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Molecular interaction of human serum albumin with paracetamol: Spectroscopic and molecular modeling studies

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ABSTRACT

The interaction between paracetamol and human serum albumin (HSA) under physiological conditions has been investigated by fluorescence, circular dichroism (CD) and docking. Fluorescence data revealed that the fluorescence quenching of HSA by paracetamol was the result of the formed complex of HSA–paracetamol, and the binding constant (K_a) and binding number obtained is 1.3×10^4 at 298 K and 2, respectively for the primary binding site. Circular dichorism spectra showed the induced conformational changes in HSA by the binding of paracetamol. Moreover, protein–ligand docking study indicated that paracetamols (two paracetamols bind to HSA) bind to residues located in the subdomain IIIA.

1. Introduction

Human serum albumin (HSA) is the most abundant protein constituent of blood plasma and serves as a protein storage component. The three-dimensional structure of human serum albumin has been determined through X-ray crystallographic measurements [1]. This globular protein consists of a single polypeptide chain of 585 amino acid residues, which has many important physiological functions [2]. HSA is a globular protein composed of three structurally similar domains (I–III), each containing two subdomains (A and B) and stabilized by 17 disulfide bridges [3–5]. Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in subdomains IIA and IIIA, which are site I and site II [3–5]. Seven binding sites are localized for fatty acids in subdomains IB, IIIA, and IIIB and on the subdomain interfaces [6]. HSA is frequently used in biophysical and biochemical studies since it has a well-known primary structure and it binds to different categories of small molecules. The role of HSA as the main carrier of free drugs in the plasma has prompted a great interest in the studies of its structure and stability.

Paracetamol (N-acetyl-p-aminophenol) (inset of Fig. 1) is a commonly used analgesic and antipyretic drug [7]. Paracetamol (PC) was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893 and has been in use as an analgesic for home medication for over 30 years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin [8].

Quenching measurement of albumin fluorescence is an important method to investigate the interactions of drugs with serum albumins. It can reveal the accessibility of quenchers to albumin's fluorophore groups, helps to understand binding mechanisms of albumins with drugs, and provide clues to the essential of the binding phenomenon [9]. Fluorescence quenching refers to any process which decreases the fluorescence intensity of a given substance. There are mainly two types of quenching. The collisional quenching (dynamic quenching) results from collision between fluorophores and a quencher. The static quenching is due to the formation of ground-state complex between fluorophores and a quencher. The quenching can be mathematically expressed by the Stern–Volmer equation, which allows for the calculating of quenching constants

Abbreviations: HSA, human serum albumin; CD, circular dichroism; FET, Forster energy transference; PC, paracetamol; Trp, tryptophan; Tyr, tyrosine; Phe, phenylalanine; Glu, Glutamic acid; Ala, alanin; Met, methionin; Asp, aspartic acid; Gln, glutamin; Arg, arginine; RMS, root mean square; LGA, Lamarckian genetic algorithm; PDB, Protein Data Bank.

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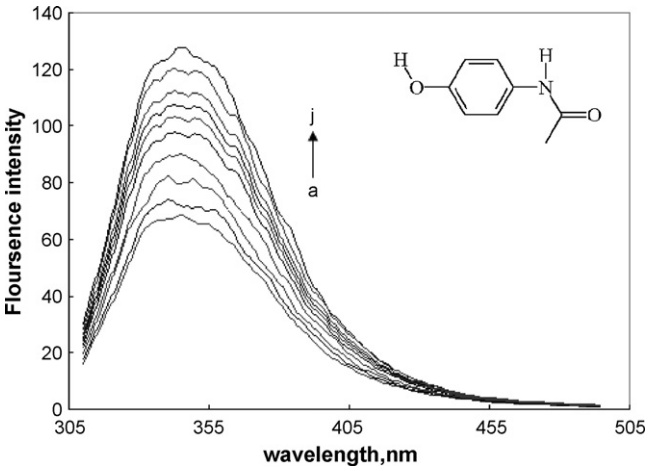

Fig. 1. The fluorescence emission spectra of the paracetamol–HSA complexes. From (a) to (j) C_{HSA} was 1.0 μM; $C_{paracetamol}$ was 3.3×10^{-5}, 2.5×10^{-5}, 2.1×10^{-5}, 1.6×10^{-5}, 1.3×10^{-5}, 1.1×10^{-5}, 0.9×10^{-5}, 6.0×10^{-6}, $4.0 \times 10^{-6}$and 2.0×10^{-6} M at pH 7.4.

[9–11]. Circular dichroism (CD) spectroscopy is a technique used most frequently for secondary-structure analysis of proteins. The affinities of drugs to proteins would straightly influence the concentration of drug in the binding site and duration of the effectual drug, and consequently contributes to their magnitude of biological actions *in vivo* [12].

Drug interactions at protein binding level will, in most cases, significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs; therefore the studies on this aspect can provide information of the structural features that determine the therapeutic effects of drugs, and have been an interesting research field in life sciences, chemistry and clinical medicine. In a series of study methods concerning the interaction of drugs and protein, fluorescence techniques are great aids in the study of interactions between drugs and plasma proteins in general and serum albumin in particular because of their high sensitivity, rapidity and ease of implementation.

HSA is the most important protein in the body because it is able to bind to the most of the drugs and carry them to the target organs. Since there was no report on the interaction of popular using drug paracetamol to HSA. In this paper we have studied the interaction of HSA and paracetamol to clarify details such as binding sites, binding forces and residues of subdomain interactions.

2. Materials and methods

2.1. Materials and preparation of solutions

Human serum albumin (HSA, 99%, fatty acid free), purchased from Sigma Chemical Company, was used without further purification. HSA was dissolved in acetate buffer solution (50 mM, pH 7.4 ± 0.1). Pure paracetamol was a gift from Drug and Food Quality Control (Tehran, Iran). The stock solution of drug was prepared by dissolving it in the acetate buffer 0.05 M pH 7.4 to form 1.0×10^{-3} M solution, and the solution of HSA was prepared at the same buffer to have a concentration of 1 mg/mL. All other chemical materials were of analytical grade.

2.2. Apparatus and methods

2.2.1. Fluorescence spectra

Fluorescence spectra were measured with a Spectrofluorimeter (Cary, Eclipse, Varian Co., Australia) equipped with a 1.0 cm optical path quartz cell and a thermostat bath. The excitation wavelength was 295 nm, and the emission spectrum was read at 300–500 nm. To quantify the binding constants of paracetamol to HSA, 700 μL solution containing 4.0×10^{-6} M HSA was titrated by successive additions of paracetamol solution using trace syringes (to give a concentration ranging from 0 to 2.0×10^{-4} M, and the fluorescence intensity was measured (λ_{ex} = 295 nm). All experiments were measured at each temperature (298, 303, and 310 K) with recycle water keeping the temperature constant. The appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence. The binding constants were calculated using the Stern–Volmer or modified Stern–Volmer equation.

2.2.2. Circular dichroism (CD)

Measurements were carried out on a Spectropolarimeter (Aviv, model 215, USA) in a 0.1 cm path-length cell at room temperature. For recording of CD spectra 20, 40 and 80 μL of paracetamol in concentration of 1.0×10^{-3} M was added to the 200 μL of HSA. The concentration of HSA in all CD experiments was 1 mg/mL. All spectra were collected with scan speed of 500 nm/min and response time of 0.5 s. Each spectrum was the average of four scans and corrected by acetate buffer background.

2.3. Docking procedure

Three-dimensional structure of paracetamol was constructed and optimized using Polak-Ribiere conjugate gradient algorithm and AMBER95 force field implemented in HyperChem (HyperCube Inc., Gainesville, FL). Then in the AutoDockTools package, the partial atomic charges were calculated using Gasteiger–Marsili method [13] and after merging non-polar hydrogens, rotatable bonds were assigned.

Of more than 50 X-ray crystallographic structures related to human serum albumin, in Protein Data Bank [14], entry with PDB ID: 1BM0 [15] was chosen for dockings because of no missing atoms, no co-crystallized ligand and having a reasonably good resolution (2.5 Å). Using a plain text editor all the water molecules were removed, then missing hydrogens and Kollman partial charges were added in AutoDockTools environment. Finally non-polar hydrogens were merged to their corresponding carbons, and desolvation parameters were assigned to each atom.

Flexible-ligand docking studies were carried out using AutoDock version 3.0.5 [16]. In order to find potential binding sites of paracetamol, the grids (one for each atom type in the ligand, plus one for electrostatic interactions) were chosen to be sufficiently large to include the whole HSA molecule. The points of the grids were thus $126 \times 126 \times 126$ with a grid spacing of 0.375 Å (roughly a quarter of the length of a carbon–carbon single bond). Of the three different search algorithms offered by AutoDock, the Lamarckian genetic algorithm (LGA) was applied and for the local search, the so-called Pseudo-Solis and Wets algorithm [17] was used. For all docking parameters, standard values were used as described before [16], except the amount of independent docking runs performed for each docking simulation which was set to maximum value supported by AutoDock (i.e. 256). Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 0.5 Å. Using smaller grid maps ($60 \times 60 \times 60$ grid points) centered on the binding site with the lowest docked energy, a next round of dockings with number of independent runs set to 150 was performed for the two candidate binding sites found in the previous round.

3. Results and discussion

3.1. Analysis of fluorescence quenching of HSA by paracetamol

Fig. 1 showed the fluorescence emission spectra of HSA with various amount of paracetamol following an excitation at 295 nm. It