XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

Thermodynamic and Kinetic Characterization of a Purified Xylanase from *Aspergillus foetidus* grown in Soybean Hulls

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ABSTRACT

A purified xylanase from Aspergillus foetidus grown on soybean hulls 2% with molecular weight estimated at 14.19 kDa was subjected to thermodynamic and kinetic trials. The K_m e V_{max} values for the testing with birchwood xylan was 26.32 mg/mL, 68.45 U/mL, respectively. The thermodynamic parameters for the hydrolysis of birch wood xylan were estimated at ΔG = - 17.56 kJmol $^{-1}$, ΔH = -26.94 kJmol $^{-1}$ and ΔS = -31.47 Jmol $^{-1}K^{-1}$. The thermodynamically favorable process allows the use of xylanase in biotechnological processes involving the hydrolysis of xylan.

Key words: *Aspergillus foetidus*; xylanase; thermodynamic; kinetic; soybean hull

INTRODUCTION

The xylanases are important enzymes due their biotechnological potential in several industrial processes such as baking, preparation of animal feed, bleaching of cellulose pulp, clarification of juices and wines, xylitol and ethanol production (Shah and Madamwar, 2005, Pal and Khanum, 2011, Seyis and Aksoz, 2005). The characterization of xylan degrading enzymes is essential for their biotechnological application. The process cost depends heavily on the cost of the enzymes. This cost reduction could be achieved by optimization of production processes, enzyme purification and enzyme characterization (Shah and Madamwar, 2005).

In industrial application enzymes should ideally be tolerant to various physical and chemical factors such as pH, temperature, metal ions, and they should present high catalytic efficiency, and low production cost. Therefore, studies that seek identification, purification and characterization of new xylanases are important (Bokhari et al., 2009).

MATERIAL AND METHODS

- **Determination of xylanolytic activity.** In order to determinate xylanolytic activity, 100 uL of substrate (1% xylan) and 50 uL of enzyme extract were incubated at 50 ° C for 30 minutes

(Ferreira et al., 1993). After the reaction time, 300 uL of dinitrosalicylic acid (DNS) were added. The reagent solutions were incubated at 100 ° C for 10 minutes (Miller, 1959). Then 1.5 mL of distilled water was added and the absorbance was read in a spectrophotometer at 540 nm (Shimadizu). One unit of enzyme activity was defined as 1 µmol of reducing sugar generate per minute (International Unit - U) per 1 mL of enzyme ($U.mL^{-1}$). All assays were performed in triplicate. Standard curves were determined with D-xylose solutions over concentration range from 0.01 to 2.0 mg/mL.

- **Determination of kinetic parameters.** The kinetic parameters (K_m e V_{max}) of the purified enzyme was determined for the substrate birchwood xylan. The enzyme assay was performed as described above using birchwood xylan in concentration range from 0 to 60 mg/mL. The Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}) were determined by the nonlinear regression method Enzffiter (Leatherbarrow, 1999).

- **Determination of thermodynamic parameters** The thermodynamic parameters of the purified enzyme were determined for the enzyme reaction with birchwood xylan. The enzyme assay was performed as described above using birchwood xylan in concentration range from 0 to 60 mg/mL, at temperatures of 20°C, 30°C, 40°C, 50°C, 60°C and 70°C. The thermodynamic parameters obtained were calculated according to the following equations:

$$Ka = 1 \div Km$$
$$R \times \ln Ka = -\Delta H \times (1 \div T) + \Delta S \text{ (approximation of van't Hoff)}$$
$$\Delta G\,(25°C) = \Delta H - T \times \Delta S$$

ΔG is the Gibbs free energy (kJ mol^{-1}), R is the gas constant (8.314 J $mol^{-1}K^{-1}$), T is the absolute temperature (K), ΔH is the enthalpy change (kJ mol^{-1}), Ka is the affinity constant (M^{-1}), Km is the Michaelis-Menten constant (M) and ΔS is the entropy change (J $mol^{-1}K^{-1}$).

RESULTS AND DISCUSSION

The thermodynamic parameters for characterization of the enzyme-substrate bond are the change in the Gibbs free energy (ΔG), the enthalpy change (ΔH) and the entropy change (ΔS) (Hegde et al., 1998).

The calculation of the parameters was performed using the van't Hoff equation showing the relation between temperature and the affinity constant (Ka). The variables for determination of the parameters are shown in Table 1.

Table 1 – Variables for thermodynamic parameters calculation

Temperature (K)	Km (M)	Ka (M^{-1})	R ln(Ka)	1/T (K)
293	0.000678	1475.05	14.498	0.0034
313	0.001519	658.46	12.895	0.0032
323	0.001855	539.13	12.498	0.0031
343	0.003471	288.12	11.253	0.0029

Km: Michaelis-Menten constant; Ka: affinity constant; R: gas constant.

The temperature dependence regarding the affinity constant is shown in Table 1, indicating Xly affinity decreases when temperature raises. At 20°C, the Ka is 1475,05 M^{-1}, while at 70°C, the affinity constant is 288,12 M^{-1}. Corroborating the fact Km, the Michaelis-Menten constant that indicates the specificity of the enzyme by the substrate, increases with increasing temperature.

The data were adjusted according to the approximation of van't Hoff resulting in the straight line equation: y = 6435.37 x - 7.51625

From these data, the thermodynamic variables ΔH and ΔS were estimated, considering ΔH corresponds to the slope of the line and ΔS corresponds to the intercept in Y. The change in Gibbs free energy was calculated from the equation y = 6435.37 x - 7.51625. The parameter values were ΔG= -17.56 kJ mol^{-1}; ΔH= -26.94 kJ mol^{-1} and ΔS= -31.47 J mol^{-1} K^{-1}.

When compared with literature, xylanase obtained from *Aspergillus foetidus* (Xly) presents an enthalpy change inferior to the estimated values for xylanase purified of *Chainia sp.* (Hegde et al., 1998). The lower the value ΔH, the better the formation of the enzyme-substrate complex (Bokhari et al., 2009). In addition, higher values of ΔH indicate that more energy is required to conformational change by distortion of the xylose ring and change to sofa conformation. Xylose presents that conformation in the transition state, which mean, in the formation of the enzyme-substrate complex (Afzal et al., 2005).

The ΔS negative value means the metabolic network is more stable, possibly for a partial aggregation of inactive enzyme molecules (Ortega et al., 2004, Bokhari et al., 2010). When ΔS increase, the enzyme assume a state of higher disorder (of the active site or of the structure), favoring the enzyme binds to the substrate - transition state (D'Amico et al., 2003). The most stable enzymes (high value of ΔH), when in their native state, are associated with higher values of ΔS in molecular interaction processes. This increase in entropy change is suggested to be a compensatory effect, favoring the formation of reaction products (Ortega et al., 2004).

Comparing Xly, ΔH = -26.94 $kJmol^{-1}$ and ΔS = -31.47 $Jmol^{-1}K^{-1}$ with xylanase obtained by Hegde et al. (1998), ΔH = - 21.00 $kJmol^{-1}$ and ΔS = -4.8 $Jmol^{-1}K^{-1}$ is observed that the higher the value of ΔH, the higher the value of ΔS (Hegde et al., 1998).

The parameter that best determines the feasibility of the enzyme reaction, however, is the change in Gibbs free energy (ΔG). The feasibility of the reaction may be defined as the conversion of the enzyme-substrate complex to products. The lower the value of ΔG, more favorable and spontaneous is the reaction to the formation of the products (Bokhari et al., 2009). For Xly, ΔG of - 17.56 $kJmol^{-1}$, indicating that the reaction of the xylanase with substrate xylan is greatly favored. The results are consistent with findings in the literature: Hegde et al. (1998) and Boraston et al. (2001) reported ΔG of - 19.45 and ΔG of - 29.02 for xylanases from *Chainia sp.* e *Thermotoga maritima*, respectively (Boraston et al. 2001, Hegde et al., 1998).

CONCLUSION

The kinetic and thermodynamic parameters of the purified xylanase was determined. The results indicate xylanase is thermodynamically favorable to form xylan degradation products and it has potential application in industrial biotechnological processes.

ACKNOWLEDGMENTS

The authors are grateful to National Counsel of Technological and Scientific Development (CNPq), Support Research of the Federal District Foundation (FAPDF), Higher Education Personnel Improvement Coordination (CAPES), Scientific and Technological Enterprise Foundation (FINATEC) and University of Brasilia (UnB) for providing funds to perform the present research.

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