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# A Comparison Study of the Interaction of Progesterone and Testosterone Drugs with Human Serum Albumin: A Fluorescence Quenching Study

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**Abstract**

The interaction of steroid hormones (testosterone and progesterone) with human serum albumin in phosphate buffer at pH 7.5 and at 25 °C have been studied using a fluorescence spectrophotometer. The results showed that fluorescence emission spectra were quenched with the increase of steroid molar ratios in fixed amount of HSA. The result indicated that progesterone and testosterone hormones have an ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. Two mathematical models Stern–Volmer and Scatchard for analysis of fluorescence spectra were used. Progesterone binding to HSA was increased by a factor of 2.6 greater than that detected for the interaction of testosterone with HSA.

The values of Stern-Volmer constants were determined to be ($2.0 \times 10^4$ L mol$^{-1}$) for progesterone–HSA complex and ($7.7 \times 10^3$ L mol$^{-1}$) for testosterone–HSA complex, Respectively. Whereas, the Scatchard constants were ($2.1 \times 10^4$ L mol$^{-1}$) and ($7.9 \times 10^3$ L mol$^{-1}$) for progesterone–HSA and testosterone–HSA complexes, respectively.

**Key Words:** Steroid hormones, Human serum albumin, Stern-Volmer, Scatchard, Fluorescence quenching spectroscopy

## INTRODUCTION

Steroid hormones are the most familiar compounds to the general public, because of the use and abuse of them for diverse purposes, such as contraception and body building [1]. Steroid hormones have many physiological effects on human body, their disorder may cause many abnormalities in human body [2, 3]. Progesterone, as a steroid hormone, is synthesized from cholesterol [1], and is secreted naturally by the corpus luteum. It is a C-21 steroid hormone; its chemical structure is shown in Fig.(1 a and b) [2].

Testosterone, a “male’’ or masculinizing sex hormone, is a C-19 steroid Fig.(1 b) is secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. It has important functions in stimulating and maintaining the secondary sex characteristics (such as beard growth), maintaining the genitals and their sperm-producing capability, and stimulating the growth of bone and muscle [4].

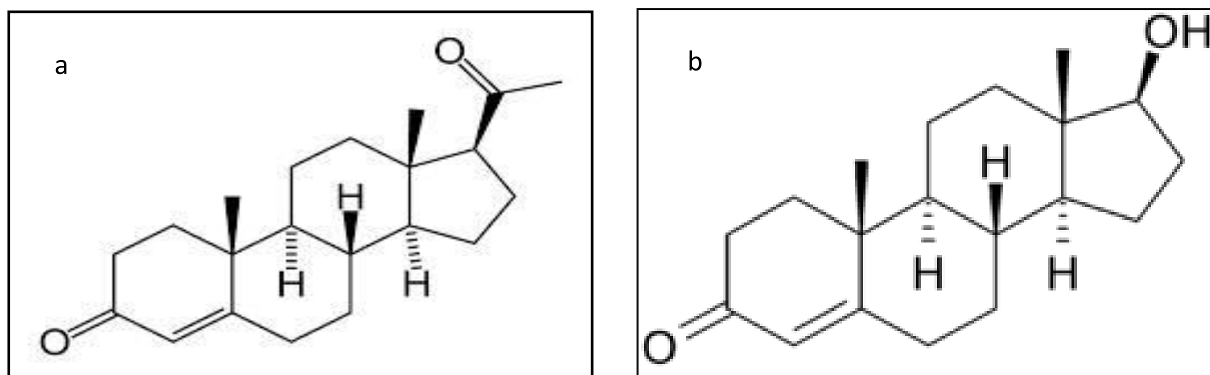


**Figure 1.** Chemical structure of steroid hormones (a): Progestrone and (b): Testosterone)

Human serum albumin (HSA) Fig. 2 is a globular single polypeptide chain protein of a molecular mass of about 67 kDa and comprises 585 amino acid residues, It is the most abundant and highly soluble plasma protein in the blood circulatory system in mammals (approximately 60% of the total protein) [6, 7] the most abundant plasma protein, greatly augments the transport capacity of blood plasma where it is present at a high concentration (≈ 600 µM). This property is exerted by reversible binding and delivering of a vast array of chemically diverse endo- and exogenous compounds [8, 9] seriously impacting their pharmacokinetic profile. HSA plays an important role in transporting metabolites and drugs through the vascular system and also in maintaining the pH and osmotic pressure of the plasma [11]. Its structure includes three homologous domains (I, II, and III) that assemble a heart shaped molecule (Fig. 2). Each domain is formed by two sub-domains (A and B) which possess common structural motifs by various forces such as salt bridges and hydrophobic interactions [12, 13]. According to the conventional view based on Sudlow's classification, drug ligands of HSA are accommodated at two main [6, 8] Crystallographic studies performed on ligand-HSA adducts have revealed the molecular details of the binding [5−9] Site IIA is a large, preformed, flexible multichamber cavity within the core of subdomain IIA which binds bulky heterocyclic compounds with a negative, often delocalized charge near to the center of a mainly nonpolar molecular framework. However, the Sudlow classification cannot account for the HSA binding of all ligands. A third binding pocket within subdomain IB (site IB) has recently been identified as the primary binding site of some compounds such as bilirubin photoisomer [14], hemin [15,16]. Crystallographic studies have also showed that the large crevice of subdomain IB harbors secondary binding sites for some additional compounds.

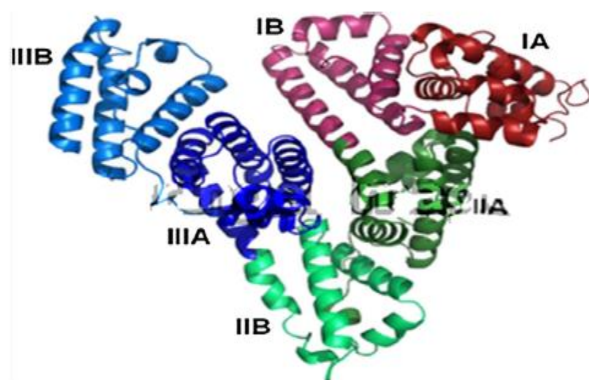


**Figure 2.** X-ray crystallographic to 2.5Å resolution of three dimensional Structure of HSA, with its subdomains [5]. The structure corresponds to the ''Protein Data Bank''.

Carter and co-workers [17, 18] have claimed that subdomain IB (site IB) is the third major drug-binding region of HS.

In this work, it is informative to study the interaction between two selected steroid hormones (testosterone and progesterone) with human serum albumin using fluorescence quenching method, and tried to do a comparison between the binding behavior of both hormones with HSA. The results will be interpreted on the basis of two mathematical models leading to estimate the binding parameters for both hormones with HSA.

# MATERIALS AND METHODS

Human serum albumin (HSA) (fatty acid free) was purchased from Sigma Aldrich company, and the hormones testosterone ampoule was purchased from Alexandria Co. pharmaceutical chemical industries and progesterone ampoule from Marcyrl pharmaceutical industries, and their solutions were prepared as 5% ethanolic phosphate buffer solutions. The other substances are of reagent grade, and were used without further purifications.

### Preparation of Stock Solutions

HSA solution was freshly prepared in phosphate buffer saline (20 mM, 0.1 M NaOH pH 7.5) and treated at final concentration of 1 µM with the previously prepared hormone solution testosterone or Progesterone. The studied hormone, was dissolved in phosphate buffer saline (5% ethanolic solution).

The concentration of HSA was determined from absorbance measurements at 278 nm using a molar extinction coefficient of 35219 $M^{-1}cm^{-1}$. [19]

Unless otherwise noted, all reagents used in this study were used as received without further purification. All solutions in this study were prepared with doubly distilled water.The phosphate buffer was 20 mM (containing 0.1 M NaCl) and its pH was adjusted to pH 7.5)

### Fluorescence Quenching Study

All fluorescence spectra were recorded on an Perkin–Elmer luminescence Spectrofluorimeter, (series no. 70412). A quartz cell of 1.00 cm width was used for the fluorescence measurements. Fluorescence spectra related to the titration of fixed volume of HSA of 1 µM concentration with concentrated stock ethanolic steroid solution (varied from 0.35 mM to 6.0 mM) were measured on the Spectrofluorimeter.

The fluorescence intensity measured were corrected for background fluorescence of buffer and the steroids studied. All samples were measured after two minutes and all binding data reported here correspond to the average values obtained after two different titrations. The instrument was thermostatically controlled by a circulatory water bath maintained at 25 °C.

The fluorescence emission spectra in absence and presence of increasing concentration of the studied steroid were recorded at 25 °C in the wavelength range of

250 – 500 nm with the excitation wavelength at 295 nm and the widths of both excitation and emission slits were fixed to 10

### Calculation of HSA Binding Constants

The apparent binding constants for both steroids with HSA were obtained after collecting fluorescence quenching data using two mathematical models, equations 1 and 2. Details of the estimation of the association constants ($K_a$) using fluorescence quenching data have been described elsewhere. Nonlinear regression analysis of the binding constant values measured at different steroid/HSA molar ratios was performed and analyzed by the Matlab software program (2008).

***Stern-Volmer***

$$F_0/F = 1 + K_q \tau_\circ [H] = 1 + \mathrm{K_{SV}} [H]_f \qquad (1)$$

Where: $F_0$ and $F$ are the fluorescence intensities in the absence and presence of the quencher, respectivety. $K_{sv}$ is

the Stern-Volmer quenching constant. and $[H]_f$ is the free hormone concentration and can be expressed as:

$$[H]_f = [H]_t - [H]_b \quad \text{..........................} (2)$$

Where: $[H]_t$ the total hormone concentration and $[H]_b$ is the bound hormone concentration.

$K_q$ is the bimolecular quenching constant, $\tau_o$ is the average lifetime of the biomolecule without quencher, and (H) is the quencher concentration

***Scatchard***

$$\frac{r}{[H]_f} = nK_{SC} - rK_{SC} \quad \text{..........} (3)$$

Where: $K_{sc}$ is the Scatchard association constant, n is the number of binding sites, and r is the number of mol of bound ligand per mol of protein,and can be calculated as:

$$r = \frac{[H]_b}{[HSA]_t} \quad \text{..............} (4)$$

Where: $[HSA]_t$ is the total concentration of protein.

## RESULTS AND DISCUSSION

**Fluorescence quenching spectra**

Fluorescence spectroscopy is one of the most widely used spectroscopic technique to study the protein ligand binding and interaction [20, 21]. Fluorescence quenching of single tryptophan residue in HSA (Trp 214) was used to monitor the steroid/HSA interaction and to measure the binding affinity of the interaction quenching spectra of HSA at various molar ratios of progesterone is shown in Fig.(3). Obviously from the results, the fluorescence intensity of

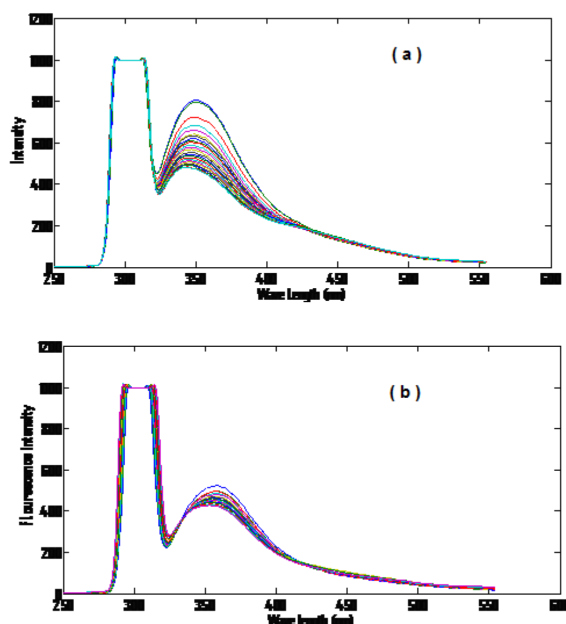


**Fig. 3** Emission spectra of HSA in the absence and the presence of hormone (a): progesterone and (b): testosterone at various hormone concentrations, $T$ = 298 K, $\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 350 nm, pH = 7.50 and the HSA concentration was 1.0 µM. The concentrations of hormone drug was varied from 0.35 to 6.0 mM in each case.

It is obviously seen from the results that the fluorescence intensity of HSA gradually quenched upon increasing the molar ratio of the studied hormone to HSA, indicating that progesterone and testosterone bind to HSA.

It is seen that, the amount of fluorescence quenching caused by the steroid molecules was dependent on the steroid type and concentration being more effective in case of progesterone/HSA. Furthermore, a slight blue shift of the maximum emission wavelength was observed at higher steroid concentrations that it could be suggested that conformational changes induced by the interaction of steroid with HSA, lead to a further hiding of tryptophan residues from the polar solvent [23-25] that is the interaction of the studied steroid with HSA induced an alteration of the tertiary structure of HSA [22] being more effective in case of progestrone.

The fluorescence emission spectra of a compound is quenched due to a variety of molecular interactions, one of which is the formation of a complex between the fluorophore and the quencher.

Stern-Volmer quenching constant $K_{sv}$ indicates the sensitivity of the fluorophore to a quencher. Figure (4 a and b) displays the Stern–Volmer plots of the quenching of HSA by the studied steroid molecules at 25 °C, in phosphate buffer solution at pH 7.5.

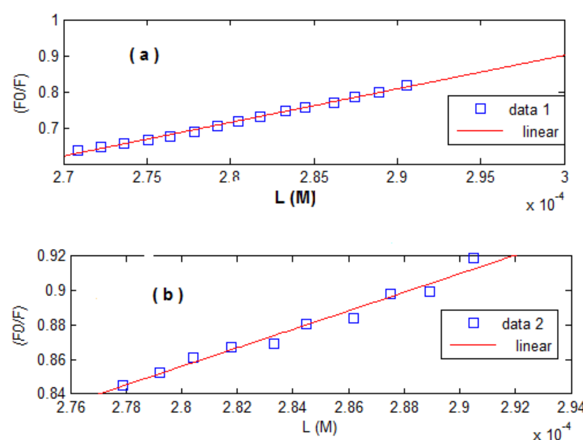


**Fig. 4.** Stern-Volmer plots of fluorescence quenching of HSA by (a): progesterone and (b): testosterone.

Table 1 lists the binding constants obtained by both models i.e Stern-Volmer and that of Scatchard ($K_{SV}$ and $K_{SC}$). It is evident that, progesterone was apparently bound more than twice as high as that for testosterone and this trend was in accordance (to some extent) with the same trend with that was obtained by [28] The differences in binding behaviors between progesterone and testosterone with HSA may be due to binding mode of interaction of different side chains in the two studied steroidal hormones and the selected binding region in HSA. [29]

**Table 1.** Association constants of progesterone and testosterone with HSA in phosphate buffer pH 7.5 and at 25°C.

| **Hormone** | **Binding constant** ($M^{-1}$) | |
|---|---|---|
| | Stern-Volmer | Scatchard |
| Progestrone | $2.0 \times 10^4$ | $2.1 \times 10^4$ |
| Testosterone | $7.7 \times 10^3$ | $7.9 \times 10^3$ |

The apparent binding constants ($K_{SV}$ and $K_{Sc}$) for the studied hormone that binds HSA can be obtained using Eqs. (1) and (3) Stern–Volmer and Scatchard, respectively [26, [27].

The fluorescence quenching data accompaining the interaction hormone/HSA in the present study were analyzed on the basis of two well known mathematical models, Stern–Volmer and Scatchard models, Eqs (1) and (2), respectively. The corresponding linear regression equations were analysed by using Matlab software.

Figs. (5 a and b) shows the Scatchard plots for the interaction of hormone/HSA at 25 °C.

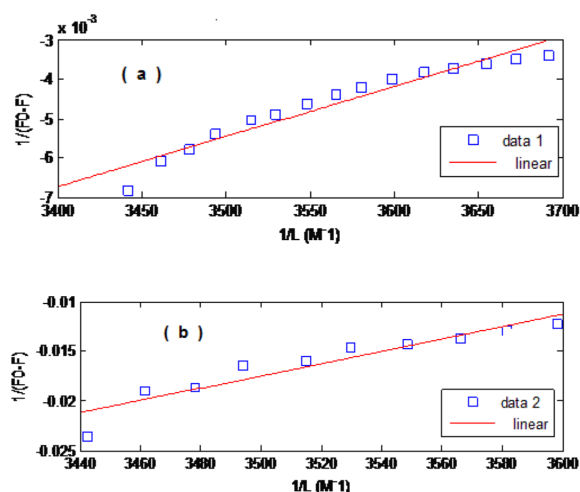


**Fig. 5.** Scatchard plots of fluorescence quenching of HSA by (a): progesterone and (b): testosterone.

## CONCLUSION

In this paper, we investigated the interaction between two steroid hormones (Progestrone and testosterone) by fluorescence spectroscopy. The experimental results indicate that both hormones can quench the intrinsic fluorescence of HSA. Our results clarified that progesterone was bound more effectively to HSA. The binding constants were calculated and analyzed according to two mathematical models (Stern-Volmer and Scatchard). This study is expected to provide important insights into the interaction of protein HSA with important drugs used in therapeutic regimes.

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