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Differential binding modes of anti-cancer, anti-HIV drugs belonging to isatin family with a model transport protein: A joint refinement from spectroscopic and molecular modeling approaches

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ABSTRACT

The present contribution reports a detailed characterization of the binding interaction of two potential anticancer, anti-HIV drugs isatin (IST) and 1-methylisatin (MI) with model transport protein Bovine Serum Albumin (BSA). Thermodynamic parameters e.g., ΔH, ΔS and ΔG for the binding phenomenon have been evaluated on the basis of van't Hoff equation to understand the force behind the binding process. A combined application of steady-state and time-resolved fluorescence spectroscopic techniques substantiate the observed drug-induced quenching of intrinsic tryptophanyl fluorescence of the protein to proceed through a static mechanism. Circular dichroic (CD), synchronous fluorescence and excitation–emission matrix fluorescence spectroscopic techniques have been exploited to delineate the secondary and tertiary conformational changes in the protein structure induced by the binding of drugs (IST/MI). The probable binding location of the drug molecules within the protein cavity (hydrophobic subdomain IIIA) has been explored from AutoDock-based blind docking simulation. Examination of drug–protein binding kinetics using stopped-flow fluorescence technique reveals that the association constants (k_a) for IST-BSA and MI-BSA interactions are 1.09×10^{-3} s^{-1} (±5%) and 1.73×10^{-3} s^{-1} (±5%), respectively, at the experimental temperature (T) of 298 K. The present study also delves into the effect of drug-binding on the esterase activity of the protein which is found to be reduced in the drug–protein conjugate system in comparison with the native protein.

1. Introduction

Isatin (1*H*-indole-2,3-dione), belonging to the well-known family of indole derivatives, was first obtained as a product from chromic acid oxidation of indigo dye by Erdmann [1] and Laurent [2] in the middle of the 19th century. However, a host of sources of isatins has been garnered by the nature also as they occur in many plants like genus *Isatis* [3], *Couroupita guianensis* Aubl [4] and *Calanthe discolor* LINDL [5]. More interestingly isatin is an endogenous indole which is widely distributed in mammalian brain, peripheral tissues and body fluid and also found as a metabolic derivative of adrenaline in the human body [6–8]. In recent time, isatins have formed the nucleus of many-faceted research activities owing to the multitude of potential applications in clinical and medicinal aspects. Isatin derivatives opened up a vista of promising prospects in synthetic organic chemistry owing to their biological and pharmacological properties [3,9]. Isatins and many analogous compounds have formed a prospective avenue of research surrounding their anticancer, antioxygenic, anticonvulsant, antibacterial properties, and sedative activities [10,11]. In addition, Mannich bases of isatin derivatives have been recognized for their antirhombotic, antiallergic, muscle relaxing, fibrinolytic activities [10,11]. In fact, some derivatives of isatins (e.g., 5-fluoro-3-substituted-2-oxoindole) are already in use for the treatment of gastrointestinal stromal tumors [12] and advanced renal cell carcinoma [13], while many other halogenated derivatives are in use for treatment of cancer and leukemia [14]. Probably, the most demanding prospect of research surrounding the isatin derivatives has evolved in the context of their antifungal [15] and antiviral [16,17] activities. Human Immunodeficiency Virus (HIV) is a lentivirus that causes Acquired Immunodeficiency Syndrome (AIDS) [18,19], in which progressive collapse of the human immune system allows life-threatening opportunistic infections and cancers to thrive. Increasing infection with HIV has already proven alarming to the human society. Moreover, the human race has faced a dire ramification for the development of drugs with anti-HIV activity which has thus been central to medicinal chemistry research. Isatin derivatives have claimed their relevance and importance in the field of anti-HIV activity [20].

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The present study is based on the interactions of two isatin drugs (*viz.*, isatin (IST) and 1-methylisatin (MI)) with the model transport protein bovine serum albumin (BSA). Serum albumins are abundantly found in blood plasma and belong to one of the most widely studied categories. They function as carrier for numerous exogenous and endogenous compounds in the body. The primary structure of BSA is composed of 583 amino acid residues and the secondary structure is characterized by ~66% α-helix [21–25]. BSA contains two tryptophan residues, TRP-134 and TRP-212 of which the former is located in hydrophilic subdomain IB and the latter in hydrophobic subdomain IIA [22].

The study of binding interactions of isatin drugs with biological and biomimicking receptors comprises a germane field of research. However, it is surprising to note that this field of research remains rather sporadically explored in the literature. Herein, we report a spectral deciphering of the binding interactions of two drugs from the isatin family, *viz.*, isatin (IST) and 1-methylisatin (MI) (*cf.* Scheme 1) with model transport protein bovine serum albumin (BSA). In order to delve into the binding mechanism and nature of the binding forces in the drug–protein interaction process the thermodynamic parameters have been evaluated from van't Hoff relationship by applying temperature-dependent fluorescence quenching. An attempt is also undertaken to unravel the effect of drug-binding on the protein secondary and tertiary structures so as to rationalize the applicability of the drug molecules as therapeutic agents. The AutoDock-based 'blind docking' strategy has been exploited to delineate the probable binding location of the drugs within the protein backbone. With a view to the prospective biological properties of isatins the characterization of binding interaction of IST and MI with a model transport protein appears to have significance in relation to further development of biomedicines and the field of safe-engineered drug delivery.

2. Experimental

2.1. Materials

Commercially available isatin and 1-methylisatin (*cf.* Scheme 1) were obtained from Sigma Chemical Co., USA and used as received. The purity of the compound was established on TLC plate before use. BSA from Sigma Chemical Co., USA was used as received. Tris buffer was purchased from SRL, India, and 0.01 M Tris–HCl buffer of pH 7.4 was prepared in triply distilled deionized water from a Milli-Q water purification system (Millipore). The solvent appeared visually transparent and the purity of the solvent was also tested by running the fluorescence spectra in the studied wavelength range. Hydrochloric acid (HCl) from E-Merck was used as obtained.

2.2. Instrumentation and methods

2.2.1. Steady state spectral measurements

The absorption and emission spectra were recorded by Hitachi UV–Vis U-3501 spectrophotometer and Perkin–Elmer LS55 fluorimeter, respectively. All spectra were recorded with appropriate background corrections. The concentrations of the protein (BSA) and the drugs (IST and MI) used in different experiments have been specified in the context of the relevant discussions.

Only freshly prepared solutions were used for all spectroscopic measurements. The temperature was kept constant at a given value by a recycling water flow accurate upto ±1.0 °C.

2.2.2. Excitation–emission matrix spectra

The excitation–emission matrix spectra (EEMS) or the three-dimensional fluorescence spectra were recorded by the same Perkin–Elmer LS55 fluorimeter when the emission wavelength range was selected from 200 nm to 500 nm and the initial excitation wavelength was set within 200 nm to 400 nm with 10 nm interval. All other parameters were adjusted to those specified previously [26].

2.2.3. Synchronous fluorescence spectra

The constant wavelength synchronous fluorescence spectra (CWSFS) presented in this study were monitored on the same Perkin–Elmer LS55 fluorimeter at a constant protein concentration (10 μM) with varying drugs (IST and MI) composition using $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm which yield characteristic information for the tyrosine (TYR) and tryptophan (TRP) microenviroment within the protein [26].

2.2.4. Time-resolved fluorescence decay

Fluorescence lifetimes were obtained by the method of Time Correlated Single-Photon counting (TCSPC) on FluoroCube-01-NL spectrometer (Horiba Jobin Yovon) using a light source of nanoLED at 291 nm and the signals were collected at the magic angle of 54.7° to eliminate any considerable contribution from fluorescence anisotropy decay [26]. The decays were deconvoluted by DAS-6 decay analysis software. The acceptability of the fits was judged by χ^2 criteria and visual inspection of the residuals of the fitted function to the data. The reported lifetimes data presented in the table are an average of five individual measurements.

2.2.5. Circular dichroism

Circular dichroism (CD) spectra were recorded by a JASCO J-815 spectropolarimeter using a cylindrical cuvette of 0.1 cm pathlength at 25 °C. The reported CD profiles are an average of four successive scans obtained at 20 nm/min scan rate with appropriately corrected baseline. The concentration of BSA and the drug during CD measurements are mentioned in the relevant discussion.

2.2.6. Kinetics measurement

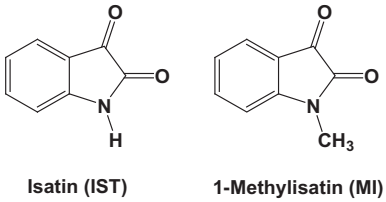
The kinetics of associations of the drugs IST and MI with the protein BSA were monitored by Perkin–Elmer LS55 fluorimeter using the stopped-flow fluorescence measurement technique. The dead time of the instrument was found to be 20 ms.

2.2.7. Esterase activity assay

The effect of the drug on the esterase activity of BSA was assayed with the synthetic substrate *p*-nitrophenyl acetate (PNPA) by following the formation of *p*-nitrophenol [27] at 37 °C. One unit of esterase activity was defined as the amount of the enzyme (BSA) required to liberate 1.0 μM *p*-nitrophenol per minute at 37 °C.

2.3. Molecular modeling: blind docking simulation

The native structure of BSA was taken from the Protein Data Bank having PDB ID: 3V03 (DOI: 10.2210/pdb3v03/pdb [28]); Docking studies were performed with AutoDock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein. For docking of IST and MI with BSA, the required files (corresponding to the three-dimensional structure of the drugs) for the ligand (IST/MI) were created through the



Scheme 1. Schematic molecular structures of the studied drug molecules.