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Human serum albumin binding to the biologically active labdane diterpene "leoheterin": Spectroscopic and in silico analysis

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A B S T R A C T

Labdane diterpenes are important substances due to their remarkable biological activities such as, antibacterial, antiprotozoal, antifungal and cytostatic and cytotoxic effects against human cancer cells. We have isolated a labdane diterpene named "leoheterin" from the aerial parts of the *Otostegia fruticosa* Forssk (Briq) obtained from south west Arabian mountains of Saudi Arabia. The isolated compound was characterized by ^{1}HNMR, ^{13}CNMR, IR and UV–visible spectroscopies. Due to the pharmaceutical importance of this class of compounds we have studied the interaction of HSA with leoheterin by using several spectroscopic methods. The change in the UV spectrum of HSA in presence of leoheterin gives a primary idea about the interaction between them. Congruently, leoheterin quenches the fluorescence of HSA with a prominent blue shift of 5 nm, reminiscent of involvement of hydrophobic interactions. There was 1:1 binding between leoheterin and albumin which was taken place via static quenching mechanism. From CD it was revealed that leoheterin induces the secondary structure of HSA which is further supported by 3-d fluorescence measurements which shows a decrease in the size of the HSA-leoheterin complex as compared to the HSA alone. Molecular docking simulations presented that among the first three conformers, which have been arranged according to the least binding energies and are also in good corroboration with the free energies of binding obtained experimentally, the first two conformers shown the binding in hemin binding site of subdomain IB while in third conformer the binding site was near to the drug binding site 1 located in subdomain IIA. All conformers exhibited the involvement of hydrogen bonding as well as hydrophobic interactions.

1. Introduction

Otostegia fruticosa Forssk (Briq). is a flowering medicinal plant in the family Lamiaceae and its greatest diversity is in neotropical region of Africa to Asia, especially in south west arabian mountains of Saudi Arabia and Sani in Egypt. The plant has a characteristic odor of thyme and is locally known as mountain thyme [1]. In Saudi folk medicine, leaves of *O. fruticose* are used to treat stomachic, asthma, fever, eye diseases, and as a disinfectant [2], whereas infusion of flowering branches as a remedy for sunstroke [3] and fumigated wood as an insecticide, specifically as a mosquito repellent [4]. The plant is also used for culinary purposes. Earlier pharmacological investigation on different extracts of *O. fruticosa* reported in the literature claims antimicrobial, antiparalytic, anticancer, and antioxidant activities [5–7]. Diterpenoids especially those with labdane skeleton, iridoid glucoside, sterols and essential oils have been isolated from *O. fruticose* [7].

Labdane diterpenes constitute an important class of natural as well as synthetic diterpenenes due to remarkable biological activities associated with them [8] which includes antibacterial, antiprotozoal, antifungal, hypolipidemic, enzyme induction, anti-inflammatory, immunomodulatory, cytostatic [9–15] and cytotoxic effects against human cancer cell lines [16], bronchodilatory effects, positive inotropic effects in cardiac muscle as well as antihypertensive properties [17,18]. In addition, diterpenoids are found to show inhibitory activities against human protein tyrosine phosphatase, a target for the treatment of effective in type-II diabetes and obesity [19].

The tile compound, leoheterin (15, 16 – epoxy- 7β, 9α- dihydroxylabdane- 13(16), 14-dien-6-one; Fig. 1A), a furanic labdane diterpene is one of major constituent that co-occur along with the diterpenoids in *O. fruticose* [7] and was isolated from the aerial parts of the *O. fruticosa*. It was first isolated and identified by Hon and co-workers in the 1990s [20] from *L. heterophyllus* and is a degradation product of prefuranic compound preleoheterin.

Attributable to the medicinal and pharmaceutical properties of

Abbreviations: HSA, human serum albumin; CD, Circular dichroism; ANS, 8-Anilinonaphthalene-1-sulfonic acid

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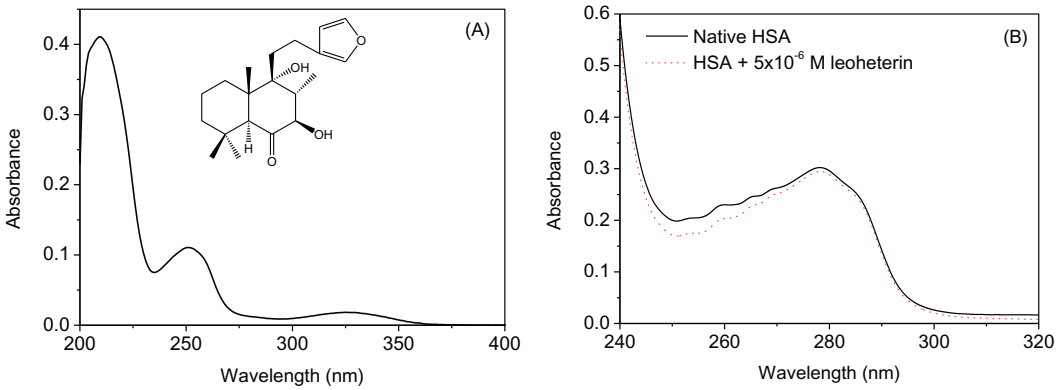

Fig. 1. (A) Chemical Structure and UV–visible spectrum of leoheterin. (B) Difference UV–visible spectra of HSA in presence of leoheterin. [HSA] = 7×10^{-6} M; [leoheterin] = 5×10^{-6} M.

labdane diterpenes understanding the interaction of these substances with biomolecules such as proteins is necessary [21–24]. Serum albumins are significant proteins which are present abundantly in circulatory system [25]. Human serum albumin is a globular protein that is responsible for carrying the substances, which are present in the blood, such as fatty acids, bilirubin and drugs, etc., throughout the body [26]. Association of any medicinal compound with serum albumin is necessary for the therapeutic effect of that compound. Similarly, the extent of association/binding is also important for a drug to work properly [27]. If a substance shows very strong interaction with albumin it might take time for its release to the site for its action which will cause a decrease in free drug concentration and a resultant decrease in therapeutic value. Alternatively, a weak interaction between albumin and drug will also need more amount for its action. Since administration of any medicinal compound to the body will more likely leads to the interaction of that with plasma and, consequently, plasma proteins which mostly contains serum albumin, understanding the mechanism of interactions of these compounds with serum albumins is necessary [28] For that reason, we have studied the mechanism of binding of leoheterin to the HSA using experimental as well as theoretical approaches. A lot of reports on the interactions with natural products of various types such as flavonoids [29], terpenoids [30], etc. are given in literature but interaction of diterpenes, in general, and labdane diterpenes, in particular, is relatively very less studied. Hence, our study will be helpful in designing the systems containing labdane diterpenes as therapeutic agents.

2. Experimental

2.1. Materials and Methods

HSA essentially fatty acid free (≥98%) was purchased from Sigma, USA. A stock solution of HSA was made in 20 mM of pH 7.4 tris-HCl buffer and protein concentration of 10×10^{-6} M was used throughout.

2.2. Isolation, Purification and Characterization of Leoheterin From O. fruticosa

The plant material in pre-flowering stage collected from Abha region of southern Saudi Arabia was identified as *O. fruticosa* by the plant taxonomist of our University. The air-dried aerial parts (stem and leaves) of *O. fruticosa* were powdered (1 kg) and percolated with neutral, acidic free ethyl acetate (3.0 L) at room temperature for 15 h and then filtered. The marc was reconstituted in ethyl acetate and extracted two more times as described above. All the drain off filtrates (3×3.0 L) were combined and concentrated under reduced pressure on rotavapor at < 50 °C to afford a dark brown residue (35.2 g) which was partitioned between acetonitrile (930 mL) and n-hexane (4×230 mL), presaturated with each other yielding two main fractions acetonitrile and n-hexane. The combined n-hexane fraction was back washed with 100 mL of acetonitrile to remove completely polar part and combined with acetonitrile fraction. Each fraction was concentrated to give acetonitrile (15.2 g) and n-hexane (20.1 g). A portion of the acetonitrile obtained above (5 g) was subjected to flash chromatography (silica gel column, 230–400 mesh, 40×2.5 cm) and was eluted with n-hexane. The polarity of mobile phase was increased by gradual addition of ether. The fractions obtained by elution with n-hexane: ether (8:2), exhibited homogenous TLC pattern, were pooled and concentrated to yield residue (2.00 g). The residue was further purified by crystallization in n-hexane to furnish a colorless compound (121 mg), $C_{20}H_{30}O_4$, R_f 0.40, mp 97.6–99.8 °C, identified as 15, 16 – epoxy- 7β, 9α- dihydroxylabdane- 13(16), 14-dien-6-one (leoheterin, Fig. 1A) by spectral analysis which is given in Supporting information along with ^{1}HNMR, ^{13}CNMR, Mass, and IR spectra (Figs. S1–S4). The UV–visible spectrum of leoheterin is given in Fig. 1A.

Rest of the details of the instruments used and the methods of study are giving in Supporting information.

3. Results and Discussions

3.1. UV–Visible Spectrophotometry

UV–visible spectroscopy is a very simple and basic technique to study the ligand-biomolecules interactions [31,32]. “HSA possesses several aromatic amino acids (tryptophan, tyrosine and phenyl alanine) which show a characteristic absorption peak around 280 nm” [31]. Fig. 1A is showing the UV–visible absorption spectrum of leoheterin while Fig. 1B is showing the UV spectrum of native HSA and the difference spectrum of HSA complexed with leoheterin [33]. The characteristic peak of HSA at 280 nm is showing a slight hypochromism in presence of leoheterin which is due to the hiding of the chromophores resultant of likely compression of the protein. However, there is no change in the wavelength of maximum absorption. “Dynamic quenching did not modify the absorption spectrum, but only affected the exited states of the quenching molecule” [32] Thus, involvement of static quenching mechanism is proposed in the interaction of HSA and leoheterin. From Fig. 1A it is also clear that leoheterin has insignificant absorption at excitation wavelength of tryptophan, therefore, the inner filter effect was considered as negligible [34,35].

3.2. Intrinsic Fluorescence

Fluorescence spectroscopy is very expedient technique to study the ligand-biomolecules interactions, particularly for the proteins which possess fluorescence amino acids. HSA is one such a protein which holds intrinsic fluorescence property because of the presence of tryptophan, tyrosine and phenylalanine amino acids among which the