We compared glycated human serum albumin by glucose ($gHSA_{GLC}$) with HSA glycated in the presence of fructose ($gHSA_{FRC}$). After parts of our work we focused on presenting the differences only between $gHSA_{FRC}$ and nonglycated (HSA) albumin used acrylamide (Ac), potassium iodide (KI) and 2-(*p*-toluidino)naphthalene-6-sulfonic acid (TNS). Motions of the microenvironment and organization of the tryptophan residue (Trp-214) of non-glycated and glycated proteins was investigated by the red-edge excitation shift method [23]. Influence glycation process on albumins binding phenylbutazone (PHB) and ketoprofen (KP), which primarily bind to subdomain II A (site I) and IIIA (site II) of human serum albumin (HSA) and are the most popular nonsteroidal anti-inflammatory drugs (NSAiDs), was investigated.

Materials and methods

Reagents

Crystallized and lyophilized human serum albumin (HSA), fraction V and ketoprofen (KP) ((*RS*)-2-(3benzoylphenyl)propanoic acid), 2-(*p*-toluidino)naphthalene-6-sulfonic acid (TNS), acrylamide (Ac) were purchased from MP Biomedicals (France). Phenylbutazone (PHB) (4-butyl-1,2-diphenyl-3,5 pyrazolidione), sodium azide (NaN_3) were provided by Sigma–Aldrich Chemical (Germany). D(+)-glucose (GLC), D(−)-fructose (FRC), Tris (hydroxymethyl)aminomethane pure p.a., Hydrochloric acid 0.1 mol L^{-1} (HCl), sodium thiosulfate anhydrous pure were obtained from POCH S.A. (Poland) and potassium iodide (KI) was purchased from Chempur (Poland). All chemicals were of the highest analytical quality. The stock solution of KP, PHB, TNS and other substances were prepared by dissolving in minimal amounts of methanol and with the use of Tris–HCl buffer (pH 7.4), respectively.

In vitro glycation of HSA

Glycated proteins ($gHSA_{GLC}$ and $gHSA_{FRC}$) were prepared *in vitro* by incubation of human serum albumin (HSA) in the presence of D(+)-glucose (GLC) and D(−)-fructose (FRC), respectively. Initial solutions HSA was dissolved in 0.05 mol L^{-1} Tris–HCl buffer containing 0.015 mol L^{-1} sodium azide (NaN_3) to yield a stock solution of 2×10^{-5} mol L^{-1}. This solution was subsequently diluted with concentrated solutions of glucose (0.1 mol L^{-1}) made in Tris–HCl buffer to get a final concentration of 1×10^{-5} mol L^{-1} HSA and 0.05 mol L^{-1} glucose. The reaction mixtures were sterilized through the membrane filter with pores of 0.20 μm size (30 mm in diameter, non-pyrogenic) into sterile glass tube capped and incubated at 310 K for 27 days. Under identical conditions solution of HSA in the presence of D(−)-fructose was prepared. Control sample of HSA (1×10^{-5} mol L^{-1}) was made and treated similarly, but without the addition of reducing sugars. After the incubation $gHSA_{GLC}$, $gHSA_{FRC}$ and control HSA were extensively dialyzed against distilled water. To perform *in vitro* glycation of the HSA samples, all glassware and spatulas that came in contact with the glycated albumins solution had been previously sterilized by auto-