SPECTROSCOPIC CHARACTERIZATIONS OF ALPHA FETO PROTEIN BINDING WITH ITS ANTIBODY BY IRMA METHOD IN TISSUE'S HOMOGENATE OF SOME TYPES OF OVARIAN TUMORS

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Abstract

Spectroscopic studies, in the ultraviolet region were carried out to characterize the binding of isolated AFP with its labeled antibody in human malignant germ cell ovarian tumor homogenates. Factors affecting the absorption properties of AFP, and the complex of isolated AFP with the labeled antibody such as pH, solvent polarity (solvent perturbation technique), spectrophotometric pH titration and thermal stability in the presence of different concentration of sodium chloride have been studied. The spectroscopic pH titration curves for human AFP and for the complex of isolated AFP with the labeled antibody gave pka of 5.7 and 7.3 for histidine residue, respectively, while 11.6 and 11.4 for tyrosine residue, respectively. Furthermore the study showed that 23.8% of histidine and 70.4 % of tyrosine residue are located on the surface of human AFP antigen, while these residues were located 38.0% and 31.0% on the surface of complex of the isolated AFP form.

الخصائص الطيفية لإرتباط البروتين الجنيني- ألفا مع ضده بإستخدام تقنية الإختبار المناعي الإشعاعي المتري في مجانسات بعض أورام المبايض

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الخلاصة

أجريت دراســات طيفيــة للمستضد البــروتيني الجنيني ومعقده ، المتكون من إرتباط الضــد الموســوم باليود المشع ذو العدد الكتلي 125 مع المستضد البروتيني الجنيني ألفــا المعزول من مجانسات أنسجة النســاء المصابات بأورام المبايض الخبيثة ذات الشكل النسيجي المبيضي الخلوي الجرثومي الخبيث باستخدام الأشعة فوق البنفسجية، حيث تضمنت الدراسة تأثير كل من الأس الهيدروجيني وقطبية المذيب ، و التسخين للمستضد والمعقد طيفياً فضلاً عن دراسة الإستقرار الحراري لكليهما بوجود تراكيز مختلفة من كلوريد الصوديوم . أوضح منحني التسخين الطيفي للبروتين الجنيني ألفا وللمعقد المتكون من إرتباط البروتين الجنيني ألفا المعــزول مــع الضــد الموسوم بنظير اليود ذي العدد الكتلي 125 قيمة لمتخلفات الهستدين pka (5.7 و7.3) علــى التــوالي ولمتخلفات التايروسين pka (11.6و 11.4) على التوالي. فضلا عن كون 23.8% من متخلفــات الهســتدين

و70.4% من متخلفات التايروسين متموضعة على سطح البروتين الجنيني ألفا بينمـــا 38% و 31 % مـــن متخلفات الهستدين والتايروسين على التوالي متموضعة على سطح البروتين المعزول في المعقد.

Introduction

A molecule or part of a molecule that can be exited by absorption of light is called a chromophore. 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 spectroscopic parameters such as λ_{max}[1].

Change in the absorption, usually at a single wavelength, is taken as a measure of a change in the protein conformation, which occurs with some variation in physical or chemical condition [2].

U.V spectral methods remain one of the most important methods in immunology because it provides a sensitive, quantitative methodology for the study of antibody structure and specific ligand binding [3, 4].

Mammalian (Alpha Feto Protein) is a single chain glycoprotein of molecular mass ranging from 66 to 72 KDa and a 3% - 5% carbohydrate content [5]. The AFP has long been considered the "gold standard " in the field of tumor markers and it is well known as a "tumor –specific embryonic antigen" or "tumor-associated fetal protein" or "tumor associated protein"[6].AFP is suggested to be the most useful markers for the diagnosis,prognosis,and monitoring of patients with malignant germ-cell tumors of the ovary[7].Very limited work concerning physical properties of AFP, especially, those related to U.V spectroscopy has been done, such as the studies on AFP in gastric cancer by Al-Tai[8] and in breast cancer by Al-Sa'adi[9]. Besides, the U.V studies on interaction of AFP with its specific antibody are not wide spread. The main goal of the present study was the U.V spectra of human AFP and complex of ^{125}I- anti AFP antibody with isolated AFP of malignant germ cell ovarian tumors [10].

Materials and methods

Chemicals

All chemical and reagents used in this study were of analar grade and were\used without further purification.Immunoradio-metric assay kit for AFP was purchased from Immunotech (France).

Patients

The study group consisted of 15 women with malignant germ cell ovarian tumors (aged range between 12-43 years). All specimens were collected during the period from June 2003 to August 2004, the histological diagnosis was available in all cases.). It was confirmed that the individuals were not suffering from any disease at time of the study, not pregnancy and did not receive any medication.

All patients were admitted for diagnosis and treatment in the following hospitals: (Medical city, Baghdad Teaching Hospital), Al-Kadhimya Teaching Hospital, Al-Yarmook Teaching Hospital, Al-Habebia General Hospital, Al-Alweya Hospital, Janeen Private Hospital and Al-Jaraah Private Hospital.All surgical operations of tumor were carried out under the supervision of surgeons.

Samples and Solutions

Collection of Ovarian Tissue Specimens

The tumor tissues were surgically removed from ovarian tumor patients by either unilateral salpinco oöphorectomy or total abdominal hysterectomy and bilateral salpingo oöphorectomy or by ovarian cystectomy[11].

The specimens were cut off, and immediately rinsed with ice-cold isotonic saline solution to remove contaminating blood components[12]. They were collected individually in plastic receptacles and stored at –20°C until homogenization.

Preparation of Ovarian Tumors Tissue Homogenate

The frozen tissues were washed with ice-cold normal saline and then dried using filter paper, weighed. The samples were minced, sliced finely, scalped in Petri dish standing on ice, and then homogenized at 4°C in Tris buffer (0.01 M, pH 7.4) with a ratio of 1:3 (weight: volume) using manual homogenizer. The homogenization of the sample was carried out in a cold medium to avoid protein denaturation and to decrease the proteolytic enzymes activity [13, 14].

The homogenate was filtered through four layers of nylon gauze to remove fiber connective tissue fragments and debris[14].The filtrate was centrifuged at 4000 r.p.m. for 45 min. at 4°C in

order to precipitate the unruptured cells and the intact nuclei. The supernatant was separated, divided in aliquots and frozen with pellet fractions at -20°C until the time of the experiments. The supernatant and pellet were considered cytosolic and nuclear fractions, respectively.

Determination of Total Protein

The total protein content of fraction of ovarian tumor tissue homogenates was determined using the method of Lowry *et. al*[15], and bovine serum albumin (BSA) as the standard protein.

Concentration by Dialysis

Peak(III) of the isolated AFP from the malignant germ cell ovarian tumors which obtained previously in our laboratory [10] using sepharose CL-6B (Pharmacia) gel filtration column(0.9×29cm) was used throughout this part of the work .This peak was used since it contained the highest level of the binding activity. The eluted fractions of this peak were collected, pooled and concentrated by dialyzing against sucrose at 4°C for 2 hrs to get the required concentration to be used in the next experiments.

Spectroscopic Studies

Gel Filtration Technique for the Separation of Free and Bound ^{125}I-Anti AFP Antibody Separation Procedure of (^{125}I-Anti AFP Antibody/ Isolated AFP) Complex.

Two hundred microliter of isolated AFP(PIII) of malignant germ cell ovarian tumor homogenate (300 $\mu g.ml^{-1}$) was
incubated with 120 µl of ^{125}I-anti AFP antibody (600 $\mu g.ml^{-1}$) and the reaction was completed to a final volume of 550 µl with Tris buffer (0.05M, pH 8.0). The tube was incubated for 180 min. at 4°C.

At the end of the incubation period, the mixture was applied onto the surface of a sepharose Cl-6B column $(0.96\times 29\,cm)$ which previously was equilibrated with Tris buffer 0.05M, pH 8.0. The bound AFP to ^{125}I-anti AFP antibody was separated from unbound antigen with flow rate of 5 $ml.hr^{-1}$ by using the same above buffer as an eluent.

The radioactivity of each fraction was counted in gamma counter for 1 min.

Calculations

Radioactivity (c .p. m.) for each fraction was counted in gamma counter type 1270 Rack Gamma II (LKB) for one minute,then the countof each fraction was plotted against the fraction number.

The U.V Spectrum of Human AFP, ^{125}I-Anti AFP Antibody, and (^{125}I-Anti AFP Antibody / Isolated AFP) Complex

The UV spectroscopic studies were carried on the two peaks obtained from the above mentioned experiment upon using the sepharose CL-6B (Pharmacia) gel filtration column(0.9×29cm).

Factors Effecting the Absorption Properties of Human AFP and (^{125}I-Anti AFP Antibody / Isolated AFP) Complex

1 - pH Effect

The pH effect was studied using (100 µl) of both the standard human AFP and the complex of the isolated AFP and its labeled antibody (peak I), where Tris buffer (0.05M) with pH of (4, 8 , 12) was used to complete the volume up to 500 µl. Then the absorption spectrum was immediately measured against the same buffer in reference beam in the region of (200-350 nm).

2- The Effect of Solvent Polarity

To study the effect of solvent polarity on the standard human AFP and the isolated complex (peak I).The above steps was repeated using (20%) of either of the following solvents:ethanol ,ethylene glycol ,glycerol and dimethyl sulphoxide (DMSO).

Spectrophotometric pH Titration of Human AFP and(^{125}I-Anti AFP Antibody / Isolated AFP) Complex

One hundred microliters of standard human AFP and of the isolated complex were placed in two tubes .Then the volume was completed to 500 µl with Tris buffer at different pH (4,5,6,7 and 8). The maximum absorbency of each sample was measured at a wavelength of 211 nm.

The same experiment was repeated using Tris buffer at different pH(9,10,11,12 and 12.5. The maximum absorbency of each sample was measured at a wavelength of 295 nm.

Calculations

The absorbance of $\lambda_{max.}$ at each pH value was plotted versus its corresponding pH.

The Effect of NaCl Concentration on the Thermal Stability of Human AFP and (^{125}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 0.1M), 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 a 0.5 cm cuvette in sample beam with the same buffer in the reference beam.
3- The absorption of the above solutions was measured at the wavelength of (292 and 295 nm) at different temperatures (30, 40, 50, 60, and 70°C).
4- The experiment was repeated with another solution (0.1 M NaCl, in 20% ethylene glycol buffer pH 8.0) at 292 and 295 nm.

Results and Discussion

Gel Filtration Technique for the Separation of Free and Bound ^{125}I-Anti AFP Antibody

(Figure 1) shows the results of the separation of ^{125}I-anti AFP antibody bound to the isolated AFP of malignant germ cell ovarian tumor homogenate by using gel chromatography. The results in (figure 1) revealed the presence of two peaks, the first one at fraction number 17 which represent the complex of isolated AFP bound to its antibody, while the second peak at fraction number 20 represents the unbound (free) ^{125}I-anti AFP antibody. First peak fractions in (figure 1) were collected and pooled.

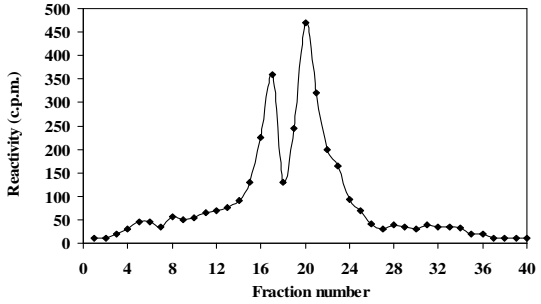

Figure 1: The elution profile of the complex of isolated AFP with its labeled antibody(fraction:14-18) and free

The U.V Spectrum of Human AFP ^{125}I-Anti AFP Antibody and (^{125}I-Anti AFP Antibody / Isolated AFP) Complex

The U.V spectra of Human AFP, ^{125}I-anti AFP antibody, and (^{125}I-anti AFP antibody / isolated AFP) complex were scanned in the region of 200-350 nm to determine the absorption spectra, and the alteration in the U.V spectra as a result of their interaction.

The U.V Spectrum of Human AFP

(Figure 2) illustrates the U.V spectrum of human AFP at pH 8.0. The spectrum shows that human AFP has two peaks at 274.2 and at 233.0 nm, where the maximal absorbance peaks at 276.8 nm is a characteristic of the side chain chromophore of tyrosyl residues, while the absorption at 233.0 nm is due to the amide group in the polypeptide bond of the isolated AFP molecule with contribution of the histidyl residues[2]. It seems that each of tyrosyl and histidyl residues in human AFP molecule is located in a way that part of it is on the surface of the protein molecule while the other part is buried.

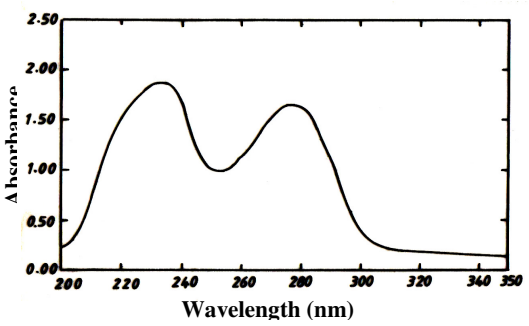

Figure 2: The U.V spectrum of human AFP at pH (8)

The U.V Spectrum of ^{125}I-Anti AFP Antibody

(Figure 3) illustrates the U.V spectrum of ^{125}I-anti AFP antibody at pH 8.0. The spectrum shows that the $\lambda_{max.}$ for ^{125}I-anti AFP antibody is consisted of 2 peaks at 218.6 nm and at 279.2 nm. The first peak at 218.6 nm is assigned to the amide groups in the polypeptide bond with contribution of histidyl residues[2], while the small peak at 279.2 nm is assigned to tyrosyl residues

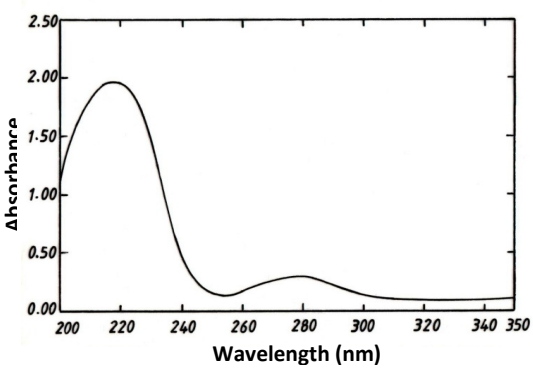

Figure 3: The U.V spectrum of ^{125}I-anti AFP antibody at pH (8)

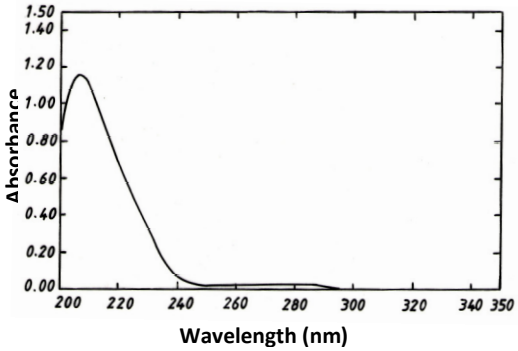

Figure 4: The spectra of isolated AFP of malignant germ cell ovarian tumor bound to ^{125}I-anti AFP antibody at pH (8)

The U.V Spectrum of (^{125}I-Anti AFP Antibody / Isolated AFP) Complex

(Figure 4) shows the spectra of isolated AFP of malignant germ cell ovarian tumor bound to ^{125}I-anti AFP antibody at pH 8.0. The spectra of the complex have a maximum absorbance at 206.8 nm. The strong absorption at 206.8 nm arises from electronic transition in the peptide backbone itself and is therefore sensitive to backbone conformation[1].

Upon the comparison of the spectra of (^{125}I-anti AFP antibody/isolated AFP) complex with the spectra of human AFP and its antibody (Table 1), it can concluded that the disappearance of the absorption at 279.2 nm and 276.8 nm of tyrosine residue is due to the interaction of AFP with its antibody. The interaction seems to involve tyrosine residues presence on the surface of the both of antigen and antibody molecules in such a way that leads to disappearance most of tyrosine residues from the surface of the complex.

Conformation changes may occur that affect the absorption of (^{125}I-anti AFP antibody/isolated AFP) complex. These changes are due to the fitting of antibody to its antigen to form (^{125}I-anti AFP antibody/isolated AFP) complex. These results are in agreement with the finding of Seinerman *et.al*[16] who found that the surface of protein interactions is polar and the complex formation leads to the burial of charged and polar residues.

Table 1: The λ_{max} of (^{125}I-anti AFP antibody/ isolated AFP) complex, human AFP, and ^{125}I-anti AFP antibody (tracer) at pH (8)

$\lambda_{max.}$ (nm)		
Human AFP	^{125}I-anti AFP antibody	(^{125}I-anti AFP antibody/ isolated AFP) complex
233.0, 276.8	218.6, 279.2	206.8

Factors Effecting the Absorption Properties of Human AFP and (^{125}I-Anti AFP Antibody/ Isolated) Complex

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 absorbance. The general features of these environmental effects are the following:

pH Effect

The pH of the solvent determines the ionization state of the ionizable chromophore in the protein molecule[1]. The U.V. spectrum of human AFP and (^{125}I-anti AFP antibody /isolated AFP) complex at different pH (4, 8, and 12) and $\lambda_{max.}$ was obtained as shown in table (2). Figure (5 A and B) shows these spectrums.

At pH (8.0), two $\lambda_{max.}$ were obtained for human AFP, $\lambda_{max.1}$ and $\lambda_{max.2}$, each of which represents the following results, respectively (233.0 nm and 276.8 nm). Whereas only one $\lambda_{max.}$ was obtained for (^{125}I-anti AFP antibody/isolated AFP) complex, $\lambda_{max.}$ was 206.8 nm.

In an acidic pH (4), there was a decrease in both $\lambda_{max.1}$ and $\lambda_{max.2}$ of human AFP with a decrease in pH. The blue shift observed in absorption of tyrosyl residues and the polypeptide bond may be attributed to conformational changes and to the chromophores that are present on the surface of native human AFP[17,18]. On the other hand, the blue shift may be due to the increasing of hydrogen bond formed in the presence of highly positively charged state[18].

The U.V spectrum of (^{125}I-anti AFP antibody/isolated AFP) complex in acidic region (pH 4.0) shows a slight shift to a shorter $\lambda_{max.}$ for the amide groups in the polypeptide bond only. This decrease was associated with the decrease of the absorbency of both human AFP and its complex.

When the pH value was increased from (8.0 to 12.0), there were an increase in both $\lambda_{max.1}$ and $\lambda_{max.2}$ of human AFP. The red shift has been observed in absorption of tyrosine residue, and this was certainly related to the ionization of side chain of the tyrosine and this lead to the availability of the lone pair on the oxygen atom to be happened easier and at lower energy level (red shift). The U.V spectrum of (^{125}I-anti AFP antibody/isolated AFP) complex in basic region at pH (12.0) shows a shift to a longer wavelength (red shift) for the amide groups in the polypeptide bond, these could be due to a conformational changes in the protein molecule[19].

Table 2: Effect of the increasing pH on the $\lambda_{max.}$ of human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex

$\lambda_{max.}$		
pH	**Human AFP**	**(^{125}I-anti AFP antibody/isolated AFP) complex**
4.0	227.2, 270.4	203.8
8.0	233.0, 276.8	206.8
12.0	241.6, 289.6	209.6, 276.2

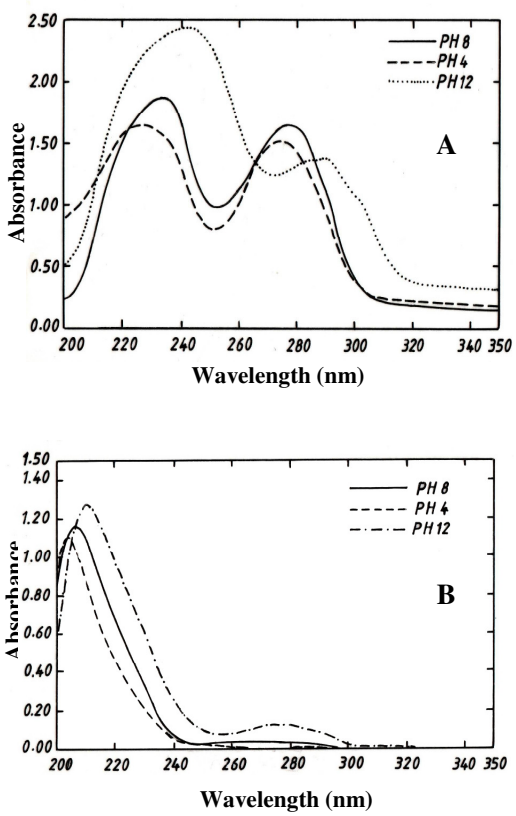

Figure 5: The pH effect on U.V spectrum of: A-Human AFP, B-(^{125}I-anti AFP/ isolated AFP) complex, at pH(4, 8, 12) (All other details are explained in the text)

A new $\lambda_{max.}$ was obtained at 276.2 nm for this complex at pH (12.0) as shown in figure (5 B). The appearance of this new $\lambda_{max.}$ may be due to that defolding of the protein a rise as a result to the change in its secondary and tertiary structure that bring the tyrosyl residues to expose to absorbance[1].

It must be noted that the spectral shifts of proteins produced by pH cannot be simply attributed to the inductive effects of vicinal charges, such spectral changes 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[20].

The Effect of Solvent Polarity

Table (3) shows the effect of 20 % ethanol and ethylene glycol at pH (8) on the human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex spectrum.

The data obtained previously from table (1) show that the $\lambda_{max.}$ of human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex at pH (8.0) were 233.0 and 276.8) nm and (206.8) nm, respectively. The $\lambda_{max.}$ value of tyrosine in human AFP shifted towards longer wave lengths (red shift)in 20 % of each ethanol, ethylene glycol and glycerol due to the hydrogen bonding of the OH groups of tyrosines with the solvent or with the π electron system of the benzene ring where tyrosine function as a hydrogen donor[1,2]. On the other hand, there is a blue shift in the $\lambda_{max.2}$ that is specific for the amide group of the polypeptide bond with contribution of histidyl residues. These shifts are attributed to the intermolecular hydrogen bonding between the amide bonds in the human AFP molecule and the solvent. Hence, additional bonds start to appear at longer or shorter wave lengths[21].

The presence of 20% DMSO has a great effect on the protein structure of human AFP molecule because of the disappearance of $\lambda_{max.}$ of tyrosyl residues (274.8) nm and a new $\lambda_{max.}$ was obtained at 266.8 nm which is assigned to the ($\pi \rightarrow \pi^*$) transition of the aromatic ring of phenylalanine[21].

(Figure 6 A) shows the spectrum of human AFP in presence of different solvent. Table (3) and figure (6 B) show the effect of different solvents on (^{125}I-anti AFP antibody/isolated AFP) complex at pH 8.0, ($\lambda_{max.}$ 206 nm as shown in previous experiments), and how the shift towards longer wavelength in presence of ethanol and ethylene glycol at a concentration of (20 %). These shifts are attributed to the amide group in polypeptide bond with the contribution of histidyl residues. Glycerol (20 %) showed an increase in $\lambda_{max.}$ from 206.8 to 214.2 nm, and a new $\lambda_{max.}$ at 280 nm was appeared. The new peak is related to tyrosine residue which exposed and become solvated with glycerol (dipole-dipole interaction)[2,20].

Table 3: Effect of solvent polarity on the $\lambda_{max.}$ of human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex

$\lambda_{max.}$ (nm)		
Solvent	**Human AFP**	**(^{125}I-anti AFP antibody/isolated AFP) complex**
Ethanol	230.8, 279.2	210.8
Ethylene glycol	222.0, 282.0	213.0
Glycerol	228.6, 284.0	214.2, 280.0
DMSO	230.4, 266.8	220.8, 263.0

In the case of DMSO (20 %), $\lambda_{max.}$ 206.8 nm shifted towards longer wavelength (220.8) nm and a newer $\lambda_{max.}$ appeared at 263.2 nm which was assigned to the ($\pi \rightarrow \pi^*$) transition of the aromatic ring of phenyl alanine residue.

The appearance of these new λ_{max} values indicates that the protein was defolded due to the change in the secondary and tertiary structure of the protein that bring the phenyl alanine and tyrosine for (^{125}I-anti AFP antibody/isolated AFP) complex and Phenyl alanine residue in human AFP to expose to absorbance while the tyrosine residue was buried inside human AFP molecule. Furthermore, it was found that human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex are highly sensitive to change in the polarity of the solvent.

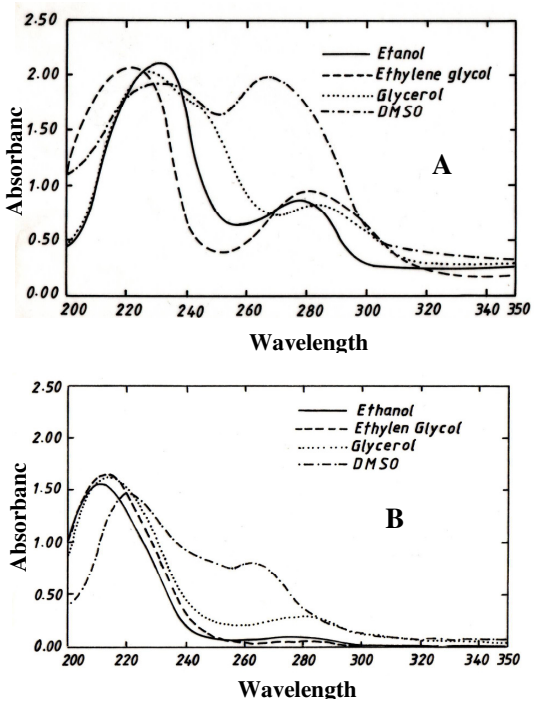

Figure 6: The effect of 20% (ethanol, ethylenglycol, glycerol and DMSO) on U.V spectrum of: A- Human AFP B-(^{125}I-anti AFP/isolated AFP) complex, at pH (8)

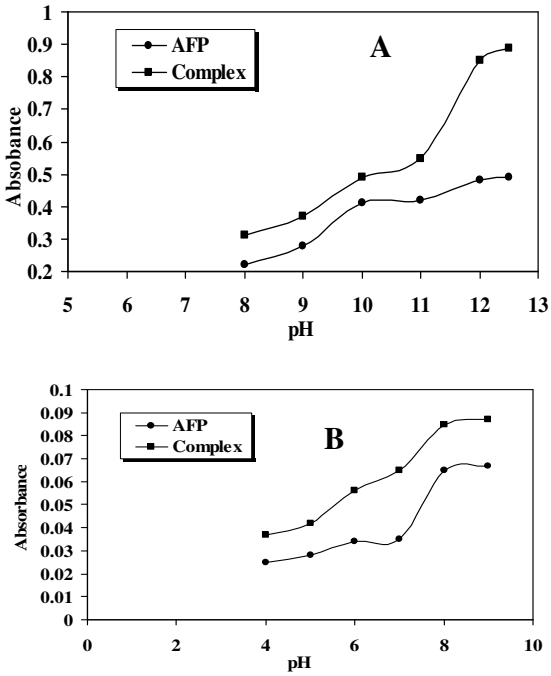

Figure 7: Spectrophotometric pH titration curve of human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex for:A- Tyrosine residues, B- Histidine residues

Spectrophotometric pH Titration of Human AFP and(^{125}I-Anti AFP Antibody / Isolated AFP) Complex

Spectrophotometric pH titration means following the change in absorbance of the chromophore with increasing pH[20].Many studies on protein structure require the determination of pK value of the protein 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 observation of histidine dissociation was carried out by measuring the absorption at 211 nm.

Figure (7A and B) show the pH titration curve of human AFP and (^{125}I-anti AFP antibody/isolated AFP) complex for tyrosine and histidine respectively.

The (A) curves show that the pKa values for tyrosine are 11.6 and 11.4 for AFP and (^{125}I-anti AFP antibody/isolated AFP) complex, respectively. While the pKa values for histidine in (B) curves were equal to 5.7 and 7.3 for AFP and (^{125}I-anti AFP antibody/isolated AFP) complex, respectively. From the same figure, it was found that:

About 70.4 and 31.0 % of tyrosine residues are located on the surface of standard human AFP and the complex of isolated AFP with its labeled antibody respectively, while about 29.6 and 69.0 % of tyrosine residues are buried interior the folded structure of the standard human AFP and the complex of isolated AFP with its labeled antibody , respectively.

Furthermore, about 23.8 and 38.0 % of hisitdine residues is located on the surface of the standard

human AFP and the complex of isolated AFP with its labeled antibody respectively, while about 76.2 and 62 % of histidine residues is embedded in the interior region of the standard human AFP and the complex of isolated AFP with its labeled antibody, respectively.

Finally, the percent of external tyrosine residues exposed to a strongly polar environment in (i.e. a tyrosine surrounded by carboxyl groups)[1] in AFP was greater than that of the complex of isolated AFP with its labeled antibody and the percent of internal histidine exposed to a nonpolar a environment [1] in AFP was greater than of the complex of isolate AFP with its labeled antibody.

Based on the fact that human AFP molecule composed of 17 tyrosine residues and 16 histidine residues [22] ,and from the results of above spectrophotometric titration studies, it can be concluded that about 12 tyrosine residues and 4 histidine residues are on the surface of AFP molecule, while about 5 tyrosine residues and 12 histidine residues are buried interior the folded structure of the AFP molecule.

The Effect of NaCl Concentration on the Thermal Stability of Human AFP

The effect of different concentrations of NaCl on the thermal stability of human AFP were examined in this experiment. The values of absorbance at $\lambda_{max.}$ (292 and 295) nm for tryptophan and tyrosine residues, respectively, in two different concentrations of NaCl 0.01M and 0.1 M in a mixture of (20 % ethylene glycol + 80 % H_2O) are shown in figure (8 A and B).

As shown in figure (8 A & B), the absorbance of both tyrosine and tryptophan reache higher absorbance at 60°C, in the presence of 0.01M NaCl and at 70°C in the presence of 0.1 M 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 [2].

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 a counter ion atmosphere enriched in appositely charged small ion, (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 decrement in the absorption intensity will be observed[19].

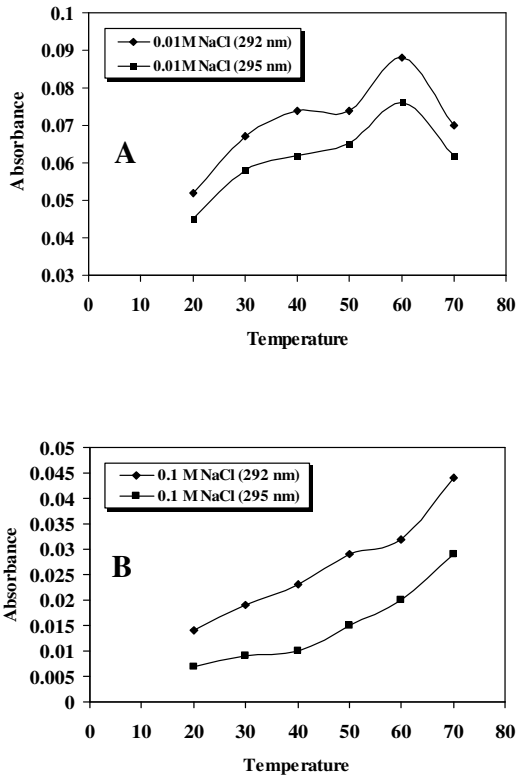

Figure 8: Effect of NaCl concentration on thermal stability of human AFP in presence of 0.01 M NaCl B. In presence of 0.1 M NaCl, at pH (8)

Conclusions

Spectroscopic studies revealed that each of AFP, and the complex of isolated AFP form with ^{125}I-anti AFP antibody have characteristic spectrum and give an idea of the location of particular amino acid in the AFP and (^{125}I-anti AFP antibody / AFP) complex molecules.

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