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

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Summary

Kinetic and thermodynamic studies were carried out on the uterine tumor homogenate binding to ^{125}I-testosterone. Time-course of the association of ^{125}I-testosterone with its receptor in human uterine tumors at four different temperatures revealed the time and temperature dependency. 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., ΔH°, ΔG°, ΔS°) and transition state (i.e., ΔH^*, ΔG^*, ΔS^*).

الخلاصة:

اجريت الدراسات الحركية والثرموديناميكية لأرتباط هورمون التيستوستيرون المعلم بنظير اليود المشع ذي العدد الكتلي 125 مع مستلماته في مجانس اورام الرحم. فقد درس المسرى الزمني لأرتباط الهورمون المعلم بنظير اليود المشع ذو العدد الكتلي 125 بمستلماته في اورام الرحم في اربع درجات حرارية مختلفة ووجد بان عملية الارتباط تعتمد على درجة الحرارة والزمن وان حركيات الارتباط تتبع حركيات المرتبة الاولى الكاذبة . ومن خلال منحنيات المسرى الزمني وسكاجرد (Scatchard) ومعادلات فانت هوف وارينوس تم قياس المعاملات الثرموديناميكية للحالة القياسية (ΔH°, ΔG°, ΔS°) والحالة الانتقالية(ΔH^*, ΔG^*, ΔS^*) .

Introduction:

Steroids 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). Human endometrium, which is the internal layer of the body of the uterus(3), changes morphologically and biochemically during the various phases of the menstrual cycle, influenced by estrogen and progesterone, and androgen(4). Some laboratory studies indicate the presence of specific binding sites for androgen receptors in endometrial cancers(5). The measurements of receptors in endometrial cancer would be clinically useful such as prognosis that could be obtained from other sources(6). The thermodynamics of the molecular interactions between testosterone and its uterine receptors were studied by measuring the temperature dependence of the rate of dissociation and association between testosterone and the receptor. Accordingly, the aim of the work in this paper includes the determination of the kinetic and thermodynamic parameters of the binding reaction of testosterone with its receptors in benign and malignant serous uterine tumors.

Experimental:

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 .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 uterine tumor patients were included in this study. Group I contained 21 patients with benign uterine tumor. Group II consisted of 9 patients with uterine cancer.
All patients were admitted for treatment to Al-Arabe Hospital and Saddam Medical City under the supervision of specialists, Dr. Ryad Mohammed Salh, Dr. Raja Al-Tikreti and Dr. Nada Al-Ubadi. They were histologically proven from the supervision of specialists Dr Luay Edward and Dr.Nabel Abdulwadoad. 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 Uterine Tissue Specimens:

The tumor tissues were surgically removed from uterine tumor patients by either hysterectomy or myomectomy. 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 UterineTumor 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, 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 and The Thermodynamic Studies

The Time-Course of ^{125}I- Testosterone Binding to Its Nuclear Receptors in Benign and Malignant Uterine Tumor

The experiment was carried out in duplicate and according to the following:

- At zero time, 100 µls. of ^{125}I-testosterone (0.9ng) was added to 200 µls. (250 µg protein) of uterine 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 a side until counting.
- After incubation, the radioactivity of bound hormone was estimated as in section (2.4.2) [7].
- Parallel experiments were performed to determine the amount of non-specific binding.
- To determine the time-course of the association of ^{125}I-testosterone with its receptor at different temperatures, the above experiment was performed at five temperatures (4, 25,37,42,50 and 60°C).

Calculations:

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

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

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

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

- 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 Uterine Tumors

The experiment was carried out in duplicate and according to the following:

- Two hundred microliters (250 µg protein) of uterine 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 malignant and 4°C for benign tumors) then the bound hormone was estimated as mentioned in section (2.4.2)[7].
- The previous steps were performed at different temperatures (4, 25,37 and 42°C).

Calculations:

- The value of ^{125}I-testosterone which is bound specifically in picomolar were calculated using the following formula:

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

- The concentration of receptors and the affinity constant were determined according to Scatchard equation:[8]

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

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

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

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 uterine 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 Uterine 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 in section (2.4.2) [7] at different temperatures.
- The rate of the association constant of (^{125}I-testosterone-receptor) complex was calculated by the following equation:

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

where:

The rate association constant k_{+1}:

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

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

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

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

- 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 of ^{125}I-Testosterone Binding to Its Receptors in Benign and Malignant Uterine Tumors Homogenates

The experiment was carried out in duplicate and according to the following:

- 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 malignant uterine tumor and 4°C for benign one for 8hrs.
- After incubation, the radioactivity of (^{125}I-testosterone-receptor) complex formed was estimated as mentioned in section (2.4.2) [7].
- 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:

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

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 constant

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:

Kinetics of the ^{125}I-Testosterone Binding to Its Receptors in Benign and Malignant Uterine Tumors

Figure (1 A & B) shows the time-course of the formation of ^{125}I-testosterone –receptor complex at six different temperatures (4, 25, 37,42,50 & 60°C). The results of time course patterns at different temperatures revealed that the binding of ^{125}I-testosterone to its receptors in uterine tumor homogenate is a temperature and time dependent process with a maximum binding occurs at 4°C for benign and 25°C for malignant after 8hrs of incubation.

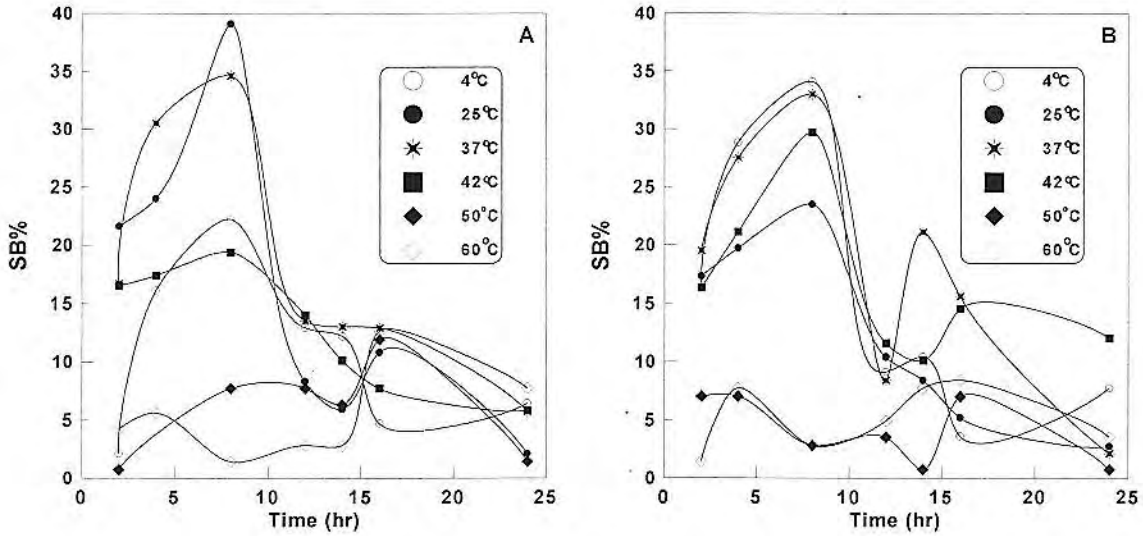


Figure (1): Time-course of ^{125}I-testosterone binding with its receptors in:

A- Malignant uterine homogenate
B- Benign uterine homogenate.

Determination of the Concentration and Affinity Constants of Testosterone Receptors

The concentration of testosterone receptors and the affinity constant of the binding have been measured in uterine 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 .

The results are summarized in table (1). Nuclear testosterone receptors binding capacities(B_{max}) of uterine cancer patients were(10.8fmol) per mg protein while benign uterine tumor patients were(8.2 fmol) per mg protein.

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

Group	No. of cases	Age (year)	Binding capacity (fmole/mg) protein	$k_a \times 10^{10}\ M^{-1}$
Benign	21	53 ± 3.4	8.2	5.7
Malignant	9	42 ± 1.9	10.8	4.6

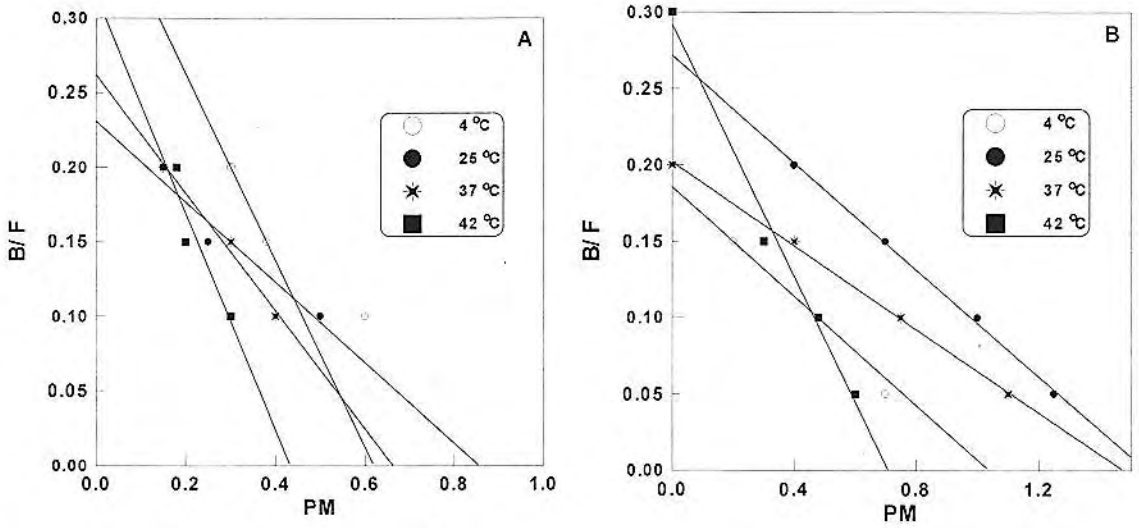


Figure (2): Scatchard analysis of the ^{125}I- testosterone binding to its receptors in (A): Benign and (B): Malignant uterine tumor.

Determination of Kinetic Parameters of ^{125}I- Testosterone Binding to Its Receptors in Patients with Benign and malignant Uterine Tumors

The time course of ^{125}I- testosterone binding to its receptors in uterine 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:

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

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).
Table (2): The kinetic parameters of ^{125}I-testosterone binding to its receptors in benign and malignant uterine 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	8.2	8.2	19.2	16.39	5.2	6.1
25	7.6	10.8	16.39	21.7	6.1	4.6
37	6.8	9.7	14.3	20.0	7.0	5.0
42	5.2	7.5	12.19	12.5	8.2	8.0

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 uterine tumors homogenates following a first order kinetic reactions but due to the bimolecularity of this reaction, the following equation(9).

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

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)(9).

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

Where k_{+1} is the kinetic association constant in $M^{-1}min^{-1}$; $(H)_T$ is the total molar concentration of ^{125}I-testosterone; $®_T$ 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

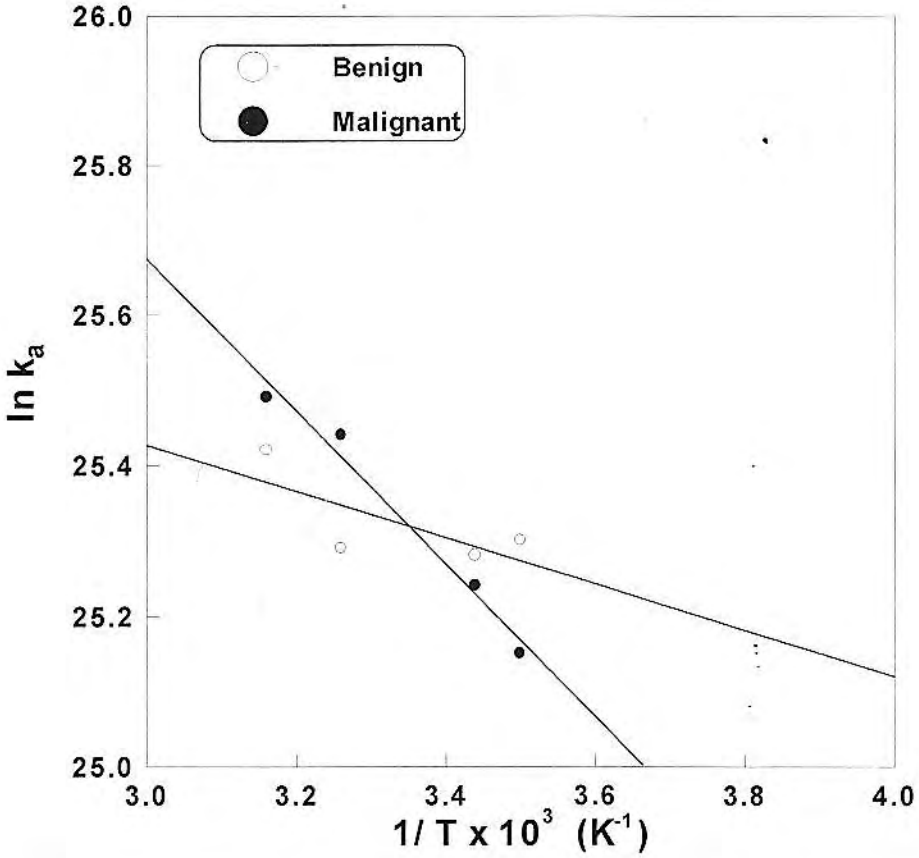


Figure (4): Van't Hoff for the ^{125}I-testosterone binding to its receptors in uterine tumors.

Table (4): Thermodynamic parameters at standard state of testosterone binding to its receptors benign and malignant uterine tumors homogenate.

	Benign			Malignant		
Temp. °C	□H° KJ/mole	□G° KJ/mole	□S° KJ/mole	□H° KJ/mole	□G° KJ/mole	□S° KJ/mole
4	+8.787	-47.85	+198.21	+9.12	-42.24	+211.22
25	+8.787	-48.8	+198.24	+9.12	-43.82	+211.42
37	+8.787	-54.21	+198.54	+9.12	-44.6	+211.62
42	+8.787	-57.32	+198.24	+9.12	-48.2	+229.82

The small positive ΔH° may indicate a favorable interactions between groups within testosterone and its receptors. These include the non-covalent interactions which are fundamentally electrostatic in nature such as charge-charge interactions which occurs in both the hormone (testosterone) and its receptors present in uterine tumor homogenate, other types of interactions include charge-dipole,dipole-dipole, charge-induced dipole, dipole-induced dipole and hydrogen bond.The contribution of any of these intraction to the enthalpy of binding is administrated by the fact when a protein molecule of the complex folds from an expanded

random coil, it gives up some favorable interaction with water[12]. The sum of these types of interactions can yield **some** stabilization to the folded structure of the complex. So, the negative value of $\Delta G°$ showed that the overall reaction was energetically favorable in the direction of complex formation.

Thermodynamic Parameters of Transition State:

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

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

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 uterine 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(13):

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

Where A is the Arrhenius constant, some times 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(14) .

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:

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

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 uterine tumor homogenates and the malignant uterine tumor homogenate at different temperatures.
The results revealed that the association rate constant k_{+1} at 25°C (according to malignant uterine tumor and 4°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). Table (3) shows that k_{-1} increases with the elevation of temperature.

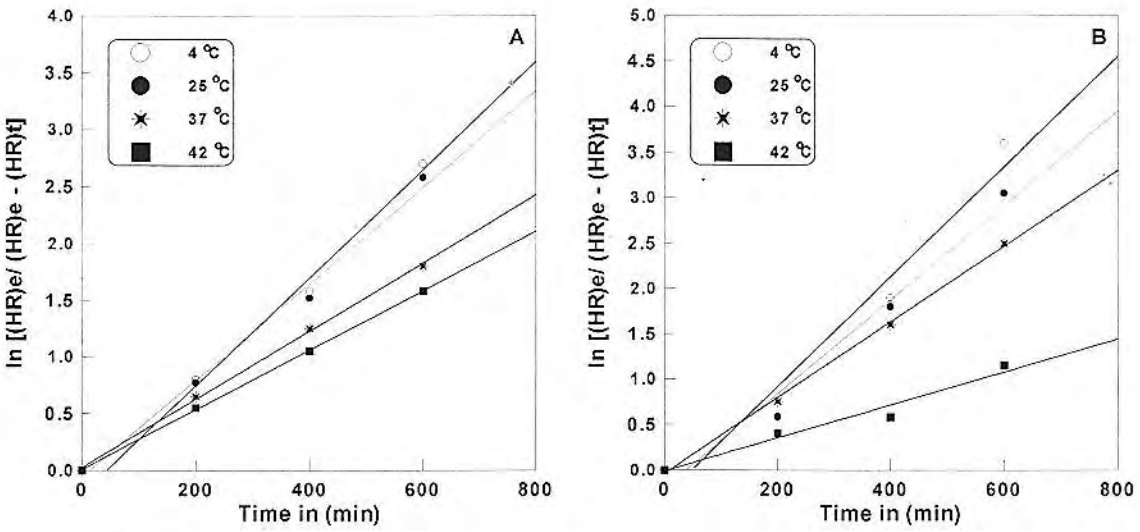


Figure (3): Pseudo-first order kinetics of ^{125}I-testosterone binding with its receptors in
A: benign uterine tissue homogenate,
B: malignant uterine tissue homogenate.

Table (3): The effect of different temperature on the kinetic parameters of testosterone binding to its receptors in benign and malignant uterine tumors.

Benign

Temp. ℃	k_{+1} $(M.min)^{-1}$ $\times 10^{8}$	k_{-1} min^{-1} $\times 10^{-4}$	$(t_{½})_{ass.}$ *hr*	$(t_{½})_{diss.}$ *hr*	k_a M^{-1} $\times 10^{10}$
4	2.91	56.1	1.66	0.012	5.2
25	4.61	75.7	1.75	0.009	6.1
37	7.8	111.4	1.80	0.006	7.0
42	11.4	139.03	3.10	0.005	8.2

Malignant

Temp. ℃	k_{+1} $(M.min)^{-1}$ $\times 10^{8}$	k_{-1} min^{-1} $\times 10^{-4}$	$(t_{½})_{ass.}$ *hr*	$(t_{½})_{diss.}$ *hr*	k_a M^{-1} $\times 10^{10}$
4	4.6	75.5	2.1	0.009	6.1
25	5.16	112.2	1.8	0.006	4.6
37	7.5	150	2.4	0.0045	5.0
42	13.56	169.5	2.65	0.004	8.0

This numerical difference may be attributed to the different types of receptor sources used and to differences in the structure of these receptors.

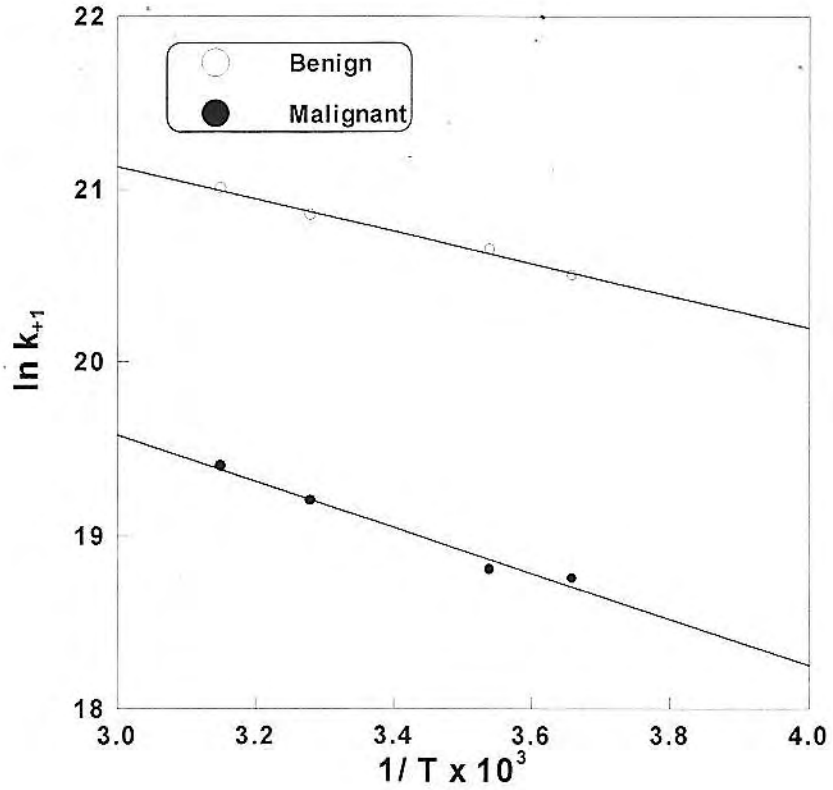
The Thermodynamic of the Binding of ^{125}I-Testosterone to Its Receptors in Benign and Malignant Uterine Tumors

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 uterine 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 ΔH° value in the case of malignant uterine tumor receptors was higher than that in the cases of benign uterine tumors receptors, so more energy was needed in case of malignant uterine tumor receptors for the reaction (binding) to occur.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(10).

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(11).



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

The activation in the thermodynamic parameters at 42°C for the standard transition states of the binding reaction in uterine 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. Comparison of the values of transition state with those of standard state in tables (4) and (5) lead us to choose a thermodynamic model shown in figure (6).

Table (5): Thermodynamic parameters at transition state of testosterone binding to its receptors in benign and malignant uterine tumors.

Benign					*Malignant*			
Temp. °C	E_a	□H* KJ/mole	□G* KJ/mole	□S* KJ/mole	E_a	□H* KJ/mole	□G* KJ/mole	□S* KJ/mole
4	4.38	2.45	27.21	-80.24	10.25	6.32	18.21	-75.72
25	4.38	2.40	29.75	-80.68	10.25	6.11	18.98	-75.52
37	4.38	2.27	30.12	-81.42	10.25	5.83	19.8	-74.18
42	4.38	2.17	32.4	-82.32	10.25	5.42	20.057	-74.62

Our model proposes that the formation of the (^{125}I- testosterone -receptor) complex undergo three thermodynamic states. Thermodynamic state (A) represents the initial energy level of the isolated ^{125}I- testosterone and its receptor®. 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). In step 1 of the reaction, the binding of ^{125}I- testosterone to its receptor was associated with positive ΔG^* value. This indicates that the initial step of the reaction requires input of energy for the system. The negative entropy change ΔS^* for this step of the reaction reflects the change of the ^{125}I- testosterone -R transition complex to a more ordered structure. The positive ΔH^* value shows that the heat content of the activated complex is more than that of the isolated species. Partial immobilization of the hydrophobically associated complex formed, in step 1, occurs when isolated hydrated species ^{125}I- testosterone and receptor ® interact partially so that there is a mutual penetration of their hydration layers to form the activated complex. This hydrophobic association is a result of the tendency of water to form a more ordered structure in the vicinity of non-polar hydrocarbon groups (e.g. the side chains of the amino acids phenyl alanine, leucine and tryptophan) this means that hydrophobic amino acid side chains which were previously accessible to solvent in the isolated species became buried upon complex formation. In step 2, the activated complex participates in further interactions, giving the fully interacting complex (^{125}I- testosterone-R). It is proposed that the formation of a testosterone - receptor complex occurs in two steps, the first step stabilized the complex by hydrophobic interactions and the second step stabilized it by short range interactions such as electrostatic interactions, hydrogen bonding and Van der Waals' interactions(15) .

Hydrophobic interactions contribute to the stability of the complex via large decrease in the excited entropy change ($\Delta S^* < 0$), while electrostatic interactions, hydrogen bonding and Van der Waals' interactions stabilize the complex via high increase in the standard entropy change ($\Delta S^o > 0$). [15, 16]

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.

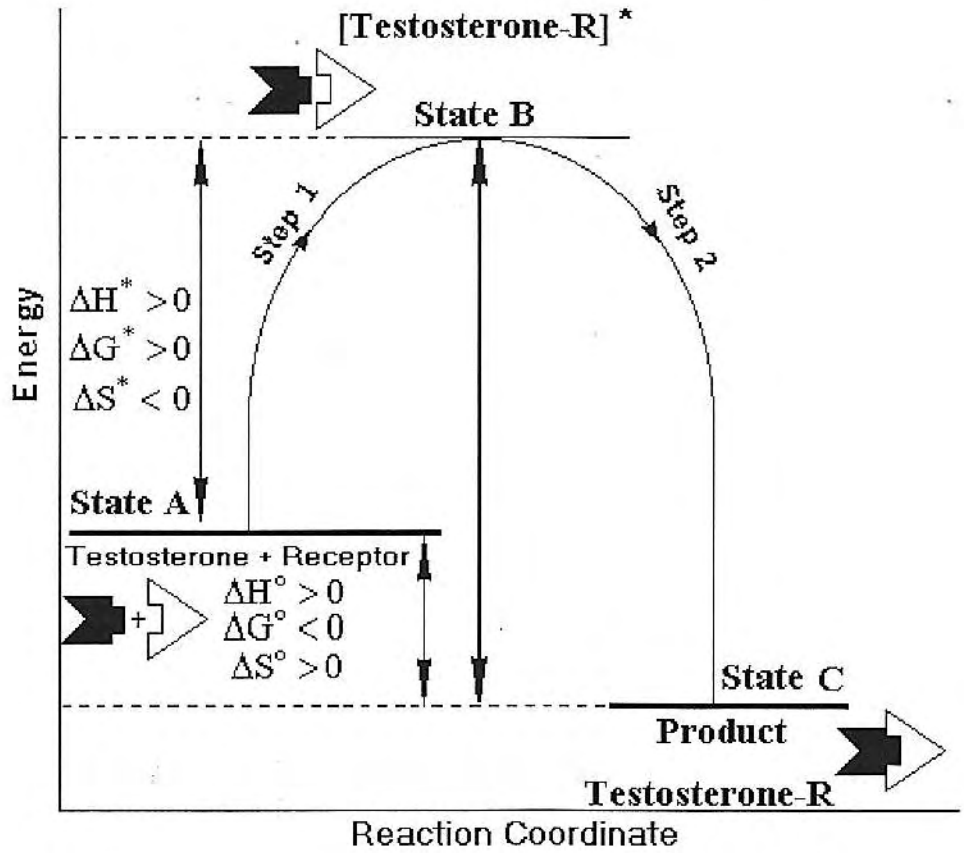


Figure (6): General energy diagram and thermodynamic model applied to the interaction of ^{125}I-testosterone with its receptor in ovarian tumors

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