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KINETIC AND THERMODYNAMIC STUDIES ON AFP BINDING TO AFP ANTIBODY IN COLORECTAL TUMOR HOMOGENATES

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ABSTRACT

Kinetic and thermodynamic parameters associated with the binding of ^{125}I- anti AFP Antibody to AFP in both crude colorectal homogenate and partially- purified fractions were investigated. It was shown that the reaction in all studied groups follow pseudo- first order reaction kinetics.

The value of kinetic parameters K_a, K_d, K_{obs}, K_{+1}, K_{-1}, $(t_{1/2})_{ass.}$, $(t_{1/2})_{diss.}$ and maximal binding capacity (B_{max}) at 25°C for the binding of anti ^{125}I-AFP antibody with its cytosolic antigen in benign colorectal tumor were found to be : 0.0543 x 10^{10} M^{-1}, 15.064 x 10^{-10}M, 0.0158 min^{-1}, 4.086 x 10^{6} $M^{-1}.min^{-1}$, 64.76x10^{-4} min^{-1}, 43 min, 104.28 min and 29.14 Pmol/mg protein respectively, while 0.0538 x 10^{10} M^{-1}, 15.142 x 10^{-10} M, 0.0163 min^{-1}, 4.301 x 10^{6} $M^{-1}.min^{-1}$, 67.63 x 10^{-4} min^{-1}, 40 min, 79.66 min and 46.25 Pmol/mg respectively for the binding of anti ^{125}I-AFP antibody with its cytosolic antigen in tissues of malignant colorectal tumor and at 4°C.

The maximum binding of partially purified AFP occurred at 25°C. The values ofK_a and K_{+1} increased with increasing temperature.

The Van't Hoff plot demonstrated linear relationship between lnKa and 1/T, using crude colorectal homogenate partially purified AFP as AFP source. Plotting between ln K_{+1} and 1/T gave linear relationship called Arrheniuns relationship. The thermodynamic parameters ΔH°, ΔG° and ΔS° for the formation of (^{125}I- anti AFP Antibody / AFP) complex at the standard state had been determinedas well asEa, ΔH*, ΔG* and ΔS* which representing the transition state.

NTRODUCTION

AFP displays both cellular uptake and transmembrane passage via specific cell surface receptors (Moro R. et al 1993), and cytoplasmic binding protein accumulating in either receptome or lysosomal pathways. Many reports have identified and characterized various binding proteins associated with AFP in different cellular compartments (Mizejewski G.J. 1995 and Mizejewski, G.J. 1995). There is a large body of literary evidence on identification and characterization of various AFP binding proteins present in the free form in physiological fluids and in the membrane bound form on the surface of certain types of human cells (Naval J. et al 1985, Kanevsky V. Y.et al 1997, and Uriel J. et al 1983).

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 (Encyclopædia Britannica. 2011, and Jasuja R.et al 2009). The study of kinetic and thermodynamics of any reaction gives the whole picture of the reaction and the application approaches of that reaction (Burley, S. K. and G. A. Petsko 1988).

Macromolecular interactions involve cooperative, independent and contiguous binding regions. The complexity caused by this is increased by the dynamic structural changes. The interaction and the equilibrium condition are influenced by the algebraic sum of the energies involved in reversible interacting energy components, namely electrostatic and hydrophobias, is best understood by study of the thermodynamic parameters of the interaction .The method most widely used for such investigations is isothermal titration calorimetry (Wiseman, T.et al 1988, Wibdenmeyer, J. A.et al 1999, and Sundberg, E. J.; et al 2000), which directly determines the heat changes during the binding process derives the thermodynamic parameters, enthalpy (ΔH), free energy (ΔG) and entropy (ΔS) . In the last two decades the method has been used to unravel the intricacies of the interaction

between several ligand – ligand pairs, like protease – protease inhibitor(Gomez, J and Freire. 1995), receptor – ligand (Myszaka, D. G.; et al. 2000), and Antigen – Antibody (Wibdenmeyer, J. A. et al 1999).

The idea of this paper is to describe the basic mathematical analysis that could be used to explain the mechanism of binding of AFP to its Antibody to form (^{125}I – anti AFP Antibody / AFP) complex in human colorectal tissues, using benign and malignant colorectal tissue homogenate and also partially purified colon tumors fractions, as AFP source.

Materials and 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, sephadex G200, and Sucrose were obtained from Fluka company, Switzerland. $CuSO_4.5H_2O$, Na,K–tartrate ,NaOH , HCl , Na_2CO_3 , and Folin – ciocalteaue were obtained from BDH limited pool, U.K. Immunoradiometric assay for AFP was purchased from ImmunotechBechman (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, Cintra 5 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.

Specimens Collection:

The specimens were surgically removed from patients of colon and rectum (CR). They were immediately rinsed with ice–cold saline solution, and immersed in the same solution. They were collected and stored at –20^{o}C until homogenization. The weight of resected tissue samples range between (1.6-18) gm.

Preparation of Tissue Homogenate:

The frozen tissue were washed with ice-cold normal saline and then weighed. The samples were minced, pulverized, with a scalpel scissors in the Petri dish on ice bath, and then homogenized at 4oC in tris buffer (0.05M , pH 7.4) with ratio of 1 : 4 (weight : volume) using normal homogenizer . The homogenates were filtered through a nylon mesh sieve in order to eliminate fiber connective tissue, and then centrifuged at 4oC. The supernatants and pellets were considered cytosolic and nuclear fractions respectively. The pellet (sediment) was discard, and the cytosolic (supernatant) was used in experiments involved cytosolic cancer antigen AFP source.

Solutions:

TES Buffer solution (0.05M, pH 7.4) was prepared as follows: (3.0285gm) of tris (hydroxy methyl amino methane), 0.93060 of Ethylene diamine tetra acetate disodium salt (EDTA) and (42.7875gm) of sucrose were dissolved in 400 mL of deionized distilled water, then the pH was adjusted with HCl (1M) at 7.4 and the solution was completed to 500 ml with deionized distilled water.

Kinetic studies

Scatchard Analysis of Determination Affinity Constant K_a and the Maximal Binding Capacity (B_{max}) of:

(A) AFP in Colorectal Tissue Homogenate associated with ^{125}I- anti AFP Antibody:

- One hundred µL of colorectal homogenate (benign and malignant colorectal tumor) containing (300 and 200 µg .ml –1) protein respectively were pipetted in each type of homogenate.
- Increased volumes of ^{125}I – anti AFP Antibody for each group of colorectal homogenate i.e., (4, 8, 12, 16, and 20) µL, in the case of benign colorectal tumors and (8, 16, 24, 32, and 40) µL for Colorectal cancer were added to each assay tubes for each case.
- The volumes of all tubes were completed to the final volume of 250 µl with tris – buffer (pH 7.0 and 7.2) respectively to the groups of tissue homogenate.
- The time of the incubation required to reach the equilibrium state are reported in table (1).
- After incubations of each group at each time and temperature required, all tubes were centrifuged at 4000 r. p. m. for one hour at 4ºC by using cooling centrifuge.

The Tables:

Table (1): The time of incubation for benign and malignant colorectal tumor homogenate at different temperatures.

Temp. °C	Time (hour)	
	Benign	Colorectal Cancer
4	4	3
25	3	6
37	3	4
45	3	5

- The supernatant was discarded and the complex formed was counted in gamma counter for one minute.

Calculation:

- The B/T ratio was computed for each tube, where:

B: is the bound radioactivity (mean counts c.p.m), which represents the ^{125}I – anti AFP / AFP) complex.

F: is the free radioactivity (mean counts c.p.m), which represents (unbound or unreacted, ^{125}I – anti AFP).

T: is the total activity (mean counts)

F= T (total counts) – B (bound radioactivity).

The concentration of (^{125}I – anti AFP / AFP) complex in mg.ml $^{-1}$ that formed after time (t) was calculated from the following equation:

$$B(mg.ml^{-1}) = \frac{\text{B (c.p.m)}}{T\,(c.p.m)} \text{ X concentration of } {}^{125}I \text{ - antibody in the incubation medium in mg.ml}^{-1}$$

The affinity constant and maximal binding capacity were determined according to Scatchard equation (Burtis, C. A et al 1999, and Kalstan, M. B. et al 1991):

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

$$Ka = \frac{1}{K_d} = \frac{K_{+1}}{K_{-1}}$$

Where:

Ka = affinity Constant or Equilibrium constant.

K_d = dissociation Constant.

B max = maximal binding capacity

The value of the affinity constant of the binding K_a at each temperature can be calculated from the slop of the straight line, while the value of the total concentration of AFP (B_{max}) in colorectal tissue for each group was calculated from the intercept on X – axis.

(B) Partially Purified AFP in Colorectal Homogenate Binding with ^{125}I – anti AFP Antibody:

- Increasing volume of ^{125}I – anti AFP Antibody (10, 15, 20, 25 and 30) µL were incubated with 35 µg.ml^{-1} of the of partially purified AFP from benign tumor tissue, while 18 µg.ml^{-1} of partially purified AFP from malignant tumor tissue were incubated with increasing volume (4, 8, 12, 16 and 20) µL of ^{125}I- anti AFP Antibody.
- All tubes were completed to 250µL with tris buffer (pH 7.0 and 7.2) for benign and malignant tumor tissue respectively.
- The time of incubation required to reach the equilibrium state were listed in table(2).

Table (2): The time of incubation for the partially purified AFP from colorectal tumor tissues.

Temp. °C	Time (hour)	
	Benign	Colorectal Cancer
4	4	4
25	5	3
37	3	5
45	3	5

- Steps 4, 5 in the experiment of (previous experiment) were repeated.

Calculation:

The steps of calculations outlined in the above experiment were followed exactly to obtain the values of K_a and B_{max} at each temperature.

The Thermodynamic Studies:

The thermodynamic of ^{125}I- anti AFP Antibody binding to its Antigen in Colorectal Homogenate and Partially Purified AFP in Benign and Malignant Colorectal Tumors:

- One hundred micro liter of colorectal tissue homogenate (benign and malignant) containing (300 and 200 µg) protein were added to (2.38 and 1.47 mg.ml^{-1}) of ^{125}I – anti AFP Antibody.
- The volume was completed to 250 µl with tris buffer (0.05 M, pH 7.0 and 7.2) respectively.
- All tubes were incubated at 25°C at different time intervals (1, 2, 3, 4, 5, 6, 7, and 8) hours.
- To determine the time course of AFP binding to ^{125}I–Antibody at different temperatures. Steps 1, 2 in the same experiment were repeated at different temperature (4, 37 and 45°C).
- Two additional tubes containing 50µl of ^{125}I–anti AFP antibody only, for total activity computation, were set aside until counting.
- After incubation, the tubes were centrifuged at 4000r.p.m for one hour at 4°C using cooling centrifuge.
- The supernatant was decanted and the radioactivity of the complex formed was counted.
- The values of (B/T)% were plotted versus the time at different temperature.

The same steps were followed for partially purified AFP.

Calculation:

- The thermodynamic parameters of the standard state obtained from Van't Hoff, the values of the natural logarithm of equilibrium constant (affinity constant Ka) obtained at different temperature were plotted against the reciprocal values of the 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 (8.31414 J. K^{-1}).

ΔH° value obtained from the slope, the linear relationship of the plot.

The change in Gibbs free energy of the standard state ΔG° was obtained from the following equation:

$$\Delta G^\circ = - R T \ln Ka$$

Where K_a is the affinity constant, while the standard state entropy change was obtained from (Adams, A.; and Karrott, D.; 1985):

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

The thermodynamic parameters of the transition state were obtained from Arrhenius plot of (ln K^{+1} values against 1/T values that gave a linear relationship according to the following equation:

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

Where A = frequency factor or per exponential 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* was obtained from:

$$\Delta H^* = Ea - RT$$

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

$$\Delta G^* = - RT \ln K_{+1} + RT \ln \frac{KT}{h}$$

Where K and h were Boltzman and Plank's constant, which equal (1.38 x 10 $^{-23}$ J. K $^{-1}$), (6.62 x 10 $^{-34}$ J. sec $^{-1}$) respectively.

The change in entropy of the transition state ΔS* was calculated from the following equation:

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

Results and Discussion:

Determination of Affinity Constant (K_a) and the Maximal Binding Capacity (B_{max}) of AFP in colorectal Tissue Homogenate Associated with ^{125}I- AFP Antibody

B_{max} and the affinity constant Ka of the binding to ^{125}I-anti AFP Antibody was measured. The experiment was carried out at the optimum conditions that were obtained in previous experiments. Scatchard plot analysis gave straight line as shown in figure(1 A&B), and the parameters obtained from Scatchard plot are shown in table(3).

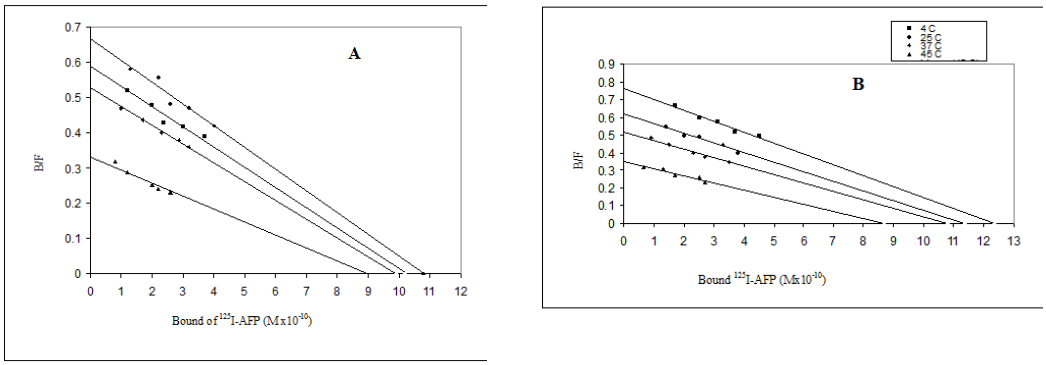

Figure (1): Scatchard plot of 125I – anti AFP binding to AFP in (A) Benign tumor (B) Malignant tumor

The values K_a and maximal binding capacity (Bmax) were calculated from Scatchard plot at four different temperatures. 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 (Rhoades R.A., and Tanner G.A. 2001).

Table (3): The Kinetic parameters of AFP binding to its ^{125}I- anti AFP Antibody in colorectal tissue homogenate

Temp. °C	Ka x 10^{10} M^{-1}		Kd x 10^{-10} M		Bmax Pmol.mg^{-1} protein	
	Benign	**Colorectal Cancer**	Benign	**Colorectal Cancer**	Benign	**Colorectal Cancer**
4	**0.0522**	**0.0578**	**16.115**	**15.142**	**27.25**	**46.25**
25	**0.0543**	**0.0532**	**15.064**	**16.554**	**29.14**	**43.28**
37	**0.0518**	**0.0514**	**16.546**	**18.556**	**25.56**	**41.35**
45	**0.0458**	**0.0509**	**17.308**	**19.485**	**24.35**	**38.64**

It is clear from table(3) that the affinity constant (K_a) is depended on the type of the tumor (i. e, benign and malignant) and on the temperature. The highest value of K_a occurred at 25ºC in the case of Benign but in the case of colorectal cancer, the optimum value of K_a was at 4ºC, while the lowest values of K_a in the both groups of colorectal tissue homogenate were at 45ºC. The kinetic and affinity constant were different due to the differences in one or more amino acid present in epitope domain (Jose, M. et al 1998).Scatchard plots analysis gave straight lines, indicating that probably only one species of binding site is present or more but with the same affinity.

The simplest proposed model representing the binding of ^{125}I – anti AFP Antibody with AFP could be expressed by the following equation

$$^{125}\mathbf{I - Ab + Ag\ (AFP)} \underset{\mathbf{k_{-1}}}{\overset{\mathbf{k_{+1}}}{\rightleftharpoons}} \left[^{125}\mathbf{I - Ab - Ag}\right]$$

Where:
K_{+1}: is the rate of the association of ^{125}I – anti AFP Antibody with AFP.
K_{-1}: is the rate of reverse reaction of dissociation of the complex formed under the same condition.
At equilibrium:

$$K_a = \frac{[^{125}I\text{ - anti AFP antibody / AFP}]}{[^{125}I\text{ - anti AFP antibody}][AFP]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\mathbf{K_d = \frac{[^{125}I - anti\ AFP\ antibody][AFP]}{[^{125}I - anti\ AFP\ antibody / AFP]}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Thus, $$\mathbf{K_a = \frac{1}{K_d} = \frac{K_{+1}}{K_{-1}}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where:
K_a: is the equilibrium constant of the association (affinity constant).
K_d: is the equilibrium constant of the dissociation of ^{125} I – anti AFP Antibody / AFP) complex.

The reorder of ^{125}I – anti AFP Antibody to AFP was determined by using the following equation (Weiland, G. A.; and Molinof, P. B. 1981):

$$\mathbf{\ln [Ab\ Ag]e\ \frac{[Ab]t - [Ab\ Ag]t\,[Ab\ Ag]e / Ag]t}{[Ab]t\,[Ab\ Ag]e - [Ab\ Ag]e} = K_{+1}\ t\ \frac{[Ab] + [Ag]t - [Ab\ Ag]e}{[AbAg]e}} \quad \ldots(4)$$

Where:
K_{+1}: is the kinetic association constant.
[AbAg]e : is the concentration of (^{125}I – anti AFP/ AFP) complex formed at equilibrium.
[AbAg]t : is the concentration of (^{125}I – anti AFP / AFP) complex after time (t).
[Ab]t : is the total concentration of ^{125}I – anti AFP Antibody.
[Ag]t : is the total concentration of AFP.
Equation (4) represents the second order kinetics, but the percent of binding was in some cases, small and most labeled Antibody remains free and only small fraction binds even at equilibrium, i.e., [Ab]t >> [AbAg]e

Thus:

$$\mathbf{[Ab]\ t >> \frac{[Ab\ Ag]t\,[AbAg]e}{[Ag]t}}$$

So that the following equation could be used in order to fit the pseudo-first order kinetics (Seely, G. A. et al 1980).

On the other hand figures (2, 3 and 4) show the plots of $\ln \frac{\mathbf{[Ab\ Ag]e}}{\mathbf{[Ab\ Ag]e - [Ab\ Ag]t}}$ against time (t) gave a straight line with a slope equal to the observed value of the first rate constant (K_{obs}) in min^{-1}. The rate of constant (K_{+1}) in mg^{-1}.ml.min was calculated at five different temperatures using the following equation (Segel, I. H. 1979).

$$\mathbf{K_{obs} = K_{+1}} \frac{^{125}\text{I - anti AFP]t [AFP]t}}{[^{125}\text{I - anti AFP / AFP}]e} \quad \ldots\ldots\ldots\ldots\ldots\ldots(6)$$

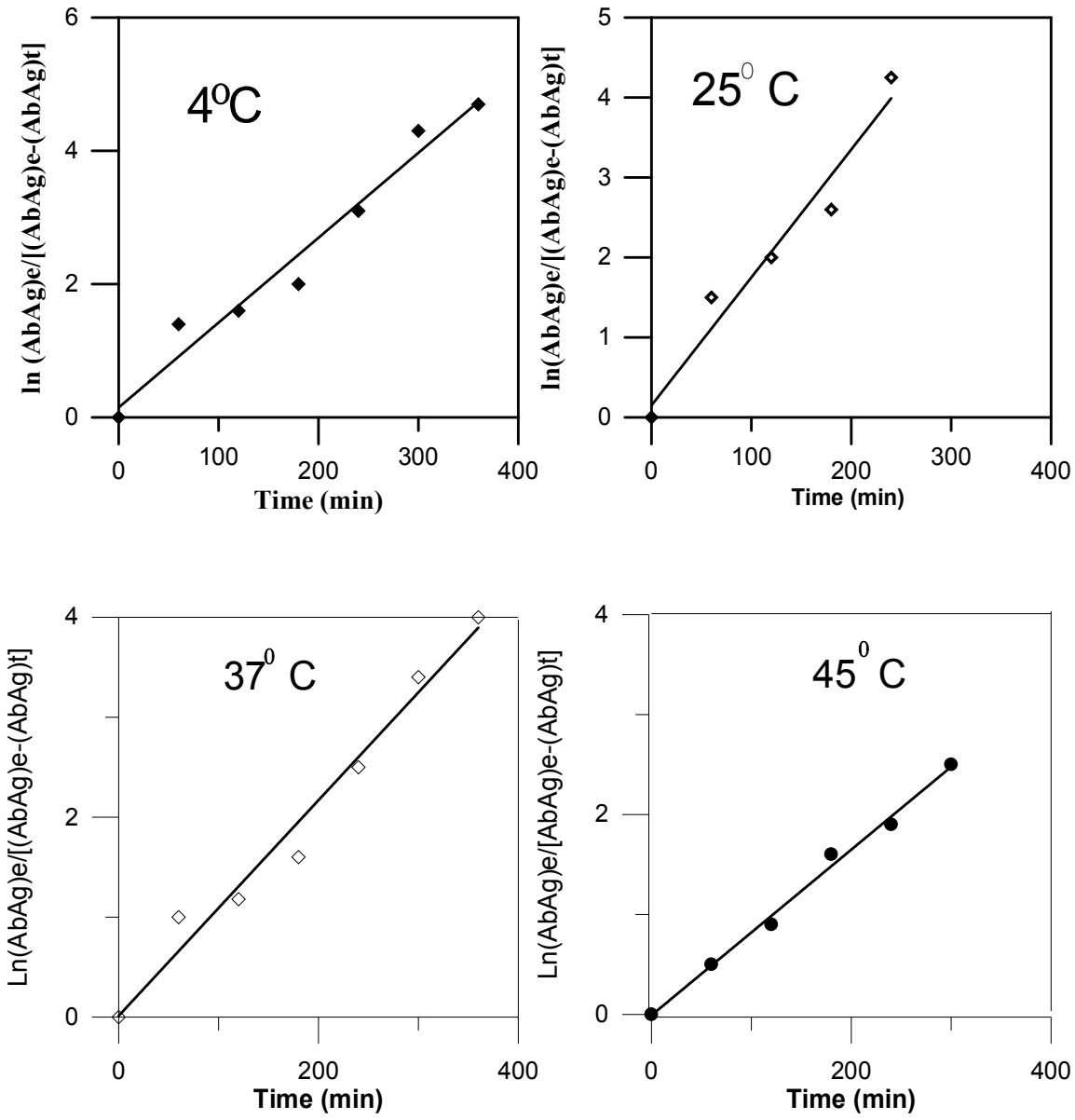

Figure (2): Kinetic of ^{125}I-anti AFP Antibody binding to AFP in Benign colorectal tumor.

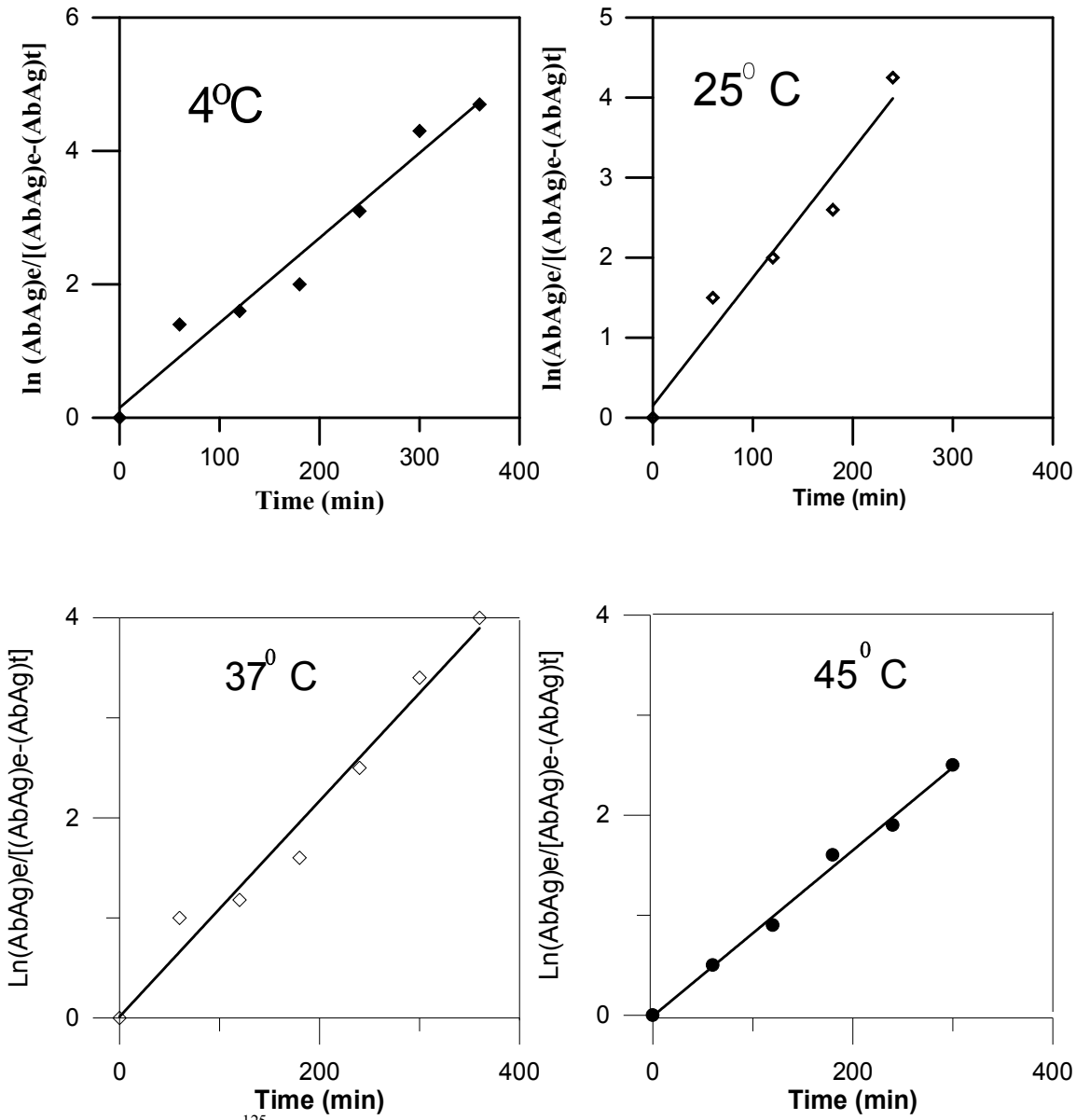

Figure (3): Kinetic of ^{125}I-Anti AFP antibody binding to AFP in Malignant colorectal tumor.

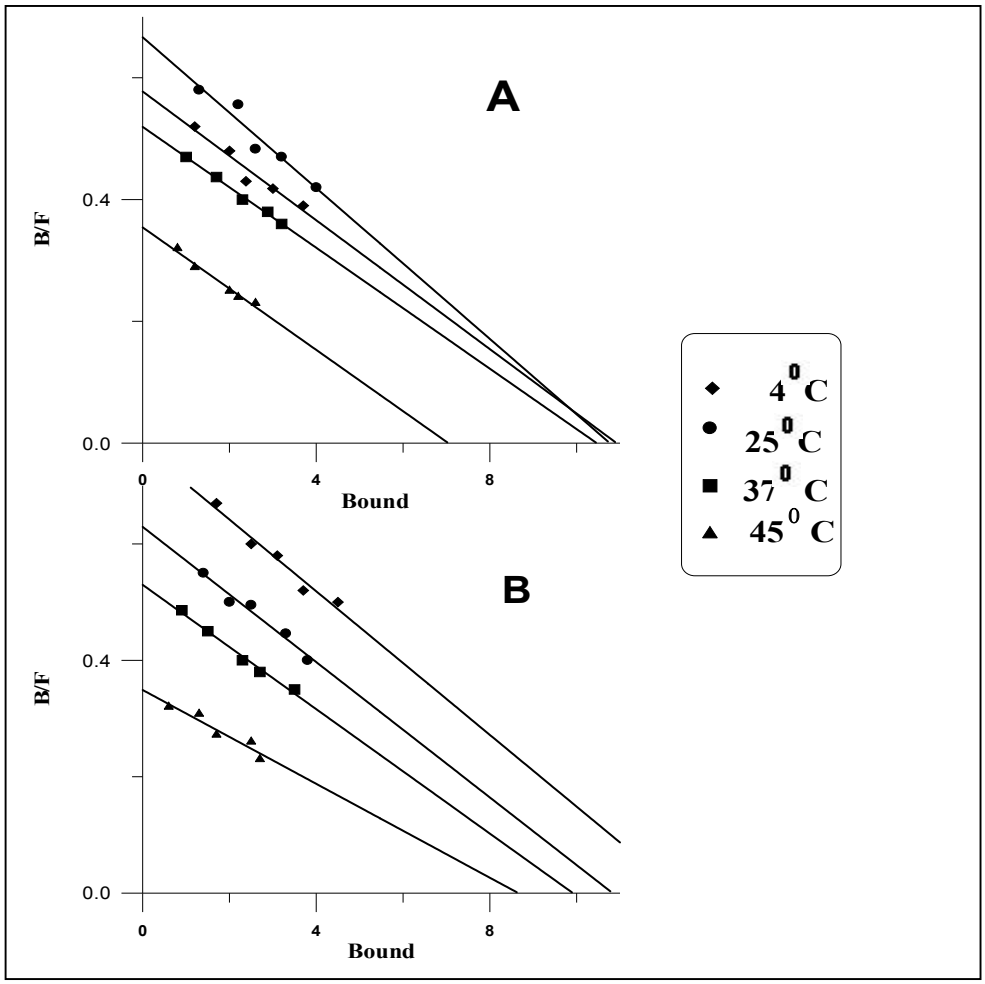

Figure (4): Scatchard plots of ^{125}I-anti AFP binding to the partially purified AFP antigen in: (A) Benign colorectal tumor and (B) Malignant colorectal tumor

Also, the value of K_{-1} at four temperature were calculated using equation (3), whereas, the half life time of association (t 1/ 2) $_{ass.}$, which represented the time needed for the formation of half amount of the complex at equilibrium was determined from the concentration of the complex at equilibrium and the time course curve. The half-life time of dissociation (t 1/2) diss., was calculated from the following equation.

$$(t_{1/2\ diss}) = \ln \frac{2}{K_{1}} = \frac{0.693}{K_{1}}$$

The values of $K_{obs.}$, K_{+1}, K_{-1}, $(t_{1/2})$ ass., and $(t_{1/2})$ diss., at different temperature are summarized in table(4). The values in this table show the highest rate for the association reaction K_{+1}, in benign colorectal tumors occurred at 25°C and at 37°C in malignant colorectal tumors, while the lowest rate occurred at 45°C, so the reaction rate is a temperature dependent, while the rate constant for the reverse reaction K_{-1} which refers to the rate of dissociation of ^{125}I– anti AFP Antibody from its AFP is temperature independent.

Kinetic of the Binding of ^{125}I – anti AFP Antibody to partially – purified AFP from colorectal tumors Homogenate

Scatchard plot analysis gave a straight line as shown in fig (4) for each partially purified AFP from benign and malignant homogenates of colorectal tumors at each temperature which indicates that the presence of single class of binding sites or more with the same

affinityfig(5).These results are summarized in Table(5).

Commonly, it can be concluded that the crude AFP had lower affinity to bind with its Antibody than the partial – purified AFP in both isolated Antigens.

The difference between benign and malignant in optimum temperature of binding relates to the differences in the whole tissues environmentfig(6)(Waelbroeck, M. et al 1979).

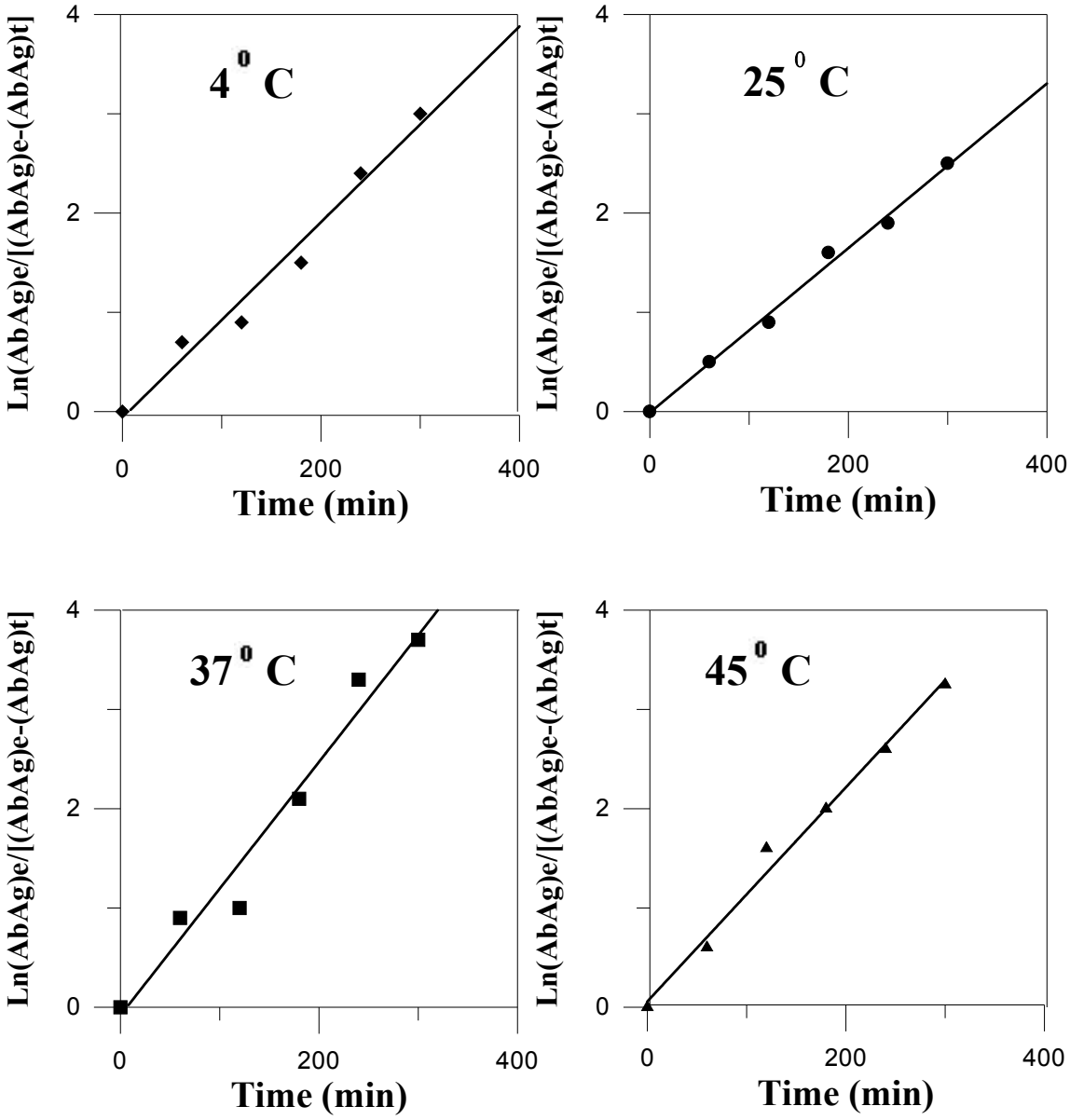

Figure (5): Kinetic of ^{125}I-anti AFP antibody binding to partially purified AFP from benign colorectal tumor homogenate.

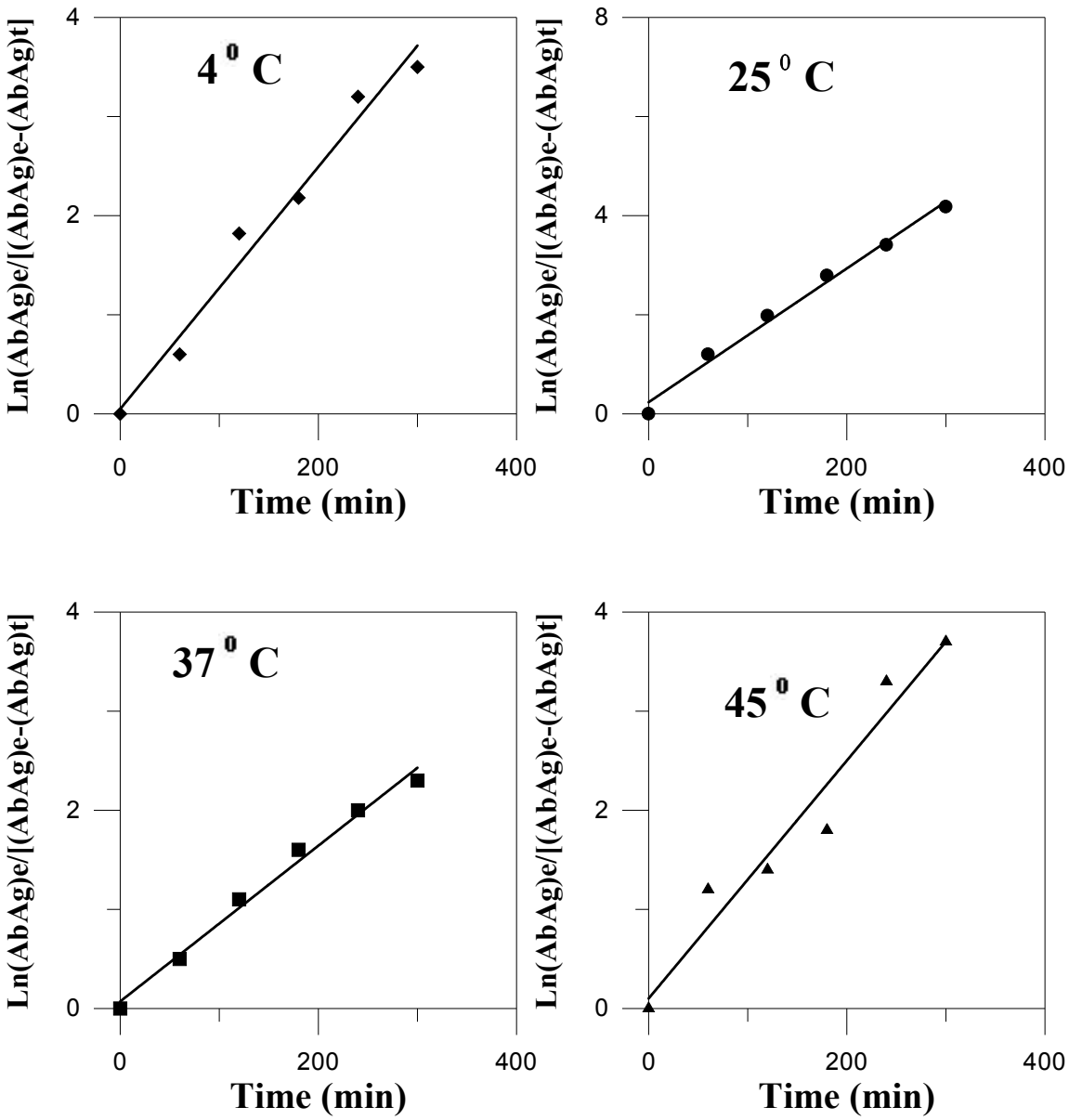

Figure (6): kinetic of ^{125}I-anti AFP antibody binding to partially purified AFP from malignant colorectal tumor homogenate.

Table (4): The effect of temperatures on the kinetic parameters of ^{125}I-anti AFP antibody binding with AFP in homogenates of colorectal tumors.

Temperature °C	$K_{obs.}$ ($min.^{-1}$)	$K_{+1} \times 10^{6}$ ($M^{-1}.min.^{-1}$)	$K_{-1} \times 10^{-4}$ ($min^{-1}.$)	$(t_{1/2})_{ass.}$ (min.)	$(t_{1/2})_{diss.}$ (min)
Benign					
4	0.0118	3.22	55.47	81	118
25	0.0158	4.08	64.76	43	104.28
37	0.102	2.428	51.32	68	128
45	0.0049	2.049	50.44	52	144
Malignant					
4	0.0163	4.301	67.63	40	99.66
25	0.0112	2.163	43.03	53	142.82
37	0.0091	1.746	38.58	65	156.74
45	0.0058	1.398	35.74	50	179.88

Table (5): The Kinetic parameters of ^{125}I- anti AFP Antibody binding to its partially – purified AFP in colorectal tissue homogenate

Temp. °C	*Ka x 10^{10} M^{-1}*		*Kd x 10^{-10} M*		*Bmax Pmol.mg^{-1} protein*	
	Benign	**Colorectal Cancer**	*Benign*	**Colorectal Cancer**	*Benign*	**Colorectal Cancer**
4	***0.2849***	***0.2621***	***36.401***	***40.823***	***35.34***	***46.66***
25	***0.2382***	***0.3179***	***38.078***	***33.312***	***31.83***	***55.24***
37	***0.2986***	***0.2545***	***33.141***	***42.867***	***43.12***	***40.11***
45	***0.2871***	***0.2766***	***35.453***	***36.419***	***38.83***	***48.12***

The thermodynamic studies of interaction of ^{125}I-anti AFP antibody with AFP in colorectal tumor tissues

(A) Thermodynamic parameters of standard state:

Figure(7) represents the dependence of the equilibrium binding constant (i.e., affinity constant) for the binding of ^{125}I-anti AFP antibody to its AFP of benign and malignant colorectal tumor homogenates on the temperature (Van't Hoff Plot).

The results obtained from Van't Hoff plot revealed that ΔH^{o} in general had small values and their positive sign ascertain that the reaction was nearly endothermic. The small positive value of ΔH^{o} may indicate a favorable interaction between ^{125}I-anti AFP antibody to both AFP in malignant breast tumor homogenate and partially purified AFP respectively.

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 (Nemethy, G. and Scheraga, H.A. 1962).

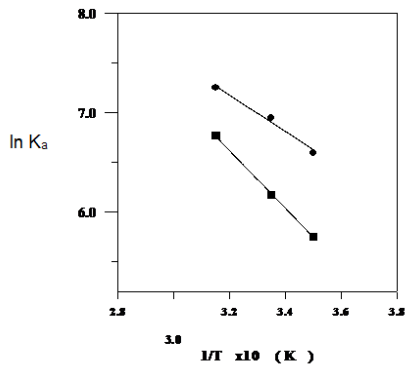

Figure (7): Van't Hoff plot for the binding of ^{125}I-anti AFP antibody with AFP of (■) benign colorectal tumor homogenates and (●) malignant colorectal tumor homogenates.

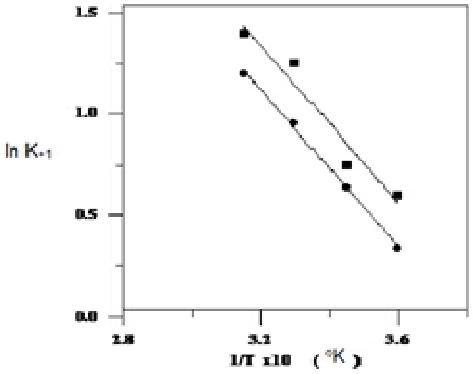

Figure (8): Arrhenius plot for binding of ^{125}I-anti AFP antibody with AFP in (■) Benign colorectal tumor homogenates and (●) malignant colorectal tumor homogenates.

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 high negative values of ΔGº for the binding reactions are controlled by small negative and high positive ΔSº values as shown in table(6). So, our system is characterized by the contribution of ΔHº and ΔSº to the stability of the complex formed.

This system is characterized by the sole contribution of ΔSº to the stability of the complex formed; while ΔHº has little or no effect (Haro, L.S.andTalamantes, F.J. 1985). The values of positive ΔSº suggest the binding spontaneity was entropically driven. Entropy was the driven force for the occurrence of the binding; this indicates that the hydrophobic interactions played an important role in stabilizing complex (Smith C. et al 2006).

Table (6): Thermodynamic parameters of standard state for the binding of ^{125}I-anti AFP antibody with AFP of colorectal tumor homogenates.

Temperature °C	ΔH° KJ. mol^{-1}	ΔG° KJ. mol^{-1}	ΔS° J. mol^{-1}. K^{-1}
	Benign		
5	*7.528*	*-18.45*	*69.28*
25	*7.528*	*-19.74*	*68.42*
37	*7.528*	*-20.97*	*69.73*
42	*7.528*	*-18.71*	*63.52*
	Malignant		
5	*8.554*	*-20.56*	*59.35*
25	*8.554*	*-21.46*	*58.34*
37	*8.554*	*-22.58*	*60.24*
42	*8.554*	*-20.65*	*55.48*

(B) Thermodynamic parameters of transition state:

The transition state theory proposes that the association of two substances to form the final product proceeds through the formation of an activated complex (transition state). Consequently, the interaction of ^{125}I – anti AFP Antibody with colorectal tissue homogenate can be represented as follows

$$^{125}\text{I Ab} + \text{Ag} \rightarrow [^{125}\text{I - Ab - Ag}]^* \rightarrow [^{125}\text{I - Ab - Ag}]$$

labeled antibody **AFP** → **an activated complex** → **Final product.**

The thermodynamic parameters of the transition state (ΔH*, ΔG*, and ΔS*) could be determined from Arrhenius equation and the Kinetic constants.

Figures (8) show the Arrhenius plot of ln K_{+1} versus 1/T values. The slop of the straight line represents the activation energy (E_a).

Tables (7) show the values of thermodynamic parameters of transition state of colorectal tissue homogenate Antigens from malignant and benign colorectal tumor at different temperature. The values of activation energy represent the required energy to overcome the energy barrier of the transition

state for the formation of ^{125}I – anti AFP Antibody / AFP complex.

Also the value of activation energy is in accordance with the high positive values of ΔG*, which indicates that the formation of the activated complex is 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 structure than the reactants.

From the results obtained for the thermodynamic parameters in the transition state, it can conclude that the positive values of ΔH* and high positive values of ΔG* are favorable to overcome the energy barrier of the transition state, the high negative values of ΔS* mean more arranged structure for the activated complex. The positive values of ΔG* is mainly attributed to the decrease in the entropy of the transition state. In addition, the positive value of ΔH* showed that the heat content of the activated complex is more than that of isolated species (Brown, E. M.et al 1976and Villacampa, M. J. et al 1984).

The values of the thermodynamic parameters of the binding reaction, gave an overall idea about the nature of forces that regulate the formation of complex.

The formation of a complex occurs in two steps, the first is the stabilization of the complex by hydrophobic interaction and the second is the stabilization by short-range interactions, such as electrostatic interaction, hydrogen bonding and Van der Waals' interactions.

Hydrophobic interactions contribute to the complex stability via high positive entropy change (ΔS* > 0), while electrostatic interactions, hydrogen bonding and Van der Waals interactions contribute to the stability of the complex via negative entropy change (ΔS* < 0) (Stull, J. T.; and Blumenthal, D. K.; Biochem; 1982 and Storm, D. I.et al 1980).

The thermodynamic data indicated that the binding of ^{125}I – anti AFP Antibody to AFP in colorectal tissue homogenate are entropy driven in agreement with the concept that hydro phobic interactions, play an important role in (^{125}I – anti AFP Antibody / AFP) interactions (Stull, J. T.; and Blumenthal, D. K.; Biochem; 1982 and Storm, D. I.et al 1980).

Table (7): Thermodynamic parameters of transition state for binding of ^{125}I-anti AFP antibody with AFP from benign and malignant colorectal tumor homogenates

Temperature °C	Ea (KJ.mol^{-1})	ΔH* (KJ. mol^{-1})	ΔG* (KJ. mol^{-1})	ΔS* (J.mol^{-1}.K^{-1})
		Benign		
5	*11.55*	*9.88*	*66.69*	*-210.11*
25	*11.55*	*9.72*	*70.87*	*-211.24*
37	*11.55*	*9.62*	*73.24*	*-211.55*
42	*11.55*	*9.55*	*77.05*	*-212.26*
		Malignant		
5	*7.5*	*10.24*	*68.69*	*-204.35*
25	*7.5*	*10.23*	*7545*	*-206.34*
37	*7.5*	*10.72*	*78.33*	*-205.44*
42	*7.5*	*1036*	*83.11*	*-209.65*

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دراسات حركية وثرموديناميكية لارتباط البروتين الجنيني الفا (AFP) مع مستضده في مجانسات اورام القولون والمستقيم

الملخص

تم دراسة كل الثوابت الحركية والثرموديناميكية المتعلقة بتفاعل الارتباط ما بين البروتين الجنيني الفا () ومستضده المعلم باليود المشع 125 في متجانسات اورام القولون والمستقيم وكذلك في المحلول المنقة جزيئياً من متجانسات اورام القولون والمستقيم. اتضح ان جميع الفاعلات تخضع لحركيات المرتبة الاولى الكاذبة.

ان قيم ثوابت الحركيات (K_a, K_d, K_{obs}, K_{+1}, K_{-1}, $(t_{1/2})_{ass.}$, $(t_{1/2})_{diss}$) وقيمة الارتباط العظمى (B_{max}) عند درجة حرارة (25^oC) للتفاعل كانت ($0.0543 \times 10^{10}\ M^{-1}$)،($15.064 \times 10^{-10} M$)،(0.0158 min^{-1})،($4.086 \times 10^6\ M^{-1}.min^{-1}$)،($64.76 \times 10^{-4}\ min^{-1}$)،(43 min)،(104.28 min) و (29.14 Pmol لكل ملغم بروتين)على الترتيب للتفاعل عند استخدام مجانسات الاورام الحميدة، بينما كانت القيم ($0.0538 \times 10^{10}\ M^{-1}$)،(15.142×10^{-10} M)،(0.0163 min^{-1})،($4.301 \times 10^6\ M^{-1}.min^{-1}$)،($67.63 \times 10^{-4}\ min^{-1}$)،(40 min)،(79.66 min) و (46.25 Pmol/mg) على الترتيب في حالة الاورام الخبيثة وعند درجة حرارة الارتباط المثلى (4^oC).

ان درجة الحرارة المثلى للارتباط للبروتين المنقى جزئياً (AFP)كانت (25^oC) كما انه اتضح ان قيم (K_a) و (K_{+1}) تزداد بازدياد درجة حرارة التفاعل. يتضح في هذه الدراسة من استخدام مخطط (Van't Hoff) ان العلاقة خطية ما بين ثابت التوازن (Ka) ومقلوب درجة الحرارة المطلقة ($1/T$). بينما العلاقة ما بين لوغريتم الثابت الحركي (K_{+1}) ومقلوب درجة الحرارة المطلقة ($1/T$) يعطي علاقة خطية وتدعى علاقة (Arrhenius). كما تمت دراسة الثوابت الثرموديناميكية في الحالتين المستقرة والمثارة لتفاعل الارتباط ما بين (AFP) والمستضد المعلم باليود المشع 125 من خلال تطبيق معدلتي (Van't Hoff) و (Arrhenius).