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INVESTIGATION IN KINETIC-THERMODYNAMIC PARAMETERS OF FREE CELLULASE PRODUCED BY LOCAL FUNGI TRICHODERMA VIRIDE

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ABSTRACT

Various Kinetic-thermodynamic parameters of cellulase from local fungi *Trichoderma viride* had been studied. Using carboxymethyl cellulose as substrate, the enzyme showed maximum activity (*V*max)75g/lmin^{-1}mg^{-1}with its corresponding *K*m value of 2.5x10^{-5}g/l.The constants of thermal inactivation/h^{-1} were1.04,2.89, 4.05, 5.24 at 40, 50,60,70 C° respctivly. Increasing the temperature to 10 C° leads to a sharp increase Eact, ΔH and ΔS**.**

Keywords: Cellulas, Enzyme Kinetics, Thermodynamic.

INTRODUCTION

There are several factors affected in cellulase production including (temperature, pH, optimal nutrition and environmental conditions for the production of the enzyme .[1] Cellulase have wide range of industrial applications such as starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry.[2-6] Cellulase enzymes from various microorganisms, mainly fungi, act on cellulosic materials and biodegrade them. Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds.[7] The enzyme kinetic constants are sensitive to various land management practices.[8] Study the enzyme kinetic is a basis of up to date knowledge of molecular mechanisms of biological processes. The kinetic parameters and mechanism of the degradation reaction were examined to provide a theoretical basis for, and assess the feasibility of, its practical application.It is necessary to know optimal conditions of enzyme active sites functioning and the effect of different physical-chemical factors on their activity so the present study focus on the investigation of kinetic and thermodynamic parameters. .

MATERIALS AND METHODS

1. Cellulase purification using ion exchange chromatography:Cellulase extracted from *Trichoderma viride* was purified by ion Exchange chromatography using DEAE-Cellulose an anionic exchanger. This matrix was used for purification because it has high capacity for bioseparation, easy to prepare, multiple use, in addition to simplicity to separate different biomolecules.[9]

2. Determination of enzyme activity: Enzyme activity was determined using spectrophotometer by the method of Mandels*et al*,.[10] The reaction mixture contained 0.9mL of carboxymethyl cellulose as substrate in 0.05 M Na–citrate buffer of pH 4.8 and finally 0.1 mL of pure enzyme and incubated at 45°C for 1 h. An appropriate control which contained 1 mL of distal water instead of pure enzyme was also run along with the test. At the end of the incubation period. The tubes were incubated for 5 min in a boiling water bath for color development and were cooled rapidly. The activity of reaction mixture was measured against a reagent blank at 540 nm. The concentration of glucose released by enzyme was determined by comparing against a standard curve constructed similarly with known concentrations of glucose. The unit of enzymatic activity defined as the amount of enzyme required to liberate Micro Mol of reducing sugars per hour under the conditions of the reaction.

3. Kinetic parameters: The kinetic parameters (Vmax and Km) were calculated by plotting the initial enzyme reaction velocities (V) against the substrate concentration (S) according to the linear Hanes-Woolf and Lineweaver-Burk transformations of the Michaelis-Menten equation**.**

$$\frac{1}{V0} = \frac{km}{Vmax}\frac{1}{[s]} + \frac{1}{Vmax}$$

In Equation above, *V*0: enzyme relative velocity, *Km*: Michaelis-Menten constant, *V*max: Maximum velocity, [S]: Substrate concentration.

RESULTS AND DISCUSSION

The calculated Michaelis constant (Km) and maximum catalytic velocity (Vmax) values of cellulase are presented in summary in Table 1.

Table 1. The values of Km and Vmax for the hydrolysis reaction of Carboxymethyl cellulose.

Enzyme	Km, g/l	V_{max} g/lmin^{-1}mg^{-}
cellulase	2.5x10^{-5}	75

values signify the extent to which the enzymes have access to the substrates ,this value of *K*m represents higher affinity between enzymes and substrates, while the value of *V*max indicates the difficulty of enzyme substrate interaction.[11] Kinetic parameters of the process of heat inactivation cellulase are shown in Table 2.

Table 2. The rate constants of thermal inactivation of cellulase.

Temperature , C°	constants of thermal inactivation/h^{-1}
40	1.04
50	2.89
60	4.05
70	5.24

Analysis of the results indicates a low thermal stability of cellulas Trichoderma viride, the inactivation rate constant at any temperature which is lower than the results in other studies for the enzyme from other sources.[12]In the study of the mechanism of thermal inactivation of proteins valuable information can be obtained by identifying some of the thermodynamic parameters: enthalpy ΔH, entropy ΔS, the activation energy Eact., free energy ΔG. Uses the theory of absolute reaction rates.[13] Central to this theory takes a provision stating that the reaction rate at a given temperature depends on the concentration of the activated complex in equilibrium with the non-activated molecules. Moreover, all of the activated complex decay at a rate determined by the ratio kvT / h, in which - the Boltzmann constant (1, 3305·10^{-23}·· J / K), h - Planck's constant (6, 6267·10^{-34}·· J · s), T - absolute temperature (K).

Thus, the reaction rate constant k was found from the equation: k = kvTK*/h.

Where K * - equilibrium constant between the activated complex and non-activated molecules.

Hence the value of K * is defined as follows: K * = kh / kv T. Knowing the value of the equilibrium constant was calculated ΔG * by the formula:

ΔG * = ΔH * - TΔS * = -RT · lnK *, where R - gas constant (8.315 J / K · mol).

To determine the ΔH * using the equation: Eact = ΔH * + RT

Activation energy (Eact) found by Arrhenius plot of the relationship:

Eact = 2,303 · R · tgα

Where α - angle between the oblique line with the abscissa.

Knowing Eact, Δ H * calculated by the formula:

Δ H * = Eact – RT

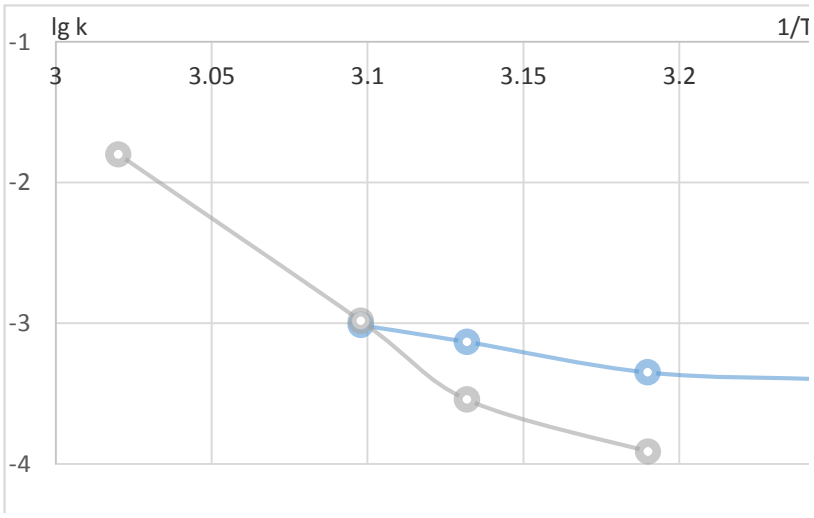
To determine the equation ΔS * used: ΔS * = (ΔH - ΔG) / T

Thermodynamic parameters for the thermal inactivation of cellulase are presented in Table 3.

Table 3. The values of thermodynamic parameters of the hydrolysis of Carboxymethyl cellulose by Trichoderma viride cellulase.

Temperature, C°	E_a(kJ/ mol)	ΔG, (kJ/ mol)	ΔH, (kJ/ mol)	ΔS, (kJ/ mol)
40	3.2	45.9	6.7	-77.4
45	4.8	46.1	6.9	-74.8
50	9.5	46.9	6.6	-78.5
55	14.1	47.7	10.3	-79.9
60	15.7	48.2	11.2	-805
65	17.6	49.5	13.4	-83.4
70	18.1	49.9	13.8	-84.8

It is shown that the effect of high temperatures on cellulase accompanied by an increase Eact, Δ H *, ΔS * hydrolysis reaction of carboxymethyl cellulose. Arrhenius plots for determining the activation energy characterized by fracture points to the serial flow of catalytic reactions (Fig.1).



Figure(1): Arrhenius plot for cellulase Trichoderma viride

Eact consequence of increasing the temperature increases the rate of reduction is the process of catalysis. Obviously, the protein globule at temperatures well below the transition temperature denaturation undergoes substantial conformational rearrangements of the type of

small local changes, taking loose metastable structure defining the steric hindrance in the formation of enzyme-substrate complex. This implies an increase of the energy barrier for the reaction catalysis (Eact) andΔ H *. Analysis of the data suggests that the conformational changes of protein molecules that affect their catalytic ability for cellulase occur at 45and 55 C° respectively.

More accurate temperatures start rearrangements in the protein globule due process of thermal inactivation can be obtained from the Arrhenius plot. Depending lg k versus 1 / T are straight, the intersection points which correspond to 55 C°. As seen from Fig.1 Arrhenius plot is convex. Concave polygonal line described by the Arrhenius equation, due to the fact that large activation energies are observed at high temperatures. Negative values of ΔS * for the hydrolysis reaction of carboxymethyl cellulose indicate that the reaction of hydrolysis of carboxymethyl cellulose proceeds with great speed and is characterized by high regularity. Increasing ΔS * with increasing temperature due to the transition from the ordered molecules cellulase globules in a chaotic tangle. Small changes in the values of ΔS * indicates a preferential destruction of weak bonds (hydrogen and electrostatic), resulting in a lower loss of catalytic activity.

CONCLUSION

Analysis the data of this study to help understanding the thermal stability of cellulase *Trichoderma viride* can make an assumption about the partial destruction of a weak electrostatic influence of temperature and, possibly, hydrogen bonds, supporting the conformation of the protein molecule, , which is accompanied by a loss of hydrolytic activity of the enzyme.It is shown that the effect of high temperatures on cellulose accompanied by an increase Eact, ΔH, ΔS hydrolysis of carboxymethyl cellulose. Arrhenius plots determine the activation energy characterized by the presence of fracture, indicating the occurrence of consecutive reaction catalysis.

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