The Kinetic and The Thermodynamic Studies on the Binding of ^{125}I-Testosterone to Its Receptors in Ovarian Tumors Homogenate

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Abstract

The aim of this work is to study the Kinetic and thermodynamic parameters of binding of ^{125}I-testosterone to its receptors in ovarian tumor homogenates. It is very important to know the kinetic and thermodynamic parameters for hormones because the binding of testosterone to the its receptor initiates a signaling cascade that results in nuclear translocation of the liganded receptor and transcriptional modulation of target genes. Two groups of ovarian tumor patients were included in this study. Group I contained 33 patients with benign ovarian tumor. Group II consisted of 22 patients with ovarian cancer. Time-course of the association of ^{125}I-testosterone with its receptor in human ovarian tumors at four different temperatures revealed the time and temperature dependency (8hrs with 25^{o}C for benign and 8hrs 37^{o}C for malignant). Association kinetics indicated pseudo first order kinetics for the binding. Time-courses, Scatchard, Van't Hoffs and Arrhenius plots led to the theoretical determination of thermodynamic parameters of both the standard state (i.e., $\Delta H°$, $\Delta G°$, $\Delta S°$) and transition state (i.e., ΔH^*, ΔG^*, ΔS^*).

دراسة الحركيات والثرموداينمك لارتباط التوستيستيرون المعلم بنظير اليود المشع 125 مع مستلماته في اورام المبيض البشرية

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الخلاصة

يهدف البحث إلى دراسة العوامل الحركية والثرموديناميكية لتفاعل ارتباط التوستيستيرون المعلم باليود المشع 125 مع مستلماته في متجانسات اورام المبيض. من المهم جداً التعرف على العوامل الحركية والثرموديناميكية للهرمونات لان ارتباط التوستيستيرون بمستلمه ينشط سلسلة من التفاعلات الحيوية التي نتيجتها تغييرات في النواة بعد تغييرات في التعبير الجيني للنسيج الهدف. تضمنت الدراسة مجموعتين من المريضات المصابات بورام المبيض. تألفت المجموعة الاولى من 33 مريضة يعانين من ورم المبيض الخبيث، بينما شملت المجموعة الثانية 22 مريضة مصابة بورم المبيض الحميد. تم دراسة تاثير الزمن ودرجة الحرارة لمعرفة الوقت الامثل ودرجة الحرارة المثلى فاتضح ان الوقت الامثل للارتباط بين الهرمون المشع والمستلم هو 8 ساعات ودرجة الحراة المثلى كانت 25 درجة مئوية للورم الحميد و 37 للورم الخبيث. دلت دراسة الحركيات على ان التفاعل يطيع المرتبة الاولى الكاذبة. تم حساب الثوابت الثرموديناميكية (ΔH, ΔG, ΔS) في الحالتين المستقرة والمثارة من خلال دراسة الزمن وطريقة فانته وف ومعادلة ارينوس.

Introduction

Steroid hormones stimulate cell growth and differentiation and regulate the synthesis of specific proteins primarily by altering the rate of transcription of specific gene. Steroids exert these actions on target cells after binding to specific receptors, which are localized primarily within the nucleus [1,2]. Testosterone, is one of steroid hormones, promotes protein synthesis in ovary and in most tissues of the body [3,4]. One reason for the importance of kinetics is that it provides evidence for the mechanisms of chemical processes. Besides being of intrinsic scientific interest, knowledge of reaction mechanisms is of practical use in deciding what is the most effective way of causing a reaction to occur [5,6]. The study of kinetic and

thermodynamics of any reaction gives the whole picture of the reaction and the application approaches of that reaction [7]. The kinetic and thermodynamics of testosterone with its receptors in ovarian tissue homogenates were not studied, therefore the aim of this study is to study the kinetic and thermodynamic parameters of binding of ^{125}I-testosterone to its receptors in ovarian tumor homogenates.

Material & Methods

Chemicals

All laboratory chemicals and reagents were of analar grade and were used without further purification. Tris (hydroxy methyl) aminomethan, were obtained from **Fluka company, Switzerland.** Hydrochloric acid, glycerol, EDTA (disodium salt) and mercaptoethanol were obtained from **BDH, England.** Kit of radioactive testosterone (^{125}I-testosterone) was purchased from CIS Bio International (**France**). The activity of the labeled testosterone was approximately 5 μci.

Instruments

The instruments used in this work were LKB gamma counter type 1270-rack gamma II, Pye-Unicom pH meter, LKB ultracentrifuge type 2332, Memmert water bath, and Memmert incubator.

Patients

Two groups of ovarian tumor patients were included in this study. Group I contained 33 patients with benign ovarian tumor. Group II consisted of 22 patients with ovarian cancer. All patients were admitted for treatment to The Medical City and Al-Arabe Hospital under the supervision of specialists . The patients were newly diagnosed and not underwent any type of therapy .Patients did not suffer from any disease that may interfere with our study were excluded.

Collection of ovarian tissue specimens

The tumor tissues were surgically removed from ovary tumor patients by oophorectomy. The specimens were cut off and immediately rinsed with ice-cold isotonic saline solution. They were collected individually in plastic receptacles and stored at -20°C until homogenization.

Preparation of ovarian tumor tissue homogenate

The frozen tissues were weighed, pulverized finely with a scalpel in petri dish standing on ice bath, and then homogenized at 4°C in buffer solution with a ratio of 1:5 (weight :volume), using a manual homogenizer .The buffer used was Tris-EDTA (Tris-HCl 0.01M, pH 7.4, containing 0.15 mM EDTA, 2-mM mercaptoethanol and 10 %glycerol) .The homogenate was filtered through several layers of nylon gauze to eliminate fibers of connective tissue, and then centrifuged at 2000 xg for 30 minutes at 4°C .The sediment was suspended in 10 volumes of TEMG buffer for 15 minutes at 4° C and then suspension was used to obtain the crude nuclear fraction.

Methods:

The Kinetic Studies:

The time-course of ^{125}I- testosterone binding to its nuclear receptors in benign and malignant ovarian tumor

- At zero time, 100 µls. of ^{125}I-testosterone (0.9ng) was added to 200 µls. (250 µg protein) of ovarian tumor homogenate. The final volume (1 ml) was made up by adding the assay buffer (0.01M, TEMG buffer pH 8.0). The assay tube was stoppered and incubated at 4°C for several time intervals (2,4,8,12,14,16, and 24 hrs).
- Another tube containing 100 µls. of ^{125}I-testosterone only, for total concentration of hormone (CPM) computation, was set aside until counting.
- The counted radioactivity which is estimated after incubation in each tube (expressed in CPM) represents the total binding (TB).
- Parallel experiments were performed to determine the amount of non-specific binding (NSB) by adding 200 fold of unlabelled testosterone.
- To determine the time-course of the association of ^{125}I-testosterone with its receptor at different temperatures, the above experiment was performed at four other temperatures (25,37,42, and 50°C).

Calculations [8]:

The value of ^{125}I-testosterone bound specifically (pico mole of ^{125}I-testosterone per mg of protein) was calculated according to the formula:

$$\begin{bmatrix} The\ value\ of\ specifically \\ bound\ ^{125}I-testosterone \\ (pmol/mg\ protein) \end{bmatrix} = \frac{\begin{bmatrix} Specifically\ bound \\ ^{125}I-testosterone\ in\ (PM) \end{bmatrix} \times \begin{bmatrix} Incubation\ volume \\ in\ Liter \end{bmatrix}}{mg\ of\ protein\ in\ incubation\ medium}$$

$$\begin{bmatrix} Specifically\ bound \\ ^{125}I-testosterone\ in\ (PM) \end{bmatrix} = \frac{[Total\ binding\ (CPM)] - [Non-specific\ binding\ (CPM)]}{Total\ counts\ (CPM)} \times \begin{bmatrix} Total\ Concentration \\ of\ labeled\ testosterone \\ in\ incubation\ medium \end{bmatrix}$$

$$(SB\%) = \frac{Total\ binding\ (CPM) - Non-specific\ binding\ (CPM)}{Total\ counts\ (CPM)\ of\ ^{125}I-testosterone\ used\ in\ each\ tube} \times 100$$

The percent of specific binding (SB%) was plotted against the different times of incubation at each temperature.

Determination of the concentration of testosterone receptors and the affinity constant of ^{125}I-testosterone association with its receptors in benign and malignant ovarian tumors

- Two hundred microliters (250 µg protein) of ovarian tumor homogenate was incubated with increasing concentration (0.18-1.08ng) of ^{125}I-testosterone with or without the addition of 200 fold excess of unlabeled testosterone in a final volume of 1 ml (completed with TEMG buffer , pH 8.0).
- The assay tubes were stoppered and incubated for (8hrs) at (25°C for benign and 37°C for malignant tumors) then the bound hormone was estimated as mentioned above.
- The previous steps were performed at different temperatures (4, 25,37 and 42°C).
- The value of ^{125}I-testosterone which is bound specifically in picomolar were calculated using the following formula:

$$B = \frac{\textbf{Total binding} - \textbf{Non specific binding}}{\textbf{Total count}} \times \textbf{Concentration of } 125I\textbf{testosterone } (PM) \textbf{in each tube assay}$$

- The concentration of receptors and the affinity constant were determined according to Scatchard equation:[9]

$$\frac{B}{F} = \frac{1}{k_d} \times (B_{max} - B)$$

$$k_a = \frac{1}{k_d}$$

B: The bound radioactivity (CPM), represents the (^{125}I- testosterone-receptor) complex.

F: The free radioactivity (CPM), represents the non-bound ^{125}I-testosterone.

T: The total activity(CPM).

F= Total count (T)- Bound radioactivity (B)

where:

K_a: Affinity constant ,K_d: Dissociation constant , B_{max}: Maximal binding capacity.

- The plot of B/F ratios vs. the B values gives a linear relationship. The value of the affinity constant of the binding k_a at each temperature can be calculated from the slope of the straight line, while the value of the total concentration of testosterone receptor in ovarian tumor homogenate can be calculated from the intercept with the x-axis.

Kinetics of the binding of ^{125}I-testosterone to its receptors in benign and malignant ovarian tumors homogenates

The experiment was carried out in duplicate at different temperatures (4, 25, 37 and 42°C).

Calculations

- The percent of specific binding (SB%) was determined according to the method mentioned previous at different temperatures.
- The rate of the association constant of (^{125}I-testosterone-receptor) complex was calculated by the following equation [10,11]:

$$\ln\left[\frac{(HR)_e}{(HR)_e - (HR)_t}\right] = K_{+1}t\left[\frac{(H)_T(R)_T}{(HR)_e}\right]$$

where:

k_{+1}: The rate association constant

$(H)_T$: The total molar concentration of ^{125}I-testosteroe

$(R)_T$: The total molar concentration of hormone receptors.

$(HR)_e$: The concentration of ^{125}I-testosterone-receptor complex formed at equilibrium.

$(HR)_t$: The concentration of the complex formed after time (t).

- The rate of the dissociation constant of the complex formed (rate of the reverse reaction constant) was calculated by using the following equation:

$$k_a = \frac{k_{+1}}{k_{-1}}$$

where

k_{-1}: The rate dissociation constant

k_a: is the equilibrium constant of the association (affinity constant)

The thermodynamic studies:

The thermodynamic of ^{125}I-testosterone binding to its receptors in benign and malignant ovarian tumors homogenates

- Two hundred microliters of the protein homogenates (250 μg) were added to (100 μls.) of ^{125}I- testosterone in a final volume of 1 ml (completed with TEMG buffer (0.01M, pH 8.0). The assay tube was stoppered and incubated at 25°C for benign ovarian tumor and 37°C for malignant tumor for 8hrs.
- After incubation, the radioactivity of (^{125}I-testosterone-receptor) complex formed was estimated.
- Parallel experiments were performed to determine the amount of non-specific binding.
- The previous steps were performed at different temperatures(4, 25, 37 and 42°C).

Calculations

- The thermodynamic parameters of standard state were obtained from ***Van't Hoff*** plot, the values of the natural logarithm of equilibrium constant (affinity constant k_a) obtained at different temperatures were plotted against the reciprocal values of absolute temperature in Kelvin (1/T), according to the following equation [12]:

$$\ln k_a = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT}$$

Where

ΔH°: The enthalpy change of the standard state.

ΔS°: The entropy change of the standard state.

R: The gas constant (8.31441 J k^{-1} $mole^{-1}$)

ΔH° Value obtained from the slope of the linear relationship of the plot. The change in Gibbs free energy of the standard state (ΔG°) was obtained from the following equation:

$\Delta G^\circ = -RT \ln k_a$

While the standard state entropy change was obtained from:

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T}$$

- The thermodynamic parameters of the transition state were obtained from ***Arrhenius*** plot of ln k_{+1} values against 1/T values, that gives a linear relationship according to the following equation:

$$\ln k_{+1} = \ln A - \left[\frac{Ea}{RT}\right]$$

where

A: Arrhenius factor

The value of apparent energy of activation (Ea) of the binding reaction can be determined from the slope of the straight line. The enthalpy of transition state ΔH^* obtained from:

$\Delta H^* = Ea - RT$

Transition state free energy change is calculated from the following equation:

$$\Delta G^* = -RT \ln k_{+1} + RT \ln\left(\frac{kT}{h}\right)$$

where k and h are ***Boltzmann*** and ***Plank's*** constants which equal (1.38×10^{-23} JK^{-1}), (0.662×10^{-33} $J\ S^{-1}$) respectively.

The change in entropy of the transition state ΔS* is calculated from the following relation:

$$\Delta S^* = \frac{\Delta H^* - \Delta G^*}{T}$$

Results and Discussions:

The Kinetic Studies:

The time-course of ^{125}I- testosterone binding to its nuclear receptors in benign and malignant ovarian tumor

Binding kinetics is also referred to as: slow offset, slow off-rate, slow dissociation, insurmountable antagonism, ultimate physiological inhibition, tight binding and non-equilibrium blockade [13]. Time is important factor of kinetic studies and the importance increased when it linked to temperature to estimate time course of defined reaction. Figure (1"A" & 1"B") shows the time-course of the formation of ^{125}I-testosterone –receptor complex at five different temperatures (4, 25, 37,42,& 50°C). The results of time course patterns at different temperatures revealed that the binding of ^{125}I-testosterone to its receptors in ovarian tumor homogenate is a temperature and time dependent process with a maximum binding occurs at 25°C for benign and 37°C for malignant after 8hrs of incubation. The events of hormone action initiated with hormone binding to its receptor and here we need to examine the best time in vitro of binding. It seems that the time of 8 hours is the best for binding, i.e. the highest SB% is proportional to this time [14]. There were values of binding at other times but the specific binding is not high. The difference between benign and malignant in optimum temperature of binding relates to the differences in the whole tissues environment [15].

Determination of the Concentration and Affinity Constants of Testosterone Receptors

Using Scatchard plots tell as much as you can about the binding reaction. In theory, a Scatchard plot of simple, reversible equilibrium binding is a straight line with the slope of the line being equal to the negative of the association constant (Ka) and the x-intercept being equal to the total receptor number (R_0). Other equally valid mathematical and graphic methods can be used to analyze hormone-receptor interactions, but the Scatchard plot is probably the most widely used [16].

The concentration of testosterone receptors and the affinity constant of the binding have been measured in ovarian tumors that show specific binding in the preliminary test. The experiment was

carried out at the optimal conditions, which were obtained in previous experiments and was repeated at different temperatures (4, 25, 37and 42°C). Scatchard plot analysis gave a straight line as shown in figure (2) at each temperature indicating the presence of only a single class of receptor site or more but with the same affinity and number of binding site [17] .

The results are summarized in table (1). Nuclear testosterone receptors binding capacities(B_{max}) of ovarian cancer patients were(9.8fmol) per mg protein while benign ovarian tumor patients were(7.8 fmol) per mg protein.

Determination of kinetic parameters of ^{125}I- testosterone binding to its receptors in patients with benign and malignant ovarian tumors:

The time course of ^{125}I- testosterone binding to its receptors in ovarian tumor homogenate was carried out to describe the kinetic parameters of the binding. The simplest proposed model representing the interaction of ^{125}I- testosterone with its receptors could be expressed by the following equation:

$$^{125}\text{I-testosterone} + \underset{\text{(receptor)}}{\text{R}} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} {}^{125}\text{I-testosterone-R}$$

Where k_{+1} is the rate of the association of ^{125}I-testosterone with its receptor and K_{-1} represents the rate of the reverse reaction of the dissociation of the complex formed under the same conditions. At equilibrium:

$$k_a = \frac{[^{125}\text{I-testosterone-R}]}{[^{125}\text{I-testosterone}]\,[R]} \qquad (1)$$

$$k_d = \frac{[^{125}\text{I-testosterone}]\,[R]}{[^{125}\text{I-testosterone-R}]} \qquad (2)$$

Thus:

$$K_a = \frac{1}{K_d} = \frac{k_{+1}}{k_{-1}} \qquad (3)$$

Where k_a is the equilibrium constant of the association (affinity constant) and K_d is the equilibrium constant of the dissociation of (^{125}I-testosterone -R) complex. The values of k_a and maximal binding capacity (B_{max}) were calculated from Scatchard plot at four different temperatures in figure (3 A and B) and table (2).

The Kinetic association rate constant, k_{+1}, can be determined from the time course of association of ^{125}I-testosterone with its receptors and verified the order of the reaction at four different temperatures. Time-course data obtained from figure (3 A and B) can be used to confirm that the binding reaction of testosterone with its receptors in benign and malignant ovarian tumors homogenates following a first order kinetic reactions but due to the bimolecularity of this reaction, the following equation [18].

$$\ln(HR)_e\left[\frac{(H)_T-(HR)_t(HR)_e/(R)_T}{(H)_T-\left[(HR)_e-(HR)_t\right]}\right] = k_{+1}t\left[\frac{(H)_T(R)_T-(HR)_e}{(HR)_e}\right] \qquad (4)$$

Equation (4) can be simplified to equation (5) when the most testosterone remained free and only a small fraction of $(H)_T$ is bound even at equilibrium (pseudo- first order conditions)[19].

$$\ln \frac{(HR)_e}{(HR)_e - (HR)_t} = k_{+1} t\left[(H)_T (R)_T / (HR)_e\right] \quad (5)$$

Where k_{+1} is the kinetic association constant in $M^{-1}min^{-1}$; $(H)_T$ is the total molar concentration of ^{125}I-testosterone; $(R)_T$ is the total molar concentration of the hormone receptors; $(HR)_e$ is the concentration of (^{125}I-testosterone-receptor) complex formed after time (t).

Figure (3 A & B) shows that the plotting of $\ln \frac{(HR)_e}{(HR)_e - (HR)_t}$ against time (t) gives a straight line with a slope equal to the observed value of first-order rate constant ($k_{obs.}$) in min^{-1}, and the association rate constant k_{+1} was calculated from the following formula:

$$k_{obs.} = k_{+1} \frac{(H)_T (R)_T}{(HR)_e} \quad (6)$$

The half-life time of association $(t_{1/2})_{ass.}$, which represents the time needed for the formation of half amounts of the complex at equilibrium, was determined from the concentration of the complex at equilibrium and the time course curve, while the half-life time of dissociation $(t_{1/2})_{diss.}$ was determined from:

$$(t_{1/2})_{diss.} = \frac{\ln 2}{k_{-1}} = \frac{0.693}{k_{-1}}$$

The k_a values were also obtained from equation (3). Figure (3 A & B) represents the kinetics of complex formation between ^{125}I-testosterone and its receptors in the two groups of benign ovarian tumor homogenates and the malignant ovarian tumor homogenate at different temperatures. The results revealed that the association rate constant k_{+1} at 37°C (according to malignant ovarian tumor) and 25°C (for benign one) were higher than that at other temperature as shown in table (3). The values of k_{-1} were obtained also from the values of k_a which have been estimated at the four different temperatures investigated. The k_{-1} was determined from the equation (3). This numerical difference may be attributed to the different types of receptor sources used and to differences in the structure of these receptors [20,21].

The thermodynamic of the binding of ^{125}I-testosterone to its receptors in benign and malignant ovarian tumors

Thermodynamic parameters of standard state:

Figure (4) represents the dependence of the equilibrium binding constant (i.e., affinity constant) for the binding of ^{125}I-testosterone to its receptors in ovarian tumor homogenate on the temperature (***Van't Hoff plot***).

The results indicated that ΔH° in general had small values and their positive sign ascertain that the reaction was nearly endothermic. The small positive value of ΔHº may indicate a favorable interaction between ^{125}I-testosterone and its receptors in ovarian tumor homogenates. The favorable interactions include the non-covalent interaction, which are fundamentally electrostatic in nature such as charge-charge, charge-dipole, dipole-dipole, charge-induced dipole, dipole-induced dipole interactions, and hydrogen bonds. The sum of these types of interactions can yield some stabilization to the folded structure of the complex [22]. The negative values of ΔGº reflect the stability of the complex hence, the high affinity of the reactants. So, the negative values of ΔGº showed that the overall reaction was energetically favorable in the direction of complex formation. The negative values of ΔG° reflect the stability of the complex, hence the high affinity of the reactants. The high negative values of ΔG° for the binding reactions are controlled by high positive ΔS° values as shown in table (4). So, our system is characterized by the sole contribution of ΔS° to the stability of the complexes formed, while ΔH° has little or no effect [23].

The high value of positive ΔS° suggests that the reaction spontaneity was entropically driven. Entropy was the driving force for the occurrence of the binding reaction. This indicates that the hydrophobic interactions played an important role in stabilizing the complex [24].

Thermodynamic Parameters of Transition State

According to the transition state theory, the interaction between the labeled hormone and its receptor leads to the formation of an activated complex (transition state), then the formation of the final product:

$$^{125}\text{I-testosterone} + \text{R} \longrightarrow \underbrace{\left[^{125}\text{I-testosterone-R}\right]}_{\text{an activated complex (Transition State)}} \longrightarrow {}^{125}\text{I-testosterone-R}$$

The transition state thermodynamic parameters ΔH^*, ΔG^*, ΔS^* and Ea could be determined from Arrhenius equation and kinetic constant. Figure (5) shows the dependence of the association rate for the binding of ^{125}I-testosterone to its receptors in ovarian tumor homogenate on temperature (***Arrhenius plot***). According to the plot of k_{+1} values vs. 1/T which gives a linear relationship (Fig.5) as in the following equation [25]:

$$\ln k_{+1} = \ln A - \frac{E_a}{RT}$$

Where A is the Arrhenius constant, sometimes called frequency factor or pre-exponential factor. The value of E_a that determined from Arrhenius plot represents the apparent energy of activation of the binding reaction.

The high positive value of ΔG^* indicated that the formation of an activated [testosterone-R] complex was a non spontaneous process and required a lot of energy (equal to E_a) to overcome the transition state energy barrier and giving the final product, whereas the high negative ΔS^* revealed that the activated complex had a more ordered structure than the reactant species ($\Delta S^* < 0$) as shown in table (5). The positive values of ΔG^* is mainly attributed to the decrease in entropy of the transition state ($\Delta S^* < 0$). In addition, the positive value of ΔH^* shows that the heat content of the activated complex is more than that of isolated species [8].

The activation in the thermodynamic parameters at 42^{o}C for the standard transition states of the binding reaction in ovarian tumor homogenate was higher than the other temperatures investigated, this could be attributed to the elevated temperature which affect the protein structure. Determination of thermodynamic parameters of the binding reaction using equilibrium data gives an overall idea about the nature of forces controlling complex formation [26,27].

Conclusions

The kinetic studies of testosterone with its receptors in ovarian tumor homogenates revealed that the reaction is pseudo first order. In thermodynamic studies there are indications to formation of testosterone – receptors complex undergo three thermodynamic states; Thermodynamic state (A) represents the initial energy level of the isolated ^{125}I- testosterone and its receptor(R). In thermodynamic state (B), the two components have come together and mutually penetrated their hydration sphere to form a partially immobilized hydrophobically associated species. Thermodynamic state (C) represents the fully interacting complex (^{125}I- testosterone -R). The thermodynamic data from this study indicate that the binding of ^{125}I- testosterone to its receptors are entropically driven and come in agreement with the concept that hydrophobic and short-range interactions have an important role in ^{125}I- testosterone -R interactions.

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The Tables:

Table (1): Concentration and affinity constant of the nuclear testosterone receptors in two groups of ovarian tumor patients.

Group	No. of cases	Age (year) ±SD	Binding Capacity (fmole/mg protein)	$k_a \times 10^{10}\ M^{-1}$
Benign	33	51±2.3	7.8	6.3
Malignant	22	43±1.7	9.8	5.1

Table (2): The kinetic parameters of ^{125}I-testosterone binding to its receptors in benign and malignant ovarian tumor homogenate.

Temp. °C	Binding capacity fmol/mg protein		$K_d = \frac{k_{-1}}{k_{+1}} \times 10^{-12} M$		$K_a = \frac{k_{+1}}{k_{-1}} \times 10^{10} M^{-1}$	
	Benign	Malignant	Benign	Malignant	Benign	Malignant
4	7.2	7.9	19.23	23.64	5.2	4.23
25	7.8	9.6	15.87	21.73	6.3	4.6
37	6.6	9.8	17.54	19.6	5.7	5.1
42	5.4	7.7	23.25	32.25	4.3	3.1

Table (3): The effect of temperatures on the kinetic parameters of ^{125}I-testosterone binding to its receptors in ovarian tumors.

Temp. °C	K_{obs} (min^{-1})	K_{+1} ($M.min^{-1}$) x10^8	K_{-1}(min^{-1}) x10^{-4}	$(t_{1/2})_{ass}$ (hr.)	$(t_{1/2})_{diss.}$ (hr.)		K_{obs} (min^{-1})	K_{+1} ($M.min^{-1}$) x10^8	K_{-1}(min^{-1}) x10^{-4}	$(t_{1/2})_{ass}$ (hr.)	$(t_{1/2})_{diss.}$ (hr.)
	Benign						Malignant				
4	0.004	7.46	143.46	1.53	0.005		0.003	6.42	151.77	1.38	0.005
25	0.005	10.95	173.81	3.05	0.0046		0.003	6.54	142.17	1.42	0.0021
37	0.004	7.52	131.93	1.49	0.0028		0.004	9.64	189.02	2.88	0.0054
42	0.002	6.43	149.54	0.94	0.004		0.002	5.22	168.39	0.87	0.0037

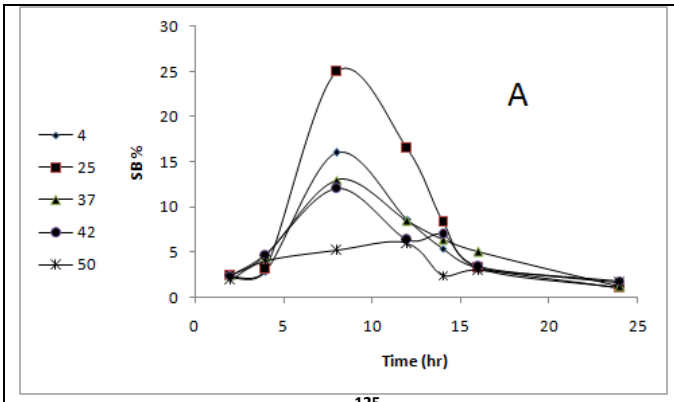
Table(4): Thermodynamic parameters at standard states of testosterone binding to its receptors in benign and malignant ovarian tumor homogenates:

Temp. °C	Benign			Malignant		
	ΔH^o KJ/mole	ΔG^o KJ/mole	ΔS^o KJ/mole	ΔH^o KJ/mole	ΔG^o KJ/mole	ΔS^o KJ/mole
4	7.516	-35.46	0.155	9.243	-42.25	0.186
25	7.516	-34.58	0.141	9.243	-43.56	0.177
37	7.516	-35.21	0.138	9.243	-40.13	0.159
42	7.516	-36.44	0.140	9.243	-42.85	0.165

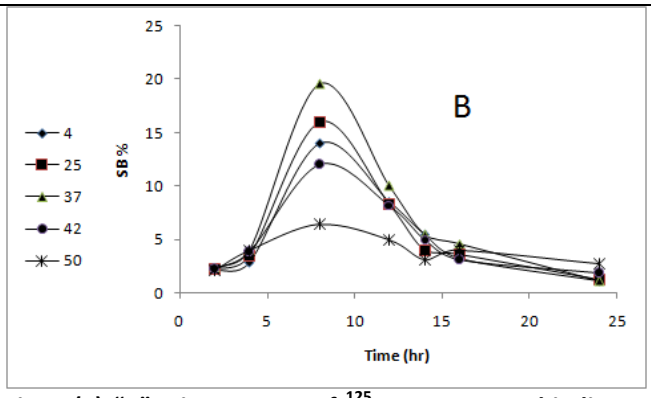
Table(5): Thermodynamic parameters at transition states of testosterone binding to its receptors in benign and malignant ovarian tumor homogenates:

Temp. °C	Benign				Malignant			
	E_a	ΔH^o KJ/mole	ΔG^o KJ/mole	ΔS^o KJ/mole	E_a	ΔH^o KJ/mole	ΔG^o KJ/mole	ΔS^o KJ/mole
4	3.356	4.652	25.64	-0.0757	8.564	6.542	17.25	-0.0386
25	3.356	4.652	26.54	-0.0734	8.564	6.542	18.46	-0.0399
37	3.356	4.652	25.46	-0.0671	8.564	6.542	20.44	-0.0448
42	3.356	4.652	24.53	-0.0631	8.564	6.542	16.78	-0.0325

The figures:



Figure(1) "A": Time course of ^{125}I-testosterone binding to its receptors in benign ovarian tumors.



Figure(1) "B": Time course of ^{125}I-testosterone binding to its receptors in Malignant ovarian tumors.

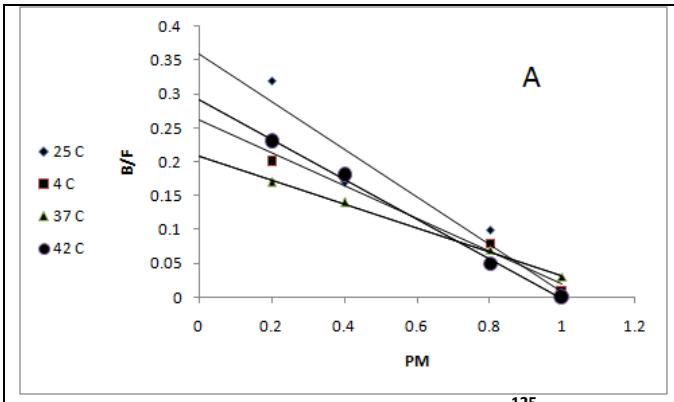


Figure (2) "A": Scatchard analysis of the ^{125}I-testosterone binding to its receptors in benign ovarian tumors.

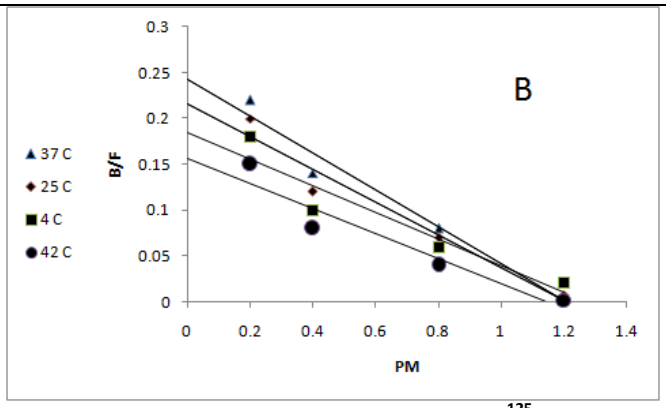
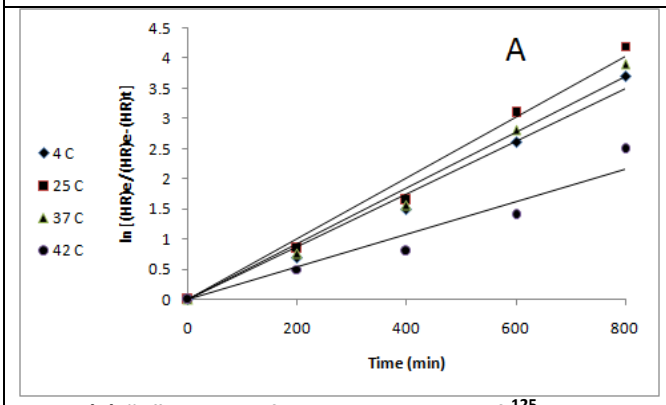
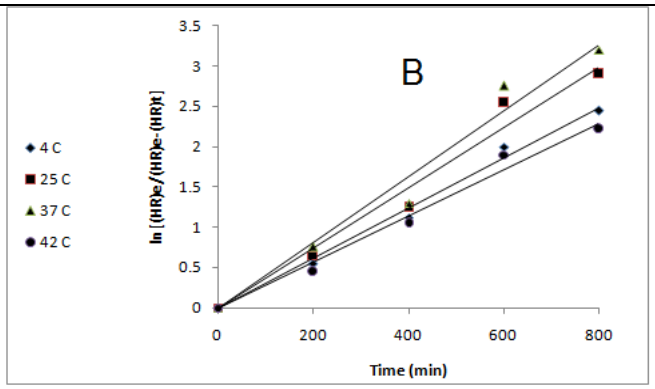


Figure (2) "B": Scatchard analysis of the ^{125}I-testosterone binding to its receptors in Malignant ovarian tumors.



Figure(3) "A": Pseudo-first order kinetics of ^{125}I-testosterone binding with its receptors in benign ovarian tumors.



Figure(3) "B": Pseudo-first order kinetics of ^{125}I-testosterone binding with its receptors in malignant ovarian tumors.

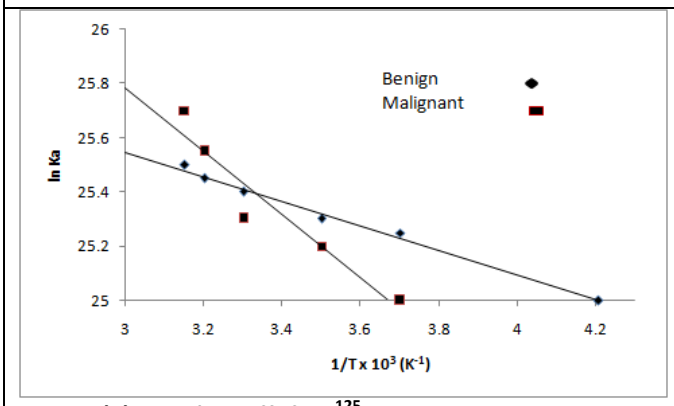


Figure (4): Van't Hoff for ^{125}I-testosterone binding to its receptors in ovarian tumors.

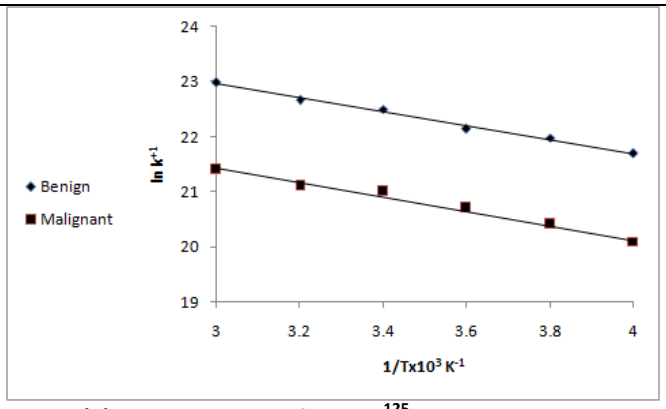


Figure (5): Arrhenius plot for the ^{125}I-testosterone binding to its ovarian receptors.