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Unraveling the binding interaction of a bioactive pyrazole-based probe with serum proteins: Relative concentration dependent 1:1 and 2:1 probe-protein stoichiometries

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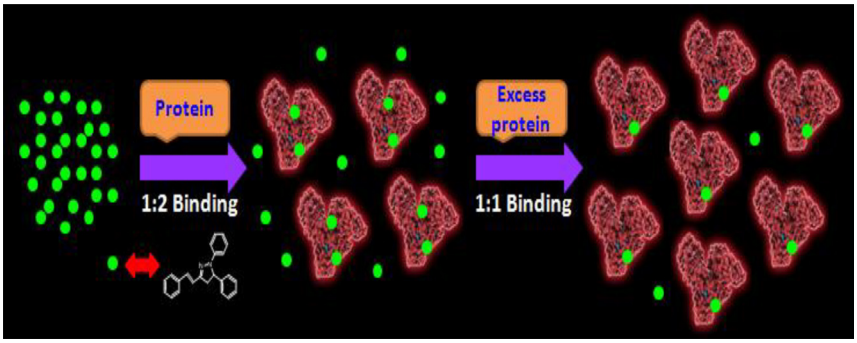
HIGHLIGHTS

- Binding of DSDP with two serum proteins, HSA and BSA, has been investigated.
- In a newer approach, binding constants are determined varying protein concentration.
- Concentration dependent 1:1 and 1:2 protein-probe stoichiometries are established.
- Hydrogen bonding and hydrophobic interactions are responsible for both bindings.
- Two plausible binding sites of DSDP in both HSA and BSA are at sites IIA and IB.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecular interactions and binding of probes/drugs with biomacromolecular systems are of fundamental importance in understanding the mechanism of action and hence designing of proactive drugs. In the present study, binding interactions of a biologically potent fluorophore, (E)-1,5-diphenyl-3-styryl-4,5-dihydro-1H-pyrazole (DSDP) with two serum transport proteins, human serum albumin and bovine serum albumin, have been investigated exploiting multi-spectroscopic techniques. The spectrophotometric and fluorometric studies together with fluorescence quenching, fluorescence anisotropy, urea induced denaturation studies and fluorescence lifetime measurements reveal strong binding of DSDP with both the plasma proteins. Going beyond the vast literature data mostly providing 1:1 probe-protein complexation, the present investigation portrays 2:1 probe-protein complex formation at higher relative probe concentration. A newer approach has been developed to have an estimate of the binding constants varying the concentration of the protein, instead of the usual practice of varying the probe. The binding constants for the 2:1 DSDP-protein complexes are determined to be $1.37 \times 10^{10}\,M^{-2}$ and $1.47 \times 10^{10}\,M^{-2}$ for HSA and BSA respectively, while those for the 1:1 complexation process come out to be $1.85 \times 10^{5}\,M^{-1}$ and $1.73 \times 10^{5}\,M^{-1}$ for DSDP-HSA and DSDP-BSA systems respectively. Thermodynamic analysis at different temperatures implies that the forces primarily involved in the binding process are hydrogen bonding and hydrophobic interactions. Competitive replacement studies with known site markers and molecular docking simulations direct to the possible locations and binding energies of DSDP with the two serum proteins, corroborating well with the experimental results.

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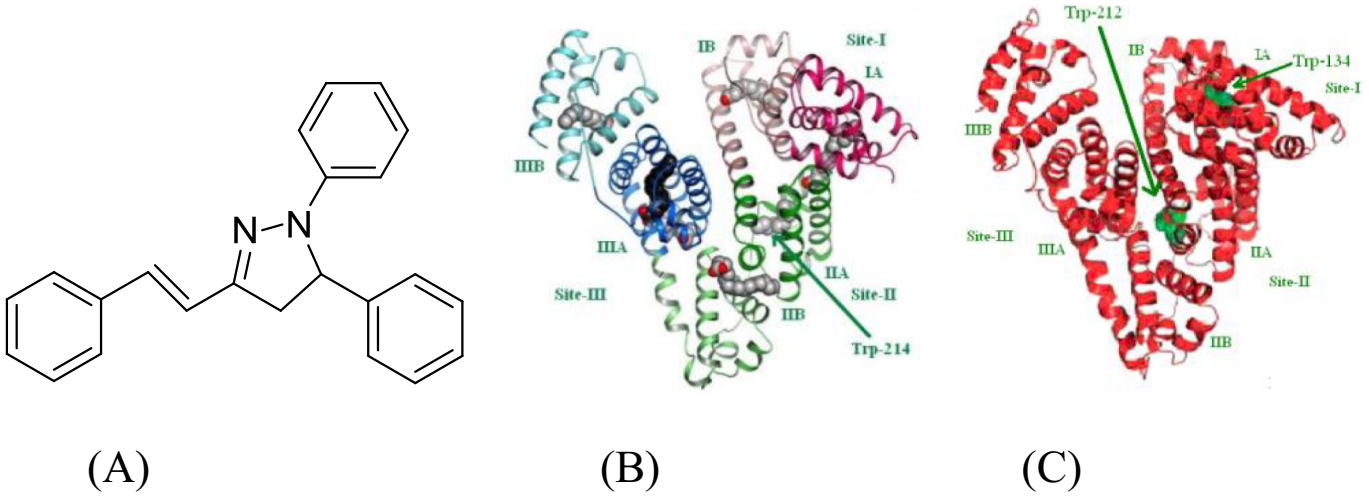

Scheme 1. Structures of (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazole (DSDP) (A), human serum albumin (B) and bovine serum albumin (C).

1. Introduction

Substituted pyrazoles and their derivatives possess a wide range of biological activities such as antimicrobial, antimycrobacterial, antifungal, antiamoebic, anti-inflammatory, analgesic, antidepressant, anticancer, anti-tubercular, anti-convulsant, angiotensin converting enzyme inhibitory, neuroprotective activities etc. [1–7] Substituted pyrazole derivatives are also used for the treatment of central nervous system (CNS), metabolic and oncological diseases [8–12]. Several pyrazole derivatives have already found their clinical applications as nonsteroidal anti-inflammatory drugs, such as antipyrine or phenazone (analgesic and antipyretic), metamizole or dipyrone (analgesic and antipyretic), aminopyrine or aminophenazone (anti-inflammatory, antipyretic and analgesic), oxyphenbutazone (antipyretic, analgesic, anti-inflammatory, mild uricosuric) etc. [2, 13] Because of the noteworthy uses of the pyrazole derivatives in medicinal and pharmaceutical fields, we have been interested in obtaining more insight into the molecular interaction of the newly synthesized fluorescent pyrazole derivative, namely, (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazole (DSDP, Scheme 1) with the most abundant plasma proteins, human serum albumin (HSA) and bovine serum albumin (BSA). An understanding of the mechanism of binding of the probe with the serum albumins at the molecular level is expected to pave a way for development of newer drugs.

Proteins play essential roles in sustaining life and are integral parts of origin, evolution and metabolism. Knowledge of mechanism of interactions between the bioactive molecules/drugs with the plasma proteins is of pivotal importance for understanding the pharmacokinetics, pharmacodynamics and bio-distribution of the drugs in the living body. The efficacy of a drug can be affected by its nature of binding to the proteins in the blood plasma like serum albumins, hemoglobin, lipoproteins, etc. [14] Therefore, unveiling the binding interaction between a drug and the protein is important to improvise the analogues of a drug with effective pharmacological characteristics. Serum albumins (SA), the most abundant transport proteins in blood plasma, assist the transportation and disposition of various endogenous and exogenous agents in the blood stream to achieve selective targeted delivery [15]. They increase the apparent solubility of hydrophobic drugs in blood plasma and modulate their delivery to cell in vivo. The structural aspects and properties of HSA and BSA are well recorded in literature [16–21]. Both these serum proteins exhibit around 80% sequence homology with a repeating disulfide pattern [18–21]. The primary protein structure has about 580 amino acid residues and is characterized by a high content of cysteine stabilizing a series of nine loops, and a low content of tryptophan [19]. The secondary structure is constituted of 67% helix of six turns and 17 disulfide bridges [20, 21]. The tertiary structure of a serum protein is composed of three linearly arranged domains (I – III), each of which is categorized by two subdomains (A and B) [19–21]. The most prominent difference between these two plasma proteins is that BSA has two tryptophan residues (Trp-134 and Trp-212) whereas HSA has only one (Trp-214). In BSA, Trp-134 and Trp-212 residues are located at the surface region (IA) and the hydrophobic pocket of the albumin (IIA) respectively; whereas, the single tryptophan (Trp-214) residue of HSA lies at the hydrophobic region (IIA, Scheme 1) [21].

The present work provides new insights on the binding interaction of the bioactive probe, DSDP, with the two most abundant plasma proteins (HSA and BSA) under physiological condition at pH 7.0. Spectral modifications in the UV–Vis absorption, fluorescence and time resolved fluorescence intensity decay measurements have been exploited to explore the binding interactions of the probe with the two serum proteins. Site selective competitive replacement studies using ibuprofen and warfarin have been performed for monitoring the binding site/s of the serum albumins for the probe DSDP [22–24]. In silico molecular docking simulations have also been carried out to predict the site/s of binding. Going beyond the existing literature, the present multi-spectroscopic study reveals two types of binding between the probe and the serum albumins. The vivid study proposes formation of 1:1 and 1:2 protein-probe complexes depending on the relative concentrations of the two interacting partners. Although the literature mostly reports 1:1 probe serum protein binding, there are a few reports of multiple binding [25–27]. The present study, thus, emphasizes on looking at the drug bindings with proteins more carefully to understand the interaction of substituted pyrazole drugs with the transport proteins for designing and pharmacokinetic studies of a vast variety of drugs containing pyrazole moiety.

2. Experimental section

2.1. Materials

(*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazole (DSDP) (Scheme 1) was received as a kind gift from Prof. G. Maiti of our department [28]. The compound was purified following the same procedure as described in one of our recent publications [29]. Fatty acid free human serum albumin (molar mass ~66.4 kDa) and bovine serum albumin (molar mass ~66 kDa) were purchased from Sigma-Aldrich, USA and were used without farther purification. Stock solutions of serum albumins were prepared by dissolving desired amounts of the proteins into 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid)