

## Spectroscopic studies of AFP, Anti AFP antibody and AFP/125I-anti AFP antibody complex.

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### ARTICLE INFO

Received: 20 / 11 /2012  
Accepted: 22 / 11 /2012  
Available online: 16/02/2014  
DOI: 10.37652/juaps.2013.84985

#### Keywords:

Spectroscopic ,  
AFP,  
Anti AFP antibody ,  
AFP/125I- anti AFP antibody complex.

### ABSTRACT

The aim of this work is to characterize spectrophotometric ally the isolated Alpha fetoprotein from human colorectal tumor homogenates and the molecules of both AFP antibody and the complex of AFP/anti AFP antibody. Gel filtration technique was used to separate <sup>125</sup>I-anti AFP antibody bound to human AFP from unbound (free) <sup>125</sup>I-anti AFP antibody. The characterization of human-AFP, anti-AFP, and (AFP/Anti-AFP) complex were carried out through the ultraviolet (U.V) spectroscopic studies. Factors affecting the light absorption properties of the molecules under investigation in this work such as pH, solvent polarity (solvent perturbation technique), spectrophotometric pH titration and thermal stability have been studied. The spectrophotometric pH titration for h-AFP, anti AFP, and (AFP/anti-AFP) complex showed that *pKa* for tyrosine was 9.5, 10.2, and 9.9, while for histidine was 5.7, 6.0, and 5.9 respectively. Spectrophotometric pH titration and several spectral changes were obtained in the presence of different polar and non-polar solvents, like the alteration of  $\lambda_{\max}$  position and intensities of protein spectrum, and the appearance of new chromophores on the surface of protein molecule. These chromophores were embedded in an interior region of the protein in the absence of the solvent. The difference in pH and polarity of the solvents is very important thing to characterize the protein molecules spectrophotometrically because they change the positions and values of molecules  $\lambda_{\max}$  in the UV region.

### Introduction:

The efficiency of absorption for molecules which absorb light depends on both the structure and environment of the molecules making absorption spectroscopy a useful tool for characterization of both small and large molecule<sup>(1)</sup>. The ultraviolet absorption of protein solutions in the regions 250 to 310 nm are contributed by phenylalanyl, tyrosyl, and tryptophanyl residues, at shorter wavelengths; the contributions come from other groups such as histidyl residues and the peptide bond<sup>(2,3)</sup>. Changes in the environment of these chromophores can lead to alteration in the absorption spectrum, and conformational changes of its chromophoric groups<sup>(4,5)</sup>.

A variety of environmental changes (e.g. pH, temperature) can affect the absorption spectrum, if the ground and excited states, the altered spectrum of the chromophore can be shifted to longer (red shift) or shorter (blue shift) wavelengths. The shift may or may not be accompanied by a change in intensity of spectrum<sup>(6-8)</sup>. Ultraviolet spectral method remains one of the most important methods in immunology for the study of antibody structure and specific ligand binding<sup>(9,10)</sup>.

The use of solvent perturbation gives an entire thought of the molecule chromophores and their position, because in perturbation technique chromophores which are in contact with the solvent are deliberately perturbed by adding on another solvent<sup>(11,12)</sup>. The characterization of Alpha fetoprotein (AFP) molecule spectroscopically and get entire picture of its

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structure are so important for a lot of biochemical reaction. So, the aim of this paper is the study of spectroscopic effects on AFP in the UV region using different techniques depending on the principle of solvent perturbation and spectroscopic pH titration.

## Materials & Methods:

### Chemicals:

All chemicals and reagents used in this study were of analar grade and were used without further purification. Bovine Serum Albumin (BSA), Tris (hydroxy methyl amino methane) hydrochloride, EDTA, Dimethyl sulphoxide (DMSO), ethanol, ethylene glycol, Glycerol and Sucrose were obtained from Fluka company, Switzerland. CuSO<sub>4</sub>.5H<sub>2</sub>O, Na,K-tartrate, NaOH, HCl, Na<sub>2</sub>CO<sub>3</sub>, and Folin - ciocalteau were obtained from BDH limited pool, U.K. Immunoradiometric assay for AFP was purchased from Immunotech Bechman (France).

### Instruments:

The instruments used in this work: LKB gamma counter type 1270-rack gamma II, Switzerland. Pye-Unicom pH meter, LKB ultracentrifuge type 2332, Shimadzu double beam UV/visible Spectrophotometer, SM-Shaker, England, PH M62 Standard pH meter, Denmark. Sartorius analytical balance BL 210 S, Germany. Memmert water bath, and Memmert incubator.

### Preparation of The Column:

The dimensions of the column were chosen according to the following equation<sup>(11, 12)</sup>:

$$\text{Diameter} = \sqrt[3]{m/10} \quad \text{Where; m: amount of protein in mg.}$$

$$L = 30 \times \text{diameter} \quad \text{Where; L: length of column.}$$

### Preparation of The Buffer:

1. Tris buffer (0.05M) was prepared by dissolving 3.0285 gm of tris (hydroxy methyl amino methane), 0.9306 gm of EDTA and 0.1 gm of sodium azide in 400 ml, the volume was completed to 500 ml with deionized distilled water, the pH was adjusted to 7.2 by adding HCl.
2. Citrate- phosphate buffer at different pH values was prepared as follows:

Solution A: 0.1 M citric acid (21.01 gm of C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>.H<sub>2</sub>O) was dissolved in 100 ml distilled water.

Solution B: 0.1M dibasic sodium phosphate (1.4198 gm of Na<sub>2</sub>HPO<sub>4</sub>) was dissolved in final volume of 100 ml distilled water.

Working buffer pH (4-6) were prepared by mixing appropriate volumes of solution A and B to reach the required pH in final volume of 100 ml.

3. Glycine- NaOH buffer was prepared as follows:

Solution A: glycine 0.1M in NaCl 0.1M (0.7507 gm glycine + 0.5844 gm NaCl) were dissolved in 100 ml of distilled water.

Solution B: NaOH 0.1N.

Working buffer pH (11-12) were prepared by mixing appropriate amount of solution A and B in a final volume of 100 ml.

### Preparation of Gel:

The gel was prepared by allowing the preswollen gel to swell again in tris buffer pH 7.2, then left to settle and the excess of buffer was decanted. The step was repeated several times. Suction was then used to degas the gel then the slurry was left for 24 hrs. to equilibrate with buffer.

The swollen gel was suspended and carefully poured into vertical glass column (0.9 x 27) down the wall using a glass rod. After the gel had settled the column was equilibrated with this buffer for 72 hrs.

### Determination of The Void Volume:

The void volume of the column was determined using blue dextran 2000 at concentration of 2mg.ml<sup>-1</sup> dissolving in tris buffer pH 7.2, the elution was carried out with the same buffer at a flow rate of 10 ml.hr<sup>-1</sup>. Fractions of 1ml were collected and their absorbance was measured at 600nm.

### Separation Procedure of (<sup>125</sup>I-Anti AFP Antibody/ AFP) Complex:

1. Standard of h-AFP was reacted with <sup>125</sup>I-anti AFP antibody at the kit condition (equipped with the kit). At the end of incubation, 720µl of the mixture was applied to surface of the gel, equilibrated with Tris buffer (0.05M, pH 7.2) with flow rate of 4ml/hr and 1ml fractions were collected.
2. The radioactivity of each fraction was counted in gamma counter.
3. Two hundred microliter of <sup>125</sup>I-anti AFP antibody was completed to 1 ml with Tris buffer (0.05M, pH 7.2), then 720µl from this volume was applied to the column as mentioned above and step 2 and 4 were repeated.
4. Radioactivity (c.p.m.) of each eluted fraction was plotted against the fraction number.

### **The U.V Spectra of (<sup>125</sup>I-Anti AFP Antibody / AFP) Complex:**

The gel filtration the previous experiment gave two peaks profile. The fractions under each peak were pooled and the absorption spectrum was scanned in U.V region using 0.5cm cuvette against Tris buffer (0.05M, pH 7.2) in reference beam.

### **Spectroscopic Studies of h-AFP, Anti AFP, and (AFP/Anti AFP) Complex:**

#### **1. h-AFP and Anti AFP:**

A volume of 20 µl each of human AFP standard and Anti AFP each were completed to 1 ml with Tris buffer pH 7.2. The samples were transferred to 0.5 cm cuvette in the sample beam then the absorption spectrum was scanned against the same buffer in reference beam in region of 200-350 nm.

#### **2. (AFP/Anti AFP) Complex:**

1. Twenty microlitter of human AFP standard provided by (AFP-IRMA Kit) was mixed with 80 µl of <sup>125</sup>I-anti AFP antibody. The volume of the mixture was completed to 250 µl with Tris buffer (0.05M, pH 7.2).
2. The mixture was incubated at 25 °C for one hour.
3. At the end of incubation, the mixture was centrifuged for 30 min. at 4000 r.p.m. at 4 °C to separate the (AFP/anti AFP) complex.
4. The supernatants were discarded by decanting the assay tube, and the precipitate formed was dissolved in 1ml Tris buffer pH 7.2 then the sample was placed in 0.5cm cuvette in the sample beam against Tris buffer pH 7.2 in the reference beam then used, the absorption spectrum was scanned.

### **Factors Affecting The Absorption Properties of h-AFP, Anti AFP, and (<sup>125</sup>I-Anti AFP Antibody/AFP) Complex:**

#### **1. The Effect of pH on The U.V Spectrum:**

##### **A. h-AFP and Anti-AFP:**

A volume of 20 µl each of h-AFP standard and Anti AFP were completed to 1ml at different pH values (4, 7, and 11) using different buffer. The samples were transferred to 0.5cm cuvette in the sample beam, and the buffer at the adjusted pH in reference beam was used, the absorption spectrum was scanned.

##### **B. (AFP/Anti AFP) Complex:**

1. Twenty microliters of human AFP standard provided by (AFP-IRMA Kit) was mixed with 80 µl of <sup>125</sup>I-anti AFP antibody. The volume of the mixture was completed to 250 µl with Tris buffer (0.05M, pH 7.2).

2. The mixture was incubated at 25 °C for one hour.
3. At the end of incubation, the mixture was centrifuged for 30 min. at 4000 r.p.m. at 4 °C to separate the (AFP/anti AFP) complex.
4. The supernatants were discarded by decanting the assay tubes, and the precipitate formed was dissolved in 1 ml with different buffer to get appropriate pH values (4, 7, and 11), then each sample was placed in 0.5cm cuvette in the sample beam. The buffer at the adjusted pH in the reference beam then used, the absorption spectrum was scanned.

#### **2. The Effect of Solvent Polarity (Solvent Perturbation) on The U.V Spectrum:**

##### **A. h-AFP and Anti-AFP:**

Twenty microliters of each h-AFP standard and Anti AFP antibody were completed to 1 ml with following solvents dissolved in Tris buffer (0.05M, pH 7.2):

20% DMSO, 20% Ethanol, 20% Glycerol, and 20% Ethylene glycol

The absorption spectrum of each sample was scanned against the corresponding solvent in reference beam using 0.5cm cuvette.

##### **B. (AFP/Anti AFP) Complex:**

The complex was prepared as mentioned previously then the complex was dissolved in the same solvent mentioned above. The absorption spectrum of each sample was scanned against the corresponding solvent in the reference beam using 0.5cm cuvette.

#### **3. The Effect of NaCl Concentrations on Thermal Stability of Human AFP, <sup>125</sup>I-Anti AFP Antibody and (<sup>125</sup>I-Anti AFP Antibody/ Isolated AFP) complex:**

In order to study the thermal stability of human AFP and its complex in presence of different concentration of NaCl (0.01M and 0.1 M), the following experiments were carried out:

1. one hundred microlitter of human AFP and its complex were completed to 500 µl with 0.01M NaCl in 20% ethylene glycol buffer pH 8.0.
2. the solution was placed in 0.5 cm cuvette in sample beam with the same buffer in the reference beam.
3. The absorption of the above solution was measured at the wavelength of (292 and 295 nm) at different temperatures (30, 40, 50, 60, and 60 °C).
4. The experiment was repeated with another solution (0.1M NaCl in 20% ethylene glycol buffer pH 8.0) at 292 and 295 nm.

#### **Solutions:**

Twenty percent ethylene glycol buffer was prepared by mixing 20ml of ethylene glycol and 80ml of Tris\_buffer pH 8.0. NaCl (0.01M) in 20% ethylene glycol buffer was prepared by dissolving 0.05844 gm of NaCl in 100 ml of 20% ethylene glycol buffer, while NaCl (0.1M) in 20% ethylene glycol buffer was prepared by dissolving 0.5844 gm of NaCl in 100 ml of 20% ethylene glycol buffer.

#### 4. Spectrophotometric pH Titration:

##### A. h-AFP and Anti-AFP:

1. One hundred microlitter each of h-AFP standard and Anti AFP were pipetted in a set of tubes. The volumes were completed to 1 ml with citrate buffer (0.05M) to get pH ranging (2-6).
2. Each sample was placed in 0.5cm cuvette in the sample beam and the buffer at the sample pH in reference beam then used. The maximum absorption of each sample was measured at 211 nm.
3. Another set each of human AFP standard and Anti AFP were pipetted in a set of tubes. The volumes were completed to 1 ml with Tris and glycine/NaOH buffer (0.05M) to get pH ranging (7-11).
4. Step 2 was repeated as above and the maximum absorbance of each sample was measured at 295nm.
5. The absorbance of  $\lambda_{max}$  at each pH values was plotted versus the corresponding pH for each h-AFP, and anti AFP.

##### B. (AFP/Anti AFP) Complex:

1. The complex (AFP/anti AFP) was prepared as mentioned in section previously.
2. A set of the complexes was dissolved in a final volume of 1 ml with buffer at pH range (7-11). The maximum absorbance of each sample was measured at 295nm.
3. The absorbance of  $\lambda_{max}$  at each pH values was plotted versus the corresponding pH.
4. Another set of the complexes was dissolved in a final volume of 1 ml with buffer at pH range (2-6). The maximum absorbance of each sample was measured at 211nm.
5. The absorbance of  $\lambda_{max}$  at each pH values was plotted versus the corresponding pH.

### Results & Discussion

#### Gel Filtration Technique for Separation of Free and Bound <sup>125</sup>I-Anti AFP Antibody:

Isolation of cytosol AFP antigens was performed by gel exclusion chromatography technique. Colorectal tumor homogenate was applied to Sephadex G 200 (0.9x27 cm). Figure (1) shows the elution profile of blue dextran 2000. The volume of the buffer required to elute

the blue dextran, which represents the void volume, was (10 ml). The use of blue dextran is because the high molecular weight (2 000 KDa) to make sure that the gel packing is suitable for isolation different protein from each other's depending on the difference in molecular weight<sup>(13)</sup>.

The elution profile of AFP from malignant colorectal tumor and benign was illustrated in figure (2). The resultant fraction of the homogenate were collected, pooled and detected for the binding with <sup>125</sup>I-anti AFP Antibody. All trials of gel filtration revealed two peaks profile. The first peak represents (<sup>125</sup>I- anti AFP antibody / AFP) complex, while the second peak represents unbound (free) <sup>125</sup>I-anti AFP antibody. The difference in molecular weight is the principle of isolation in gel filtration and depend on the exclusion. So the first peak is for the complex of AFP with its antibody which have high mass comparing with AFP which has the second peak. The binding method in Immuno Radio Metric Assaay IRMA is highly specific and highly sensitive because it established on the immune reaction and monitored by estimating the radioactivity<sup>(14)</sup>. Accordingly, only two peaks had been got.

#### Spectroscopic Studies of h-AFP, Anti AFP, and (AFP/Anti AFP) Complex:

The U.V spectrum of h-AFP, anti AFP, and (AFP/anti AFP) complex were scanned to determine their maximum wavelength, and the alteration in the U.V. spectra as a result of their interaction.

##### The U.V Spectra of h-AFP:

Figure (4) illustrated the U.V spectra of h-AFP provided with kit at pH 7.2. As shown, the spectrum consisted of one broad peak at 219 nm represents the amide bond with contribution of tyrosyl residue<sup>(7)</sup>. It seems that each of tyrosyl residues in human AFP molecule is located in a way that part of it is on the surface of protein molecule.

##### The U.V Spectra of Anti AFP:

Figure (5) illustrated the U.V spectra of anti AFP at pH 7.2. The spectrum shows that the  $\lambda_{max}$  is consisted of two peaks at 218 and 280 nm. The first peak represents the amide bond which assigned to tyrosyl residue, while the second peak represents to the side chain chromophore of tryptophyl residue<sup>(15)</sup>.

##### The U.V Spectra of (AFP/Anti AFP) Complex:

The U.V spectra of (AFP/anti AFP) complex appeared two peaks at 218.2 and 279 nm respectively as shown in figure (6). These two peaks were characteristic of the amide bond, which assigned to tyrosyl residue and

tryptophyl residue respectively. The value of  $\lambda_{\max}$  for the spectrum of h-AFP, anti AFP, and (AFP/anti AFP) complex are illustrated in table (1).

#### **Factors Affecting The Absorbance of h-AFP, Anti AFP, and (AFP/ Anti AFP) Complex:**

The absorbance of a chromophore is primary determined by the chemical structure of the molecule. However, a large number of environmental factors produce detectable change in the  $\lambda_{\max}$  <sup>(15,16)</sup>.

#### **The Effect of pH:**

The pH of the solvent determines the ionization state of the ionizable chromophore in the protein molecule. Table (2) shows the effect of increasing pH on the  $\lambda_{\max}$  value of h-AFP, anti-AFP, and (AFP/anti-AFP) complex.

The  $\lambda_{\max}$  was measured at different range of pH (2, 4, 7, and 11). From this table, two peaks were almost observed at different pH. There were a significant changes in  $\lambda_{\max 1}$  compared with  $\lambda_{\max 1}$  in table (1). There was disappeared of  $\lambda_{\max 2}$  at pH 4, 7, and 11 of anti AFP antibody molecule, this is may be due to conformational changes of this molecule <sup>(17)</sup>.

It was found that h-AFP has a remarkably hydrophilic exposed molecular surface at neutral pH and possesses extensive hydrophobic binding sites located in crevices. Conformational changes occur in h-AFP in the acid and alkaline pH regions; extensive, hydrophobic areas of AFP are exposed by both acid and alkaline transitions <sup>(18, 19)</sup>. At low pH the AFP molecule is transformed into the molten globule state <sup>(20)</sup>. In the case of tyrosine, it was found that the -OH group can dissociate a proton at high pH, producing a red shift. Below pH 9, where the -OH group is unionized, it could be as a donor in hydrogen bond with a suitable solvent or solute acceptor. It has even been suggested that the  $\pi$ -electron system of the benzene ring may serve as a weak proton acceptor, so that the -OH group might form a hydrogen bond with the tyrosine ring, hence this would also produce a red shift <sup>(7,21)</sup>.

#### **The Effect of Solvent Polarity (solvent perturbation) on The U.V Spectrum of h-AFP, Anti-AFP, and (AFP/Anti-AFP) Complex:**

The spectra of chromophores depend on the polarity of their environment, therefore the determination of whether an amino acid is internal or external by measuring the spectra of a protein in a polar and nonpolar solvent is called the solvent-perturbation method <sup>(15)</sup>. The nonpolar solvent itself does not introduce conformational changes. In fact, proteins are rarely studied in completely nonpolar solvents because

most proteins are either insoluble or denatured in these solvents <sup>(15)</sup>.

Table (3) shows the effect of different solvent on  $\lambda_{\max}$  of h-AFP, anti AFP, and (AFP/anti AFP) complex. There was a shift in  $\lambda_{\max 1}$  toward longer wavelength (red shift) in the presence of 20% ethanol for h-AFP, anti AFP, and (AFP/anti AFP) from 219, 218 and 218.2 nm to 222, 221 and 221.5 nm in each molecule.

Ethylene glycol showed no significant changes in  $\lambda_{\max 1}$  in both h-AFP and anti AFP, while for (AFP/anti AFP) complex there was an alteration from 218.2 to 221.4 nm. Also  $\lambda_{\max 2}$  appeared in h-AFP, while a decrease in  $\lambda_{\max 2}$  from 279 to 273 nm was occurred in the complex.

In the case of 20% glycerol; there was a red shift in  $\lambda_{\max 1}$  for h-AFP and the complex suggested that the buried tyrosyl regions of the molecules are more readily disrupted <sup>(21)</sup>. For anti AFP,  $\lambda_{\max 1}$  was disappeared and no significant change in  $\lambda_{\max 2}$ , while  $\lambda_{\max 2}$  appeared in h-AFP which the assigned to tyrosine.

The effect of 20% DMSO showed a red shift in  $\lambda_{\max 1}$  for the three molecules under investigation and no significant change in  $\lambda_{\max 2}$  for anti AFP and the complex which is related to tyrosin and phenyl alanine and there was appearance of  $\lambda_{\max 2}$  in h-AFP.

The several spectral changes obtained in the presence of the perturbants, like the alteration of  $\lambda_{\max}$  position and disappearance or appearance of new chromophore on the surface of the molecules. These chromophores were embedded in an interior region of the protein in the absence of the solvent <sup>(223-224)</sup>. One of the main assumptions of the solvent perturbation was that the perturbing solvent does not alter the conformation of the protein but solvent alter the peak positions and intensities by altering the energy and probability of electronic transitions changes in permanent dipole moment during excitation, i.e. the dipole hydrogen bonding which will tend to produce either a short wave or long wave shift depending on the electronic transition and whether the solute is hydrogen donor or hydrogen acceptor <sup>(25-28)</sup>.

#### **The Effect of NaCl Concentration on The Thermal Stability of Human AFP:**

The effect of different concentration of NaCl on the thermal stability of human AFP were examined in this experiment. The value of absorbance at  $\lambda_{\max}$  292 and 295 nm for tryptophan and tyrosine residues, respectively, in two different concentration of NaCl 0.01M and 0.1M in a mixture of (20% ethylene glycol + 80% H<sub>2</sub>O) are shown in figures 7 and 8.

As shown in figures 7&8, the absorbance of both tyrosine and tryptophane reach higher absorbance at

60°C, in the presence of 0.01M NaCl and at 70°C in the presence 0.1M NaCl for human AFP. Therefore higher concentration of NaCl causes more stabilization for protein molecule. The increment in the absorbance of both tyrosine and tryptophan residues with increasing temperature could be due to the fact that buried chromophores become exposed to the solvent during thermal denaturation<sup>(29)</sup>.

The decrease of absorbance in presence of 0.1M NaCl as compared with that in 0.01M NaCl could be due to salt concentration. Each protein in solution containing salts will collect about it counter ion atmosphere enriched in oppositely charged small ions (chloride ion and sodium ion), and such a cloud of ions will tend to screen the protein, the larger concentration of small ion present the more effective this electrostatic screening will be, and the decrease in the absorption intensity will be observed<sup>(30)</sup>.

#### **Spectrophotometric pH Titration:**

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 amino acid location 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 295nm ( $\lambda_{max}$  for the ionized form of tyrosine, and the observation of histidine dissociation was carried out by measuring the observation at all 211nm).

Figure (9) shows the pH titration curve of h-AFP, anti-AFP, and (AFP/anti AFP) complex for tyrosine. It was shown that the pKa values of tyrosyle residue were (9.5), (10.2), and (9.9) respectively.

Figure (10) illustrated the pH titration of h-AFP, anti-AFP, and (AFP/anti AFP) complex for histidine. It was shown that the pKa values of histidyl residue were (5.7), (6.0), and (5.9) respectively.

In AFP the tyrosine residues were largely present on the surface of the molecule and the internal tyrosines are in a strongly nonpolar environment<sup>(31,32)</sup>. While the internal tyrosine residues in (<sup>125</sup>I-antiAFP antibody/AFP) complex were in strongly polar environment (i.e. a tyrosine surrounded by carboxyl groups)<sup>(31)</sup>. On the other hand, the histidine residues are slightly present on the molecular surface of AFP and (<sup>125</sup>I-anti AFP antibody/ AFP) complex.

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**Table (1): The  $\lambda_{max}$  of the U.V. spectrum of h-AFP, anti AFP, and (AFP/anti AFP) complex:**

Sample	$\lambda_{max}$ (nm)
h-AFP	219
Anti AFP	218, 280
(AFP/anti AFP) complex	218.2, 279.0

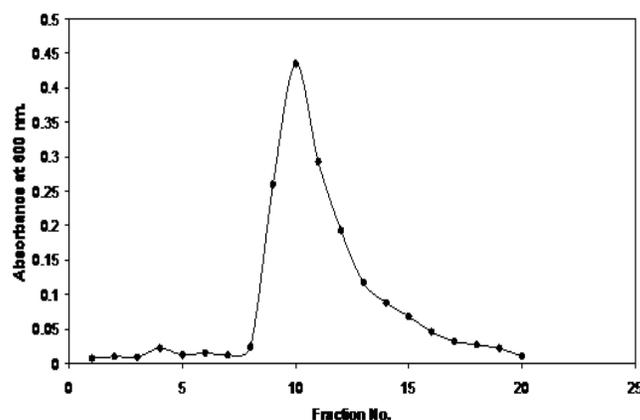
**Table (2): The effect of different pH on h-AFP, anti-AFP, and (AFP/anti-AFP) complex:**

pH	$\lambda_{max}$ (nm)					
	h-AFP		Anti-AFP		AFP/Anti-AFP complex	
2	217	277	208	272	220	273

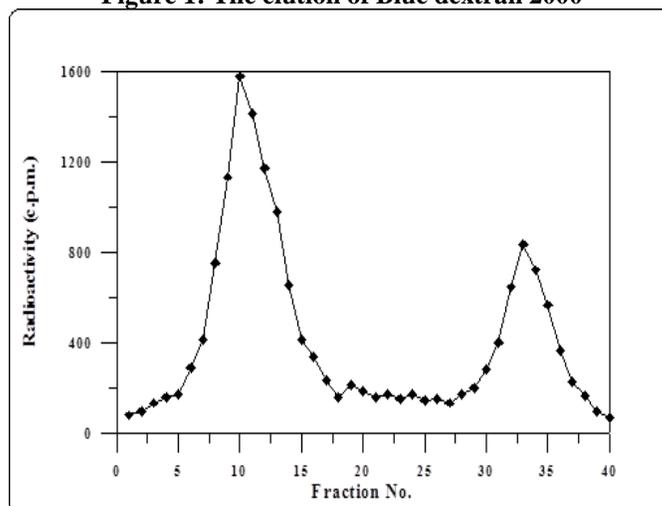
4	217	275.2	208	270	227	274
7	221.4	275.3	214.2	--	224	272
11	227.5	274.4	221.1	--	227	274

**Table (3): The effect of solvent polarity, solvent perturbation on h-AFP, anti-AFP, and (AFP/anti-AFP) complex on U.V spectrum:**

Solvent	$\lambda_{max}$ (nm)					
	h-AFP		Anti-AFP		AFP/Anti-AFP complex	
20% Ethanol	222	274	221	277	220.5	276
20% Ethylene glycol	222	275.6	218.8	278	221.4	273
20% glycerol	227	278	---	277	227	271
20% DMSO	243.2	277	234.6	278	244	274.6



**Figure 1: The elution of Blue dextran 2000**



**Figure 2: elution profile of the prepared standard complex.**

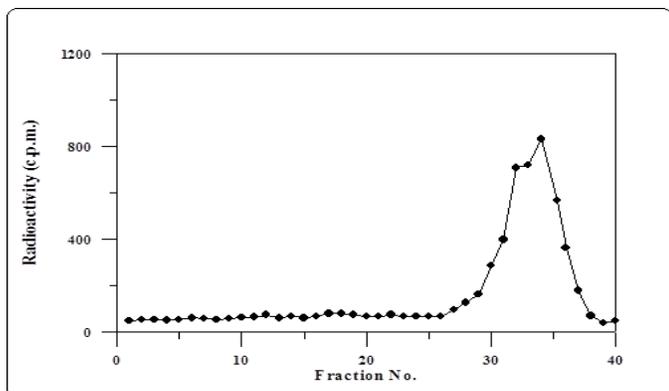


Figure 3: The elution profile of 125I-anti AFP antibody

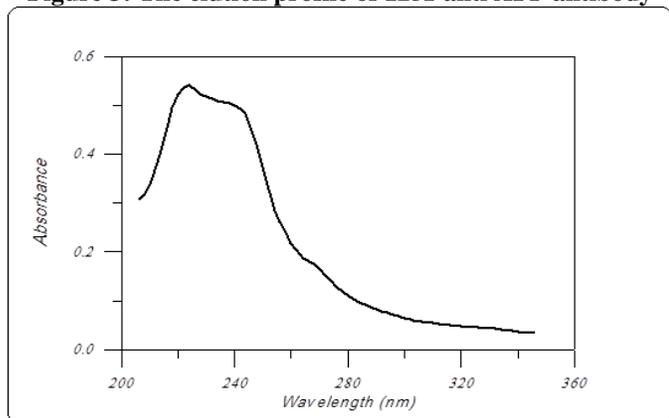


Figure 4: The U.V Spectrum of h-AFP.

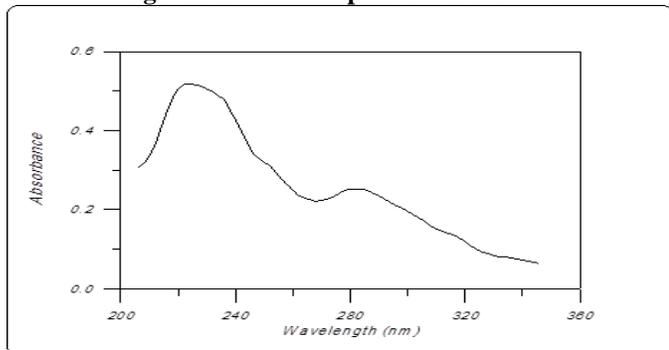


Figure 5: The U.V Spectrum of anti AFP.

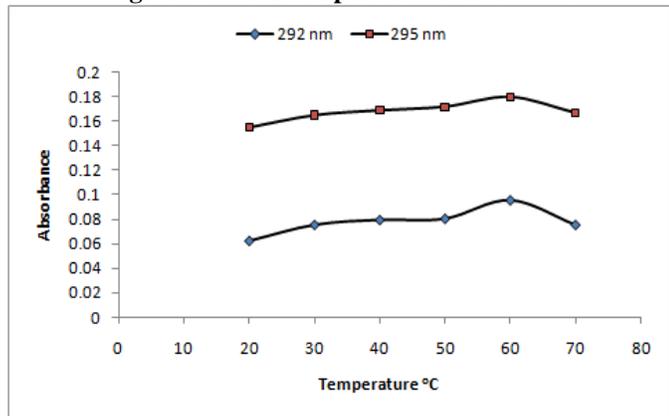


Figure 6: The U.V Spectrum of (AFP/anti AFP) complex.

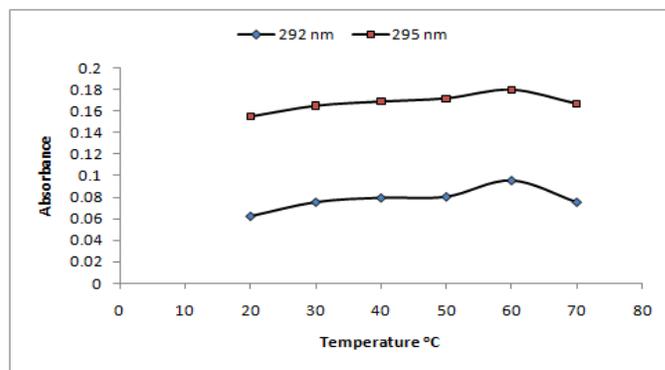


Figure 7: Effect of NaCl Concentration on thermal stability of human AFP in presence of 0.01 M at pH 8.

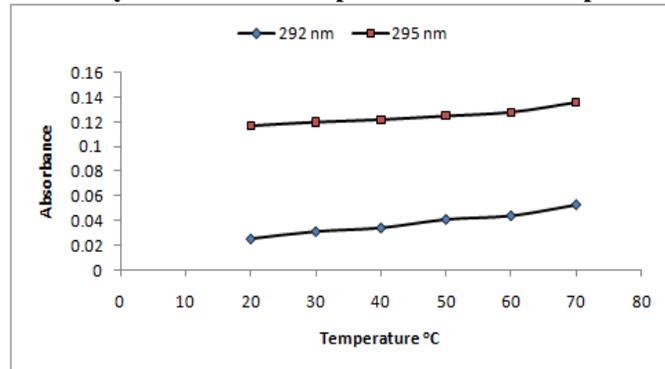


Figure 8: Effect of NaCl Concentration on thermal stability of human AFP in presence of 0.1 M at pH 8.

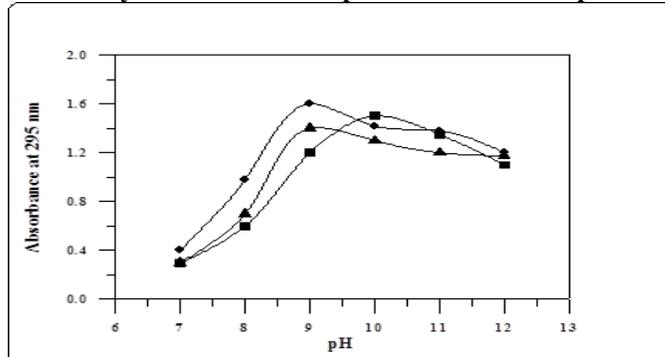


Figure 9: Spectrophotometric pH titration of: (●) h- AFP, (■) anti-AFP, and (▲) (AFP/anti AFP) complex for tyrosine.

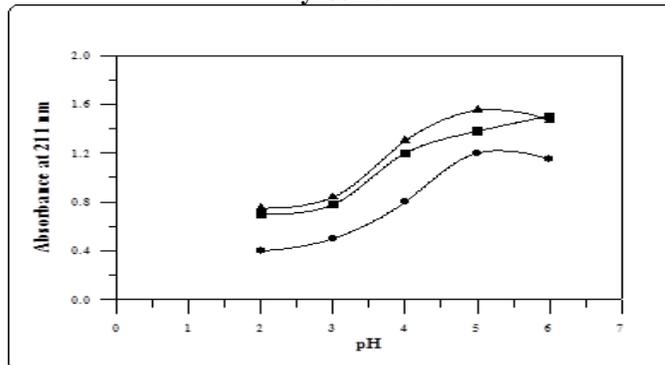


Figure 10: Spectrophotometric pH titration of: (●) h- AFP, (■) anti-AFP, and (▲) (AFP/anti AFP) complex for histidine.

## دراسات طيفية لجزيئة البروتين الجيني الفا (AFP) جزيئة الضد لـ (AFP) والمعقد بين AFP وجزيئة الضد المعلم باليود المشع ١٢٥

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### الخلاصة

الهدف من هذه الدراسة هو للتوصيف الطيفي لجزيئة البروتين الجيني الفا (AFP) المنقى من متجانسات اورام القولون والمستقيم البشرية وكذلك توصيف مستضد الـ (AFP) والمعقد المتكون من الجزيئة والمستضد. استخدمت تقنية الترشيح الهلامي لفصل المعقد عن المستضد المعلم باليود المشع ١٢٥. تم التوصيف الطيفي في منطقة الاشعة فوق البنفسجية وتم دراسة المؤثرات على امتصاص الضوء وهي الدالة الحامضية وقطبية المذيب (اي تقنية التشويه بالمذيب) والتسحيح الطيفي والاستقرارية الحرارية بوجود تراكيز مختلفة من ملح (NaCl). اظهر التسحيح الطيفي ان قيم ثابت التاين لمتخلفات الحامض الاميني التايروسين لـ (AFP) والمستضد والمعقد كانت ٩.٥ و ١٠.٢ و ٩.٩ على الترتيب بينما كانت للهستيدين ٥.٧ و ٦.٠ و ٥.٩ على الترتيب. تم ملاحظة التغيرات الطيفية الحاصلة على الجزيئات عند استخدام مذيبات مختلفة اذ تغيرت قيم قمم الامتصاص العظمى ( $\lambda_{max}$ ) وكذلك مواقع القمم. كما تم ظهور مجموعات قابلة للامتصاص (الكروموفورات) نتيجة للاختلاف في قطبية المذيبات ونتيجة للتسحيح الطيفي دلالة على ان الكروموفورات كانت ضمن المناطق الداخلية للبروتين ويتاثر المذيب وصلت للسطح واصبحت معرضة للشعاع الطيفي. الاختلاف في قيم (pH) والقطبية عوامل مهمة جدا لتوصيف جزيئة البروتين طيفيا لما لها من تاثيرات على قيم حزم الامتصاص ومواقعها ضمن منطقة الاشعة فوق البنفسجية.