Kor. J. Appl. Microbiol. Bioeng.
Vol. 9, No. 4, 179—183 (1981)

Some Kinetic Properties of an Extracellular Chitinase from *Streptomyces* sp. 115-5

Yong Ki Hong and Jung Hwn Seu
Department of Agricultural Chemistry, College of Agriculture,
Kyungpook National University, Taegu, 635, Korea
(Received June 26, 1981)

*Streptomyces*속 115-5 균주로부터 생성된 *Chitinase*의 저해작용기작

홍용기 · 서정훈

경북대학교 농과대학 농화학과

(1981년 6월 26일 수리)

Abstract

An extracellular chitinase was purified from the culture fluid of *Streptomyces* sp. 115-5, and its inhibition mechanism by end product was studied. The activity of chitinase was suppressed by the reducing sugar as the reaction proceeded, and the activity was inhibited by the addition of D-glucose. Besides D-glucose, the rate of chitin hydrolysis was inhibited by D-glucuronic acid, D-sorbitol and D-xylose in the reaction system of the enzyme. It was found that the hydroxyl groups at the C-2, C-3 and C-4 position of D-glucose molecule play an important role in the inhibition of the chitinase activity. D-glucose was found to inhibit the enzyme activity by mixed type of competitive and non-competitive mode.

Introduction

Chitinase (E.C. 3.2.1.14) can not only be used in food and feed industry and as an insecticidal aid, but used in lysis of fungal cell walls. This system is useful in studying the lytic phenomena responsible for cell wall decomposition and hypal destruction where chitin is a major structural component,[1-4] and also in studying on the formation of protoplasts of fungal cells.[5-7] The previous papers[8,9] described the production, purification and some properties of an extracellular chitinase produced by *Streptomyces* sp. 115-5. The reaction of chitinase was gradually suppressed by the increase of product substances according to the action of enzyme. In this paper, studies were made on the enzyme inhibition by D-glucose and its mode of inhibition.

Materials and Methods

Preparation of Chitinase

Streptomyces sp. 115-5 was cultured at 30°C for 48 hrs in the medium described previously.[9] The enzyme

was purified by ammonium sulfate fractionation, 1st gel filtration using Sephadex G-100, DEAE-cellulose column chromatography, and 2nd gel filtration using Sephadex G-100.

Preparation of Chitin

Chitin as substrate was prepared by the treatment of conc-HCl as described previously.[8]

Measurement of Enzyme Activity

The activity of chitinase was assayed by the method described previously.[9] The amount of reducing sugar released from the reaction mixture was determined by the Somogyi-Nelson method.[10] One unit of the chitinase is defined as the amount of enzyme which releases 1 μ-mole of reducing sugar equivalent expressed as glucose per hour.

Chemicals used

The chemicals are of the purest grades commercially available. 3-0-methyl-α-D-glucopyranoside was purchased from Sigma Chemical Company.

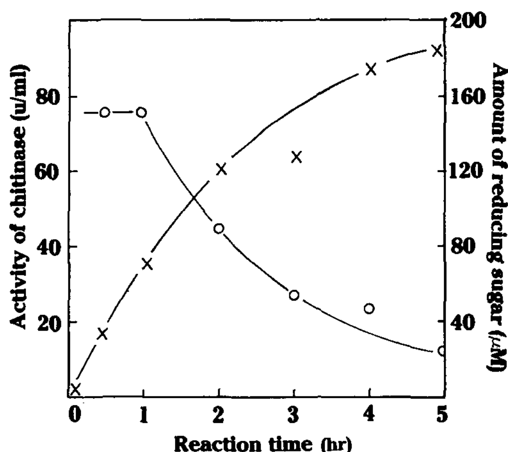

Fig. 1. Time Course of Reaction Catalyzed by Chitinase.

o—o; activity of chitinase, x—x; amount of reducing sugar released by chitinase.

Results and Discussion

Time Course of Reaction Catalized by Chitinase

Fig. 1 indicates that the release of reducing sugar catalized by the crude chitinase is linear with time to one hour before the rate gradually decreased as the reaction proceeded. This result shows that the chitinase activity is suppressed by the accumulation of the reaction product. Cohen[11] reported that the release of N-acetylglucosamine catalized by the soluble chitinase produced by *Phycomyces blakesleeanus* is linear with time to 8 hrs to release 200 n-mole, and the rate gradually decreases thereafter. In this work, the reaction was started to slow down when 80 μ-mole of reducing sugar was accumulated, and 180μ-mole of reducing sugar inhibited the chitinase by 83%.

Effect of D-Glucose in the Activity of Chitinase

From the above result, it was revealed that the chitinase was inhibited by end product and it was a first enzyme in multi-enzyme system. In such system, the end product of the sequence of reactions can inhibit the first enzyme, with the result that the rate of the entire sequence is determined by the steady-state concentration of the end product. So the purified chitinase was reacted in the reaction system containing D-glucose in order to know the effect of D-glucose as an end product. The enzyme reaction mixture was incubated for 2 hrs at 50°C with the addition of various concentrations of D-glucose. As shown in Fig. 2, the addition of 100μg (185μM) of D-glucose per 3 ml of reaction mixture inhibited 76% of the enzyme activity, and no enzyme activity was found in the reaction mixture added by 200μg (370μM) of D-glucose. The enzyme inhibition by glucose might be caused because of the sugar's structure as a inhibitor and/or because the sugar is a metabolite of the substrate. While on the other, it was already reported that the pro-

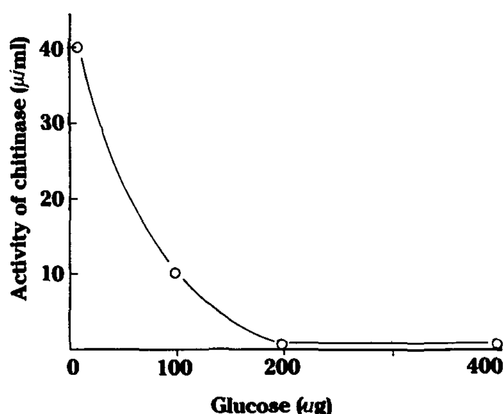

Fig. 2. Effect of Glucose on the Activity of Chitinase.

Table 1: Effect of Several Sugars on the Activity of Chitinase.

Sugars (2 mM)	Relative activity (%)
D-glucose	0
D-glucuronic acid	9.0
D-sorbitol	14.5
D-xylose	36.4
D-mannitol	75.5
D-fructose	90.9
D-arabinose	113
D-mannose	113
D-ribose	125
D-galactose	125
DL-glyceraldehyde	133
3-O-CH_3-D-glucose	136
Control	100

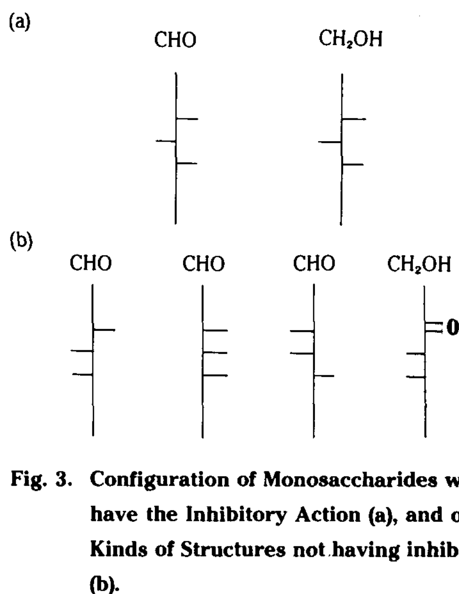

Fig. 3. Configuration of Monosaccharides which have the Inhibitory Action (a), and other Kinds of Structures not having inhibition (b).

duction of chitinase was repressed from the strain of *Streptomyces* sp. 115-5[8] or *S. marcescens*[13] growing on chitin by addition of D-glucose to the culture. Not only the production of chitinase was repressed by the addition of D-glucose, but the action of chitinase was inhibited by D-glucose.

Inhibition of Chitinase Activity by Several Sugars

Table 1 shows the effect of several sugars on the activity of chitinase. 2 m-mole of each sugar was added to the reaction mixture and incubated for one hour at 50°C. The activity of chitinase is expressed as a relative activity against that of normal reaction which does not contain any monosaccharides. As shown in the table, D-glucose gives complete inhibition of the activity, and other sugars show also inhibitory activities in the following order; D-glucose , D-glucuronic acid , D-sorbitol. A 5-carbon sugar, D-xylose also gives an inhibitory effect on the chitinase activity. A derivative of D-glucose, 3-O-methyl-D-glucopyranoside does not inhibit the activity. Therefore it can be said that the hydroxyl groups at the C-2, C-3 and C-4 position play an important role in the inhibition of chitinase as shown in Fig. 3, because the positions of hydroxyl groups at C-2, C-3 and C-4 are common to those of D-glucose, D-glucuronic acid, D-sorbitol and D-xylose. D-xylose which is a pentose without the hydroxyl group of C-5 position inhibits weakly, and D-sorbitol which is the C-1 substituent of glucose inhibits more strongly than D-xylose. D-glucuronic acid which is the C-6 substituent of glucose inhibits more strongly than D-sorbitol. From the results, it was concluded that the conformation of hydroxyl groups of C-2 to C-5 position is most competent in the inhibitory action, the aldehyde group of C-1 position is next followed by the primary hydroxyl group of C-6 position.

Inhibition Mode by D-Glucose

In order to study the behaviour type of inhibition by D-glucose, the initial velocities were measured using reaction mixtures containing different amount of substrate and 25 or 50 μg of glucose as inhibitor. The inhibition of chitinase by D-glucose is expressed as following. Many situations of inhibition are covered by the general inhibition equation;[13]

$$\nu = \frac{V[A]}{K_A\left(1+\frac{[Q]}{K_Q^s}\right)+\left(1+\frac{[Q]}{K_Q^i}\right)[A]}$$

which in reciprocal form becomes

$$\frac{V}{\nu} = \frac{K_A}{[A]}\left(1+\frac{[Q]}{K_Q^s}\right)+1+\frac{[Q]}{K_Q^i}$$

ν is the velocity of the inhibited reaction, V (100μ-mole of glc/ml·hr) is the rate of the uninhibited reaction at high substrate concentrations, and K_A (3.6 mg of chitin/ml) is the Michaelis constant for the reaction in the absence of

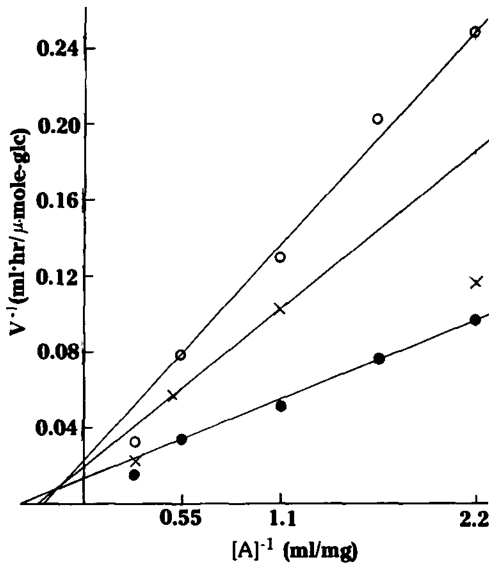

Fig. 4. Double Reciprocal Plots of Reaction Velocity against Glucose Concentrations.
o—o; 50 μg of glucose, x—x; 25 μg of glucose, ●—●; without glucose.

inhibitor Q. A lineweaver-Burk plot of 1/V against 1/[A] for this equation is shown schematically in Fig. 4. The slope of this plot for a given value of inhibitor concentration depends upon the magnitude of inhibition constant of slope K_Q^s, and the intercept on the magnitude of inhibition constant of intercept K_Q^i, namely

$$\text{slope} = \frac{K_A}{V}\left(1+\frac{[Q]}{K_Q^s}\right),$$

$$\text{intercept} \fallingdotseq \frac{1+\frac{[Q]}{K_Q^i}}{V}$$

At first, in the case of inhibition by 50 ug (92.6 *u*M) of glucose,

slope = 0.101 hr·mg-chitin/*u*M-glc.

So the slope is given by;

$$\text{slope} = \frac{K_A}{V}\left(1+\frac{[Q]}{K_Q^s}\right) = \frac{3.6\ \text{mg-chitin/ml}}{100\ \mu\text{M-glc/ml·hr}}$$

$$\left(1+\frac{92.6\ \mu\text{M-glc}}{K_Q^s}\right) \doteqdot 0.101\ \text{hr·mg-chitin/}$$

μM-glc

Therefore, the value of K_Q^s is about 50.94 μM-glucose. Next,

intercept = 0.023 ml·hr/*u*M-glc.

So the intercept is given by;

$$\text{intercept} \fallingdotseq \frac{1+\frac{[Q]}{K_Q^i}}{V} = \frac{1+\frac{92.6\,\mu\text{M-glc}}{K_Q^i}}{100\ \mu\text{M-glc/ml·hr}}$$

$$\doteqdot 0.023\ \text{ml·hr/}\mu\text{M-glc}$$

Therefore, the value K_Q^i is about 71.23 *u*M-glucose. It is now clear that the value of constant K_Q^i is more than that of constant K_Q^s, and the degree of inhibition is reduced as the concentration of substrate is increased. Conclusionly, the inhibition has some competitive character, and it will therefore be described in the present work as mixed type of competitive and non-competitive inhibition. Also, in the case of inhibition by 25 ug-glucose, the value of constant K_Q^i is 55.11 *u*M-glucose and that of constant K_Q^s is 42.19 *u*M-glucose. It therefore confirms that the constant of K_Q^i is more than that of K_Q^s.

요 약

진균류의 세포벽제거 및 이에 따른 protoplast 생성등에 많이 이용되어지고 있는 chitinase를 *Streptomyces* sp. 115-5 균주로 부터 생산하여 순수정제한 다음, 이 chitinase의 작용을 저해하는 포도당의 저해양상을 조사하였다. 포도당외에 D-glucuronic acid, D-sorbitol 및 D-xylose등도 chitinase의 활성을 저해하였다. 그런고로, 포도당 분자에 의한chitinase의 활성 저해 효과에는 위의 분자들의 공통부분인 2번, 3번 및 4번 탄소의 hydroxyl group들의 구조위치가 중요한 영향을 가진다는 것을 알 수 있다. 그리고 포도당에 의한 chitinase의 저해양상은 competitive inhibition과 non-competitive inhibition 과의 혼합 저해형으로 나타났다.

References

1) Skujins, J.J., Potgieter, H.J., and M. Alexander : *Arch. Biochem. Biophys.*, **111**, 358 (1965)

2) Monreal, J. : *Arch. Biochem. Biophys.*, **126**, 960 (1968)

3) Toyama, N. and K. Ogawa : *J. Ferment. Technol.*, **46**, 626 (1968)
4) Tanaka, H., Ogasawara, N., Nakajima, T., and T. Tamari : *J. Gen. Appl. Microbiol.*, **16**, 39 (1970)
5) Anné, J. and J.F. Poberdy : *J. of Gen. Microbiol.*, **92**, 413 (1976)
6) Villanueva, J.R. and I.G.A. Acha : Methods in Microbiology, (Booth, C., ed.) Vol. 4, Academic press, New York, p. 665 (1971)
7) Starr, M.P. : Ann. Review Microbiolo., Vol. 33, Annual reviews inc., California, p. 21 (1979)
8) 김광현, 서정훈 : 한국산업미생물학회지, **6**, 149 (1978)
9) 홍용기, 서정훈 : 한국산업미생물학회지, **7**, 149 (1979)
10) 安藤鋭郎, 寺山宏, 西澤一俊, 山川民夫 : 生化学研究法 I , 朝倉書店, 東京, p126 (1967)
11) Cohen, R.J. : *Life Sciences*, **15**, 289 (1974)
12) Monreal, J. and E.T. Reese : *Can. J. Microbiol.* **15**, 689 (1969)
13) Laider, K.J. and P.S. Bunting : The Chemical Kinetics of Enzyme Action, 2nd ed., Clarendon press, Oxford, p. 94 (1973)