Pak. j. life soc. sci. (2004), 2(1): 1-4

Pakistan Journal of Life and Social Sciences

Kinetic Study of Carboxymethylcellulase from *Trichoderma reesei*

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Abstract

Kinetics of carboxymethyl cellulase (CMCase) from Trichoderma reesei was studied. The enzyme showed maximum activity at pH 3.0 to a temperature of 30°C. Lower value of Michealis constant (Km = 0.769) obtained from Lineweaver Burk plot is indicative of higher affinity of the enzyme for the substrate. The value of energy of activation (Ea) obtained from the Arhenius Plot was very small (30 Kj K-1 mol-1). This may be interpreted in terms of good relationship between enzyme and the substrate. Enthalpy for the hydrolysis of cellulose by CMCase at optimum temperature obtained was 27.56 Kj K-1 mol-1. The low value of Q10 (1.43) shows very high catalytic activity of the enzyme. Dependence of the enzyme activity on substrate concentration gave a good agreement between the theoretical and experimental values.

Keywords: Kinetic study, CMCase, *Trichoderma ressei*

Introduction

Cellulose is the major structural polysaccharide of plants (Gosh *et al.*, 1984). It is formed from linear chains of glucose units linked by glycosidic bonds into β-1, 4-glucan chains that can interlink by hydrogen bonding to produce an insoluble crystalline polymer (Preston, 1986). The polymer has both crystalline and amorphous regions. The former referring to the portion more resistant to chemical/biochemical attack and the latter to the portion of the cellulose chain that is prone to easy hydrolysis.

Crystalline cellulose allows the penetration of exoglucanase, while amorphous cellulose allows the penetration of endoglucanase that catalyzes the hydrolysis of internal bonds.

Enzymatic conversion of cellulose to metabolizable sugars is an essential step, if further conversion to useful products is required such as ethanol production (Rajoka *et al.*, 1997).

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Cellulases are used in the manufacturing of pharmaceuticals, beverages, paper and textiles. Bacterial and fungal cellulases now days are used in animal feed industry, grain alcohol fermentation, brewing, malting and extraction of fruit and vegetable juices (Deshpande and Erickson, 1984). Conversion of agricultural residues to useful products is also an attractive option as a remedy for air pollution, energy production and other environmental concerns (Doran *et al.*, 1994). Furthermore, fast growing population of the world is becoming a permanent threat to the natural resources (Yaqub; 1990, 1996). Under such conditions, an alternative strategies amongst be laid down to meet our future energy demands. Therefore efficient methods of recycling of waste materials into useful products must be found out. Cellulose biodegradation is mediated by several enzymes, which have been extensively studied because they are secreted in large quantities. The extra cellular cellulases of Trichoderma reesei have been studied as a model system for fungal cellulases. These enzymes act synergistically for the complete hydrolysis of cellulose into glucose (Fan *et al.*, 1987). CMCase (endoglucanase) converts the polymeric form, then avicelase (exoglucanase) separates cellobiose by acting on non-reducing end. Finally, β-glucosidase changes cellubiase into glucose units.

The aim of present study was to study the effects of pH, temperature and substrate concentration on CMCase activity and to determine energy of activation, enthalpy of activation and Q10 of the enzyme isolated from Trichoderma reesei.

Materials and Methods

Carboxymethyl cellulase (CMCase)) isolated from *Trichoderma reesei*, obtained form National Institute for Biotechnology and Genetic Engineering Faisalabad, was subjected to kinetic studies. Carboxymethyl cellulose was used as substrate that is hydrolyzed by CMCase to produce free carboxymethyl glucose units. The free caboxymethyl glucose forms a colored complex, which is detected spectrophotometrically at 550 nm (Gadgil *et al.*, 1995). Enzyme solution (1 mL) was incubated for 30 minutes with 1 mL of 3.0 percent CMC and 1 ml of glutamic acid buffer (pH 3.0) at 30°C. The reaction was terminated by adding 3 ml of DNS reagent and mixture was boiled for 10 minutes, cooled in ice and absorbance was noted at 550 nm. Enzyme activity was determined by using standard factor obtained from standard curve (Fig 1).

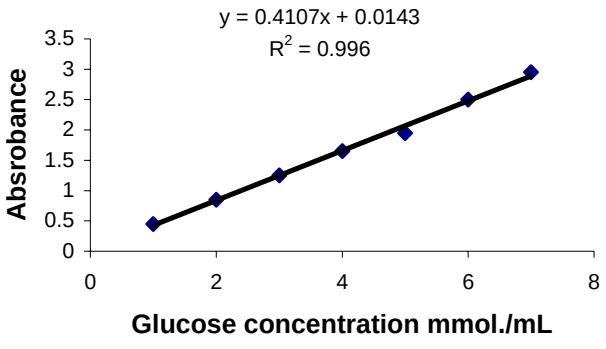

Figure 1: Standard curve of glucose

Optimum pH and optimum temperature

Optimum pH for CMCase activity was determined using buffer of different pH (2-6). Optimum temperature for maximum activity of cellulase was determined as described by Sanyal *et al.*, (1998). The assay was made at different temperatures (20 to 400C).

Activation energy and enthalpy of activation (ΔH)

Activation energy of CMCase was determined by using the data from optimum temperature assay as in following equation:

Ea = -Slope x R (Atkins, 1985)

Where

R = Molar gas constant (8.314 jk-1/mol)

Increase in reaction rate per 10°C rise in temperature (Q10)

The value of activation energy was also used to calculate the increase in reaction rate for every 10°C increase in temperature.

Effect of substrate concentration

CMCase was assayed in glutamic acid buffer (pH 3.0) with variable amounts of 3% CMC as substrate. The values of Vmax and Km were calculated from the plot of 1/V vs 1/[S] (Lineweaver-Burk plot).

Results and Discussion

Carboxylmethyl cellulase (CMCase) obtained from fungal strain Trichoderma reesei was used in the present study for the catalytic hydrolysis of carboxymethyl cellulose (CMC) under different conditions of temperature, pH, and substrate concentration.

Optimum pH and Temperature

Twelve duplicate experiments were carried out to optimize pH for normal enzyme activity of CMCase from *Trichoderma reesei*, Maximum activity was obtained at pH 3 (Fig 2).

Our results are similar to that of Vidya *et al.*, (1984) who reported high enzyme activity (6.181U/mL) produced from Fusarium lini.Similarly Ghori *et al.*, (2001) reported maximum CMCase activity (0.87 1U/ml) at pH 3.5. It has been found that CMCases from Aspergillus terrcus; Aspergillus niveus; and Aspergillus niger had pH optima of 3.8; 4.8 and 4.4 respectively (Bastawade; 1992, Taj *et al.*, 1993 and Siddique *et al.*, 2000).

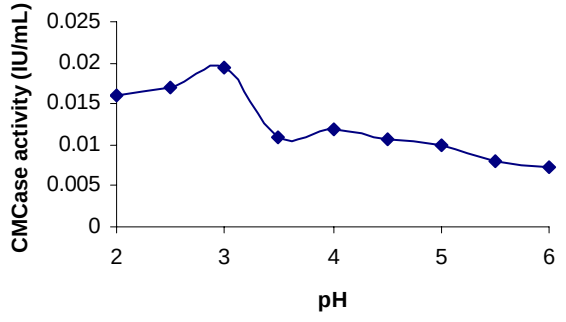

Fig 2. Effect of pH on CMCase activity at 30°C

The experiments at varying temperatures viz, 20°C, 30°C and 40°C were performed to find out the optimum temperature for CMCase activity. It was observed that optimum temperature for the enzyme from Trichoderma reesei was 30°C (Fig 3). At 25°C, activity of enzyme was lower; it was increased gradually per 10°C rise in temperature. At 30°C enzyme showed maximum activity, which was further decreased at 40°C.

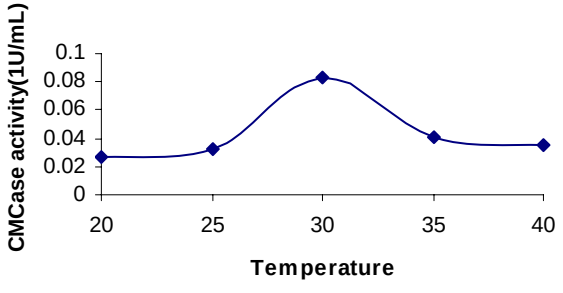

Figure 3: Effect of temperature on CMCase activity at pH 3.0

The work was in accordance with earlier work conducted (Fauth *et al.*, 1991; Lucas *et al.*, 2001) who observed that endoglucanase from different microbial origins had different temperature optima e.g., CMCase from *Streptomyces lividans* and *Chalara paradora* had their temperature optima of 37°C, respectively. Similarly Ghori *et al.* (2001) reported maximum CMCase activity (0.870 1U/mL) at 30°C. The results are comparable with Rajoka and Malik (1984) who reported temperature optima of different enzymes from *C. biozota* mainly between 30-50°C. They showed that the enzyme retained 100% original activity upto 50°C, and all the enzymes showed a tendency to decrease activity above 50°C and to a great extent at 60°C.

Energy of activation and enthalpy of activation

Energy of activation of endoglucanases from Trichoderma reesei was 30kJK-1 mole-1 (Fig 4).

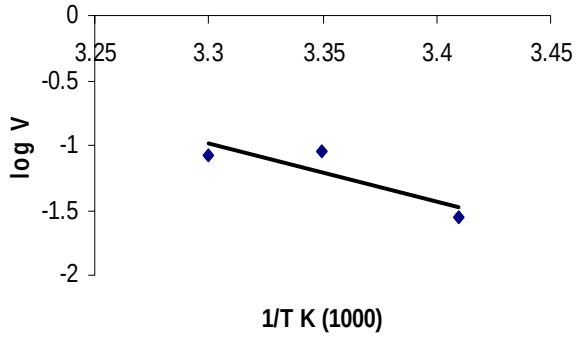

Fig. 4. Arhenius plot for activation energy of CMCase catalyzed reaction

It was observed that at 30°C CMCase had maximum catalysis for the conversion of CMC into glucose. After this temperature the enzyme starts becoming denatured and shows less activity towards the conversion of substrate into product. This small quantity of activation energy highlights a good coordination between enzyme and substrate. These results are similar to those by Sanyal *et al.* (1988) who reported energy of activation 34.276 kJ mol-1 for CMCase. Similarly Ghori *et al.*, (2001) reported energy of activation 22.52 kJ mol-1 for CMCase from Trichoderma reesei. Earlier Siddique *et al.* (1997) reported that CMCase from Cellulomonas biozotea had Ea 35 KJ mol-1.

Enthalpy of activation (ΔH) for CMCase was found to be 27.5 KJ K-1/mol-1. It is therefore interpreted that kinetically CMCase of Trichoderma reesei is favorably good for the conversion of cellulose into glucose.

Increase in reaction rate per 10°C (Q10) in temperature

The Q10 value obtained for CMCase was 1.43. This value indicates that there was, on average, 1.43 times increase in reaction rate of this enzyme when the temperature was increased from 20°C to 30°C. Lower Q10 values demonstrate high catalysis, as a distinctive feature of enzyme catalysis is that the Q10 of a catalyzed reaction is lower as compared to the same reaction uncatalyzed (Segal, 1975).

Effect of substrate concentration

The dependence of the reaction rate on the concentration of carboxymethyl cellulose (CMC) was calculated. Using increasing amounts of CMC as substrate, the Km and Vmax values of endoglucanase from Trichoderma reesei at 30°C were found to be 0.769 and 0.1 mM/mL/min respectively as obtained from Lineweaver Burk plot (Fig 5).

Our results indicate small Km values of CMCase, which demonstrates high affinity of the enzyme with the respective substrates. (Palmer, 1987). A good agreement between the theoretical and experimental data shows that CMCase obtained from *Trichoderma reesei* followed Michealis-Menton kinetics.

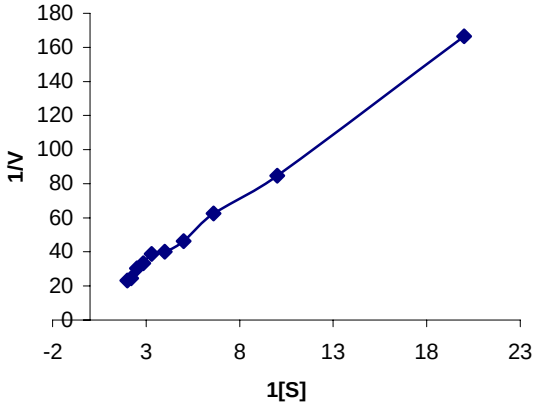

Figure 5: Effect of substrate concentration on CMCase activity (Lineweaver-Burk plot)

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