RESEARCH PAPER

Interaction of Sophoridine to Human Serum Albumin: Spectroscopic Approach

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ABSTRACT

Sophoridine is an important traditional medicinal herb, which has been effectively used in the treatment of dysentery, diarrhea, stomatitis, throat infections, and hepatitis in folk medicine. In this study, the interaction between sophoridine and human serum albumin (HSA) was investigated by fluorescence spectroscopy and UV−Vis absorbance spectroscopy. In the mechanism discussion, it was proved that the fluorescence quenching of HSA by sophoridine is a result of the formation of sophoridine−HSA complex. Fluorescence quenching constants were determined using the Stern−Volmer equation to provide a measure of the binding affinity between sophoridine and HSA. Human serum albumin (HSA) is one of the main protein components of the circulatory system. Its well characterized physiological role is to carry numerous ligands to their target site. Overall pharmacokinetic profile of a drug is deliberately influenced by its affinity towards plasma proteins, especially with albumins. Alkaloids as small molecules are natural nitrogenous organic compounds that have significant medicinal properties that bind to HSA. There are three sites viz., I, II and III, on HSA molecule where the drug/small molecule binds based on their molecular size, structure and hydrophobicity. The major driving forces for the interaction of alkaloids-HSA are non-covalent interactions. CD spectra analysis suggests very little conformational changes in HSA on sophoridine binding.

Keywords: Alkaloids, Human Serum Albumin, Fluorescence, Quenching, Absorption Spectroscopy.

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INTRODUCTION

Plasma protein binding is an important factor to understand the pharmacokinetics and pharmacodynamic properties of drug candidates, as it strongly influences drug distribution and determines the free fraction, which is available to the target (1). Human serum albumin (HSA), the most prominent protein in plasma, has the capability to bind a wide range of endogenous and exogenous compounds such as nonesterified fatty acids, heme, bilirubin, thyroxine, and bile acids, as well as an extraordinarily broad range of drugs (2-6). The crystallographic analyses of HSA revealed that the protein, a 585 amino acid residue monomer, contains three homologous α-helical domains (I−III), each of which is composed by two subdomains A and B. The protein is stabilized by 17 disulfide bridges (7-8). The crystal structure analyses also indicate that the principal regions of ligand binding sites in albumin are located in hydrophobic cavities in subdomains IIA and IIIA (9-10). The hydrophobic cavities in HSA often increase the apparent solubility of hydrophobic drugs in plasma and

modulates their delivery to cells in vivo and in vitro; they can play a dominant role in drug disposition and efficacy. Drug-HSA interaction is an admired area of research since it has been lately employed for both therapeutic and investigative reasons and is one of the main elements determining the pharmacokinetic and pharmacodynamic profiles of the therapeutic molecules.

This paper investigates the association of HSA with sophoridine. Sophoridine (fig. 1) an alkaloid isolated from Chinese herbs, is an important traditional medicinal herb, mainly grows in Asia and Europe, which has been found to have analgesic, antibacterial, antimalarial, antipyretic, antitubercular, antiphotooxidative, antileish manial, antisecretory and antitumour activities in vitro and in vivo. In addition to its medicinal uses, sophoridine is also used as a fluorescent probe of cells, DNA, and energized mitochondria in biochemical researches (11-12).

Sophoridine hydrochloride (sophoridine) was selected to investigate because of its physiological and pharmacological effects mentioned above. It is widely accepted in the pharmaceutical industry that the overall distribution, metabolism, and efficacy of many drugs can be altered based on their affinity to serum albumin (13-14). In addition, many promising new drugs are rendered ineffective because of their unusually high affinity for this abundant protein (15). Obviously an understanding of the chemistry of the various classes of pharmaceutical interactions with albumin can suggest new approaches to drug therapy and design. Despite much information on the potential of sophoridine, the interaction of HSA with sophoridine has not yet been detailed investigated. This study enhances current understanding by elucidating the binding mechanism, and site specificity nature of the sophoridine–HSA interaction, thereby providing critical insights into its transport behavior and pharmacokinetic properties.

Spectral methods are powerful tool for the study of the reactivity of chemical and biological systems since it allows nonintrusive measurements of substances in low concentration under physiological conditions, and there are several studies of albumin induced by drugs or other bioactive small molecules using spectral methods (16-21).

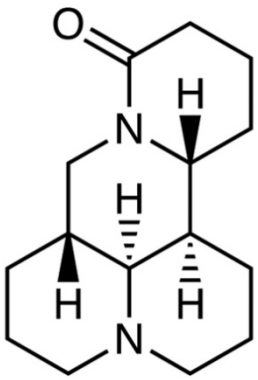

Fig. 1 Chemical structure of quinolizidine-based alkaloid, sophoridine

MATERIALS AND METHODS

Materials

Chemical such as sophoridine was purchased from TCI, USA. Disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from SRL India. Human serum albumin (HSA) was purchased from Calbiochem, India. All the other chemicals and solvents were of reagent grade and were used as supplied without further purification.

Equipments and Spectral Measurements

The UV spectrum was recorded at room temperature on a Shimadzu UV mini visible spectrophotometer UV-1700 equipped with 1.0 cm quartz cuvette. All fluorescence spectra were recorded on Shimadzu RF-5301 spectrofluorophotometer (Tokyo, Japan) in a quartz cell of path length 10 mm. The widths of both the excitation slit and the emission slit were set to 2.5 nm. An excitation wavelength of 295 nm was chosen since it provides no excitation of tyrosine residues and therefore neither emission nor energy transfer to the lone indole side chain would be nonnegligible. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background. Far-UV CD spectra of HSA and sophoridine (4-24 μM) were recorded using Jasco spectropolarimeter, model jasco 815 and serial no B069061168. Far-UV CD spectra (190–250 nm) were taken in cuvette of 0.1 cm path length. The protein concentration was 8μM (22).

Principles of Fluorescence Quenching

Fluorescence quenching is described by the well-known Stern−Volmer equation (18).

$$F0/F = 1 + Ksv[Q] \qquad (1)$$

where F0 and F denotes the steady-state fluorescence intensities in the absence and in the presence of quencher (sophoridine), respectively, K_{SV} is the

Stern−Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, equation 1 was applied to determine K_{SV} by linear regression of a plot of F0/F against [Q].

RESULTS & DISCUSSION

UV–visible absorption studies

UV–visible absorption measurement is a very simple technique to explore the structural changes and the complex formation. The absorption spectra of native HSA and in the presence of varying concentrations of sophoridine are shown in fig. 2. On addition of sophoridine increase in absorbance was observed with red shift in the peaks, which is indicative of the formation of HSA-sophoridine complex.

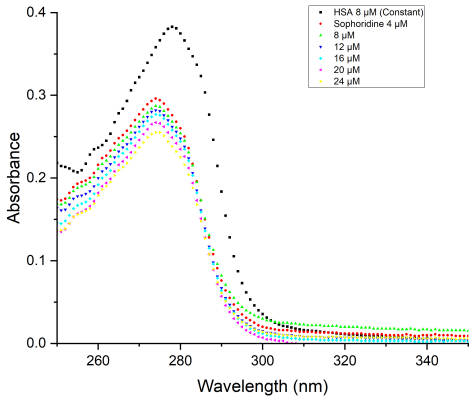

Fig. 2 UV–visible spectra of HSA in the absence and presence of sophoridine.

Circular dichroism (CD) measurements

Circular dichroism is an imperative technique to study the changes in the secondary structure of proteins. Spectra in the far-ultraviolet wavelength region (from ~190 to 250 nm) provide information on the polypeptide backbone conformations of proteins. Binding of ligand to protein may result in the rearrangement of intermolecular forces liable for sustaining the secondary and tertiary structures, triggering a conformational alteration of the protein.

To gain a better understanding of the changes in the secondary structure of HSA upon the complex formation with sophoridine, far-UV circular dichroism was performed. Fig. 3 shows the far-UV CD spectra of HSA in the absence and presence of sophoridine at physiological pH (7.2) in various molar ratios of HSA to sophoridine (1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3). Far-UV CD spectra of HSA show a presence of the α-helical structure in HSA is reflected by the appearance of two negative peaks at 208 and 222 nm, which are contributed by π-π^* and n- π^* transition in the peptidebond. From the results, it was concluded that there is increase in the intensity in both the peaks without any shift in the peak position. The CD data was obtained in ellipticity and were further converted into molar residual ellipticity (MRE) by using the given equation.

$$MRE = observed\ CD\ (mdeg)/cp\ nl\ X\ 10 \quad (2)$$

Where C_p is the molar concentration of HSA, n represents the number of amino acid residues present in the BSA i.e. 585 and path length is denoted as 'I' which is equal to 0.2 cm.

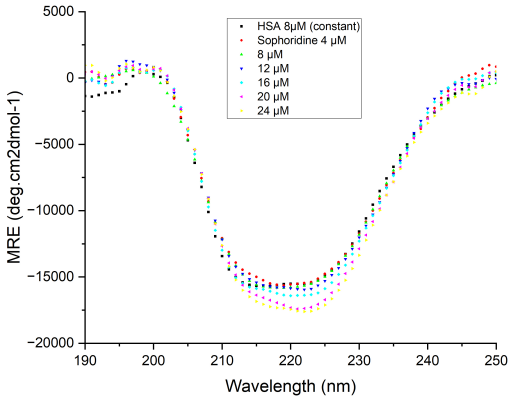

Fig. 3 Far-UV circular dichroism spectra of HSA (8 µM) in the presence of different concentrations of sophoridine (4-24 µM).

Fluorescence quenching of HSA by sophoridine

Fluorescence quenching of protein could be used to retrieve many drug-protein binding information. Tryptophan, tyrosine and phenylalanine are the three aromatic fluorophores that are used to study conformational changes in the structure of a protein. Fluorescence quenching of aromatic fluorophores in HSA by sophoridine at three different temperatures viz. 30, 40 and 50 °C, was studied in the presence of varying concentrations of sophoridine. As shown in fig. 4, HSA has a strong emission peak at ~335 nm on excitation at 295 nm, which decreased gradually with increase in concentration of sophoridine, at 30 °C. Similarly, a progressive decrease in the fluorescence intensity was observed with increase in the concentration of sophoridine at 40 and 50 °C also (not shown).

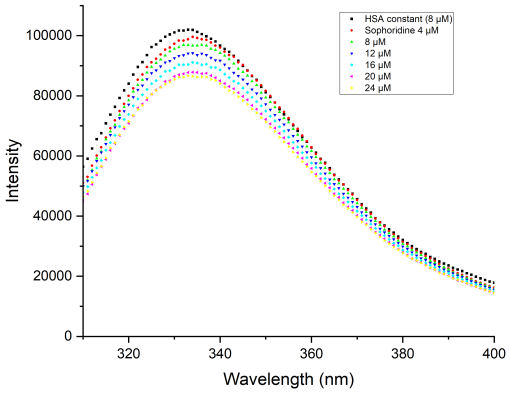

Fig. 4 Fluorescence emission spectra of HSA in the presence of different concentrations of sophoridine (4-24 μM). at 303 K HSA (8 μM) was excited at 295 nm.

Binding affinity

To obtain the Stern-Volmer quenching constant (Ksv) and binding constant (Kb), the fluorescence quenching data of HSA-sophoridine binding at different temperatures were analyzed by the linear and modified Stern-Volmer equation, respectively:

$$F0/F = 1 + Kq\tau_o[q] = 1 + Ksv[Q] \quad (1)$$

$$log\ (F0/F)/F = logK + n\ log\ [Q] \quad (3)$$

where, in Equation (1), Fo and F are the fluorescence intensities in the absence and presence of the quencher (sophoridine), Ksv and kq are the Stern-Volmer quenching and biomolecular rate constants, respectively, and τ_o is the average lifetime of fluorophores without quencher, which is 10^{-8} s. Ksv is calculated from the slope of plot of Fo/F vs [Q], where [Q] is the concentration of the quencher (fig. 5A). The results in table 1 indicate that the probable quenching mechanism of HSA by sophoridine involves dynamic quenching, because Ksv is increasing with increase in temperature. The increase in Stern–Volmer quenching constant with temperature confirms a dynamic quenching mechanism, indicating that sophoridine interacts with HSA through transient collisional encounters rather than stable complex formation, which has important implications for its rapid transport, bioavailability, and reduced protein-binding constraints in physiological systems.

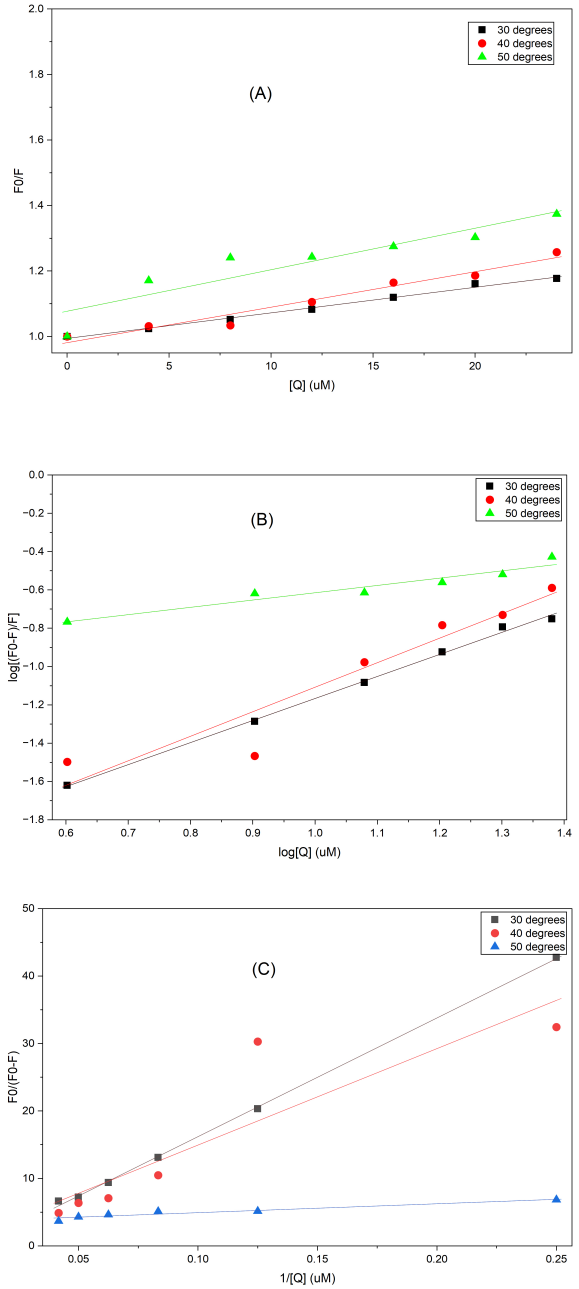

Fig. 5 Stern-Volmer (A), plot of log(F_0-F)/F versus log[Q] (B) and modified Stern-Volmer (C) plot of fluorescence quenching of HSA by sophoridine at different temperatures.

Table 1. Binding and thermodynamic parameters for the interaction of sophoridine with HSA at different temperatures obtained from fluorescence quenching experiments.

		$F0/F = 1 + Kq\tau_o[q] = 1 + Ksv[Q]$		$log\ (F0/F)/F = logK + n\ log\ [Q]$		
		Slope of graph $F0/F\ vs\ [Q]$	$Kq = Ksv/\tau_o$; where, τ_o = 10^-8	Intercept of graph $log(F0 - F)/F\ vs\ log[Q]$		Slope of graph $log(F0 - F)/F\ vs\ log[Q]$
TEMPERATURE(° C)	TEMPERATURE(K)	Stern-Volmer Dynamic Quenching Constant (Ksv)	Bimolecular Quenching Rate Constant (Kq)	Log Kb	Binding Constant (Kb)	No. of Binding Sites (n)
30	303	0.0078	780000	-2.31715	0.098554065	1.18073
40	313	0.01082	1082000	-2.38771	0.096839756	1.18021
50	323	0.01267	1267000	-1.99548	0.091954602	1.25121

Binding constants

In Equation (3), Kb is the binding constant and n is the number of binding sites per HSA molecule. The values of Kb and n were calculated from the intercept and slope of the plot log(Fo/F - 1) vs log[Q], respectively (Fig. 5B). As shown in Table 1, the observed decrease in binding constant (Kb) with increasing temperature indicates reduced stability of the HSA–sophoridine complex, suggesting partial thermal dissociation. However, the binding stoichiometry (n ≈ 1) confirms the formation of a single-site complex. These results, together with temperature-dependent dynamic quenching behavior, imply that sophoridine interacts with HSA through a combination of transient collisional interactions and weak, reversible ground-state complex formation.

CONCLUSION

In this study, the biophysical interaction between the quinolizidine alkaloid Sophoridine and Human Serum Albumin (HSA) was systematically elucidated through an integrated spectroscopic approach. Analysis of fluorescence quenching data via the Stern-Volmer equation confirmed a predominantly dynamic quenching mechanism, indicating that sophoridine interacts with the protein through transient molecular collisions rather than the formation of a stable, static ground-state complex. This finding is critical as it suggests a highly reversible interaction within the systemic circulation.

From a pharmacokinetic standpoint, this "intermediate" strength is ideal for a bioactive compound like sophoridine, it ensures that the drug is sufficiently bound for stable transport through the blood, yet maintains a high enough free-drug fraction to remain bioavailable for therapeutic action at target tissues. Furthermore, Circular Dichroism (CD) and UV-Vis absorption spectra demonstrated that the binding of sophoridine induces only minimal perturbations to the secondary structure of HSA, suggesting that the protein's native transport capacity and physiological stability remain intact.

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Conflict of interest

The authors declare that there is no conflict of interest.

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