# Spectroscopic Studies of the Purified Testosterone Receptors in Human Benign and Malignant Prostatic Tumors

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## ABSTRACT:

**OBJECTIVE:**

To study the human benign and malignant prostatic testosterone receptors spectroscopically in the UV region.

**MATERIALS AND METHODS:**

In this study, spectroscopic characterization in the UV region was carried out on purified testosterone receptors by determining the polarity and pH effects on receptor UV spectra. Spectrophotometric pH titration and the observation of the thermal stability of testosterone receptors were also included in the present study.

**RESULTS:**

The UV spectra of purified testosterone receptors separated from benign prostatic tumors. BI receptor has two peaks at 196nm and 256.4nm, BII purified receptor gives one peak at 195.5nm, MI purified receptor gives one peak at 194nm and MII purified receptor gives one peak at 193.1nm.

**CONCLUSION:**

This study provides useful information about the spectroscopic characterization of testosterone receptors isolated from two types of human prostatic tumors.


***KEY WORDS:*** Benign, Malignant, Prostatic tumors, Ultra-Violet, Perturbation, pH titration.

## INTRODUCTION:

Androgens play a crucial role in several stages of male development and act on their target cells via an interaction with the androgen receptor resulting in direct regulation of gene expression [(1)]. One of the target organs of androgen is the prostate. Development and maintenance of differentiated function of the normal prostate gland require androgen[(2-4)].

Androgen has also been implicated in the abnormal growth, since neither cancer nor hyperplasia develops in castrates. The understanding of the hormonal regulation of normal and diseased human prostates is incomplete [(3,4)].

Molecules absorb light. The wavelengths that are absorbed and the efficiency of absorption depends on both the structure and the environment of the molecule, making absorption spectroscopy a useful tool for characterizing both small and large molecules [(5)].

The ultraviolet absorption maxima of proteins undergo shifts to shorter wavelengths (blue shift) and slight decreases in intensity[(6,7)]. When the secondary and tertiary structures of the molecules are disrupted. Attention has been directed particularly to the tyrosyl side chains, because of the possible contribution of their phenolic hydroxyl groups to the stability of the tertiary structure of proteins via hydrogen bonding [(8)]. The absorption spectrum of a chromophore is primarily determined by the chemical structure of the molecule. However, a large number of environmental factors produce detectable changes in $\lambda_{max}$ and $\varepsilon$. Environmental factors consist of pH, the polarity of the solvent or neighboring molecules, and the relative orientation of neighboring chromophores. It is precisely these environmental effects that provide the basis for the use of absorption spectroscopy in characterizing macromolecules [(5)].

For polar chromophores, the value of $\lambda_{max}$ for $n \longrightarrow \pi^*$ transitions occurs at a shorter wavelength in polar hydroxylic solvents ($H_2O$ and alcohols) than in non-polar solvents. The shift is toward longer wavelengths for $\pi \longrightarrow \pi^*$ transitions. The $\pi \longrightarrow \pi^*$ transition is the more common transition for biological molecules. An exception are the amino acids that are usually studied in conformational analysis; these are $n \longrightarrow \pi^*$ transitions, so that the spectrum is shifted toward the blue region[(5)].

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## MATERIALS AND METHODS:

### PATIENTS:

The benign prostatic hyperplasia (BPH) patients group comprised twenty five men, aged 64-68 years [mean age 65.96±1.15 (SD) years], while prostatic adenocarcinoma (PCA) patients consisted of thirteen men, aged 72-75 years [mean age 73.66±0.62 (SD) years]. All tumors are without any type of prostatitis. All patients underwent transurethral resection prostatectomy (TURP). Non of the patients had a history of chronic illnesses like diabetes mellitus, hypertension and cardiovascular diseases.

Preparation of prostatic tumors tissues homogenates

The human prostatic tumor tissues were weighed, sliced finely with a scalpel in Petri dish standing on ice bath, the slices were thawed and further minced with scissors then homogenized in TEMG buffer with a ratio of 1:5 (weight: volume) using a manual homogenizer. The homogenate was filtered through four layers of nylon gauze in order to eliminate fibers of connective tissues, then centrifuged at 2000 xg for 75 min at 4°C. The sediment was suspended in 10 volumes of the TEMG buffer for 15 min at 4°C and then the suspension was used to obtain the crude nuclear fraction [9,10].

Nuclear testosterone receptors were purified by gel filtration technique according to a procedure adapted in a previous work[11]. The elution profiles of benign prostatic tumors gave two different peaks with different molecular weights corresponding to different testosterone receptors (BI & BII), malignant prostatic tumors also gave two different peaks with different molecular weights corresponding to different testosterone receptors (MI & MII) [11].

The U.V. spectra of purified nuclear testosterone receptors

One hundred micro liters (350 µg protein) of each purified nuclear receptor was completed to 0.5 ml with distilled water pH 7.4, then placed in a 0.5cm cuvette in sample beam and the absorption spectrum was immediately measured against the adjusted pH. The distilled water as a reference.

Factors affecting the absorption properties of purified nuclear testosterone receptors

- pH effect

One hundred micro liters (350 µg protein) of purified receptors were completed to 0.5 ml with distilled water at different pH (2,6,7.2,8.2,9.2,and 12) then each of which was placed in the test cell and the adjusted pH distilled water was placed in the reference cell and the absorption spectra of different purified receptors were measured immediately.

- Polarity effect

**a.** The effect of 20% ethanol on the testosterone receptors spectra:

One hundred micro liters (350 µg protein) of purified receptors were completed to 0.5 ml with distilled water contains 20% ethanol at pH 7.2 then each of which was placed in the test cell and the 20% ethanol adjusted pH was placed in the reference cell using 0.5 cm cuvette. The absorption spectrum of each sample was measured immediately.

**b.** The effect of 20% ethylene glycol on the testosterone receptors spectra:

One hundred micro liters (350 µg protein) of purified receptors were completed to 0.5 ml with distilled water contains 20% ethylene glycol at pH 7.2 then each of which was placed in the test cell and the 20% ethylene glycol adjusted pH was placed in the reference cell using 0.5 cm cuvette. The absorption spectrum of each sample was measured immediately.

**c.** The effect of 20% urea on the testosterone receptors spectra:

One hundred micro liters (350 µg protein) of purified nuclear receptors were completed to 0.5 ml with distilled water at pH 7.2 containing 20% urea then placed in the test cell against the 20% urea adjusted pH in the reference cell using 0.5 cm cuvette. The absorption spectra of different purified receptors were measured immediately.

**d.** The effect of NaCl and $CaCl_2$ on the testosterone receptors spectra:

One hundred micro liters (350 µg protein) of purified nuclear receptors were completed to 0.5 ml with distilled water at pH 7.2 containing separately 0.1 M NaCl and 25 mM $CaCl_2$, then each of which was placed in a 0.5 cm cuvette in the test beam against an appropriate blank in the reference beam. The absorption spectra were measured immediately.

Spectrophotometric pH titration of purified nuclear testosterone receptors

A series of purified nuclear receptors (350 µg protein in 100 µl) were completed to 0.5 ml with distilled water at pH ranging from 9.0 to 12.5. The

maximum absorbance of each sample was measured at a wavelength of 295 nm, the absorbance of $\lambda_{max}$ at each pH value was plotted versus the corresponding pH.

Another series of purified receptors were completed to 0.5 ml with distilled water at pH range from 4 to 8.0. The maximum absorbance of each sample was measured at a wavelength of 210 nm. The absorbance of $\lambda_{max}$ at each pH value was plotted against the corresponding pH.

Observation of the helix coil transition of the purified nuclear testosterone receptors

One hundred micro liters (350 µg protein) of purified nuclear receptors were completed to 0.5 ml with 20% ethylene glycol and 0.01 M NaCl dissolved in distilled water at pH 7.2. Each mixture was placed in 0.5 cm cuvette in the sample beam and the maximum absorbance of each purified receptor was measured at a wavelength of 292 nm at increasing temperatures (20 to 70°C) against the reference (ethylene glycol-NaCl adjusted pH solution).

The maximum absorbance of each purified receptor was plotted against the different temperatures. The experiment was repeated for each purified receptor with another solution (20% ethylene glycol-0.1M NaCl).

The U.V. spectra of $^{125}$I-testosterone and the different $^{125}$I-testosterone receptor complexes

- The U.V. spectra of different human testosterone-receptors complexes

The binding experiment of different purified nuclear testosterone receptors with $^{125}$I-testosterone was carried out at the optimum conditions. Half milliliter of the $^{125}$I-testosterone-receptor complex supernatant of each type of purified receptors was placed in 0.5cm cuvette in the sample beam and the absorption spectrum was measured immediately against an appropriate blank in the reference beam.

- The U.V-spectrum of $^{125}$I-testosterone

Half milliliter of $^{125}$I-testosterone was placed in a 0.5cm cuvette in the sample beam and the absorption spectrum was measured immediately against an appropriate blank in the reference beam.

**RESULTS:**

Figure (1 A&B) illustrates the U.V. spectra of purified testosterone receptors at pH 7.2. The U.V. spectra show that the $\lambda_{max}$ for the purified receptor BI is consisted of two peaks; at 196 nm and 256.4 nm, BII-purified receptor gives one peak at 195.5 nm, MI-purified receptor gives one peak at 194 nm and MII-purified receptor gives one peak at 193.1 nm.

A trial to calculate the specific absorption coefficients ($a_s$) of human testosterone receptors revealed that $a_{s(196\ nm)}$ was found to be $8.85\times10^{4}\,cm^{-1}.g^{-1}.L$ and $a_{s(256.4\ nm)}$ was equal to $3.34\times10^{4}\,cm^{-1}.g^{-1}.L$ for BI-purified receptor, $a_{s(195.5\ nm)}$ was found to be $4.53\times10^{4}\,cm^{-1}.g^{-1}.L$ for BII-purified receptor, $2.61\times10^{4}\,cm^{-1}.g^{-1}.L$ was the $a_{s(194\ nm)}$ value for MI-purified receptor and $a_{s(193.1\ nm)}$ value for MII-purified receptor was found to be $3.06\times10^{4}\,cm^{-1}.g^{-1}.L$ according to the Lambert-Beer's law[12].

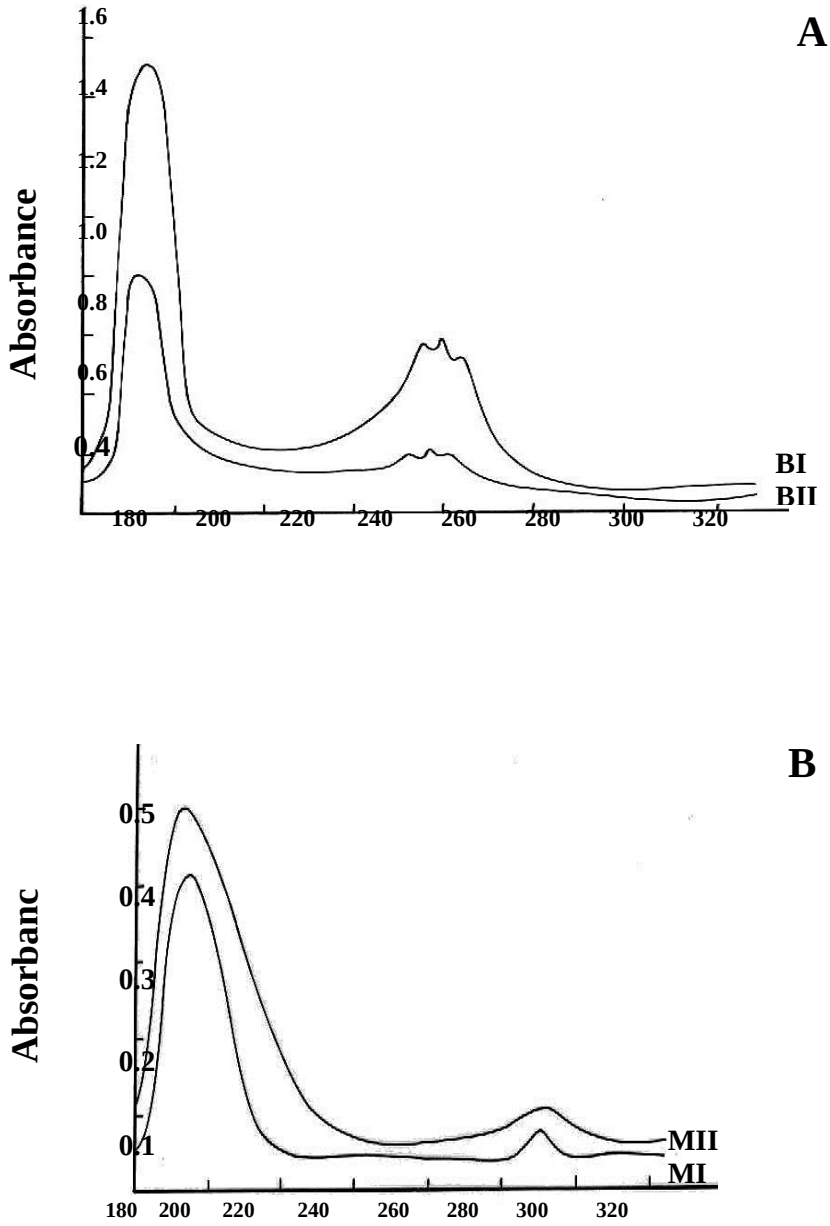


**Figure (1): The U.V. spectra of purified nuclear testosterone, A) BI & BII receptors, B) MI & MII receptors.**

Table (1) lists the effect of pH on the $\lambda_{max.}$ values of different testosterone receptors spectra.

**Table (1): The effect of pH on the $\lambda_{max}$ of testosterone receptors spectra.**

| pH | BI-purified receptor | BII-purified receptor | MI-purified receptor | MII-purified receptor |
|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) |
| 2 | 206.1, 255.6, 273, 293 | 209, 275, 293.5 | 221, 276, 294 | 295 |
| 6 | 192.8 | 196.2 | 196.4 | 193.2 |
| 7.2 | 196, 256.4 | 195.5 | 194 | 193.1 |
| 8.2 | 193.2 | 193.1 | 192.8 | 192.4 |
| 9.2 | 193.5 | 193.8 | 192.8 | 195.4 |
| 12 | 295.5 | 295.1 | 295.2 | 295.3 |

Table (2) shows the effect of 20% ethanol, 20% ethylene glycol and 20% dimethylsulfoxide at neutral pH on the testosterone receptors spectra. In 20% ethanol, it was found that one $\lambda_{max}$ was obtained for each receptor, at 214, 213.8, 216.2 and 214.5 nm for BI, BII, MI and MII–purified receptors respectively which were assigned to tryptophan residues, while in the case of 20% DMSO, a newer $\lambda_{max}$ was appeared for each purified receptor, these are 281.6, 282.6, 283 and 283.4 nm for BI, BII, MI and MII respectively which were assigned to tryptophan residues.

**Table (2): The effect of 20% ethanol, ethylene glycol and dimethylsulfoxide on the $\lambda_{max}$ of testosterone receptors spectra.**

| Solvent | BI-purified receptor | BII-purified receptor | MI-purified receptor | MII-purified receptor |
|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) |
| 20% ethanol | 214 | 213.8 | 216.2 | 214.5 |
| 20% ethylene glycol | 247.8, 222 | 257 | - | - |
| 20% DMSO | 281.6 | 282.6 | 283 | 283.4 |

Figure (2 A&B) illustrates the $\lambda_{max}$ of human testosterone receptors BI and BII in 20% ethylene glycol at neutral pH.

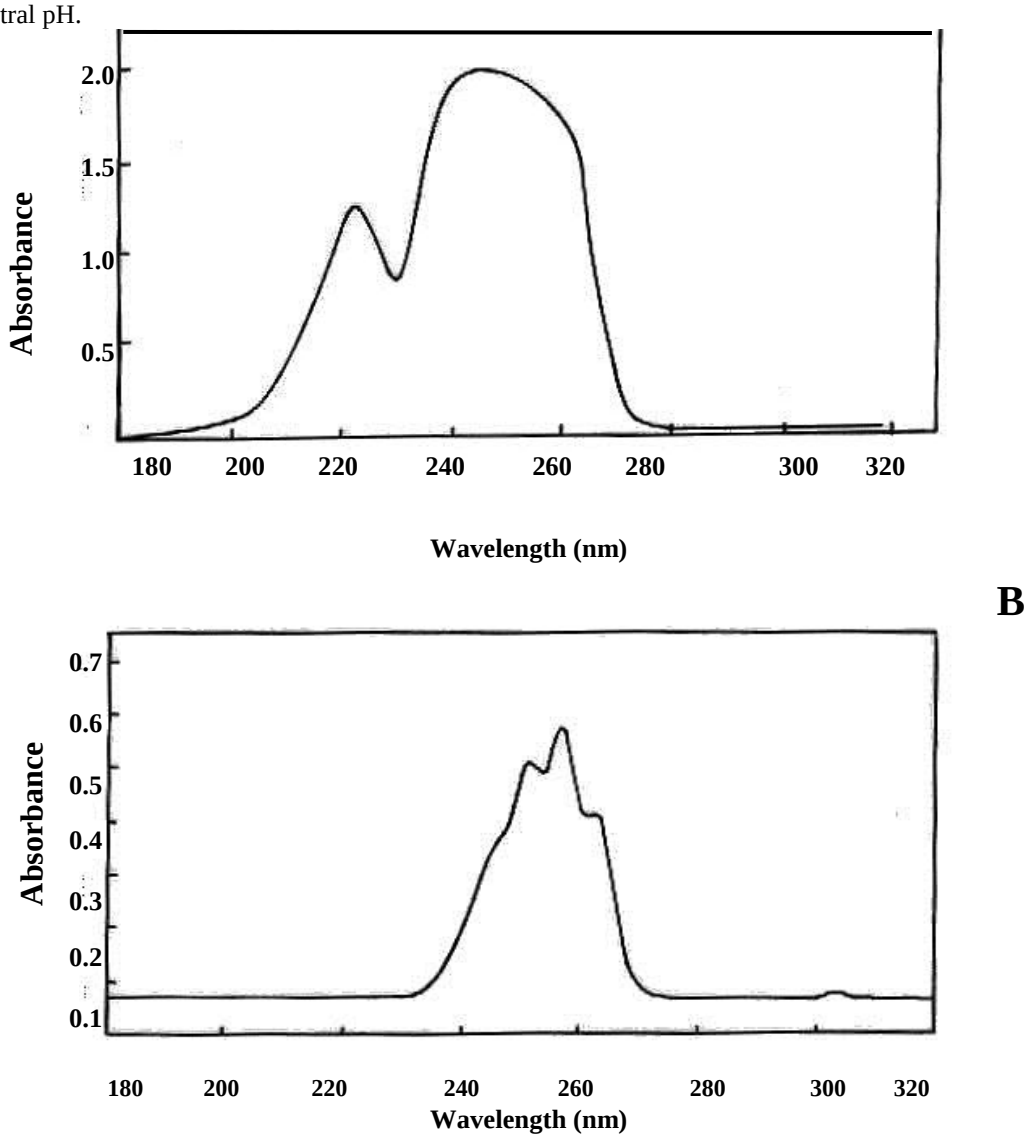


**Figure (2): The effect of 20% ethylene glycol on, A) BI-purified receptor, B) BII-purified receptor.**

Table (3) shows the effect of urea on the testosterone receptors U.V. spectra at pH 7.2.

**Table (3): The effect of 20% urea on the $\lambda_{max}$ values of testosterone receptors spectra at neutral pH.**

| Purified receptor | $\lambda_{max}$ (nm) |
|---|---|
| BI | 293.21 |
| BII | 222.35 |
| MI | 295.28 |
| MII | 222.02 |

The effect of sodium chloride and calcium chloride salts on the $\lambda_{max.}$ values of testosterone receptors was also studied as shown in table(4).

**Table (4): The effect of sodium chloride and calcium chloride on the $\lambda_{max}$ values of testosterone receptors spectra.**

| Solvent | BI-purified receptor | BII-purified receptor | MI-purified receptor | MII-purified receptor |
|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) | $\lambda_{max}$ (nm) |
| 100 mM NaCl | 205.4 | 204.8 | 205.0 | 205.4 |
| 25 mM $CaCl_2$ | 204.4 | 204.0 | 203.6 | 203.6 |

Figure (3 A&B) shows the pH titration curves of testosterone receptors for tyrosine and histidine respectively. (A) curves show that the $pk_a$ values for tyrosine are 10.1, 11.5, 11.5 and 11.6 for BI, BII, MI and MII–purified receptors respectively, while the $pk_a$ values for histidine in (B) curves were equal to 5.5, 7.48, 6.1 and 7.5 for BI, BII, MI and MII–purified receptors respectively.

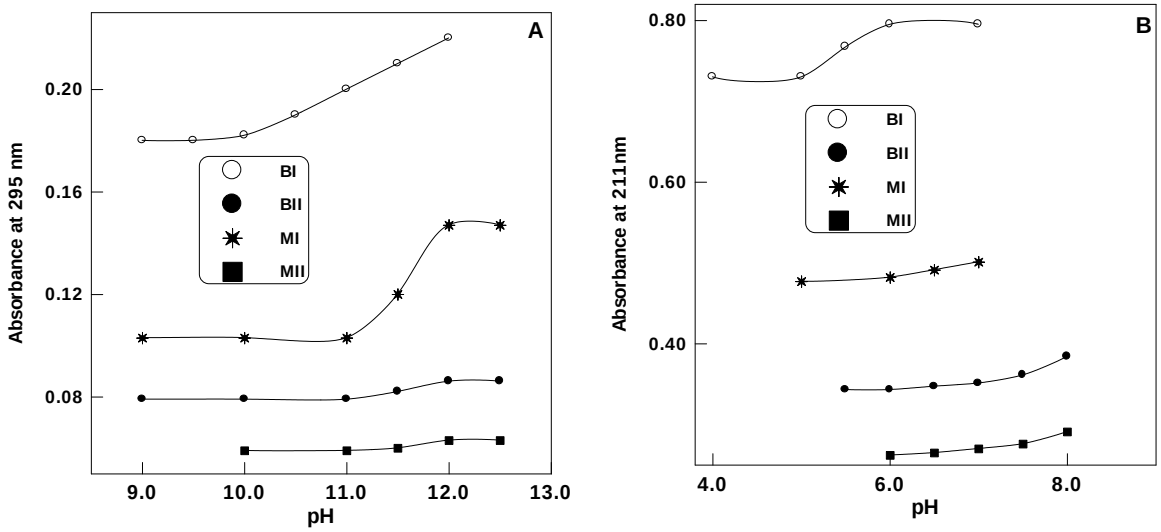


**Figure (3): Spectrophotometric pH titration of purified nuclear testosterone receptors for, A) tyrosine residues, B) histidine residues**

Table (5) shows the $\lambda_{max}$ and absorbance values in the absence and presence of different perturbants (20% ethanol, 20% ethylene glycol and 20% urea).

**Table (5): Solvent perturbation on purified testosterone receptors.**

| Perturbing substance | BI-purified receptor | | BII-purified receptor | | MI-purified receptor | | MII-purified receptor | |
|---|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | A | $\lambda_{max}$ (nm) | A | $\lambda_{max}$ (nm) | A | $\lambda_{max}$ (nm) | A |
| Without | 196<br>256.4 | 1.5<br>0.566 | 195.5 | 0.802 | 194 | 0.440 | 193.1 | 0.5 |
| 20% ethanol | 214 | 0.081 | 213.8 | 0.072 | 216.2 | 0.080 | 218.5 | 0.076 |
| 20% ethylene glycol | 247.8<br>222 | 1.998<br>1.285 | 257 | 0.574 | - | - | - | - |
| 20% urea | 293.21 | 0.133 | 222.35 | 0.740 | 295.28 | 0.099 | 222.02 | 0.471 |

Thermal stability analysis of human testosterone receptors was carried out at two different sodium chloride concentrations (0.01 and 0.1 M) as depicted in figure(4).

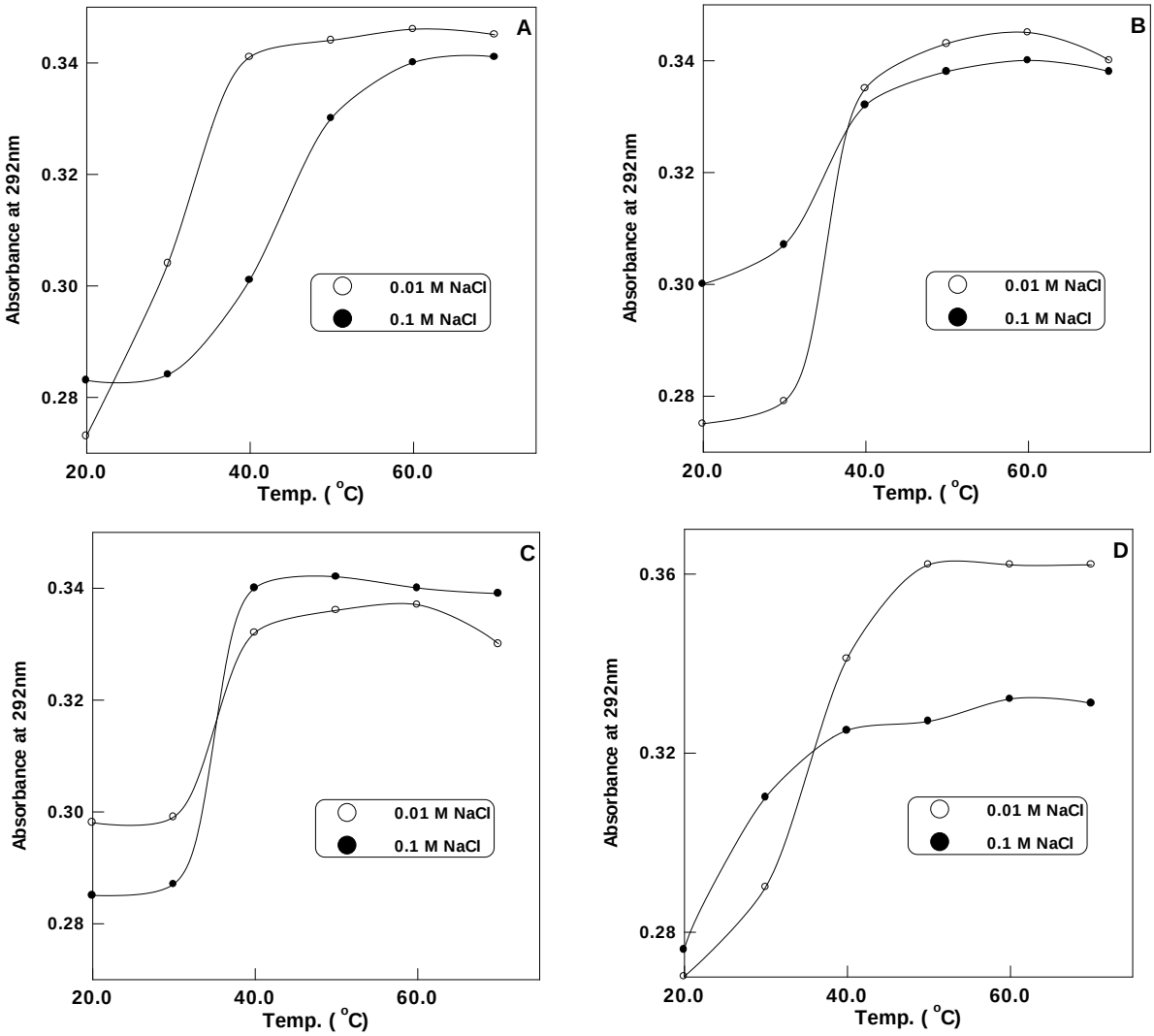


**Figure (4): Helix-coil transition of purified nuclear testosterone, A) BI-receptor, B) BII-receptor, C) MI-receptor, D) MII-receptor with different NaCl concentrations.**

The $\lambda_{max.}$ values of the U.V. spectra of $^{125}$I-testosterone and their complexes with different human testosterone receptors were recorded and listed in table (6)

**Table (6): The $\lambda_{max}$ values of the U.V. spectra of $^{125}$I-testosterone and its complexes with purified receptors.**

| Purified receptor | $\lambda_{max}$ (nm) |
|---|---|
| $^{125}$I-testosterone –R (BI) | 204.6 |
| $^{125}$I-testosterone –R (BII) | 204.4 |
| $^{125}$I-testosterone –R (MI) | 205.6 |
| $^{125}$I-testosterone –R (MII) | 205 |
| $^{125}$I-testosterone | 213.6, 267 |

## DISCUSSION:

Figure (1) shows that each human testosterone receptor has a characteristic spectrum and can be identified by their peaks. Maximum absorptions at 196, 195.5, 194 and 193.1 nm are assigned to tyrosine residues, while the vibrational structure as a small "wiggles" at 256.4 nm is assigned to phenylalanine [5,8]. Also it was found from the Figure (1 A&B) that tryptophan residues does not occur on the surface of benign receptors while it slightly occur on the surface of malignant receptors at 295 nm [8]. It seems that in BII, MI and MII–purified receptors, tyrosines are located in a way that part of it, is on the surface of the receptor molecules and the other parts are buried, whereas in BI–purified receptor, all tyrosine residues seem to be on the surface, exposed to absorbance. On the other hand, phenylalanine residues of the BI–receptor molecule seem to be on the surface, these residues partially buried in BII–receptor molecule while in MI and MII–receptors, these residues may be completely buried[5,8-11].

The absorption spectrum of a chromophore is primarily determined by the chemical structure of the molecule. However, a large number of environmental factors produce detectable changes in $\lambda_{max}$ and ε. Environmental factors such as pH and polarity of the solvent provide the basis for the use of absorption spectroscopy in characterizing macromolecules[5].

The pH of the solvent determines the ionization state of ionizable chromophores. Table (1) shows the $\lambda_{max}$ values for human testosterone receptors at different pH (2-12). At an acidic pH 2. BI–purified receptor has four $\lambda_{max}$ values at 206.1, 255.6 nm which were assigned to phenylalanine, 273 nm which was assigned to tyrosine and 293 nm which assigned to tryptophan residues.

In BII–purified receptor three $\lambda_{max}$ were obtained at 209, 275 and 293.5 nm which were assigned to phenylalanine, tyrosine and tryptophan respectively. In MI–purified receptor, three $\lambda_{max}$ were obtained, the first one at 221 nm, the second at 276 nm while the third at 294.0 nm, the first and the second peaks were assigned to tyrosine residues and the third one was assigned to tryptophan residues. In MII–purified receptor, one $\lambda_{max}$ was obtained at 295 nm which was assigned to tryptophan.

At neutral pH 7.2, BI–purified receptor spectrum consists of two $\lambda_{max}$, the first at 196 and the second at 256.4 nm, these $\lambda_{max}$ are assigned to tyrosine and phenylalanine respectively. In BII, MI and MII–purified receptors, there were one $\lambda_{max}$ at 195.5, 194 and 193.1 nm respectively which were assigned to tyrosine residues.

When the pH was increased from 8.2 to 9.2, there were no significant change in the $\lambda_{max}$ obtained for each receptor type and the tyrosine is the only residue present in all cases but a further increase in pH value from 9.2 to 12 has shown an increase in the $\lambda_{max}$ of tyrosine residues in all receptor types, this result is due to the dissociation of the phenolic OH of tyrosine ($pk_a$ = 10.07) giving an ionized form of this amino acid which absorps at higher wavelength (red shift) [5].

In general, these results may be explained partly by the induction effects, so when the pH lowered from 12 to 6, the spectral maxima of tyrosine residues shift toward shorter wavelength (blue shift). This shift is due to slight increases in the energies of electronic transitions of the tyrosine aromatic ring, resulting from the formation of the electron–withdrawing ammonium group. Many researchers underlined that these inductive effects of vicinal charges are quite small to account for the changes occurring in protein spectra and the spectral shifts of proteins produced by changing pH must therefore be attributed mainly to rearrangements of secondary and tertiary structure, although the possibility of field effects, due to unusually close conjunction of charges to aromatic groups is not excluded [8].

Values listed in table (2) reveal the vanish of phenylalanine and tyrosine absorbances and appearance of a new $\lambda_{max}$ value corresponding to tryptophan. These observations indicate that the protein was defolded due to change in the secondary and tertiary structure of the protein that bring the tryptophan to expose to absorbance while phenylalanine and tyrosine residues were buried inside the receptor molecule, also it was found that testosterone receptors are highly sensitive to change in the polarity of the solvent.

Table (2) and figure (2A and B) show that the $\lambda_{max}$ value of tyrosine was shifted towards longer wavelengths (red shift) in 20% ethylene glycol due to the hydrogen bonding of the OH groups of tyrosines with the solvent or with the $\pi$ - electron system of the benzene ring where tyrosine was functioned as a hydrogen donor, while the $\lambda_{max}$ value of phenylalanine was shifted towards shorter wavelengths in 20% ethylene glycol, this shift was attributed to $\pi \rightarrow \pi^*$ transitions [5,13]. These two shifts in $\lambda_{max}$ were accompanied with an increase in the absorbency of phenylalanine and a decrease in the absorbency of tyrosine, these findings could be attributed to a change in the protein structure that bring the phenylalanine residues to the surface of the protein while tyrosine residues were partly embedded in a hydrophobic region of the protein molecule.

The changes in the protein structure for BII-receptor may bury tyrosine residues in the internal region of the protein and bring phenylalanine to the molecule surface. Also, it was found that malignant receptors were very high sensitive to ethylene glycol, this solvent can bury all absorbing amino acids inside the hydrophobic region of the protein molecule.

Data listed in table ( 1 ) show two peaks in BI-purified receptor spectrum, $\lambda_{max1}$ at 196 nm and $\lambda_{max2}$ at 256.4 nm at pH 7.2. These peaks were assigned to tyrosine and phenylalanine respectively. In the presence of 20% urea pH 7.2 as shown in table(3), these two amino acids were buried inside the receptor molecules and tryptophan residues were appeared on the surface. Similar effect was obtained on the MI-purified receptor molecules, since tyrosine residues were buried and tryptophan residues were appeared on the surface of molecule with a new absorption peak. The results indicate that urea affects the testosterone purified receptors BI and MI structurally, since many chromophores which were embedded in an interior region of the receptor molecule where they were inaccessible to the solvent came into contact with it due to the unfolding of the molecule, and hence, different spectra were obtained [13]. The $\lambda_{max}$ of tyrosine residues in BII and MII-purified receptors were shifted towards longer wavelengths without affecting the structure of these receptors, the shift indicates that at 20% urea, the exposed tyrosines become solvated with urea (dipole-dipole interaction) [8,13].

Table (4) illustrates the effect of sodium chloride and calcium chloride salts on the U.V. spectra of testosterone receptors. The effect of these salts on increasing the binding extent of testosterone with its receptors in benign and malignant prostatic tumors ascertain us to study the effect of these salts on the testosterone receptors spectra. In BI-purified receptor, a blue shift was obtained in the $\lambda_{max}$ of phenylalanine residues in the different salts used. This blue shift is due to the negative or positive charges of the salt anions and also cations which might interact directly with the $\pi$-electron system of the benzene ring of phenylalanine amino acids [13]. In all purified receptors, it was found that tyrosine residues were buried inside the interior region of the protein molecule and phenylalanine residues were appeared on the molecular surface of these receptors.

Spectrophotometric pH titration is the following of the change in absorbance of the chromophore with increasing pH [5]. Many studies of protein structure require the determination of pk values for proton dissociation from ionizable amino acid side chains, because these values give an indication of the location of the amino acid in the protein. This can often be done spectrophotometrically because dissociation often changes the spectrum of one of the chromophores, the observation of tyrosine dissociation was performed by measuring the absorption at 295 nm ($\lambda_{max}$ for the ionized form of tyrosine), and the observation of histidine dissociation was carried out by measuring the absorption at 211 nm.

From the spectrophotometric pH titration curves in figure (3) , it was found that:

1) About 83.7, 91.8, 70 and 93.6% of tyrosine residues are located on the surface of the BI, BII, MI and MII-purified receptors molecule respectively.
2) About 16.3, 8.2, 30 and 6.4% of tyrosine residues are buried interior the folded structure of

the BI, BII, MI and MII-purified receptors respectively.

3) About 91.8, 89.33, 99.2 and 90 % of histidine residues are located on the surface of the BI, BII, MI and MII-purified receptors molecule respectively.

4) About 8.2, 10.68, 0.8 and 10% of histidine residues are embedded in the interior region of the BI, BII, MI and MII-purified receptors molecule respectively.

5) In BI-purified receptor the tyrosine residues were largely present on the surface of the molecule and the internal tyrosines are in a strongly nonpolar environment, while the internal tyrosine residues in BII, MI and MII purified receptors were in a strongly polar environment (e.g. a tyrosine surrounded by carboxyl groups). On the other hand, the histidine residues are largely present on the molecular surface of BI and MI-receptors and the internal residues are in a nonpolar environment whereas the internal histidine residues of BII and MII-purified receptors are likely to be in a strongly polar environment.

Finally, the percent of external tyrosine residues in MII-purified receptor was greater than that of BII-purified receptor and the percent of internal tyrosine in MI-purified receptor was greater than that of BI-purified receptor, on the other hand the percent of internal histidine in BI-receptor was greater than that of MI-receptor and the percent in BII-receptor was greater than that of MII-receptor.

The determination of whether an amino acid is internal or external by measuring the spectra of a protein in a polar and nonpolar solvents is called the solvent perturbation method. In fact, proteins are rarely studied in completely nonpolar solvents because most proteins are either insoluble or denaturated in these solvents; therefore, mixtures of 80% water and 20% of reduced polarity solvent were used [(5)]. Solvents alter the peak positions and intensities by altering the energy and probability of electronic transitions and this alteration arises from a difference in the solvation energies of the ground state and the first excited singlet state [(8)].

From the results of solvent perturbation experiment listed in table(5), it was found that several spectral changes were obtained in the presence of these perturbants, like the alteration of the $\lambda_{max}$ positions and intensities of testosterone receptors spectra, and the appearance of new chromophores on the surface of the receptor molecule. These chromophores were embedded in an interior region of the protein in the absence of solvent.

From the solvent perturbation studies, the following remarks could be drawn:

1) About 83, 93.2, 70 and 93.3% of tyrosine residues are on the surface of BI, BII, MI and MII-receptor molecule respectively. So, about 25, 28, 21 and 28 tyrosine residues are on the surface of BI, BII, MI and MII–purified receptor molecule, while 19, 18, 21 and 19 histidine residues may be considered as external amino acids [(14)].

2) There are about 5 tryptophan residues in the benign receptor active site and 2 tryptophan residues in the malignant receptor active site. These results are in accordance with those obtained previously. Many investigators underlined that tryptophan residues present in the active site of androgen receptor and play an important role in the interaction with testosterone [(15,16)].

Because buried chromophore becomes exposed to the solvent during denaturation, by monitoring the absorbance of these chromophores, one can observe the helix-coil transition (denaturation) for proteins [(17)]. For example, if a protein contains tryptophans, some of which are internal, the unfolding as a function of temperature could be detected by measuring the absorbance at 292 nm in a 20% ethylene glycol solution, also this could then be used to examine the effects of other agents such as NaCl concentration on the thermal stability. Figure (4 A, B, C&D) shows the thermal stability curves of purified testosterone receptors. The results obtained from these curves indicate that protein denaturation in 0.01 M-NaCl occurred at 50, 60, 40 and 50ºC for BI, BII, MI and MII-purified receptors respectively, while in 0.1 M-NaCl the denaturation occurred at 60, 60, 40 and 60ºC for BI, BII, MI and MII-purified receptors respectively, therefore, higher NaCl concentration causes more stabilization for the purified receptors. From the thermal stability curves, it was found that at 0.01 M-NaCl, BII-purified receptor was more thermostable than BI and MII-purified receptors

and those were more stable than MI-purified receptor.

These results are in accordance with those reported previously by many investigators on nuclear androgen receptor of rat ventral prostate which indicated that these proteins are heat labile at temperatures above 50ºC [18], also their heat-denaturation curve is similar to those obtained in our work.

Thermal stability analysis at 0.01 M-NaCl revealed that 6 tryptophan residues were embedded inside the internal region of BI and MII-purified receptors while 7 tryptophan residues were buried in an iterior region of the BII and MI-purified receptors.

The binding of ligand to the active site of a receptor frequently produces spectral changes in chromophores in or near the active site by affecting the polarity of the region or the accessibility to solvent this means that chromophores on the surface become inaccessible to the solvent by being buried in the region in which binding takes place or because a conformational change that buries or exposes a chromophore in another part of the molecule can accompany binding[5].

Table (6) shows the $\lambda_{max}$ values of $^{125}$I-testosterone and its complexes with purified receptors. These results indicated that the binding of testosterone with its receptors abolished the $\lambda_{max}$ values of free testosterone and also tyrosine residues were embedded in an interior region of the protein and appearance of phenylalanine residues on the molecule surface in all testosterone receptor types. The present study results supported the previous steroid enveloping concept which suggests that testosterone is bound by its receptors from multiple sides (α, β and peripheral) and testosterone then is being "enveloped" in the hydrophobic cavity [18].

The absorbances at 213.6 and 267 nm obtained for testosterone may be attributed to the $n\rightarrow\sigma^*$, $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions respectively.

## CONCLUSION:

Each human testosterone receptor has a characteristic spectrum and can be identified by their peaks at 196nm, 195.5nm, 194nm and 193.1nm which are assigned to tyrosine residues, while the vibrational structure as a small "wiggles" at 256.4nm (fingerprint of phenylalanine) is assigned to phenylalanine. Solvent perturbation studies demonstrate that about 83, 93.2, 70 and 93.3% of tyrosine residues are on the surface of BI,BII,MI and MII receptor molecule respectively and there are about five tryptophan residues in the benign receptor active site and two tryptophan residues in the malignant receptor active site.

## REFERENCES:


1. Kuil CW and Brinkmann AO : Androgens, antiandrogens and androgen receptor abnormalities. Eur. Urol. 1996; 29(suppl. 2): 78-82.
2. Lin MF, Meng TC, Rao PS, Chang C, Schönthal AH and Lin FF: Expression of human prostatic acid phosphatase correlates with androgen-stimulated cell proliferation in prostate cancer cell lines. J. Biol. Chem. 1998;273, 5939-5947.
3. Tenniswood M :Role of epithelial- stromal interactions in the control of gene expression in the prostate: An hypothesis. Prostate. 1986. 9: 375-385.
4. Kwon HC, Choi SH, Kim YU, Son SO and Kwon JY :Androgen action on hepatic vitellogenin synthesis in the ecl, Anguilla japonica is suppressed by an androgen receptor antagonist. The Journal of Steroid Biochemistry and Molecular Biology. 2005;96,175-178.
5. Freifelder D; Physical Biochemistry; 2nd ed.; 1982,500-517.
6. Beaven GH, Holiday ER and Jope EM ; Discussions Faraday Soc.; 1950; 9: 406.
7. Beaven GH and Holiday ER. "Advances in Protein Chemistry"; Academic Press, Inc.; New York, 1952 ; 7,319.
8. Yanari S and Bovey FA: Interpretation of the ultraviolet spectral changes of proteins. J. Biol. Chem. 1960; 235, 2818-2826.
9. Green B. and Leake R.E.; Steroid Hormones A Practical Approach; IRL Press Limited; 1987, 29, 30, 76-82.
10. Juzumiene D, Chang CY, Fan D, Hartney T, Norris JD and McDonnel DP: Single step purification of full-length human androgen receptor. Nuclear Receptor Signaling (NRS). 2005; 3,1-5.
11. Abdul-Rasheed of. Molecular Characterization of Testosterone Receptors in Prostatic Tumors by Radireceptor technique [M.S.c thesis]. Baghdad University- Iraq; 2000.
12. Segel I.H.; Biochemical Calculations; 2nd ed.; John Wiley and Sons; 1976, 327.

**13.** Leach SJ and Scheraga HA :Ultraviolet difference spectra and the internal structure of proteins. J. Biol. Chem. 1960; 235, 2827-2829.

**14.** Heyns W, Peeters B, Mous J, Rombauts W and De Moor P : Purification and characterization of prostatic binding protein and its subunits. Eur. J. Biochem. 1978; 89, 181-186.

**15.** King R.J.B. and Mainwaring W.I.P.; Steroid–Cell Interactions; 1st ed.; The Butter Worths Company; 1974, 10,11,18.

**16.** Abelson D, Depatie C and Craddock V: Interactions of testosterone with amino acids. Arch. Biochem & Biophys. 1960;91, 71-74.

**17.** Leach S.J.; "Physical Principles and Techniques of Protein Chemistry"; 1969; New York; Academic Press; Part A; pp. 102-170.

**18.** Litwack G.; Biochemical Actions of Hormones; Volume IV; Academic Press; 1977, 358-394.