P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2013,(7), (3) :50-53

Study of Optimum Condition for Xanthine Oxidase Activity in patients type two Diabetes Mellitus.

Samar M. Abd Al-elah

University Of Anbar - College of Science

ARTICLE INFO

Received: 2 / 1 /2013
Accepted: 22 / 5 /2013
Available online: 19/7/2022
DOI:

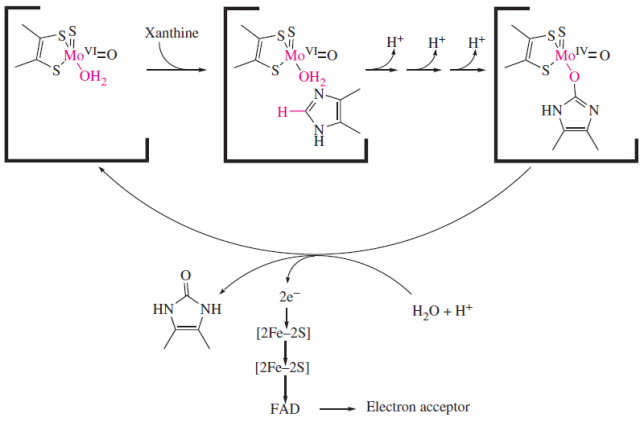
Keywords:
xanthine oxidase ,
kinetic enzyme,
Optimum condition ,
Arrhenius equation.

ABSTRACT

This study is to determine of optimum conditions of xanthine oxidase activity diabetes mellitus type two for ten patients. The results of conditional enzyme work (substrate concentration [S], temperature , pH) were 0.1 mmol/L, 313 K ,7.8 respectively and the kinetic constant (Km ,V max) were 0.0443 mM , 23.31 U / ml respectively using Lineweaver-Burk plot and 0.0341mM ,20.313 U / ml respectively using Eadie - Hofstee . The result of activation energy ΔE***** was 458.9099 Cal / mol by Arrhenius equation and thermodynamic constants (ΔH***** , ΔG***** , ΔS*****) were -1080.8409 cal/mol , 16210.632 cal/mol , -55.2443 cal/mol respectively.

Introduction

Xanthine oxidase (Ec 1.1.3.22) [(1)] is a molybdenum- containing enzyme that plays an important role in the metabolism of many xenobiotics and drugs, such as purines and pyrimidines[(2)].It is a highly versatile enzyme that is widely distributed among species(from bacteria to man) and within the various tissues of mammals[(3)]. Xanthine oxidase contains two Fe_2S_2 clusters and bound FAD. The enzymes can also oxidize xanthine further by a repetition of the same type of oxidation process at positions 8 and 9 to form uric acid[(4)]. One of the mechanisms proposed for xanthine oxidase includes the initial ionization of the C8(H) from the purine ring show figure (1)[(5)].



* Corresponding author at: University Of Anbar - College of Science .E-mail address: dean_coll.science@uoanbar.edu.iq

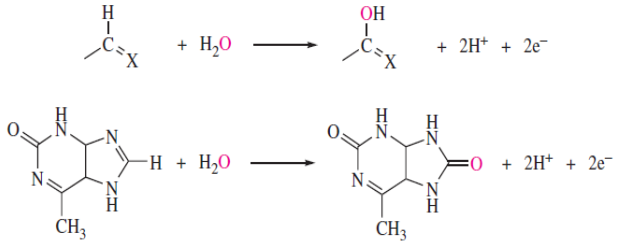


Figure (1): The reaction cycle of xanthine oxidase to product uric acid .

Generally The xanthine oxidase family catalyzes the oxidation of H—C=X groups in molecules and transforms them into HO—C=X groups, where X may be O or N.the general chemical equations for these reaction is shown above [(5)].

Material & methods:

Samples:

Venous blood samples were collected from (10) patients. The collected blood was 10 ml, and then was centrifuged. The remainder of blood was centrifuged at (3000 xg) for 10 minutes to separate the serum. The obtained serum was stored in frozen at -30°C until used to estimate optimum condition of the enzyme activity included in the study.

Effect of substrate concentration on xanthine oxidase activity:

XO enzymatic reaction was carried out under optimum reaction condition using different concentrations of xanthine as a substrate (0.01, 0.02,

0.03, 0.05, 0.08, 0.1, 0.14 mmol/L) for XO activity,the relationship between each substrate concentration and the enzyme activity was plotted in order to determine the optimum substrate concentration for enzymatic rate. Also Km and Vmax values were determined using the Lineweaver-Burk plot. The relationship between 1/v versus 1/[S] and Eadie – Hofstee plot V versus V/[S] [6] .

Effect of temperature on xanthine oxidase activity:

XO activity toward (0.14 mmol / L) was measured as described at different incubation temperature (15, 25,30,35,40, 45, 55C°). The optimum temperature of XO activity it should be enzymatic rate vs temperature[6].

Effect of pH on XO activity:

The enzymatic reaction for XO was carried out by using sodium phosphate buffer 0.4M at different pH (6, 6.8, 7, 7.4, 7.8, 8.2, and 8.6). The pH optimum of XO activity was estimated by plotting the relationship between the enzyme activity Vs pH values [6].

Determination of the activation energy (Ea) of the enzyme reactions:

The XO activities as indicated were determined at different temperatures. A curve was plotted activities and temperature. Then log K vs 1/T depending on Arrhenius equation [7].

$\text{Log } K = - Ea / 2.303RT + \log A$

or

$K = A\, e^{-Ea/RT}$

Where R = 1.987 cal. / mol

A straight line was obtained and from the slope, energy of activation Ea* of the enzymatic reaction was estimated.

Determination of Ea and thermodynamic constants (ΔH* , ΔS* , ΔG*):

The evaluation of thermodynamic parameters of transition state ΔH*, ΔG*, ΔS*. ΔH* of transition state of enzyme substrate complexes were calculated from the following equation :

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

While transition state free energy changes ΔG* was found by using the following equation:

$\Delta G^* = - RT \ln k + RT \ln k\, T/h$

Where (k) is the boltzmann constant and is equal to (0.33×10^{-23} cal/mol) ,(h) is the planks constants and equals to 1.58×10^{-34} cal.sec the changes in entropy of the transition state ΔS* were obtained from the following relation [7].

$\Delta S^* = (\Delta H^* - \Delta G^*) / T$

Result and Discussion:

Determination of xanthine oxidase (XO) activity:

In recent years, free radicals and other reactive intermediates produced in normal metabolic processes have been implicated in the pathogenic mechanism of a wide range of diseases. Free radical sources include Xanthine Oxidase-derived superoxide exerts its actions on various endogenous oxidant and antioxidant systems. XO-derived superoxide can combine with endothelial derived NO role in diabetes and its pathophysiology has been less explored[8] .This study is analyzing the activity of XO in type two diabetes,

increase in activity in diabetes prompts the question of the origin of this increased XO activity in diabetic patients . That found that diabetes causes an increase of xanthine oxidase activity in liver. Moreover, found that xanthine oxidase is released by the liver of diabetic [9] .

Determination of Km , Vmax and optimum [S] for XO enzyme activity:

A protocol of article [6] has been adopted using different concentrations of the substrate (Xanthin). In order to evaluate the two parameters (Km and Vmax) values, the linear relationship by adopting two types of plotting, these were:

Lineweaver-Burk and Eddie-Hofstee.

Table (1) reflects the values of Km and Vmax for the two types of plotting that expressed in figure (2 & 3) Values of the kinetic constant Km and Vmax can be determined from the slop and the intercept values of the linear relationship obtained. The important of Km and Vmax values is that their level variations can be presumed to be a parameter for diagnostic. In addition a comparison of the two methods of plotting; the Lineweaver-Burk and Eddie- Hofstee was found to be more convenient. This experiment gives an idea about the affinity of the enzyme to work on the substrate. This can give a good tool for determination of the chemical structure of the active site, which become suitable for the substrate binding [10].

Table (1): Km and Vmax values by two types of plots

plot type	Vm U / ml	Km mM
XO activity at linwaver berk	23.31	0.0443
XO activity at Eadie – Hofstee	20.313	0.0341

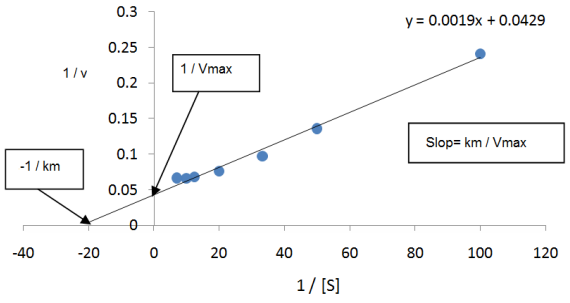


Figure (2) Lineweaver-Burk plot

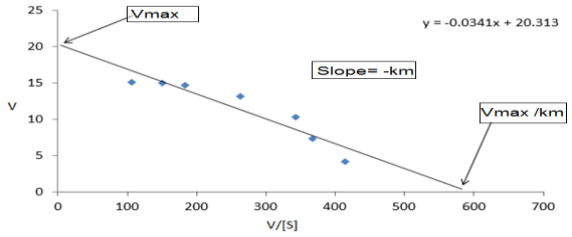


Figure (3) Eadie – Hofstee plot

Temperature Effect for XO Activity:

Figure (4) show the effects of different temperature on XO activity. Results show that the highest XO activity was obtained at temperature 313K. Effect of temperature on the activity of the enzyme reaction may be explained due to its effect on the enzyme stability, or on the enzyme-substrate affinity or on the actual velocity of breaking down of the active E-S complex of the transition state.

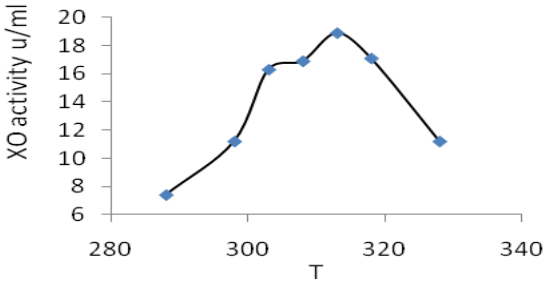


Figure (4) Temperature effect for XO activity

pH Effect XO Activity:

Figure (5) show that a pH value of (7.8) insures a highest activity of XO, as an optimum pH value for serum; it has its own effect on the tertiary structure of the XO enzyme on its activity. Results show that a highest XO activity was obtained at pH 7.8 An extreme pH value can be denaturing the XO activity irreversibly [(11)]. The pH optima were greatly varied with different enzymes, and are dependent on the particular ionized state of the substrate that will be optimally bound to the enzyme. Therefore, ionization at the specific amino acid provided by the E activities are greatly influenced the substrate binding site [(12)].

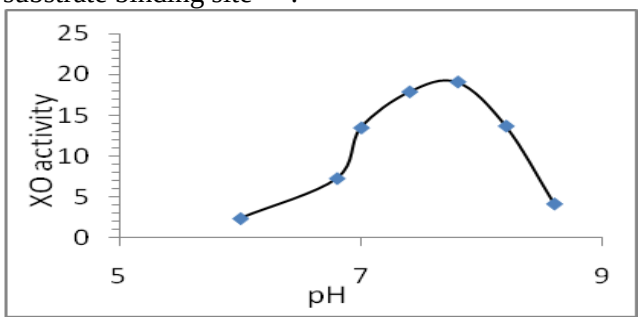


Figure (5) pH effect for XO activity

Determination of the Thermodynamic Parameters ΔS*, ΔH*, ΔG* and Ea*:

Table (2) and figure (6) show the thermodynamics parameters of the transition state. The activation energy Ea is a useful parameter for quantitative measurement of the thermodynamics barrier to be overcome in the course of catalysis.

Table (2): Thermodynamic parameters

	Ea cal / mol	ΔG* cal / mol	ΔH* cal / mol	Δs* cal / mol
XO	458.909	16210.632	-1080.840	-55.244

The activation energy is useful to relate conformational changes during catalytic events and to compare homologies enzyme of divers' phylogenetic origin. Arrhenius plot, (6), was seen to obey Arrhenius equation in the temperature range used and no refraction has been observed in the plot.

Nonlinear plots may reflect temperature-induced structural charges in the enzyme molecule. The effect of temperature on the rate of the reaction is frequently expressed A positive ΔH* value indicate a large amount stretching or even breaking of chemical bond occurred during the formation of transition state. It also indicates that the enzyme reaction was endothermic and the heat content of the activated complex [ES] was greater than that of isolated species E and S [(13)]. The negative value of ΔS reflect a change to the most ordered structures as a measure of inherent probability of the occurrence of the transition state, ΔS indicate the formation of the complex XO-xanthine .A positive value of ΔG indicate that the active complex XO-xanthine formation require input of energy[(7)] .

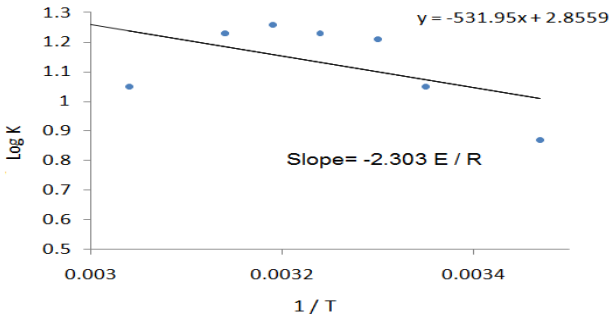


Figure (6) Arrhenius plot of the effect of temperature on maximum velocity of an enzyme reaction .

Reference:

1- Luisa M and Lurdes M (2002), Xanthine Oxidase and Aldehyde Oxidase: A Simple Procedure for the Simultaneous Purification from Rat Liver, Vol. 400, No. 1, April 1, pp. 48–53.

2- Rashidi M. R. , Soruraddin M. H, Taherzadeh F , and Jouyban A (2009), Catalytic Activity and Stability of Xanthine Oxidase in Aqueous- Organic Mixtures. Puplished in Russian in Biokhimya ,Biochemistry(Moscow), Vol.74,pp.97-101.

3- Evans C. E ,Mohammed A. VUNCHI and Patience O. EGWIM(2005), Comparism of xanthine oxidase activities in cow and goat milks. BIOKEMISTRI 17(1):1-6(June 2005).

4- Muthanna O. Hussein AL-sabhany (2009), Determination of xanthine oxidase activity , related with Nitric oxide (NO) and lipid profile in serum of patients with atherosclerotic diseases . Journal of Al-Anbar University for pure science Vol.3 No.2 P. 88-94.

5- Perry A. Frey and Adrian D. Hegeman (2007), ENZYMATIC REACTION MECHANISMS , Copyright © by Oxford University Press , ch4, pp222-226.

6-John F. Robyt ; Bernard J. White (1987). BIOCHEMICAL TECHNIQUES THEORY AND PRACTICE .BROOKS/COL PUBLISHING COMPANY MONTEREY, CALIFORNIA . ch 9 ,pp291-306.

7-IRWIN H. SEGEL (1976) BIOCHEMICAL CALCULATIONS 2ND EDITION .copyright by John Wiley and Sons, Inc. ch 4 ,p 197-281.

8- Suriyajothi M.A., Sangeetha .R., Venkateswari R. (2011). ACTIVITY OF XANTHIN OXIDASE DIABETICS: ITS CORRELATIO WITH AGI G. Pharmacologyonline 2: 128-133 .

9- Marı´.C. D, Miguel .A, Rafael M , Jose´ Ma, Ma´ximo .V, Federico V. P , Juan S (2002) . Xanthine Oxidase Is Involved in Free Radical Production in Type 1 Diabetes. DIABETES journal , VOL. 51, p 1118-1124.

10- Erric E. Connand and Stumpf PK; "Outline of Biochemistry "3rdedition, (1972), John Wiley and sons publication

11- Michael L. Bishop, MS, CLS, MT (ASCP).(2010) CLINICAL CHEMISTRY TECHNIQUES, PRINCIPLES, CORRELATIONS. Sixth edd. ch.12 p281-288.

12-A.Nease C ,Ferhan E. Tezcan (1993). Inhibition kinetics of brain butyrylcholinesterase by Cd+2 and Zn+2 , Ca+2 or Mg+2 reactivates the inhibited enzyme.International journal of biochemistry V 25, August 1993,pages 1115-1120.

13- Robert S.A and Claude B. K (1981) Purification and Characterization of Smooth Muscle Myosin Light Chain Kinase. JOURNAL OF BIOLOGICA CHLEMISTRY Vol 256,No.14,Issue of July 25.pp 7501-509.

دراسة الظروف المثلى لإنزيم الزانثين اوكسيديز للمرضى المصابين بداء السكري من النوع الثاني

سمر محمد عبد الإله

E.mail: dean_coll.science@uoanbar.edu.iq

الخلاصة:

تضمنت الدراسة تحديد الظروف المثلى لفعالية أنزيم الزانثين اوكسيديز لمرضى داء السكري من النوع الثاني والثوابت الحركية والثرموديناميكيه لها ، وكانت نتائج الظروف المثلى من تركيز المادة الأساس ودرجة حرارة وقيمة الدالة الحامضية هي (1,0 mmol/L ، K313 ، 7.8) على التوالي،وقيم الثوابت الحركية لكل من Km, Vmax كانت(0.0443 mM ، 23.31 U / ml) على التوالي بتطبيق معادلة لنيوفر بيرك وكانت قيمها (0.341 mM ، 20.313 U / ml) بتطبيق معادلة ايدي هوفست.وكانت قيمة طاقة لتنشيط (ΔE^*) هي 458,9099 cal/mol المحسوبة بمعادلة ارهينوس والقيم الثرمو دينامكيه لكل من(ΔS^* , ΔG^* , ΔH^*) كانت كالآتي (−55,2443 cal/mol ، 16210,632 cal/mol ، −1080,8409 cal/mol) على التوالي .