Effect of Activators and Inhibitors on Lactase Activities to Determine its Kinetic Model

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Summary
Lactase activity in presence of mono and divalent metal ions, as its cofactors, and some reducing agents was studied. Activation effects of k^{+} and Mn^{2+} were better than other cations and dithiotrethiol was more inhibitor. By inhibition constant, 12.15mMol, the type of inhibition was non-competitive. The kinetic model of Lactase hydrolysis reaction also was determined. Among glucose and galactose as its products, galactose had more inhibition effect and the type of inhibition was non-competitive. Galactose inhibition constant in lactose substrate was 78.7mMol.

Key words: lactase(β-galactosidase), lactase activity, lactase kinetic

Introduction

Lactase (β-galactosidase) is an induced protein for lactose, the sugar of milk. This enzyme which removes galactose from lactose (Bohle 1978, Gruegor 1984, Takasa 1988) has an important role on digestion in the gastrointestinal tract. In fact, the chemical changes incident to digestion are with the aid of hydrolase enzymes of digestive tract. The lack of this enzyme causes digestion problems (Bohle 1978, Palumbo 1995).

In biologic systems, enzymes are protein catalysts for chemical reactions. Thereby, understanding of kinetic properties of enzymes and effects of some biological and chemical materials on activity of them is important. The effect of various metal ions on this enzyme on activity of lactase was studied (Palumbo 1995, Van Griethyusen 1988, Zadaw 1992). Depending on nature and structure of this enzyme and also kind of buffer the effect of metal ions were differed. K^{+} and Mn^{2+} for the enzyme, which extracted from *kluyveromyces fragilis* and Na^{+} and Mn^{2+} for the enzyme that extracted from *Escherichia coli* act as cofactors. In this study effects of some metal ions and reducing agents on activity of lactase and also the kinetic of reactions were determined.

Materials and Methods

Enzyme. β-galactosidase was purchased from Lactaid Co.(Canada) originated from *Aspergilus orizae* (EC: 3.2.1.23).

Chemicals. Citric acid, sodium hydrogen phosphate, sodium chloride, potassium chloride, manganese chloride, magnesium chloride, calcium chloride, lactose, cystein, glutathion, dithiotreitol were obtained from Merck.

β-galactosidase activity. ONPG and lactose tests can be used for measuring of β-galactosidase activity. Here lactose test was carried out according to Wendroff (1971). 0.5ml of enzyme solution (1g/l) was added to 4.5ml of citrate buffer (0.1M, pH 4.8), which contain 272mg lactose and incubated for 10 min at 30C. To stop the reaction tubes were incubated in boil water bath for 5 min. Amount of glucose was measured by glucose oxidase test. One unit of β-galactosidase is the amount of the enzyme, which will be produced 10^{-6} mole of glucose in one min at 30C.

Effect of activators and Inhibitors. As inhibitors 10^{-1}-10^{-3}M of cystein, dithiotreitol and reduced glutathion were added to 100mM lactose substrate. 0.25, 10^{-1}-10^{-3}M of NaCl, KCl and 10^{-1}-10^{-4}M of $MnCl_2$, $MgCl_2$ and $CaCl_2$ were added as activators. The enzyme activity was measured.

Kinetic model of β-galactosidase. *a) Initial velocity.* A substrate concentration in which enzyme activity was high selected and series of substrate concentration were made again. Enzyme activity was measured for minimum substrate concentration in one min alternatively.

b) Km of Michaeles-Menten and inhibition constant. The enzyme was added to different concentration of lactose and enzyme activity in optimum conditions (pH 4.8, 30C) were measured. Initial velocity for each concentration of substrate was calculated. Lineweaver-Burke curve was plotted, Vm and Km were calculated. Inhibition constant for each inhibitor was measured. To determine enzyme kinetic in presence of inhibitors three tests were carried out. In the first test enzyme activity with different concentrations of lactose were measured and Km, Vm (without presence of inhibitor) determined. In the second test, inhibitor concentration was kept constant while substrate concentrations were changed. In third test, they were inversely.

Results and Discussion

The result showed that K^+, a monovalent, and Mn^{2+}, a bivalent, cations in commparation to others were more effective on the lactase activity (Table 1).

Relative activity of the enzyme without presence of these cations was 48.95 %.

Table 1. *Relative activity (%) of lactase in presence of mono and bivalent cations*

	Monovalent cation			Bivalent cation		
Con. (M)	NaCl	KCl	Con. (M)	$MnCl_2$	$MgCl_2$	$CaCl_2$
0.25	53.02	61.46	0.1	71.80	67.58	52.03
0.1	50.83	56.02	0.01	64.45	60.66	50.81
0.01	50.50	51.40	0.001	56.01	52.72	49.50
0.001	49.60	50.02	0.0001	50.92	49.06	48.97
0.0001	48.97	48.98				

The inhibition of enzyme activity was determined according to Yang (1984). The results summarized on table 2. The inhibition effect of dithiotreitol in 10^{-1}M concentration was more.

Table 2. *Relative activity (%) of lactase in presence of reducing agents*

Con. (M)	Cystein	Reduced glutathion	Dithiotreitol
0.1	27.35	20.28	18.01
0.01	48.30	37.64	20.78
0.001	74.52	63.20	42.45
0.0001	92.45	86.79	47.16

Enzyme kinetic constants. To determine the enzyme kinetic constant in presence of inhibitors, Linewearver-Burke curve plotted. In control condition, Km and Vm were 83mMol and 1.92μMol min^{-1}, respectively. By decrease in maximum velocity, Km was also decreased (Figure 1). This indicated that inhibition effect of these reducing agents were non-competitive (Wierzbicki 1974).

In presence of 100mMol cystein, Km_{app}=13.69mM, $Vmax_{app}$= 0.32μMol min^{-1} and Ki=20.5mM were calculated.

In presence of 100mM reduced glutathion, Km_{app}=10mM, $Vmax_{app}$=0.23μMol min^{-1} and Ki=13.94mMol were calculated.

In presence of 100mM dithiotreitol, Km_{app}= 8.85mM, $Vmax_{app}$= 0.2μMol min^{-1} and Ki= 2.15mMol were calculated.

Effect of product as inhibitor on enzyme activity. Glucose and galactose are products of lactose hydrolysis by β-galactosidase. Glucose dose not has any effect on the enzyme activity while galactose inhibits it (Yang 1984). If 100mMol galactose was added to different concentration of lactose, it was seen that max velocity

decreased and Km had not changed. By increasing concentration of inhibitor, Vmax became smaller but Km had not changed (Figure 2). By knowing the values of inhibitors concentration, inhibition constant of galactose were estimated 78.7mMol.

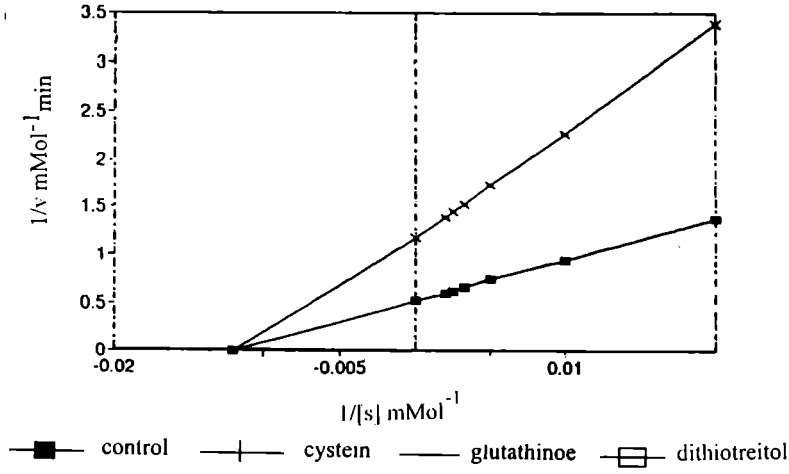

Figure 1. *Plot of lineweaver-burk for inhibition of lactase by reducing agents*

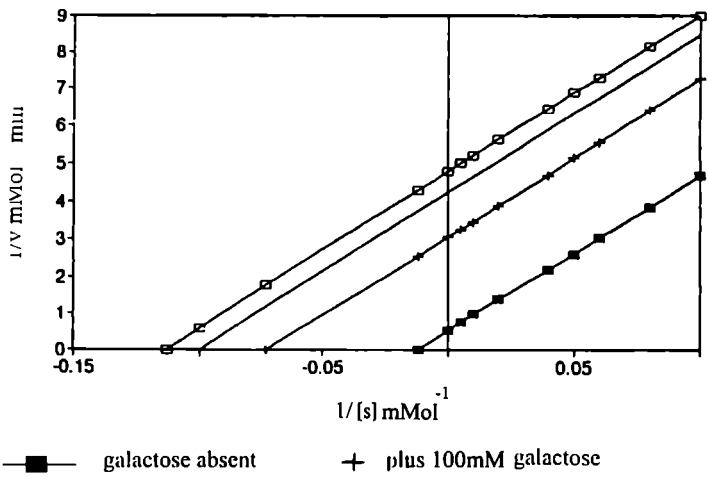

Figure 2. *Plot of lineweaver-burk for inhibition of lactase by galactose*

Kinetic model of enzyme hydrolysis reaction. This model which were suggested, with respect to K_4constant, was different with Michaeles model. In hypothesis of Michaeles-Menten, velocity of converting substrate to product depends on the

velocity of degrading of enzyme substrate complex. According to this hypothesis, the velocity of forming ES would be equal to the velocity of its consumption.

$$E+S \rightleftharpoons ES \rightleftharpoons E+P$$

E=Free enzyme, S=Initial material concentration, ES=Enzyme-Substrate complex, P=Product, Vm= K_3 E_t max velocity, E_t=concentration of whole enzyme used, Km=Michaele's constant, Keq=Equilibrium constant, Kp=Inhibition constant of product, Keq=0.204 according to references.

$K_1(S)(E)+K_4(E)(P)=K_3(ES)+K_2(ES)$

$E_t=E+ES$

$Ki\ S(E_t-ES)+K_4\ P(E_t-ES)=(ES)(K_3+K_2)$

$$ES = \frac{KiS+K_4P}{K_3+K_2+K_1S+K_4P}$$

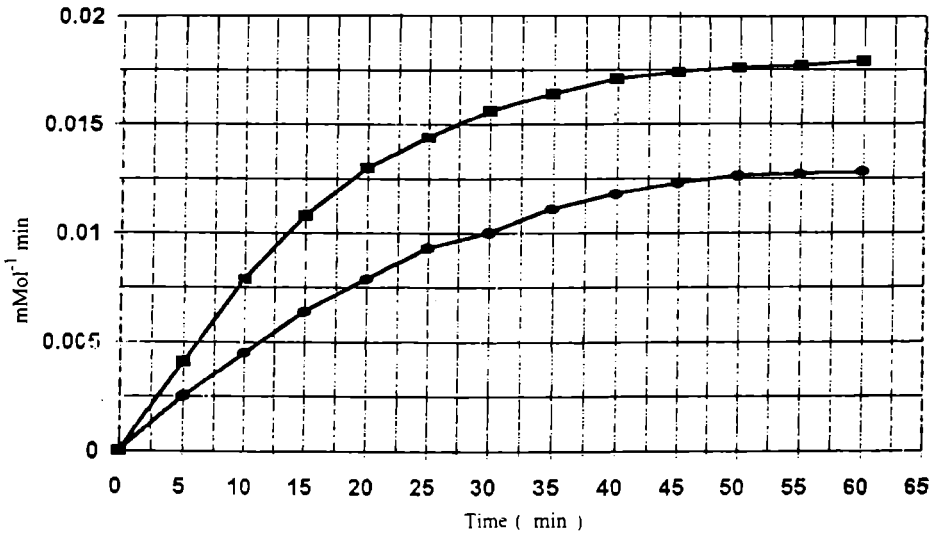

Figure 3. *Kinetic model for lactose enzymatic hydrolysis*

By calculation of different mathematical equations and placing, kinetic amounts from test concentrations of initial compounds in final equation the parameters were appeared.

Keq=0.204, Kp=78.7mM, Km=83mM, Vm=1.92 10^{-3}μMol min^{-1}, S_0=200mM

$$t=-4.28\ P-24973.72-163.39\ \ln(1-0.03\ P)$$

With respect to the calculated equilibrium in known time, the amount of P would be found. The obtained reaction velocity could comparable with the amount, which

obtained from test. According to figure 3 the suggested kinetic model was different with results of experimental tests, which showed non-linearity of reaction velocity. This was because of inhibition effect of product on enzyme activity. (Bohle 1978, Wierzbicki 1974). Studying on enzyme kinetic constants showed max velocity and max velocity constant in presence of inhibitors were non-competitive, which proved this kinetic model was different from Michaele's model.

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