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 Mn²⁺ were better than other cations and dithiothreitol 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, Gruengor 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 hydrolyase 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 differred. K⁺ and Mn²⁺ for the enzyme, which extracted from kluyveromyces fragilis and Na⁺ and Mn²⁺ 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 Aspergillus oryzae (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 10⁻¹⁻¹⁻⁰⁻⁵ M of cystein, dithiotreitol and reduced glutathion were added to 100 mM lactose substrate. 0.25, 10⁻¹⁻¹⁻⁰⁻⁵ M of NaCl, KCl and 10⁻¹⁻¹⁻⁰⁻⁵ M of MnCl₂, MgCl₂ and CaCl₂ 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 Michaelis-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 Mn²⁺, 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

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Monovalent cations</th>
<th>Bivalent cations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>KCl</td>
</tr>
<tr>
<td>0.25</td>
<td>53.02</td>
<td>61.46</td>
</tr>
<tr>
<td>0.1</td>
<td>50.83</td>
<td>56.02</td>
</tr>
<tr>
<td>0.01</td>
<td>50.50</td>
<td>51.40</td>
</tr>
<tr>
<td>0.001</td>
<td>49.60</td>
<td>50.02</td>
</tr>
<tr>
<td>0.0001</td>
<td>48.97</td>
<td>48.98</td>
</tr>
</tbody>
</table>

The inhibition of enzyme activity was determined according to Yang (1984). The results summarized on table 2. The inhibition effect of dithiotreitol in 10⁻³M concentration was more.

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

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Cystein</th>
<th>Reduced glutathion</th>
<th>Dithiotreitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>27.35</td>
<td>20.28</td>
<td>18.01</td>
</tr>
<tr>
<td>0.01</td>
<td>48.30</td>
<td>37.64</td>
<td>20.78</td>
</tr>
<tr>
<td>0.001</td>
<td>74.52</td>
<td>63.20</td>
<td>42.45</td>
</tr>
<tr>
<td>0.0001</td>
<td>92.45</td>
<td>86.79</td>
<td>47.16</td>
</tr>
</tbody>
</table>

Enzyme kinetic constants. To determine the enzyme kinetic constant in presence of inhibitors, Lineweaver-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 cysteine, 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 $K_a$ constant, was different with Michaelis model. In hypothesis of Michaelis-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.

\[ \text{E} + \text{S} \rightarrow \text{ES} \rightarrow \text{E} + \text{P} \]

E=Free enzyme, S=Initial material concentration, ES=Enzyme-Substrate complex, P=Product, Vm=\( K_3 \) E\(_{\text{max}}\) max velocity, E\(_{\text{i}}\)=concentration of whole enzyme used, Km=Michaelis’s constant, Keq=Equilibrium constant, Kp=Inhibition constant of product, Keq=0.204 according to references.

\[ K_1(S)(E)+K_4(P)(P)=K_3(ES)+K_4(ES) \]

\[ E=E+ES \]

\[ Ki S(ES) + K_4 P(ES) = (ES)(K_3+K_2) \]

\[ ES = \frac{Ki S + K_4 P}{K_3 + K_3 + K_4 + S + K_4 P} \]

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=78.7 mM, Km=83 mM, Vm=1.92 \( 10^3 \) \( \mu \)Mol min\(^{-1}\), S\(_{\text{f}}\)=200 mM

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

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 Michael’s model.

References


