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Interaction of glutathione with bovine serum albumin: Spectroscopy and molecular docking

Ali Jahanban-Esfahlan [a,b,*], Vahid Panahi-Azar [c], Sanaz Sajedi [c]

[a] Biotechnology Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Drug Applied Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

This study aims to investigate the interaction between glutathione and bovine serum albumin (BSA) using ultraviolet–visible (UV–vis) absorption, fluorescence spectroscopies under simulated physiological conditions (pH 7.4) and molecular docking methods. The results of fluorescence spectroscopy indicated that the fluorescence intensity of BSA was decreased considerably upon the addition of glutathione through a static quenching mechanism. The fluorescence quenching obtained was related to the formation of BSA–glutathione complex. The values of K_{SV}, K_a and K_b for the glutathione and BSA interaction were in the order of 10^5. The thermodynamic parameters including enthalpy change (ΔH), entropy change (ΔS) and also Gibb's free energy (ΔG) were determined using Van't Hoff equation. These values showed that hydrogen bonding and van der Waals forces were the main interactions in the binding of glutathione to BSA and the stabilization of the complex. Also, the interaction of glutathione and BSA was spontaneous. The effects of glutathione on the BSA conformation were determined using UV–vis spectroscopy. Moreover, glutathione was docked in BSA using ArgusLab as a molecular docking program. It was recognized that glutathione binds within the sub-domain IIA pocket in domain II of BSA.

1. Introduction

As a tripeptide, glutathione (D-γ-glutamyl-L-cysteinylglycine) is composed of three amino acids of glycine, cysteine and glutamic acid (Fig. 1). This hydrophilic antioxidant shows two characteristic features in its structure: a sulfhydryl group and a glutamyl linkage (Wang, Guan, & Yang, 2012). Glutathione is an endogenous antioxidant present in mammalians cells. In the living cells, glutathione is mainly present in two forms: one is a reduced form (GSH) and the other one is in oxidized form as glutathione disulfide (GSSG) (Noh, Chandra, Moon, & Shim, 2012). Recently, it is in considerable demand as an effective therapeutic drug, due to its multiple biological functions in different tissues and, also, its involvement in malnutrition and numerous diseases (Chen, He, Liu, & Cha, 2006). It has been shown that the senescence process and several human diseases, such as immune, neurodegenerative, cardiovascular and inflammatory diseases, is associated with the reduction in the level of GSH (Koo, Lee, Kim, & Lee, 2011). More than 90% of glutathione in healthy living cells is GSH. During oxidative stress, GSH can be easily converted to GSSG. In such case, two GSH molecules form a molecule of GSSG via the formation of double-sulfur bond (Chen et al., 2006). Then, by the action of glutathione reductase enzyme, the GSSG is reverted to GSH (Pastore, Federici, Bertini, & Piemonte, 2003). Therefore, the main and important physiological function of GSH is cellular defense by scavenging free radicals and toxins (Niu et al., 2012). The reduced form of glutathione is also an essential cofactor in different cellular functions, including apoptosis, proliferation, differentiation and metabolism (Ballatori, Krance, Marchan, & Hammond, 2009).

It is well-known that the binding properties of a molecule to proteins in the serum broadly affect its biological activity. Serum albumin is the most abundant protein in the blood stream (~60%) and acts as a carrier and depot protein for various substances (Xu et al., 2012). Bovine serum albumin (BSA), a widely considered protein, is one of the most important transporters for a wide spectrum of endogenous and exogenous molecules in the blood and thus, is extensively employed in the protein binding investigations as a model protein (Jahanban-Esfahlan, Panahi-Azar, & Sajedi, 2015; Kratochwil, Huber, Müller, Kansy, & Gerber,

* Corresponding author at: Biotechnology Research Centre and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail addresses: jahanbana@tbzmed.ac.ir, a.jahanban@gmail.com (A. Jahanban-Esfahlan).

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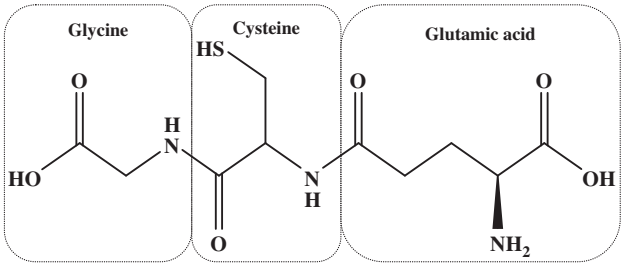

Fig. 1. The chemical structure of glutathione.

2002; Sharma, Choudhary, & Kishore, 2012). It is a globular heart-shaped macromolecule with a molecular weight of 66.4 kDa and is composed of about 583 amino acid residues. BSA includes three homologous domains (I, II, and III), which are divided by 17 disulfide bonds into nine loops (L1–L9) and each domain is composed of two subdomains (IA and IB, IIA and IIB, IIIA and IIIB). X-ray crystallographic studies have shown that the structure of this serum albumin is principally α-helix, and the remaining polypeptide occurs in turns and extended or flexible regions between subdomains with no β-sheet (Carter & Ho, 1994; Curry, Brick, & Franks, 1999; He & Carter, 1992; Majorek et al., 2012). The binding sites of albumin for various exogenous or endogenous molecules can be placed in subdomains IIA and IIIA, known as Sudlow's sites I and II, respectively, and thus, the main binding sites are situated in these domains (Tayeh, Rungassamy, & Albani, 2009). The existence of hydrophobic binding pockets on serum albumin enables it to increase the solubility of the hydrophobic substances in the plasma and control their delivery to the cells of target tissues in the body (Khan, Hossain, & Kumar, 2013). Consequently, the absorption, distribution, metabolism, and excretion properties of various molecules could be considerably influenced as a result of their interaction with serum proteins. BSA has two tryptophan residues, namely as Trp134 and Trp213, that possess intrinsic fluorescence (Samari, Hemmateenejad, Shamsipur, Rashidi, & Samouei, 2012). Trp134 is placed on the surface of the molecule, and Trp213 is situated within a hydrophobic binding pocket of the protein (Sułkowska, 2002). There have been a number of research reports about the interaction between glutathione and serum albumins. In an earlier study, Libenson and Jena (1963) considered the interaction of human plasma albumin and reduced glutathione. In another investigation, Yang, Zhou, Zhu, and Chen (2013) synthesized glutathione modified CdTe quantum dots (CdTe@GSH QDs) in an aqueous solution and studied the binding of the CdTe@GSH QDs to human serum albumin (HSA) using fluorescence spectroscopy. Recently, Li and Wang (2015) studied the binding of glutathione and melatonin to HSA using isothermal titration calorimetry (ITC) in combination with UV–vis absorption, Fourier transform infrared (FT-IR), and circular dichroism (CD) spectroscopies. However, up to now, the protein binding of glutathione using spectroscopic techniques, particularly fluorescence spectroscopy and molecular docking, has not been reported. Nevertheless, to the best of our knowledge, perfect and complete basic information for clarifying the binding mechanisms of glutathione to plasma proteins remain unclear. Information about the binding mechanism between glutathione and serum albumin is very significant to know the pharmacodynamics and pharmacokinetics properties of glutathione. The present study examines the thermodynamic properties of the binding of glutathione to BSA and the consequent conformational changes have been monitored using UV–vis absorption spectroscopy. Spectroscopic observations are then combined with molecular docking results to understand those mechanisms underlying the interactions.

2. Materials and methods

2.1. Materials

BSA fraction V and glutathione were purchased from Sigma–Aldrich Chemical Company (USA) and used as supplied. All other reagents and solvents were all of analytical reagent grade and were used as purchased without further purification. Double distilled water was used throughout the experiments.

2.2. Preparation of stock solutions

The appropriate amount of BSA protein was dissolved in 10 mM phosphate buffer (pH 7.4). A stock solution of 10 mM glutathione was directly prepared by dissolving certain amount of its powder in phosphate buffer at pH 7.4 and then appropriately diluted to prepare working solutions for fluorescence and UV–vis experiments. Prepared solutions of glutathione were immediately used because of its oxidation under light and air.

2.3. Fluorescence study

All fluorescent spectra measurements were taken on a Jasco FP-750 fluorescence spectrophotometer (Kyoto, Japan) quipped with a xenon lamp source and 1 cm quartz cell. Temperature was controlled at three different temperatures (17, 27 and 37 °C) using a temperature controller instrument with a water jacket cell holder and stirrer. The excitation wavelength was set at 290 nm and the fluorescence intensity was measured at 349 nm. The excitation and emission slit widths were set at 5 nm. The scan speed was 1200 nm/min. The fluorescence measurements were obtained at pH 7.4 using a fixed concentration of BSA (5 μM) in the presence of different concentrations of glutathione (0, 2.5, 5, 10, 20, 40, and 80 μM).

2.4. UV–vis absorption experiment

The UV–vis absorption spectrum of BSA in the presence and absence of glutathione was obtained in the range of 200–350 nm at room temperature using T70 UV/VIS spectrophotometer (PG Instrument Ltd., UK). The concentration of BSA and glutathione was 40 μM.

2.5. Molecular docking

Molecular docking study was performed in order to find out the binding sites on BSA and the binding energy of protein–ligand complex. The structure of glutathione as ligand was generated in ArgusLab followed by energy minimization. The crystal structure of BSA with PDB ID 3VO3 was obtained from Protein Data Bank (http://www.rcsb.org). All the ligands and water molecules were removed, and then hydrogen atoms were added to the protein structure. The docking experiments were performed with ArgusLab 4.0.1 docking software (Thompson, 2004). For the identification of the binding sites in BSA, docking was performed by using a box size of 80 × 80 × 80 Å with grid resolution of 0.4 Å. The docking runs were performed on the Argus Dock docking engine with a maximum of 200 candidate poses and ligand was selected as flexible. The conformations were ranked using the Ascore scoring function, which estimates the free binding energy. After the molecular docking, the ligand-receptor complexes were further analyzed using Pymol (DeLano, 2002).