Journal of Applied Biological Sciences 5 (1): 35-47, 2011
ISSN: 1307-1130, E-ISSN: 2146-0108 www.nobel.gen.tr

Study of Progesterone interaction with Human Serum Albumin: Spectroscopic approach

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Received : November 01, 2010
Accepted : January 23, 2011

Abstract:
The interaction between progesterone and human serum albumin has been investigated. This interaction was studied by UV-absorption and fluorescence spectroscopy. From spectral analysis progesterone showed a strong ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. The binding constant (K) is estimated 6.56×10^2 M^{-1} at 293 K. FT-IR spectroscopy with Fourier self-deconvolution technique was used to determine HSA secondary structure and progesterone binding mechanisms. The observed spectral changes indicate the formation of H-bonding between progesterone and HSA molecules which can be related to the intensity decrease in the absorption band of α-helix relative to that of β-sheets.

Keywords: progesterone; amide I-III ; binding mode; binding constant; protein secondary structure; Fourier transform IR; UV–spectroscopy, Flurosence spectroscopy.

INTRODUCTION

Steroid hormones are the most familiar compound to the general public, because of the use and abuse of it 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, is one of the steroid hormones, and is synthesized from cholesterol [1], and is secreted naturally by the corpus luteum. Progesterone is a C-21 steroid hormone; its chemical structure is shown in Fig.(1) [2].

If progesterone level is low, this hormonal deficiency may produces a variety of symptoms such as bloating, anxiety, irritability, moodiness, food cravings, crying, breast tenderness, fatigue, depression and anxiety [3]. Progesterone, in general, has opposite effects on lipid metabolism compared with estrogen. That is, progestins decrease high-density lipoprotein (HDL) cholesterol and increase lowdensity lipoprotein (LDL) cholesterol, both (HDL and LDL) increase risks of cardiovascular disease [4].

Human serum albumin (HSA) is the most abundant carrier protein of the body with a high affinity for a wide range of metabolites and drugs. The most important physiological role for the protein is to bring such solutes into the blood stream and then deliver them to the target organs, as well as to maintain the pH and osmotic pressure of plasma [5]. The molecular interactions between HSA and many compounds have been investigated successfully [6,7,8]. It has been recently proved that serum albumin plays a decisive role in the transport and disposition of a variety of endogenous and exogenous compound such as fatty acids, hormones, bilirubin, drugs…[9] The distribution and metabolism of many biologically active compounds in the body whether drugs or natural products are correlated with their affinities toward serum albumin. Thus, the study on the interaction of such molecules with albumin is of imperative and fundamental importance [5]. In recent years, many investigations on the binding of drugs, natural products to protein were carried out. Progesterone, as a possible ligand, was not studied in details upon its binding reaction with HSA. It has been reported that progesterone binds to HSA [10]. So far, none of the investigations determine in details the Progesterone–HSA binding constant and the effects of progesterone complexation on the protein structure. In this work, we have investigated the interaction of progesterone with HSA by means of FT-IR, UV/VIS, and fluorescence spectrophotometer. Infrared spectroscopy provides measurements of molecular vibrations due to the specific absorption of infrared radiation by chemical bonds. It is known that the form and frequency of the Amide I band, which is assigned to the C=O stretching vibration within the peptide bonds is very characteristic for the structure of the studied protein . From the band secondary structure, components peaks (α-helix, β-strand) can be derived and the analysis of this single band allows elucidation of conformational changes with

high sensitivity [11]. This work will be limited to the mid-range infrared, which covers the frequency range from 4000 to 400 cm-1. This wavelength region includes bands that arise from three conformational sensitive vibrations within the peptide backbone (Amides I, II and III) of these vibrations, Amide I is the most widely used and can provide information on secondary structure composition and structural stability [12,13,14]. One of the advantages of infrared spectroscopy is that it can be used with proteins that are either in solution or in thin film. In addition there is a growing body of literature on the use of infrared to follow reaction kinetics and ligand binding in proteins, as will as a number of infrared studies on protein dynamics.

Other spectroscopy techniques are usually used in studying the interaction of drugs and proteins, fluorescence and UV spectroscopy are commonly used because of their high sensitivity, rapidity and ease of implementation [15,16,17].

MATERIALS AND METHODS

HSA (fatty acid free), and progesterone in powder form were purchased from Sigma Aldrich chemical company and used without further purification.

2.1 preparation of stock solutions

HSA was dissolved in phosphate buffer saline, at physiological pH 7.4, to a concentration of (80mg/ml) based on its list molecular weight 66 kDa. Progesterone with molecular weight of (314.47 g/mol) was dissolved in phosphate buffer saline (0.7622 mg/ml), the solution was placed in ultrasonic water path (SIBATA AU-3T) for six hours to ensure that all the amount of progesterone was completely dissolved.

The final concentrations of HSA-progesterone solutions were prepared by mixing equal volume of HSA and the hormone. HSA concentration in all samples was fixed at 40mg/ml. However, the concentration of progesterone in the final protein hormone solutions was decreased gradualy such that the molecular ratios of (HSA:progesterone) are (10:18, 10:14, 10:10, 10:6, and 10:2). The solution of progesterone and HSA were incubated for 1 h (at 20oC) before spectroscopic measurements were taken.

2.2 UV-absorption spectra

The absorption spectra were obtained by the use of a NanoDrop ND-1000 spectrophotometer. The absorption spectra were recorded for free HSA (40 mg/ml) and for its complexes with progesterone solutions with the molar ratios of (HSA:progesterone) (10:18, 10:14, 10:10, 10:6, and 10:2).. Repeated measurements were done for all samples and no significant differences were observed.

2.3 Fluorescence

The fluorescence measurements were performed by a Nano- Drop ND-3300 Fluorospectrphotometer at 25oC. The excitation source comes from one of three solid-state light emitting diodes (LED's). The excitation source options include: UV LED with maximum excitation 365 nm, Blue LED with excitation 470 nm, and white LED from 500 to 650 nm excitation. A 2048-element CCD array detector covering 400–750 nm, is connected by an optical fiber to the optical measurement surface. The excitation is done at the wavelength of 360 nm and the maximum emission wavelength is at 439 nm.

2.4 FT-IR Spectroscopy Experimental Procedures

The FT-IR measurements were obtained on a Bruker IFS 66/S spectrophotometer equipped with a liquid nitrogen-cooled MCT detector and a KBr beam splitter. The spectrometer was continuously purged with dry air during the measurements.

The absorption spectra were obtained in the wave number range of 400–4000 cm-1. A spectrum was taken as an average of 60 scans to increase the signal to noise ratio, and the spectral resolution was at 4 cm-1. The aperture used in this study was 8 mm, since we found that this aperture gives best signal to noise ratio. Baseline correction, normalization and peak areas calculations were performed for all the spectra by OPUS software. The peak positions were determined using the second derivative of the spectra.

The infrared spectra of HSA, and Progesterone–HSA complex were obtained in the region of 1000–1800 cm−1. The FT-IR spectrum of free HSA was acquired by subtracting the absorption spectrum of the buffer solution from the spectrum of the protein solution. For the net interaction effect, the difference spectra {(protein and Progesterone solution) – (protein solution)} were generated using the featureless region of the protein solution 1800-2200 cm–1 as an internal standard [18]. The accuracy of this subtraction method is tested using several control samples with the same protein or drug concentrations, which resulted into a flat base line formation. The obtained spectral differences were used here, to investigate the nature of the drug–HSA interaction.

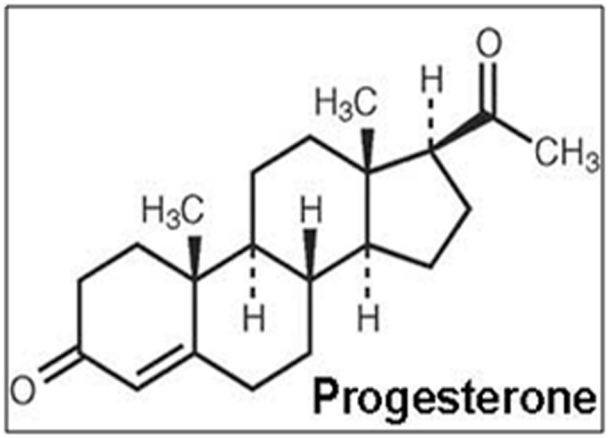

Figure 1: Chemical structure of progesterone.

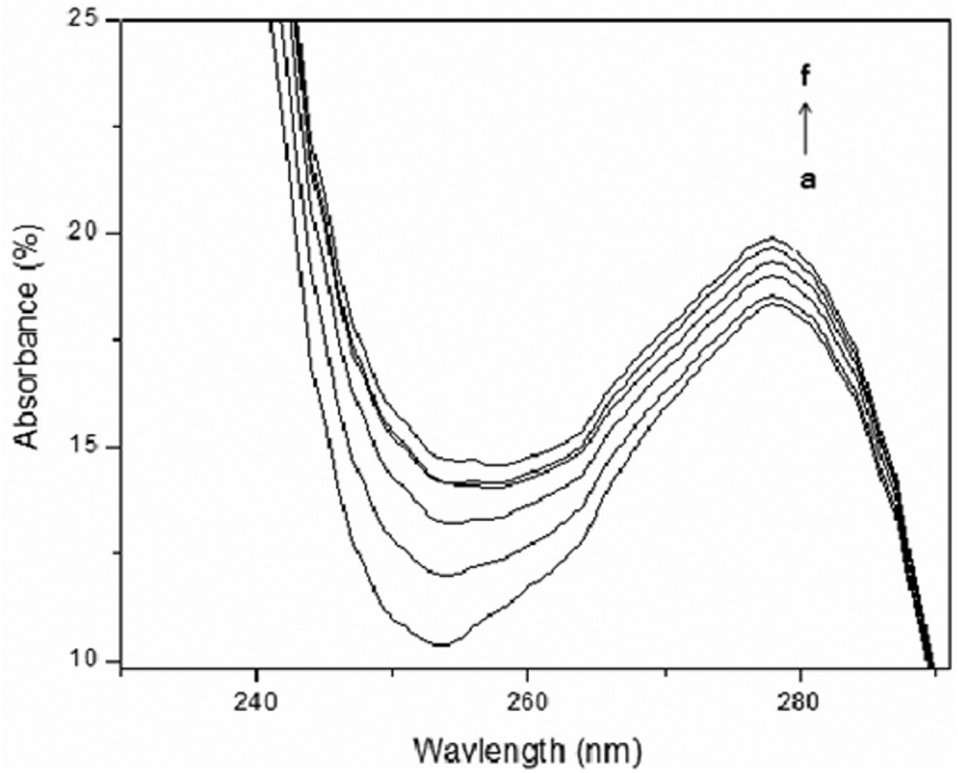

Figure 2: UV-Absorbance spectra of HSA with different molar ratios of progesterone (a=0:10, b=2:10, c=6:10, d=10:10, e=14:10, f=18:10).

RESULTS AND DISCUSSION

3.1 UV-absorption spectroscopy

Many investigators have reported the effectiveness use of UV-VIS spectroscopy to investigate the interaction of drugs with HSA [19,20,21]. The excitation has been done on 210 nm and the absorption is recorded at 278 nm. The absorption spectra of different ratios of progesterone with fixed amount of HSA are displayed in Fig.(2). The figure shows that the UV-VIS absorbtion intensity of HSA increases with the increasing of progesterone molar ratios, and that the absorption peaks of these solutions showed moderate shifts indicating that with the addition of progesterone, the peptide strands of HSA molecules extended more and the hydrophobicity of progesterone was decreased [22]. The results indicated that an interaction occurred between progesterone with HSA. Obviously, it was seen from the spectrum that pure progesterone have little or no UV absorption. This result supports that the peaks are due to the interaction between progesterone with HSA. Repeated measurements were done for all samples showing consistent results and no significant differences were observed.

The progesterone - HSA complexes binding constants were determined using UV-VIS spectrophotometer according to published method [23,24]. By assuming that there is only one type of interaction between progesterone and HSA in aqueous solution, leads to establish Equations. (1) and (2) as follows:

(1) HSA + Progesterone ↔ Progesterone: HSA

(2) K = [Progesterone: HSA]/ [Progesterone][HSA]

The absorption data were treated using linear double reciprocal plots based on the following equation [25,26]:

(3) $$\frac{1}{A-A_0}=\frac{1}{A_\infty-A_0}+\frac{1}{K[A_\infty-A_0]}\cdot\frac{1}{L}$$

where A_0 corresponds to the initial absorption of protein at 280 nm in the absence of ligand, A∞ is the final absorption of the ligated protein, and A is the recorded absorption at different progesterone concentrations (L). The double reciprocal plot of $1/(A - A_0)$ vs. $1/L$ is linear (Fig.(3)) and the binding constant (K) can be estimated from the ratio of the intercept to the slope to be $6.354\times10^2 M^{-1}$. The value obtained is indicative of a weak progesterone-HSA interaction with respect to the other drug-HSA complexes with binding constants in the range of 10^5 and 10^6 M^{-1} [27]. The reason for the low stability of the progesterone HSA complexes can be attributed to the presence of mainly hydrogen bonding interaction between protein donor atoms and the progesterone polar groups or an indirect progesterone- protein interaction through water molecules. Similar weak interactions were observed in cis- Pt(NH3)2–HSA and taxol–HSA complexes [28,29].

3.2 Fluorescence spectroscopy.

Fluorescence spectroscopy is one of the most widely used spectroscopic techniques in the fields of

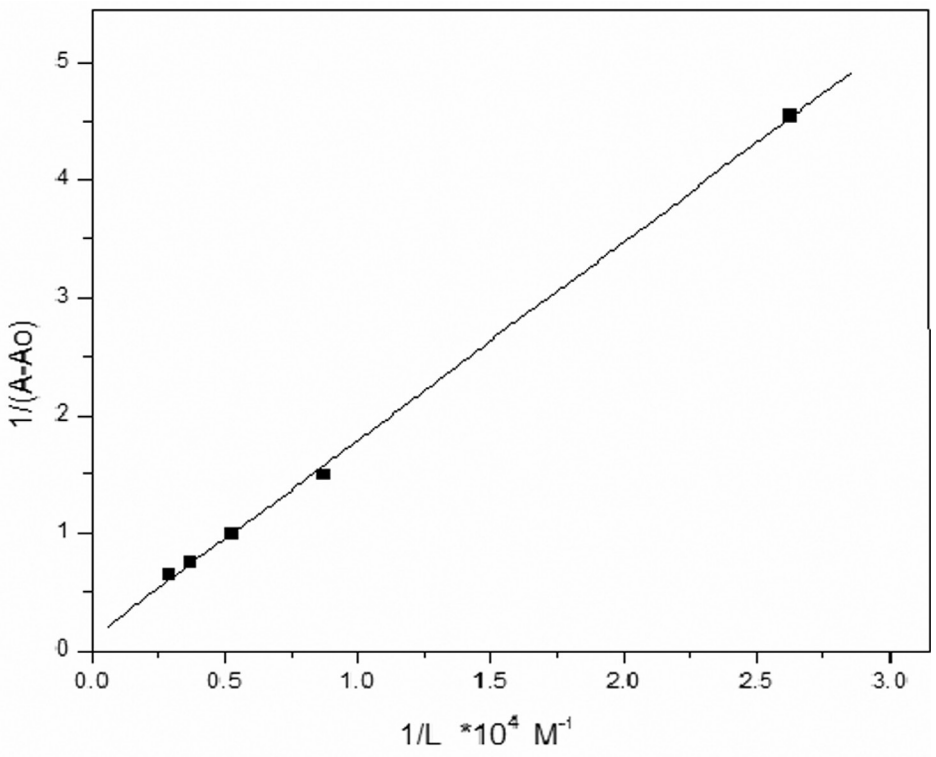

Figure 3: The plot of 1/(A-Ao) vs 1/L for HSA with different concentrations of progesterone.

biochemistry and molecular biophysics today [30].The fluorescence of HSA comes from tryptophan, tyrosine and phenylalanine residues. Actually, the intrinsic fluorescence of HSA is almost contributed by tryptophan alone, because phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized or near an amino group, a carboxyl group or a tryptophan [31].

In our work the fluorescence sensor is based on intramolecular charge transfer (ICT), which is highly sensitive to the polarity of microenvironment. Therefore, it is expected to act as fluorescent probe for some biochemical system like proteins [32].

The fluorescence quenching spectra of HSA at various molar ratios of progesterone is shown in Fig.(4). Obviously from the results, the fluorescence intensity of

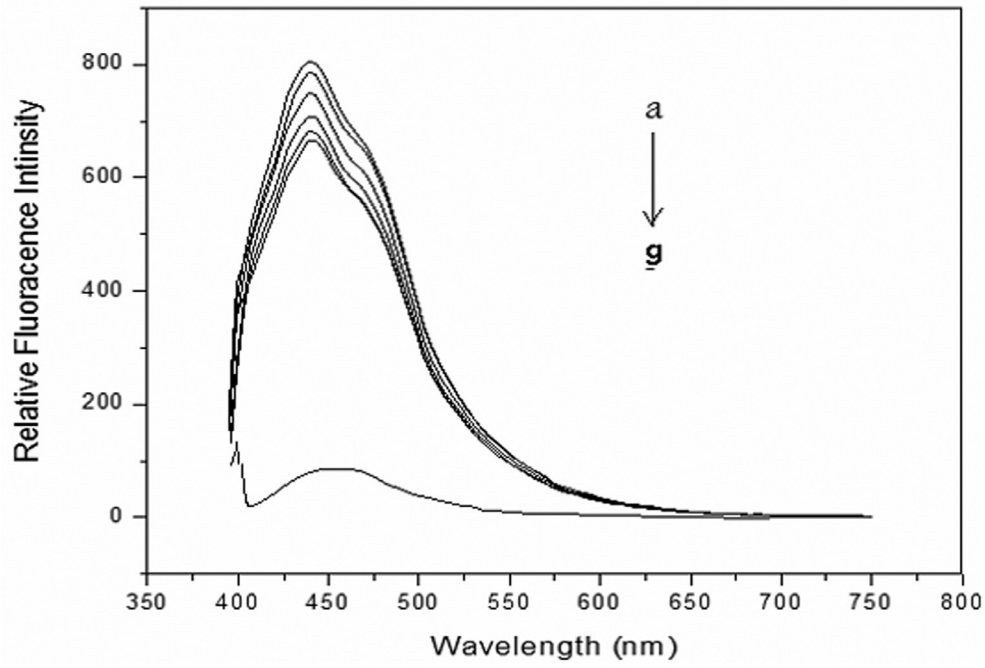

Figure 4: Fluorescence emission spectra of HSA in the absence and presence of progesterone in these ratios (Prog:HSA a=0:10, b=2:10, c=6:10, d=10:10, e=14:10, f=18:10, g= free progesterone).

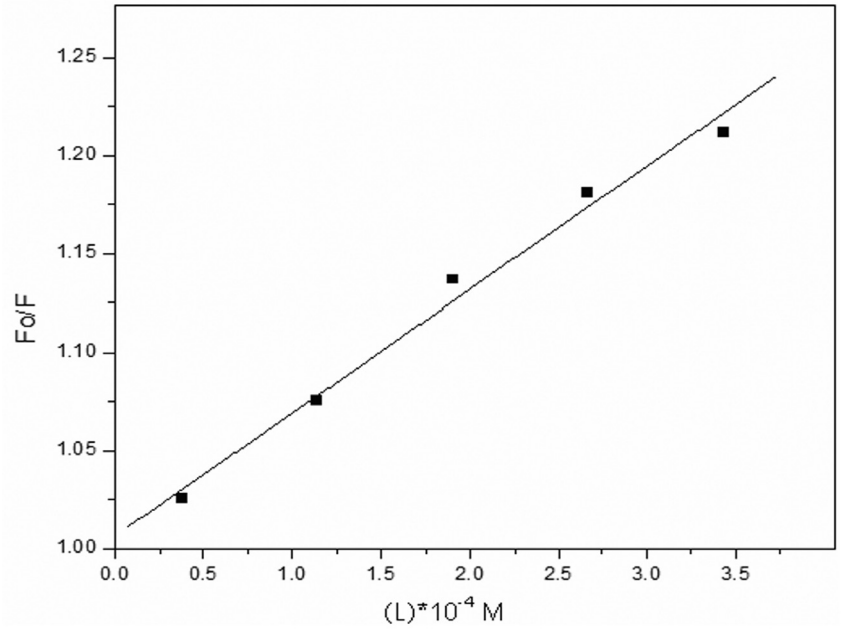

Figure 5: The Stern-Volmer plot for progesterone- HSA complexes.

HSA gradually decreased while the peak position shows little or no change upon increasing the molar ratio of progesterone to HSA, indicating that progesterone bind to HSA. Under the same condition, no fluorescence of progesterone was observed. Which indicates that progesterone could quench the auto fluorescence of HSA, and that the interaction between both progesterone from one hand, and HSA from the other indeed exists, leading to a change in the microenvironment around the tryptophan residue and further exposure of tryptophan residue to the polar solvent [33,34,35].

Fluorescence quenching can be induced by different mechanisms, usually classified into dynamic and static quenching [36]. Assuming dynamic quenching is dominating then the decrease in intensity is described by the well-known Stern-Volmer equation:

(4) $$\frac{F_0}{F} = 1 + K_q \tau_0 (L) = 1 + K_{sv} (L)$$

In this expression F and F_0 are the fluorescence intensities with and without quencher, respectively Ksv is the Stern-Volmer quenching constant, Kq is the bimolecular quenching constant, τ_0 is average lifetime of the biomolecule without quencher, and (L) is the quencher concentration. The Stern-Volmer quenching constant Ksv indicates the sensitivity of the fluorophore to a quencher.

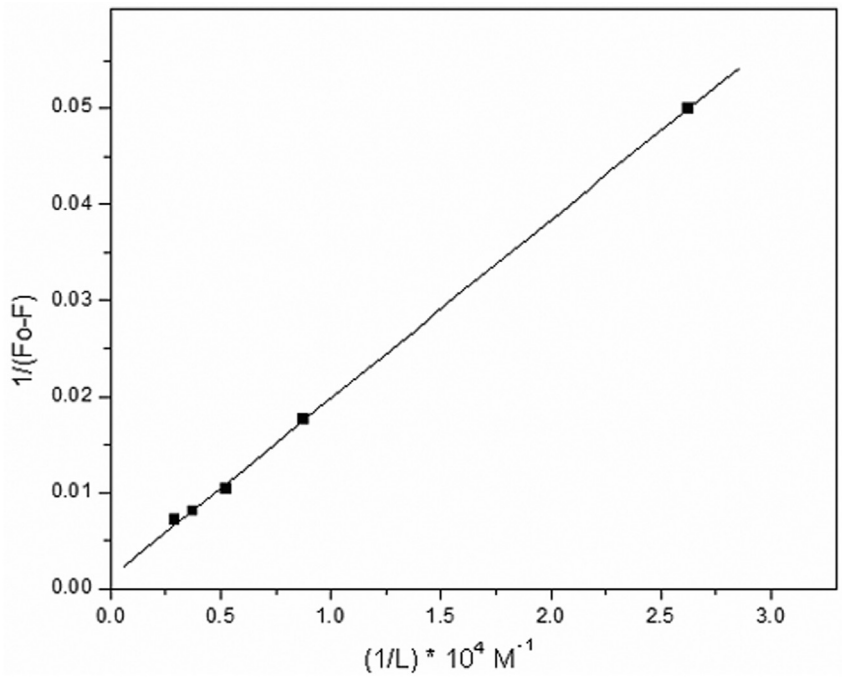

Figure 6: The plot of 1/(Fo-F) vs (1/L)×104 for progesterone- HSA complexes.

Linear curves were plotted according to the Stern–Volmer equation as shown in Fig.(5) for progesterone-HSA complexes. The Stern–Volmer quenching constant Ksv was obtained by the slope of the curve obtained in Fig.(5), and its value equals (6.26×10^2 L mol^{-1}). Obviously from equation 4 the value of Ksv=Kq τ_0 , from which we can calculate the value of Kq using the fluorescence life time of 10^{-8} s for HSA [37,38], to obtain Kq value of (6.20×10^{10} L mol^{-1}s^{-1}) for progesterone- HSA complexes. Which is larger than the maximum dynamic quenching constant for various quenchers with biopolymer (2×10^{10} L mol^{-1} s^{-1}) [39]. Which implies that the quenching is not initiated by dynamic collision but from formation of a complex, so static quenching is dominant in our case[36,40].

When static quenching is dominant the modified Stern-Volmer equation could be used [41]:

The acting forces between a small molecule substance and macromolecule mainly include hydrogen bond, van der Waals force, electrostatic force and hydrophobic interaction force. It was more likely that hydrophobic and electrostatic interactions were involved in the binding process. However, progesterone might be largely unionized under the experimental conditions, as expected from its structure. Hence, electrostatic interaction could be precluded from the binding process. Thus, the binding of progesterone to HSA includes the hydrophobic interaction [42].

This can be deduced using FTIR from the impact of complexation on progesterone antisymmetric and symmetric CH_2 stretching vibration in the region 3000-2800cm^{-1} see Fig.(7). Free progesterone CH_2 bands at 2933, 2872 and 2856 cm-1 shifted to 2959, 2937 and 2873 cm-1 after complexation (progesterone-HSA).

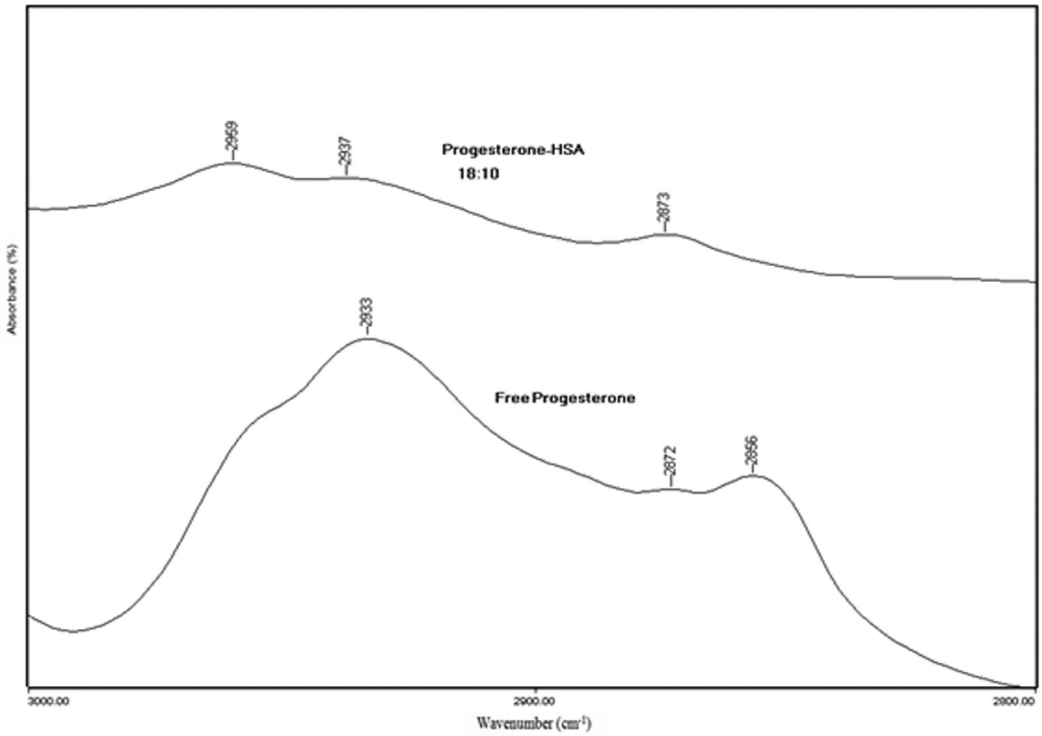

Figure 7: Spectral changes of progesterone CH2 symmetric and antisymmetric stretching vibrations upon HAS complexation.

(5) $$\frac{1}{F_0-F}=\frac{1}{F_0 K\ L}+\frac{1}{F_0}$$

Where K is the binding constant of progesterone with HSA, and can be calculated by plotting 1/(F_0-F) vs 1/L, see Fig.(6). The value of K equals the ratio of the intercept to the slope. The obtained values of K equals (6.56×10^2 M^{-1}), which agrees well with the value obtained earlier by UV spectroscopy and supports the effective role of static quenching. The highly effective quenching constant in this case has lead to a lower value of binding constant between progesterone and HSA, due to an effective hydrogen bonding between progesterone and HSA [11].

Major shifting of progesterone antisymmetric and symmetric CH_2 stretching vibration suggests the presence of hydrophobic interaction via progesterone aliphatic tails and hydrophobic region in HSA. The observed spectral shifting for progesterone CH_2 stretching modes is similar for that observed for its parent compound "cholesterol" [43].

3.3 FT-IR Spectroscopy.

FT-IR spectroscopy is a powerful technique for the study of hydrogen bonding [44], and has been identified as one of the few techniques that is established in the determination of protein secondary structure at different physiological systems [45,46]. The information on the

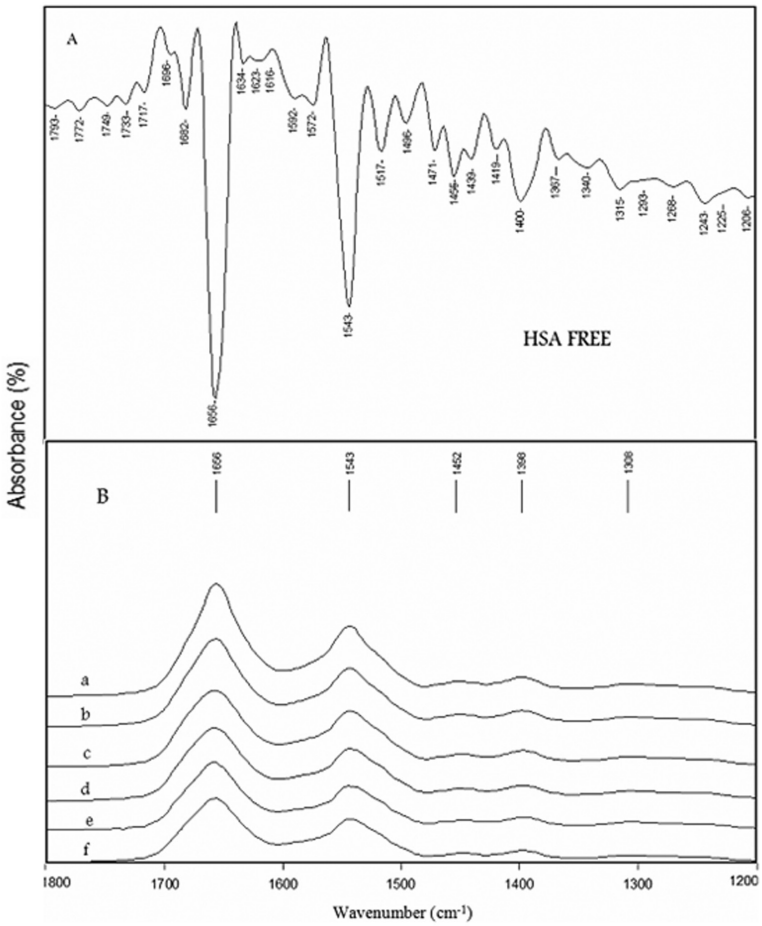

Figure 8: A: The spectra of HSA free (second derivative) And B: (a, b, c, d, e, f) Progesterone-HSA with ratios (0:10, 2:10, 6:10, 10:10, 14:10, 18:10), respectively.

secondary structure of proteins arises from the amide bands which results from the vibrations of the peptide groups of proteins. When drugs bind to a globular protein like HSA, changes in hydrogen bonding which is involved in the peptide linkages would occurs, resulting in changes in the vibrational frequency of the different amide modes [47,48].

The modes most widely used in protein structural studies are amide I, II and III. Amide I band ranging from 1700 to 1600 cm-1 and arises principally from the C=O stretching [49], and has been widely accepted to be used [50]. The amide II band is primarily N-H bending with a contribution from C-N stretching vibrations, amide II ranging from 1600 to 1480 cm-1.And amide III band ranging from 1330 to 1220 cm-1 which is due to the C-N stretching mode coupled to the in-plane N - H bending mode [46,51].

The second derivative of free HSA is shown in Fig. (8.A), where the spectra is dominated by absorbance bands of amide I and amide II at peak positions 1656cm-1 and 1543cm-1, respectively. Fig.(8.B), shows the spectrum of progesterone- HSA complexes with different molar ratios of progesterone. It is obviously seen as progesterone molar ratios was increased the intensities of the amide I, amide II and amide III bands decreased further in the spectra of all progesterone- HSA complexes. The reduction in the intensity of the three amide bands is related to the progesterone- HSA interactions.

In Table (1) the peak positions of HSA with different molar ratios of progesterone are listed. The amide bands of HSA infrared spectrum shifted as listed in Table (1), for amide I band the peak positions have shifted as follows: 1616 to 1615cm-1, 1634 to 1628cm-1, 1656 to 1658cm-1, 1682 to 1681cm-1, and 1696 to 1692cm-1. Also a peak at 1623cm-1 has disappeared after the interaction of progesterone with HSA. In amide II the peak positions have shifted as follows: 1496 to 1498cm-1, 1517 to 1516cm-1, 1543 to 1548cm-1, 1572 to1569cm-1, and 1592 to 1596cm-1. In addition a new peak at 1584cm-1 appeared after the interaction of progesterone with HSA. In amide III region the peak positions have also been shifted in the following order: 1225 to 1226cm-1, 1243 to 1241cm-1, 1268 to 1266cm-1, and 1315 to 1311cm-1. In addition a new peak has appeared at high molecular ratios of progesterone at 1276cm-1, also a peak at 1293cm-1 has disappeared after the interaction of progesterone with HSA.

The shifts in peak positions and shape of HSA amides after complexation with progesterone come from the changes in protein secondary structure. The minor changes in peak positions can be attributed to the effect of the newly imposed H-bonding between progesterone

molecules with the protein. It is suggested that, the shift to a higher frequency for the major peak in amide I region (1656–1658cm-1) came as a result of stabilization by hydrogen bonding by having the C–N bond assuming partial double bond character due to a flow of electrons from the C=O to the C–N bond [52].

The difference spectra for [(HSA + progesterone) - (HSA)] were obtained in order to monitor the intensity variations of these vibrations; and results are shown in Fig.(9). Clearly in amide I region there is a strong negative feature at 1656cm-1, and in amide II region one negative feature was also observed at 1544cm-1.

It is clearly shown that the strong negative features in the difference spectra became stronger as molar ratios of progesterone were increased with a little shift in their positions, which are attributed to the intensity decrease in the amide I and II bands in the spectra of the progesterone–HSA complexes, that is due to the interaction (H bonding) of progesterone with protein C=O and C-N groups, and the reduction of the proteins α-helix structure upon progesterone–HSA interaction [28,53].

The band at 1523 cm-1 of the free HSA, related to the tyrosine amino acid side-chain vibration [54,55,56,57], exhibited no spectral changes upon progesterone complexation, which is indicative of no perturbation of the tyrosine residue in the progesterone– HSA complexes.

Determination of the secondary structure of HSA and its progesterone complexes was carried out on the basis of the procedure described by Byler and Susi [58]. In this work, a quantitative analysis of the protein secondary structure for the free HSA and progesterone– HSA complexes in dehydrated films is determined from the shape of Amide I, amide II and amide III bands. Infrared Fourier self-deconvolution with second derivative resolution and curve fitting procedures, were applied to increase spectral resolution and therefore to estimate the number, position and area of each component bands. The procedure was in general carried out considering only components detected by second derivatives and the half widths at half height (HWHH) for the component peaks are kept around 5cm-1 , the above procedure was reported in our recent publication [11].

The component bands of amide I, II, and III regions were assigned to a secondary structure according to the frequency of its maximum a raised after Fourier self deconvolution have been applied; for amide I band ranging from 1610 to 1700cm-1 generally assigned as follows: 1610–1624 cm-1 are generally represented to β-sheet, 1625–1640 cm-1 to random coil, 1646–1671 cm-1 to α-helix, 1672–1787 cm-1 to turn structure, and 1689-1700cm-1 to β-antiparallel. For amide II ranging from 1480 to 1600cm-1, the absorption band assigned

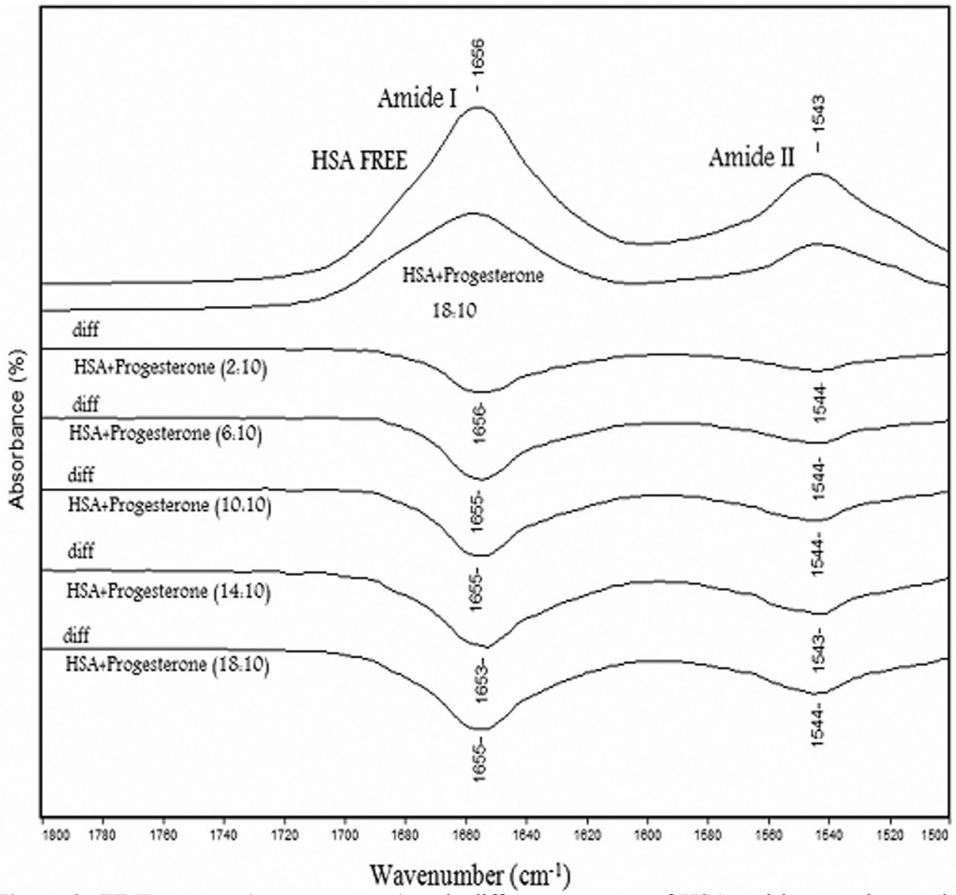

Figure 9: FT-IR spectra (top two curves) and difference spectra of HSA and its complexes with different progesterone concentrations in the region 1800-1500 cm-1.

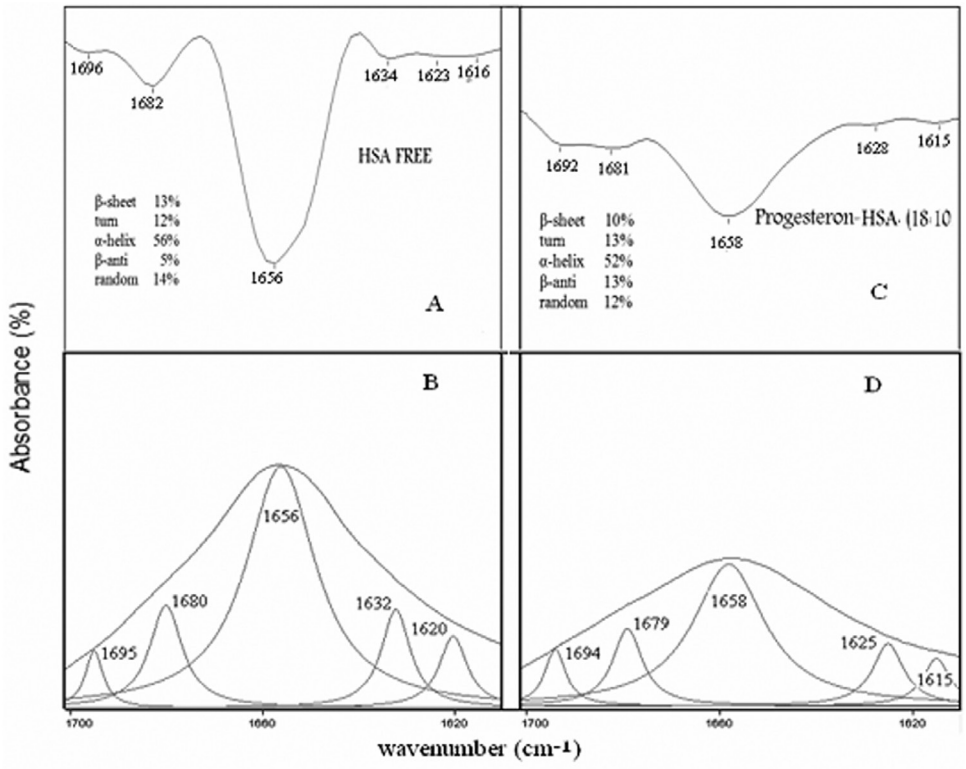

Figure 10: Second-derivative enhancement and curve-fitted Amide I region (1610-1700 cm-1) and secondary structure determination of the free human serum albumin (A and B) and it progesterone complexes (C and D) with 18:10 progesterone: HSA ratios.

in the following order: 1488–1504 cm-1 to β-sheet, 1508–1523 cm-1 to random coil, 1528–1560 cm-1 to α-helix, 1562–1585 cm-1 to turn structure, and 1585-1598cm-1 to β-antiparallel. And for amide III ranging from 1220 to 1330cm-1 have been assigned as follows: 1220–1256 cm-1 to β-sheet, 1257–1285 cm-1 to random coil, 1287–1301 cm-1 to turn structure, and 1302–1329 cm-1 to α-helix. Most investigations have concentrated on Amide I band assuming higher sensitivity to the change of protein secondary structure [59]. However, it has been reported that amide II and amide III bands have high information content and could be used for prediction of proteins secondary structure [60,61,62].

Based on the above assignments, the percentages of each secondary structure of HSA were calculated by integrated areas of the component bands in amide I, II, and III then summed and divided by the total area. The obtained number is taken as the proportion of the polypeptide chain in that conformation.

The Secondary structure determination for the free HSA and its progesterone complexes with different progesterone molar ratios are given in (Table 2). The second derivative resolution enhancement and curve – fitted Amide I, region and secondary structure determinations of the free human serum albumin (A, B) and its progesterone complexes (C, D) with the highest molar ratio of progesterone in dehydrated films are shown in Fig.(10).

The percentage values for the components of Amide I of free HSA are consistent with the results of other recent spectroscopic studies [63]. The results of Amide II and Amide III showed similar trends in their percentage values to that of Amide I. The reduction of α-helix intensity percentage in favor of the increase of β-sheets are believed to be due to the unfolding of the protein in the presence of progesterone as a result of the formation of H bonding between HSA and the hormone. The newly formed H-bonding result in the C–N bond assuming partial double bond character due to a flow of electrons from the C=O to the C–N bond which decreases the intensity of the original vibrations [52], the hydrogen bonds in α-helix are formed inside the helix and parallel to the helix axis, while for β-sheet the hydrogen bonds take position in the planes of β-sheets as the preferred orientations especially in the anti-parallel sheets, so the restrictions on the formation of hydrogen bonds in β-sheet relative to the case in α-helix explains the larger effect on reducing the intensity percentage of α-helix to that of β-sheet. Similar conformational transitions from an α-helix to β-sheet structure were observed for the protein unfolding upon protonation and heat denaturation [64,65].

Table 1: Band assignment in the absorbance spectra of HSA with different progesterone molecular ratios for amide I, amide II, and amide III regions.

Bands	HSA free	Prog:HSA 2:10	Prog:HSA 6:10	Prog:HSA 10:10	Prog:HSA 14:10	Prog:HSA 18:10
	1616	1616	1615	1613	1612	1615
	1623					
Amide I	1634	1632	1630	1627	1627	1628
(1700-1600)	1656	1654	1653	1658	1659	1658
	1682	1682	1682	1680	1679	1681
	1696	1692	1691	1691	1691	1692
	1496	1497	1497	1498	1498	1498
	1517	1516	1515	1515	1515	1516
Amide II	1543	1546	1548	1548	1549	1548
(1600-1480)	1572	1572	1570	1569	1567	1569
			1589	1586	1584	1584
	1592	1594	1595	1595	1596	1596
Amide III	1225	1226	1226	1226	1226	1226
	1243	1243	1243	1242	1241	1241
(1330-1220)	1268	1269	1269	1267	1266	1266
					1276	1276
	1293					
	1315	1314	1313	1312	1311	1311

Table 2: Secondary structure determination for Amide I, amide II, and amide III regions in HSA and its Progesterone complexes.

2nd Structure	HSA free(%)	HSA-Prog 2:10(%)	HSA-Prog 6:10(%)	HSA-Prog 10:10(%)	HSA-Prog 14:10(%)	HSA-Prog 18:10(%)
Amide I						
β-sheets (cm^{-2}) (1610-1624) (1689-1700)	17	31	31	30	30	34
Random(cm^{-2}) (1625-1640)	8	3	4	4	3	2
α-hilex (cm^{-2}) (1646-1669)	57	51	49	52	53	49
Turn (cm^{-2}) (1672-1687)	18	15	15	14	16	15
Amide II						
β-sheets (cm^{-2}) (1488-1504) (1585-1598)	25	30	33	32	33	33
Random(cm^{-2}) (1508-1523)	7	10	11	10	10	10
α-hilex (cm^{-2}) (1528-1560)	53	46	43	45	43	43
Turn (cm^{-2}) (1562-1585)	15	14	13	13	14	14
Amide III						
β-sheets (cm^{-2}) (1220-1256)	40	41	41	46	44	45
Random(cm^{-2}) (1257-1285)	8	9	13	7	11	10
Turn (cm^{-2}) (1287-1301)	6	6	3	4	4	4
α-hilex (cm^{-2}) (1302-1329)	46	44	43	43	41	41

CONCLUSION

The binding of progesterone to HSA has been investigated by UV-absorption spectroscopy, fluorescence spectroscopy and by FTIR spectroscopy. From the UV and Fluorescence study we determined values for the binding constant and the quenching constant for progesterone-HSA complexes. The results indicate that the intrinsic fluorescence of HSA was quenched by progesterone through static quenching mechanism. Analysis of the FTIR spectra reveals that HSA – progesterone interaction results in major protein secondary structural changes in the compositions of α-helix to that of the β-sheets.

Acknowledgements

This work is supported by the German Research Foundation DFG grant No. DR228/24-2

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