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The interaction studies between isobutyl derivative of thiosalicylic acid and human serum albumin

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pharmaceuticals MDPI

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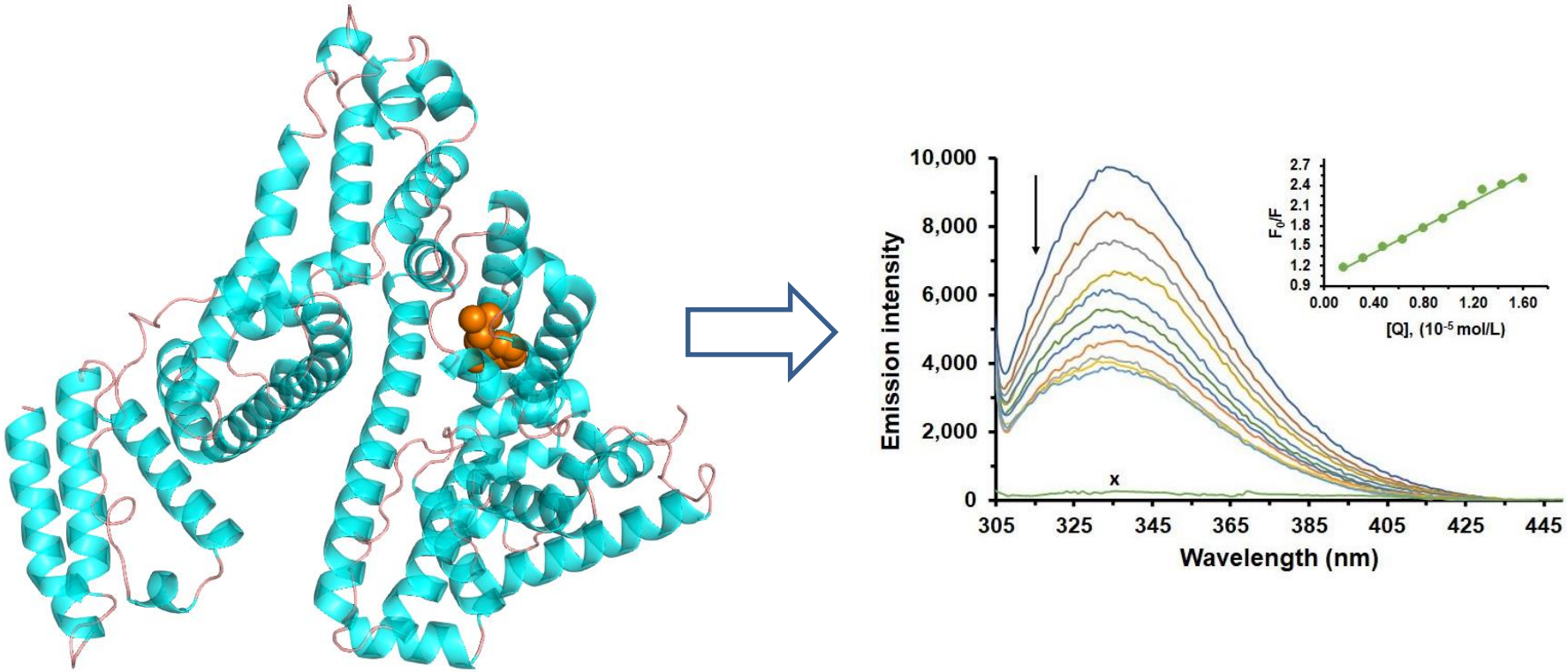
Graphical Abstract



Abstract:

It is well known that thiosalicylic acid and its S-alkyl derivatives have found applications in medicinal inorganic chemistry. Here are the study results on the interactions between the isobutyl derivatives of thiosalicylic acid (ligand, L) and human serum albumin (HSA). In particular, serum albumin is the primary soluble protein found in the human circulatory system. The metabolism of drugs, their distribution, and effectiveness strongly depend on the drug-albumin interaction. This interaction also affects the concentration of free drugs in the body. The interactions of the isobutyl derivatives of thiosalicylic acid (L) with HSA under physiological conditions was investigate by spectroscopy measurements and molecular docking. The results suggest that ligand could interact with HSA and influenced a slight change in the conformation of HSA through the static quenching mechanism. The analysis revealed that the HSA molecule has a moderate reaction to the ligand, as there is only one binding site for the ligand on the protein.

Keywords: Thiosalicylic acid; Human Serum albumin; Fluorescence spectroscopy; Docking simulations

Introduction

HUMAN SERUM ALBUMIN	Site I	**A**
		B
	Site II	**A**
		B
	Site III	**A**
		B

It's interesting to note that certain drugs exhibit a high level of binding specificity to particular site. For instance, warfarin is known for its characteristic binding to site I, while ibuprofen is commonly associated with site II. Understanding these unique binding properties can help inform drug development and treatment strategies.

The substantial binding capacity of human serum albumin plays a crucial role in the pharmacokinetics of numerous drugs [1-6]. Examining the binding capability of molecules to HSA is of paramount importance for drug transport and targeted tissue delivery.

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Introduction

HSA is composed of three homologous domains (I, II, III), with each domain further divided into two subdomains (A and B). The amino acid residues constituting these domains are as follows: IA 1-112, IB 113-195, IIA 196-303, IIB 304-383, IIIA 384-500, and IIIB 501-585.

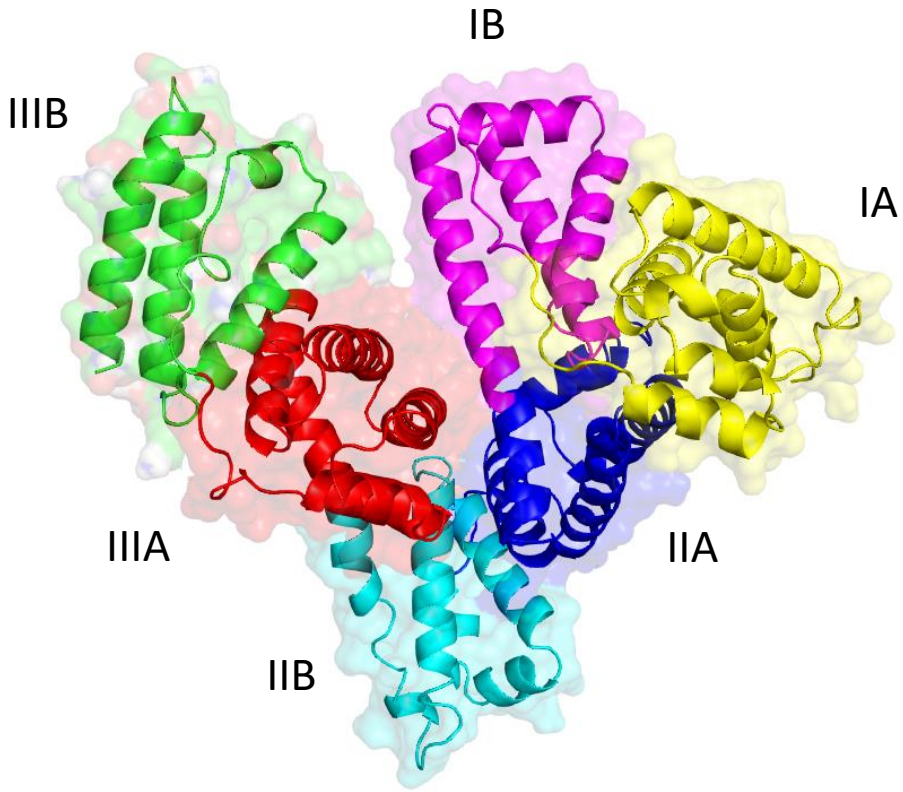

Figure 1. Crystal structure of HSA with subdomains

Introduction

Over the past few years, there has been a growing interest among scientists in the synthesis of small organic molecules that can exhibit notable biological activity on their own or serve as potential ligands for metallopharmaceuticals. Thimerosal has been used as constituent in various medical and biological formulations since the beginning of 20th century.

The thiosalicylate component found in thimerosal has the ability to suppress the release of vascular endothelial growth factor, thereby diminishing the toxicity associated with mercury(II)-chloride in vaccines and other formulations [7,8]. *In vitro* investigations into the antitumor properties reveal that S-alkyl derivatives of thiosalicylic acid demonstrate moderate cytotoxic effects on human colon and lung carcinoma cells, with the degree of cytotoxicity depending on the dosage [9].

In this ongoing study, we aim to experimentally determine the interactions and binding characteristics of the tested compound. This will be achieved through the application of spectroscopic methods, including fluorescence spectroscopy and molecular docking calculations.

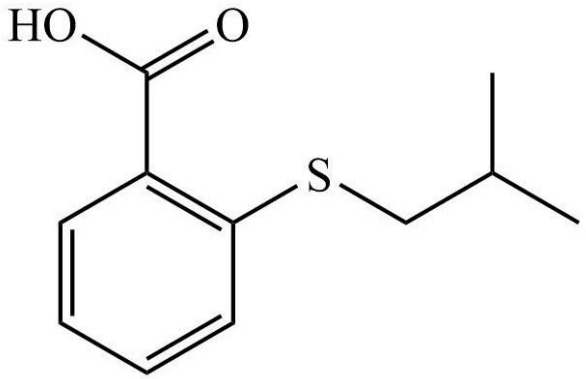

Figure 2. Structural formula of examined ligand.

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Results and discussion

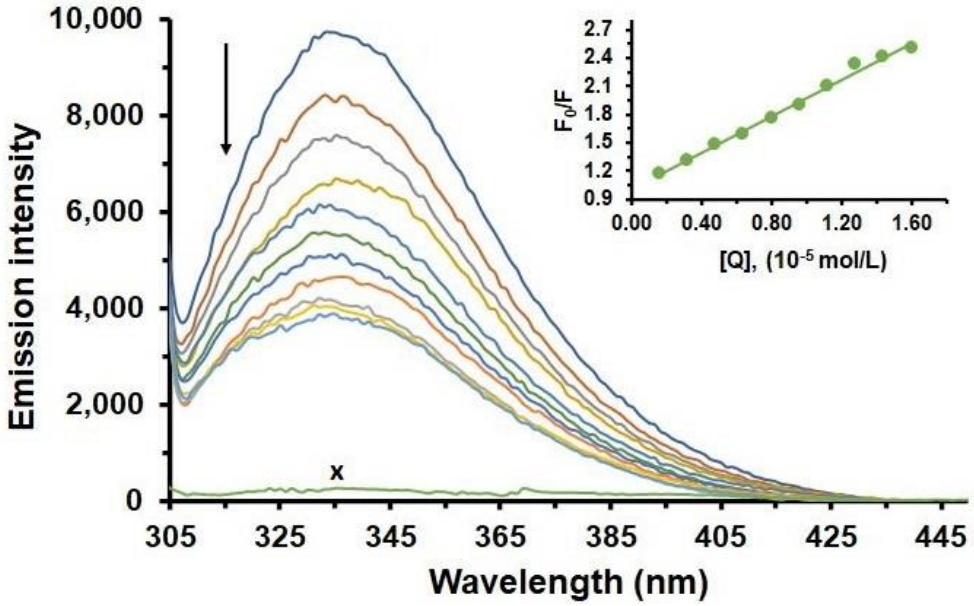

Figure 3. Emission spectra of HSA in the presence of various concentrations of S-isobutyl derivative of thiosalicylic acid (L) (T = 296 K, pH = 7.4). [HSA] = 1.6 µM; [ligand] = 0 - 16 µM. x represents a 16 µM ligand only. The inset: plot of F_0/F vs. [ligand].

Fluorescence measurements were employed to investigate the interactions between HSA and the tested compound, L. As a result, calculated values for the Stern-Volmer constant (9.72 x 10^4), binding constant (*K*a, 8.74 x 10^4), and the number of binding sites (n, 0.99) were determined. Linear Stern-Volmer plot and values of *K*q (9.72 x 10^{12}) indicate that the probable quenching mechanism of the intrinsic fluorescence of HSA was by static mechanism.

Results and discussion

Table 1

Stern-Volmer quenching constants (K_{SV}), quenching rate constants (K_q), binding constants (K_a), and number of binding sites (n) for the interaction of ligand (L) with HSA.

Ligand	K_{SV} (M^{-1})	K_q (M^{-1} s^{-1})	R^{2a}	K_a (M^{-1})	n	R^{2a}
L	9.72 x 10^4	9.72 × 10^{12}	0.9963	8.74 x 10^4	0.99	0.9968

[a]R is the correlation coefficient

The calculated value of n is closely to 1 for the investigated ligand (L), indicating the existence of a single binding site of L in HSA. According to the K_a values, it can be concluded that ligand L forms stable complex with HSA, with the K_a value of 8.74 x 10^4 M^{-1}. Binding constants (K_a) for the HSA-L system are significantly greater than 10^4, implying a strong binding affinity of towards HSA [10].

References:

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Results and discussion

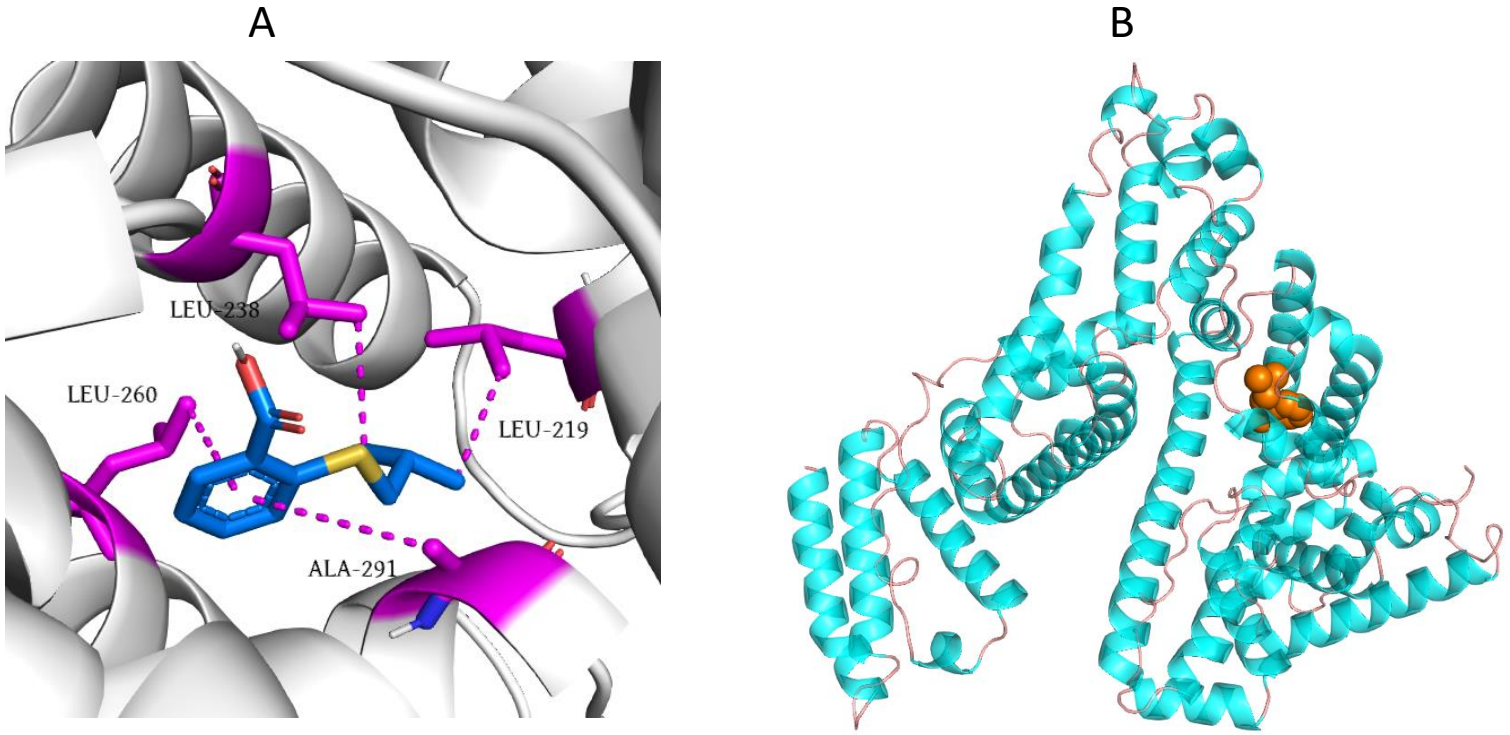

Figure 4. A) Molecular docking of L into the HSA. Hydrophobic interactions are shown as magenta dashed lines. B) Schematic representation of L bound to the HSA.

During molecular docking of L into the HSA, investigated compound forms four hydrophobic interactions. Carboxyl group of L does not interact with amino acid residues of HSA.

Results and discussion

The results of molecular docking confirmed the results of tested compound obtained by spectroscopic studies. The binding affinity of the investigated compound was evaluated using three criteria: category, number of crucial interactions, and free binding energy. Molecular docking analysis indicates that L spontaneously interacted with the hydrophobic cavity of HSA, exhibiting a minimal binding energy of -6.7 Kcal/mol. To determine the binding constant, we employed the following equation, which relies on the value of Gibbs free energy:

$$\Delta G = -RT \ln K$$

In this equation R represents the universal gas constant, while T denotes the absolute temperature. The calculated binding constant value was found to be 8.08×10^4 M^{-1}, which is consistent with the finding obtained through the fluorescence quenching analysis.

Conclusions

The obtained results suggest:

- ✓ The ligand has the capability to interact with HSA, inducing a subtle change in HSA's conformation through the static quenching mechanism.
- ✓ The HSA molecule exhibits a moderate response to the ligand due to the presence of only one binding site for the ligand on the protein.
- ✓ The interaction of L and HSA is predominantly characterized by the formation of multiple hydrophobic contacts.

Acknowledgments

ИНСТИТУТ ЗА ИНФОРМАЦИОНЕ ТЕХНОЛОГИЈЕ КРАГУЈЕВАЦ
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