A Study Of Kinetic And Thermodynamic On Plasma Ceruloplasmin In Patients With Bladder Cancer

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Abstract

The levels of plasma ceruloplasmin (CP) in patients with bladder cancer, bladder stones as pathological control and normal subjects were determined .The study include the kinetic and thermodynamic properties. The study include (35) patients suffer from bladder cancer, (25) patients suffer from bladder stones disease and (25) normal subjects. The statistical analysis showed a significant increase ($P < 0.05$) in the activity of plasma CP in patients with bladder cancer as compared to pathological control and normal subjects , the optimum conditions of enzyme activity showed a 9.26 mmole/L concentration of p-phenylene diamine (PPD) (substrate) under incubation time of 15 min & 37^0C . Estimation of some kinetic & thermodynamic parameters of plasma CP enzyme reaction, indicated that : the km values for the plasma CP were lower in bladder cancer patients than in normal subjects, while the V_{max} values for the CP reaction found to be higher in bladder cancer patients than in normal, the forward reaction rate constant (k_1) for CP decreased in bladder cancer patients. The disease affected (increased) the half-life time ($t_{1/2}$) of CP reactions , the Hill coefficient (n) values revealed that there was a significant difference in the case of bladder cancer patients as compared with those of the control groups, the activation energy Eact.* for CP reaction decreased in bladder cancer patients compared with normal group subjects , the ΔH* values for CP enzyme in normal subjects and bladder cancer patients were positive that confirmed the reaction to be endothermic. The ΔS* values for CP enzyme in normal subjects and bladder cancer patients were negative ,whereas the ΔG* values for CP enzyme-substrate reactions in normal and bladder cancer patients groups were positive.

الخلاصة

تضمن البحث تقدير فعالية إنزيم السيرياوبلازمين في بلازما المرضى المصابين بسرطان المثانة، حصى المثانة كمجاميع سيطرة والاشخاص الأصحاء ودراسة صفاته الحركية والثرموديناميكية. شملت الدراسة (35) مريضاً مصاباً بسرطان المثانة، (25) مريض بحصى المثانة و(25) من المتبرعين الأصحاء. أظهرت النتائج: إن هناك زيادة معنوية ($P < 0.05$) في فعالية الإنزيم في بلازما مرضى سرطان المثانة مقارنة بمجاميع السيطرة والأصحاء , حددت الظروف المثلى لعمل الإنزيم،حيث استعملت تركيز الركيزة 9.26mmol/L, ودرجة الحرارة (37م^0) وزمن حضن(15دقيقة) , قدرت بعض القيم الحركية

والثرموديناميكية للإنزيم في بلازما مرضى سرطان المثانة والأصحاء وأظهرت النتائج:- إن قيم (km) للإنزيم كانت أقل في المرضى المصابين بسرطان المثانة مقارنة بالأصحاء. أما (Vmax) كانت عالية مقارنة بالأصحاء , قيم ثابت معدل السرعة الأمامية (k_1) للتفاعل الإنزيمي للسيروبلازمين كانت عالية في الأصحاء ، أما نصف العمر ($t_{1/2}$) لتفاعل الإنزيم كانت أعلى في مرضى سرطان المثانة مقارنة بالأصحاء , أظهرت قيم معامل هل (n) الى وجود فرق ملحوظ بين قيم مرضى سرطان المثانة والأصحاء , انخفاض طاقة التنشيط (Eact.*) لتكوين المعقد (ES*) في مرضى سرطان المثانة مقارنة بالأصحاء , أظهرت قيم (ΔH^*) للتفاعل الإنزيمي للسيروبلازمين في الحالتين بأن هذه التفاعلات ماصة للحرارة وان المحتوى الحراري للمعقد (ES*) أعلى من المحتوى الحراري لكل من الإنزيم والركيزة بينت القيم السالبة للـ (ΔS^*) بأن المعقد (ES*) أكثر ترتيباً من الإنزيم والمادة الأساس, أشارت القيم الموجبة (ΔG^*) لتفاعل إنزيم - ركيزة في الحالتين الى تكوين المعقد (ES*) بحاجة الى طاقة (غير تلقائي).

Introduction

Bladder cancer is the most common malignant tumor of the urinary system [1]. It affects twice as many men as women , it is rare in people under 55 years of age and most common among those over 70 years [2].Ceruloplasmin(CP) the copper binding protein[3].Its M.wt.is a 132 KDa [4], it is a plasma glycoprotein that is primarily synthesized by the liver and secreted into the blood [5]. CP acts as an antioxidant by several mechanisms [6 - 10] , inhibiting iron – dependant lipid peroxidation and $HO^{\bullet}$ formation from hydrogen peroxide (H_2O_2) via its ferroxidase activity [8] reacting with and scavenging H_2O_2 and superoxide anion and inhibiting copper – induced lipid peroxidation by binding copper ions [6,8]. CP synthesis and / or secretion is altered by inflammation , hormones and copper. Physiological factors like cancer , exercise , chronic inflammation , pregnancy increase its level [11]

Discussing enzyme – catalytic reaction properties , specific parameters should be concern and their mathematical values must be calculated in order to specify the behavior of the enzyme substrate [ES] complex formation. These were kinetically followed and include maximal velocity Vmax ,Michaels constant Km and turn over number [12].

In order to understand fully molecular mechanism that control enzymatic reactions complexation with its substrate . This requires an inhibition studies aimed at giving an explanation of the [ES] complex formation. In addition, a performed transition state analysis of the complex [ES] was found to require an analysis

of the thermodynamic parameters ΔS,ΔH and ΔG together with the description of the energy content and energy of activation [13, 14].

To our knowledge , there is no available data on plasma CP kinetic and thermodynamic in patients with bladder cancer & normal subjects ,therefore , in the present study , we aimed to determine CP activity in bladder cancer , pathological controls & normal subjects and investigate kinetic and thermodynamic properties of CP enzyme in plasma of patients with bladder cancer & normal subjects.

Materials and Methods

All common laboratory chemicals and reagents were of analar grade , Twenty five samples of blood (15) male, (10) female were taken from physically normal volunteers used as controls age between (25-71) years. Thirty five samples of blood were taken from patients with bladder cancer aged (48-75) years. After being classified by senior surgery (patient suffering from any disease, that may interfere with our study were excluded). Blood samples were also collected from twenty five pathological controls (patients with bladder stones).

All patients were admitted for treatment to specialized surgical hospital. Five milliliters (ml) of venous blood sample were taken by using plastic disposable syringes, and were added to EDTA tubes.

Plasma was separated from blood by centrifugation at (1000Xg) for 15 mints. Plasma samples were aspirated and stored in caped sterilized tubes at -20^{o}C until time of analysis. The host information is shown in table (1).

Table (1): The host information of all patients, pathological & healthy subjects.

Groups	No.	Mean age (year)	Range of age (year)
Bladder cancer	35	62	48-75
Bladder stones	25	56	38-73
Control (healthy)	25	48	25-71

The activity of CP enzyme was determined according to the method of Menden, et al[15].

Statistical Analysis

Descriptive statistics were used in analyzing the patients characteristics and laboratory parameters for each groups. In addition , unpaired student t – test was used to assess group differences , where appropriate .A statistical significant difference was accepted as p value less than 0.05 . All the statistical analysis in this study were made using SPSS 10.0 for windows program.

Results

The activity of CP expressed as (mean+SD)mg/L was estimated in plasma of patients with bladder cancer, pathological control and normal subjects as clear in table (2):

Table (2): Ceruloplasmin activity in plasma of patients with bladder cancer, pathological control & normal subjects.

Groups	No.	CP mg/L Mean ± SD
Bladder cancer	35	532.718 ± 35.73
Pathological control	25	275.370 ± 18.79
Normal control	25	222.207 ± 9.593

figures (1A+B) shows that the optimum concentration of substrate equal to 9.26 mmole/L in patients with bladder cancer and normal subjects.

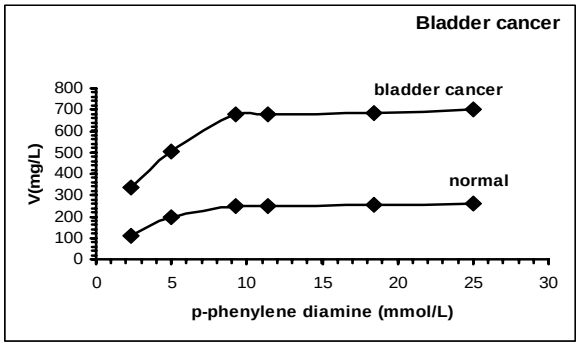

Figure (1): Effect of substrate concentration on the activity of CP in patients with bladder cancer and normal subjects.

Figures(2A+B) shows that the K_m value of CP is equal to (4.762 mole/L) and (7.692 mole/L) in plasma of patients with bladder cancer & normal subjects respectively. While V_{max} value for CP enzyme is equal to (1052.6315 mole/L/min.) & (416.666 mole/L/min.) in patients with bladder cancer & normal subjects respectively.

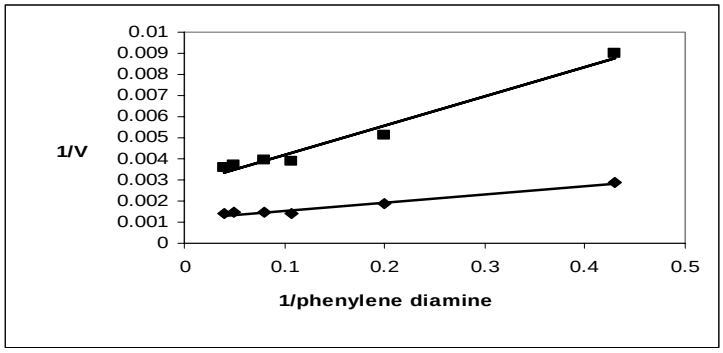

Figure (2): Lineweaver-Burk plot for CP activity in patients with bladder cancer and normal subjects.

Figures (3), (4), (5) show the K_m and V_{max} values of plasma CP activity in bladder cancer patients and normal subjects in different temperatures (15, 25,30,37,40)^{0}C.

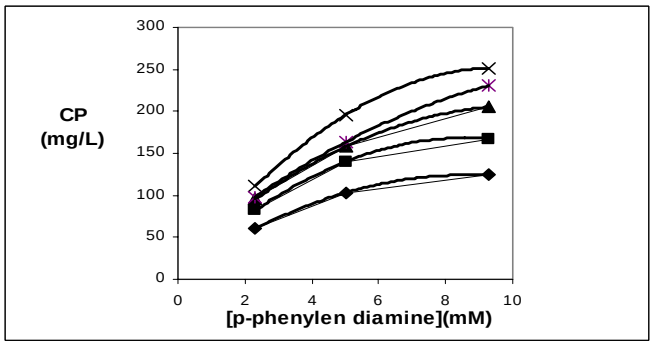

Figure (3): Michaelis-Menten relationship for CP activity at different temperatures in normal subjects.

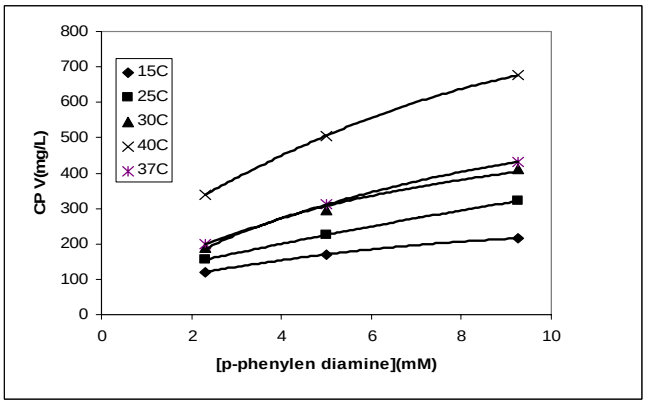

Figure (4): Michaelis-Menten relationship for CP activity at different temperatures in patients with bladder cancer.

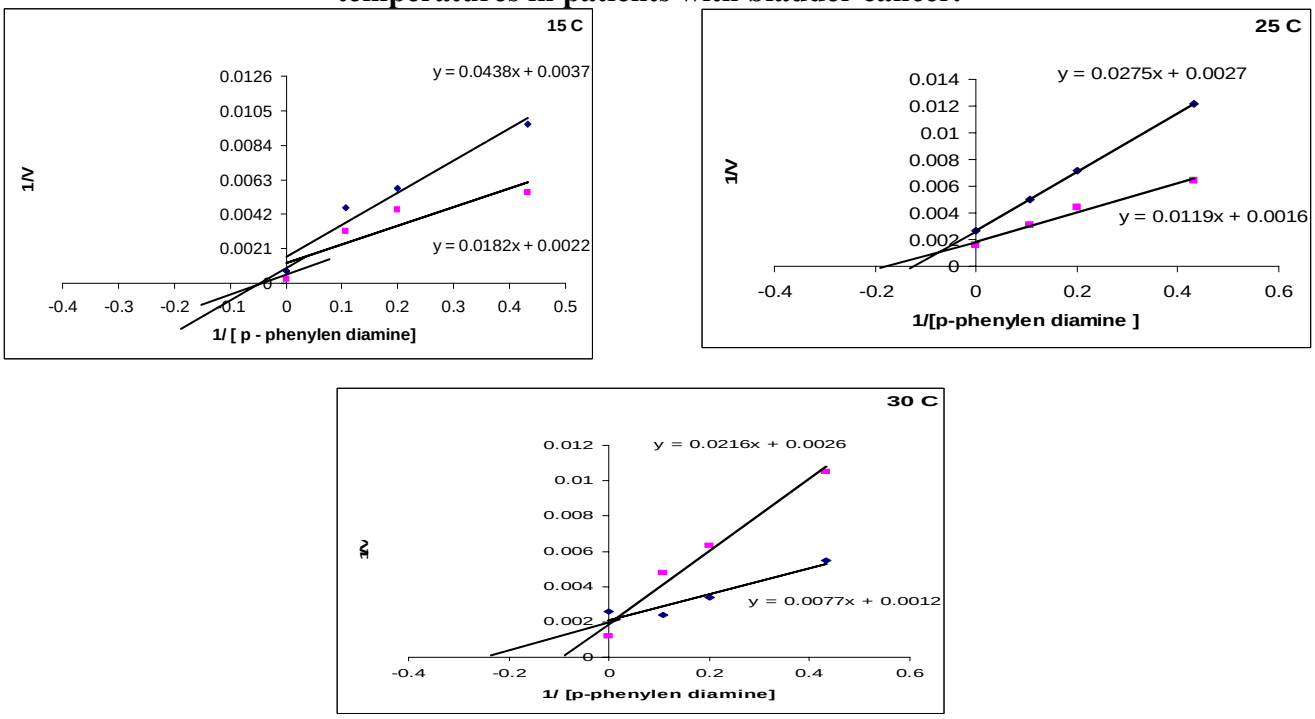

Figure (5): Lineweaver-Burk relationship for CP activity at different temperatures in normal subjects and patients with bladder cancer.

Figure (6) show the time required to obtain higher activity for CP enzyme in plasma of patients with bladder cancer and normal subjects is equal to(15min.).

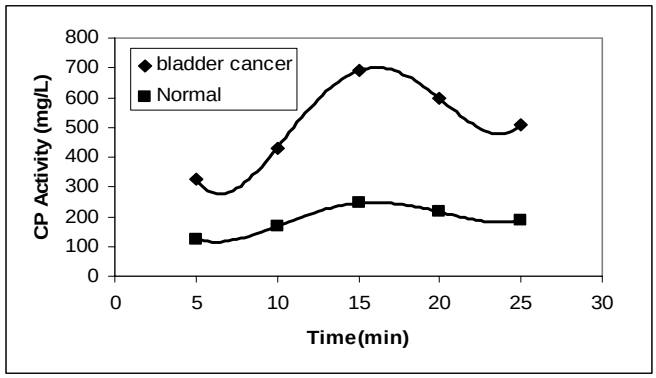

Figure (6): Effect of incubation time on ceruloplasmin activity in patients with bladder cancer and normal subjects.

Figures (7A+B) shows the optimum temperature for CP enzyme in plasma of patients with bladder cancer and normal subjects respectively.

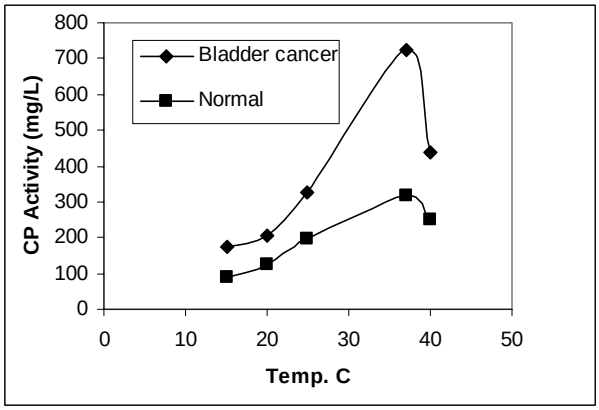

Figure (7): Optimum temperature of ceruloplasmin activity in patients with bladder cancer and normal subjects.

Figures (8 A+B) show that in patients with bladder cancer and normal subjects, the enzyme is more stable. when stored at (-20 ^{0}C) than (4 ^{0}C) and (37 ^{0}C) in both groups.

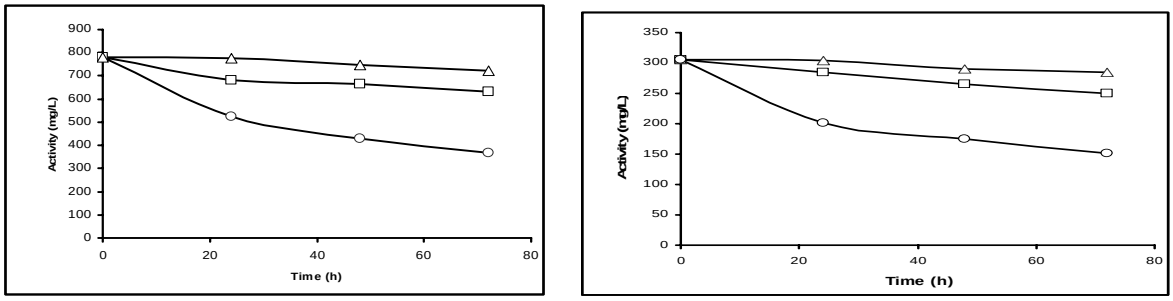

Figure (8): Effect of temperature and incubation time on CP stability in patients with bladder cancer and normal .

Table (3) shows the value for forward reaction rate constant(K_1) and half life time($t_{1/2}$)were determined also shows the pseudo first order reaction for bladder cancer patients and normal subjects.

Table(3): Rate of reaction velocity for CP in patients with bladder cancer and normal subjects.

Bladder cancer	Normal

$t_{1/2}$ (min.)	K_1(min.$^{-1}$)	$t_{1/2}$ (min.)	K_1(min.$^{-1}$)
21.792	0.0318	21.656	0.032

Table (4) shows that Hill-coefficient value for CP enzyme in plasma of patients with bladder cancer were (0.878) while for normal subjects were (1.044),

Table (4): Hill coefficient values of CP in bladder cancer patients and normal subjects.

Groups	Hill coefficient (n)
Bladder cancer	0.878
Normal	1.044

Table (5) clear the values of ΔH*,ΔS* and ΔG* in transition state for [ES] complex reaction.

Table (5): Thermodynamic parameters at transition state in patients with bladder cancer and normal subjects

Case	Ea* (KJ/mol)	ΔH* (KJ/mol)	ΔG* (KJ/mol)	ΔS* (J/mol.K)
Bladder cancer	**60.12**	**57.54**	**84.90**	**-88.27**
Normal	**62.25**	**59.67**	**84.89**	**-81.339**

Discussion

The main finding of the present study is that a significant increase in the main value of CP activity in patients with bladder cancer and pathological control as compared to normal subjects. Increase in plasma CP could be attributed to the following reasons:CP is an acute phase reactant that increases in circulation in chronic disease including tumors[16] and CP is considered as a storage marker of copper in circulation and over 95% of copper is bound to CP[4], these results are in agreement with (Christine et al 2004)[17],(BOZ,etal2005)[18], (Gundogdu,et.al2007) [19],they reported a significant increase in plasma CP activity in ovarian , gastrointestinal tract and Chronic Lemphocytic Leukemia cancer patients respectively.

Kinetic properties of CP :

1- Effect of different substrate concentrations on CP activity

According to the world recommendation optimum concentration Must be used for substrate when measuring the activity of different enzymes in order to obtain the maximum velocity Vmax in this study the optimum concentration of substrate equal to 9.26 mmol/L in patients with bladder cancer and normal subjects , when velocity of reaction reach to maximum level , this indicates saturation of enzyme by substrate[12].

2- Michaelis-Mentin constant Km & maximum velocity Vmax value:

Km and Vmax values were determined for CP in plasma of patients with bladder cancer and normal subjects according to Linweaver-Burk equation. Km value for CP is lower in bladder cancer patients as compared to normal subjects this mean that infection by disease may effect the enzyme affinity to its substrate and this may be due to a change in chemical structure the ionic state of the active sites that become more suitable for conjugation with substrate[19]. Vmax for enzymatic reaction of patients with bladder cancer was higher as compared to normal subjects and this means probability of change in active sites number of CP bladder cancer patients which leads to increase its activity[20].

Km and Vmax values of plasma CP activity in bladder cancer patients and normal subjects in different temperatures (15,25,30,37 and 40)C° were measured, Km values for both cases will be decline with the increase of temperature from (15 to 37) C° , data obtained that 37 C° temperature is more suitable for increasing enzyme affinity to its substrate while in 40C° affinity decreases perhaps due to the change occurring in enzyme structure[12]. Vmax value for enzyme-substrate reaction in both cases increase with increasing temperature from(15 to 37) C° and temperature37 C° is suitable for increasing the activity of enzyme while enzyme activity decrease in temperature 40C° , this may be due to the occurring denaturation for some enzyme molecules , that lead to make changes occur in catalytic site , which decreases active site number which decreases Vmax value. From above this may represent that enzyme

affinity for its substrate in normal subjects is lower , while Vmax value will be increased in patients with bladder cancer as compared to normal subjects which leads to increase enzyme activity can be attributed to : the stability of enzyme , therefore, velocity be hydrolysis of [ES*] complex , the affinity of enzyme to its substrate , the pH of compound that change pKa and the enzyme affinity and its activity[21].

3- Optimum time for CP activity :

Time required to obtain higher activity for CP in plasma of patients with bladder cancer and normal subjects which are indicated to

be 15 min. , there are several factors contributed in these changes which include : with progressing the velocity of enzymatic reaction is decreased by decreasing the substrate concentration , reversing rate is cleared with increasing the concentration of enzymatic reaction products, inhibition of enzyme by reaction products[22] and the presence of change in the concentration of oxygen as a result of

reaction , therefore , the catalytic amplitude of enzyme will be effective[23].

4- Optimum temperature for CP activity :

Highest activities obtained at incubation temperature of 37 C° this reflecting great affinity of the CP toward its substrate in patients and normal subjects . It was obvious that raising temperature to 40C° cause decline in the CP activity suggesting either a degradation of the complex [CP-PPD] or CP denaturation may occurs or possibility of the breakdown of hydrogen bond and hydrophobic bond , which is responsible for maintaining the secondary and tertiary structure of protein , which gives rise to the denaturation of proteinic structure of CP enzyme[19] , so temperature effect on the speed of enzymatic reaction either by effect on the stability of enzyme or effect on affinity of [ES] complex or on disintegration of the complex.

5- Enzyme stability :

CP is more stable when stored at -20 C° than 4 C° and 37 C° in both patients and normal subjects.

6- Determination of K_1 ,$t_{1/2}$ and Hill coefficient :

Results shows the pseudo first order reaction for bladder cancer patients and normal subjects , revealed that K_1 values decrease in plasma of patients with bladder cancer as compared to normal subjects, so bladder cancer patients not affect of Keq for [ES*] formation . Also results indicated that there was an increase in $t_{1/2}$ for CP in plasma of patients with bladder cancer as compared to normal subjects so the effect of bladder cancer patients on $t_{1/2}$ by decreasing the affinity of enzyme concentration[12].The value of Hill coefficient was determined from the following equation : log(V/Vmax-V)=n log[S]-log K_1 .The Hill coefficient can be used as a convenient index of cooperatively , the utility of such plots is limited because the linear relationship in the equation hold over only a limited range of substrate concentrations, results clear that there was a significant difference in Hill coefficient in patients with bladder cancer as compared to normal subjects.

Thermodynamic studies :

1- Activation energy Eact.* of enzymatic reaction :

Results indicated that there was decrease in activation energy in plasma of patients with bladder cancer as compared to normal subjects , the above mentioned results can high light after the reaction mechanism of enzyme effect during infection with bladder cancer[12] .

2- ΔH*, ΔS* and ΔG*:

ΔH* value of CP enzyme in bladder cancer patients and normal subjects were positive and this indicates that this enzyme reaction is endothermic and need energy for the formation of [ES*] complex , also the value of ΔH* in patients with bladder cancer is lower than that in normal subjects, this indicates that the formation of [ES*]complex needs temperature in patients with bladder cancer than that in normal subjects. .ΔG* has a positive value in patients with bladder cancer and normal subjects , this indicates that the reaction of ES formation is not spontaneous , but needs energy so called endergonic reaction. ΔG* value of this reaction is independent on molecular pathway of mechanism of transformation[24]. ΔS* value of CP enzyme in bladder cancer patients and normal subjects is negative , so indicate that the complex [ES*] more arranged than enzyme and substrate ΔS* in bladder cancer patients is lower than that in normal subjects ,where the negative value of ΔS* reverse during increasing the structure arrangement[25]

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