

# Kinetic Characterization of a Multifunctional $\beta$ -Xylosidase from Mollusca, *Pomacea canaliculata*

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## Abstract

The enzymatic properties of a  $\beta$ -xylosidase from *Pomacea canaliculata* for trifunctional glycosidase was investigated using artificial substrates. The glycosidase have broad substrate specificity which hydrolyze *p*-nitrophenyl (*p*NP-)  $\beta$ -D-xylopyranoside (Xyl), *p*NP-  $\beta$ -D-glucopyranoside (Glu), and *p*NP-  $\alpha$ -L-arabinofuranoside (Araf). The relative activities ( $\beta$ -xylosidase:  $\beta$ -glucosidase:  $\alpha$ -arabinofuranosidase) were given as almost 100:40:10. All three enzyme activities have a same optimal pH (4), similar pH stability (at 24°C after 15h of preincubation at the pH ranging from 2 to 11), and similar thermal stability (after 55 min of preincubation at 50 °C , 55 °C , and 60 °C ). The kinetic features when mixed substrates (*p*NP-Xyl and -Glu, *p*NP-Xyl and -Araf, and *p*NP-Glu and -Araf) were used with different molar ratio, fitted to theoretical curves for a single catalytic site on the enzyme catalyzing the hydrolysis of the two substrates, respectively. The effect of temperature on  $\beta$ -xylosidase,  $\beta$ -glucosidase, and  $\alpha$ -arabinofuranosidase activities was quite different. The activation energies were 68kJ/mole ( $\beta$ -xylosidase), 42kJ/mole ( $\beta$ -glucosidase), and 33kJ/mole ( $\alpha$ -arabinofuranosidase). D-Glucono-1,5-lactone to all three activities shown almost same inhibition, though methyl xylose and methyl glucose inhibited with different dose responses. The ratio of the three activities changed with different NaCl concentration. These results suggest that the enzyme activities to the three artificial substrates originate from a common catalytic site, however the sugar binding site to the three substrates are slightly different. It is considered that some conformational changes approximately derive the changes of the enzymatic character.

Key word :  $\beta$ -Xylosidase  
 $\beta$ -Glucosidase  
 $\alpha$ -Arabinofuranosidase  
Mollusca; *Pomacea canaliculata*

## Introduction

$\beta$ -Xylosidase (BXase; EC3.2.1.37) is a hemicellulase that hydrolyze xylans, which are composed of  $\beta$ -1,4- and/or 1,3-linked xylopyranose with branches containing L-arabinofuranosyl and glucopyranosyl residues. Several different xylosidases are described, which are especially important for various industrial applications, currently under the development such as pulp preparation processes<sup>1-4</sup>, food process<sup>5-7</sup>, the synthesis of oligosaccharide<sup>8-10</sup>, and drug design technics. Some BXases has broad substrate specificity which has another catalytic activity of  $\beta$ -glucosidase,  $\alpha$ -arabinosidase or levansucrase as bifunctional glycosidase<sup>11-14</sup>. Some BXases are also reported which has both  $\beta$ -glucosidase (BGase) and  $\alpha$ -arabinofuranosidase (AFase) activities, in which the relative activities (BXase/ BGase/ AFase) of *Aspergillus niger* xylD<sup>15</sup>, *Butyrivibrio fibrisolvens* xylB<sup>16</sup>, and *Trichoderma ressi* BXLI<sup>14</sup> are reported as almost (100/ 0.4/ 5), (100/ 174/ 39), and (100/ 32/ 15), respectively. Although many fungal and bacterial  $\beta$ -xylosidases were studied so far, their catalytic properties and amino acid sequences were recently classified<sup>17</sup>. However, the information of animal  $\beta$ -xylosidases from rat<sup>18,19</sup>, *Charonia lampas*<sup>20</sup>, and *Pomacea canaliculata*<sup>21</sup> are still limited.

In a previous paper, the purification and some properties of BXase from the molluscan *Pomacea canaliculata* were reported.<sup>21</sup> The BXase released a xylose residue from high mannose type oligosaccharides branching  $\beta$ -1,2-Xyl and from  $\beta$ -1,3 or 1,4-xylooligosaccharides. The enzyme was purified to homogeneity, which was obvious from the SDS-polyacrylamide gel electrophoresis (85kDa), isoelectric focusing (pI4.5), and ultracentrifuge analysis (5.75S<sub>20W</sub>). The single N-terminal amino acid sequence (DYPPFR-) of the purified protein was detected. The enzyme hydrolyzed *p*-nitrophenyl (*p*NP-)  $\beta$ -D-xylopyranoside (Xyl) and *p*NP-  $\beta$ -D-glucopyranoside (Glu). In addition,  $\alpha$ -L-arabinofuranosidase (AFase) activity of the enzyme had been detected. However, the multifunctional mechanism was not clarified enough. The glycon specificity for a multifunctional enzyme has been focused so far. Several kinetic properties of *P. canaliculata* BXase were presented at the academic meeting<sup>22,23</sup>.

In this study, the enzymatic properties are further

considered including unpublished data. The enzymatic properties, BXase, BGase, and AFase activities, which provide evidence for a multifunctional enzyme from the kinetic studies, that all the three activities derive from the same single catalytic site, and also consider some conformational properties of the substrate binding site.

## 2. Materials and Methods

### 2.1. Materials

*p*NP-Glycosides were purchased from Sigma Chemicals Co. or Nakarai Tesuqu Co. D-Glucono-1,5-lactone (GluLac), and methyl  $\beta$ -D-glucopyranoside (mGlu) and methyl  $\beta$ -D-xylopyranoside (mXyl) were purchased from Tokyo Kasei Co. All other chemicals of analytical grade were from Nakarai Tesuqu Co. or Kanto Kagaku Co. The BXase was purified to homogeneity from the extract of the molluscan hepatopancreas of Apple snails (*Pomacea canaliculata*) by the same method described previously<sup>21</sup>. The enzyme was additionally purified by passing through the gel filtration column equilibrated with 10mM sodium acetate buffer (pH4.0).

### 2.2. Determination of protein

Protein concentration was measured using bovine serum albumin as a standard protein by the method of Lowry et al.<sup>24</sup>

### 2.3. Enzyme assay

BXase, BGase, AFase, and the other glycosidase activities were measured at 37°C for 15min in 300  $\mu$ l of 0.33M NaCl-0.136M sodium acetate buffer (pH4.0) containing 0.61mM *p*NP-glycosides as standard assay<sup>21</sup>. The temperature, pH, NaCl concentrations, and the reaction time were varying during some experiments, as indicated in the text or the figure legend. The glycosidase activity indicated the rate of reaction as the released *p*-nitrophenol/ min/ mg of protein.

### 2.4. Determination of kinetic constants

Kinetic data was initially plotted as *S/V versus S* plot fitted by a least-squares treatment. The kinetic constants and IC<sub>50</sub> were calculated from the theoretical curve fitting or the linear fitting, to the respective data plots by using Origin software (Microcal Co.) on a personal computer.

## 2.5. Analysis of data

Mixed substrates reaction of the enzyme was analyzed as the function of  $K_m$ , depending on  $F$ , molar ratio of two substrates ( $S_1$  and  $S_2$ ), which was varying from 0 to 1.  $K_{m(1)}$  and  $K_{m(2)}$  are shown as  $K_m$  with  $S_1$  and  $S_2$ , respectively. Competition between two substrates was analyzed by the method of Uziie et al.<sup>25)</sup>. The theoretical  $K_m$  curve was given as a formula:

$$K_m = \frac{K_{m(1)}K_{m(2)}}{K_{m(1)}(1-F) + K_{m(2)}F}$$

where the molar ratio ( $F$ ) is given as follow

$$F_{(S_1/S_2)} = \frac{[S_1]}{[S_1] + [S_2]}$$

Activation energy ( $E_a$ ) of the enzyme activity was calculated from Arrhenius equation given as follow

$$d \ln k = \frac{E_a}{R} d \left( \frac{1}{T} \right)$$

where  $k$  is the rate constant of the reaction,  $R$  the gas constant, and  $T$  the absolute temperature.

## 3. Results

### 3.1. Enzyme purification

The purified BXase showed a single band of 85kDa on SDS-PAGE consistent with the retention time upon gel filtration and anion-exchange column chromatography as previously reported (data not shown)<sup>21)</sup>. BXase, BGase, and AFase activities in the purified enzyme were  $1.75 \pm 0.17$ ,  $0.61 \pm 0.05$ , and  $0.30 \pm 0.08$   $\mu$  mole of the released *p*-nitrophenol/ min/ mg of

protein, respectively. The other glycosidase activities,  $\beta$ -galactosidase,  $\alpha$ -/ $\beta$ -mannosidase,  $\alpha$ -fucosidase,  $\beta$ -N-acetylglucosaminidase and  $\alpha$ -arabinopyranosidase activities, neglected in the purified BXase were less than 1% of the control.

### 3.2. Effect of pH

Effect of pH on the BXase, BGase, and AFase activities was measured in 0.133M citrate-phosphate buffer. In this buffer BXase, BGase, and AFase activities were  $1.02 \pm 0.08$ ,  $0.39 \pm 0.05$ , and  $0.13 \pm 0.01$  ( $\mu$  mole of *p*-nitrophenol/ min/ mg of protein at pH4.0), respectively. The pH dependence of these three glycosidase activities showed the same bell shapes with the maximum activity at around pH 4.2. At any given pH (between pH 3.0 and 5.0) the average ratio between the three activities was the same: 100: 31  $\pm$  7: 11  $\pm$  1 for BXase, BGase, and AFase, respectively.

The pH stability of the activity was measured as the residual activity after preincubation in 0.1M of sodium acetate, citrate phosphate, or glycine-NaOH buffers (pH 2.0-11.0) for 15hr at 24°C. The BXase, BGase, and AFase activities showed the same stability, at least between pH 3.5-8.0.

### 3.3. $K_m$ with mixed substrates

Glycosidase activity was measured in 10 mM sodium acetate buffer (pH 4.0).  $K_m$  values to each substrate were calculated from  $S/V$  versus  $S$  plot as  $1.9 \pm 0.1$ mM for BXase,  $6.7 \pm 0.1$ mM for BGase, and 130mM overreached for AFase (data not shown). As shown in Fig. 1, the experimental values of  $K_m$ s for different  $F$  closely fit the theoretical curves typical for a single

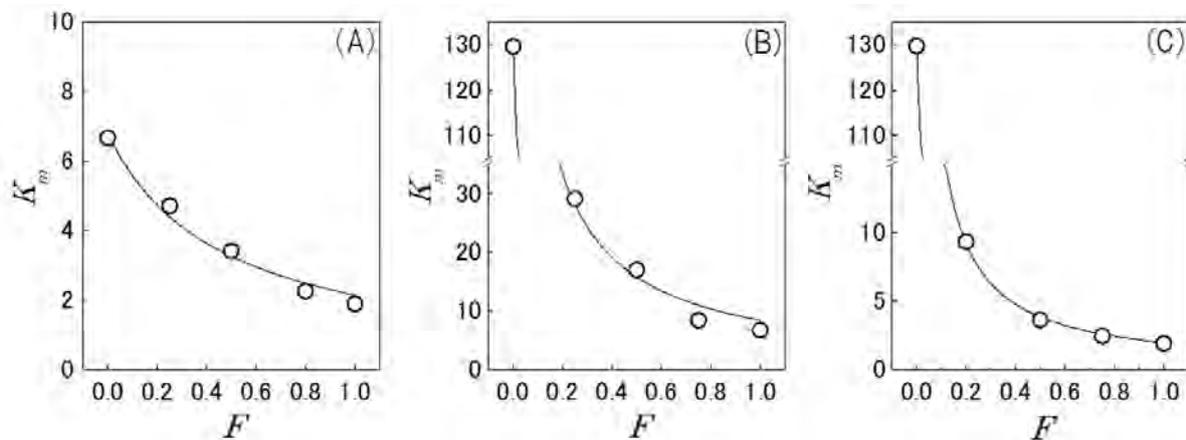


Fig. 1. Dependencies of  $K_m$ s on  $F$ s

The experimental  $K_m$  value was taken with  $S$  versus  $V$  plot from the activity of 4.2  $\mu$ g enzyme which was measured in 0.33M NaCl-0.133M sodium acetate buffer (pH4.0) with 0.32-4.42mM of substrates for 1.5-25min.

(A),  $F_{(pNP-Xyl/pNP-Glu)}$ ; (B),  $F_{(pNP-Glu/pNP-Ara)}$ ; (C),  $F_{(pNP-Xyl/pNP-Ara)}$ ; —, theoretical curves; ○, experimental values.

active site utilizing two substrates. The theoretical  $K_m$  values to each substrate were calculated as  $2.0 \pm 0.1$  (mM) for BXase,  $7.4 \pm 0.3$  for BGase, and  $130 \pm 0.2$  for AFase.

### 3.4. Effect of temperature

The thermal stability was given as the residual activity after 55min preincubation at respective temperature in 10 mM sodium acetate buffer (pH 4.0). The three stabilities were almost identical, 100% at  $50^\circ\text{C}$ , 80% at  $55^\circ\text{C}$ , and 10% at  $60^\circ\text{C}$ .

In contrast, the temperature dependency of the BXase, BGase, and AFase activities was completely different. These three activities showed the catalytic rates, 7.19 ( $\mu\text{mole}$  of *p*-nitrophenol/ min/ mg of protein) for BXase, 1.75 for BGase, and 0.62 for AFase, at  $57.5^\circ\text{C}$  ( $30.24 \times 10^{-4} \text{ K}^{-1}$ ). The activities (BXase, BGase, and AFase) increased with different slope, with increasing temperature up to the denaturation (almost  $75^\circ\text{C}$ ), as shown in Fig. 2. The activation energies<sup>26)</sup> to the three different substrates were calculated as 68kJ/mole (BXase), 42kJ/mole (BGase), and 33kJ/mole (AFase).

### 3.5. Inhibition studies

The effects of inhibitors, mXyl, mGlu and GluLac were examined on each of the three activities BXase, BGase, and AFase. All three inhibitors exhibited the

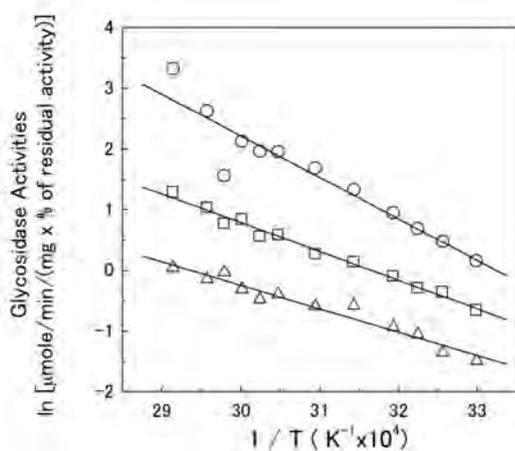


Fig. 2. Effect of Temperature on the Activities.

The activity of  $3.5\text{--}7.0 \mu\text{g}$  enzyme was measured in 0.133M sodium acetate buffer at various temperatures for 5-10min. The amount of the enzyme in the glycosidase activity was considered to avoid the effect of heat denaturation. The amount of the active protein was approximated to measure the remaining activity (% of control) which was measured after incubation at various temperatures for 3min (data not shown).

○, BXase; □, BGase; △, AFase.

different dose response as shown in Fig. 3. The  $\text{IC}_{50}$  values from the fitting of logistic dose response curve for mXyl, mGlu and GluLac were calculated as 17.7mM, 218mM and 0.370mM (BXase), 56.5mM, 35.5mM and 0.159mM (BGase), and 3.84mM, 61.2mM and 0.180mM (AFase), respectively. GluLac inhibited BXase, BGase, and AFase activities with the  $\text{IC}_{50}$  values in the same order of magnitude, but mXyl and mGlu showed different dose response, depending on the particular enzyme activity. For BXase activity  $\text{IC}_{50}$  for mXyl was lower than for mGlu, but for BGase activity this ratio was reversed, with both inhibitors showing almost total inhibition at maximal tested concentration. These two inhibitors tested at concentrations up to 550 mM showed only partial inhibition to the AFase activity by 51% and 46%, respectively.

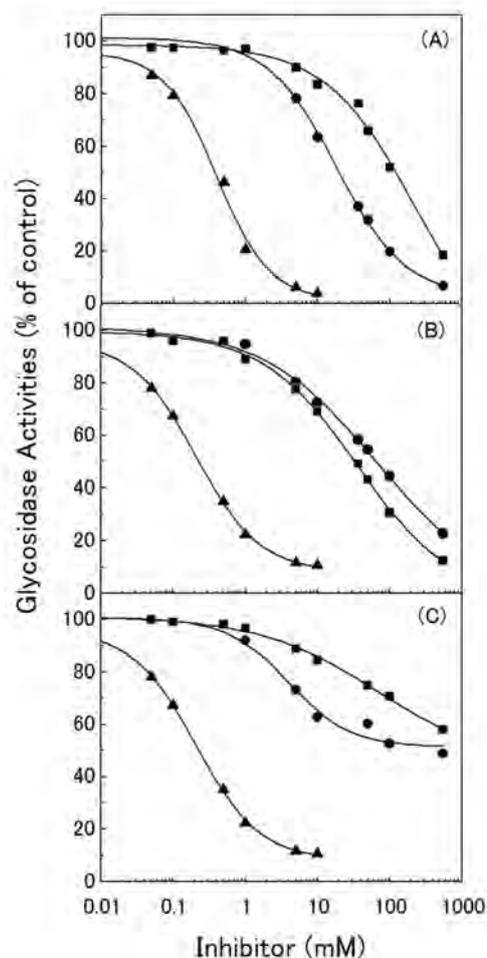


Fig. 3. Effects of Inhibitors on the BXase, BGase, and AFase activities.

The activity of  $7.0 \mu\text{g}$  enzyme was measured in 0.133M sodium acetate buffer (pH4.0). The activity was shown as the remaining activity as a percent of control (no inhibitor). The logistic dose response curve was used for the theoretical curve fitting and the calculation of  $\text{IC}_{50}$  value.

(A), BXase; (B), BGase; (C), AFase; ●, mXyl; ■, mGlu; ▲, GluLac.

Effects of inhibitors on the catalytic rates of BXase, BGase, and AFase activities were examined by using 0.1mM GluLac, 20mM mXyl, and 30mM mGlu. The linearity of  $S$  versus  $S/V$  toward respective glycosidase activity is shown in Fig. 4. The three tested inhibitors act competitively toward BXase activity. The BGase activity was inhibited competitively by mGlu and GluLac and noncompetitively by mXyl. However, the inhibition of AFase was not fitting to any standard inhibition graph. The slopes on the graph depicting  $S/V$  versus  $S$  for all three inhibitors to the AFase activity were not shown in the range of 0.32-4.42mM, because of the high  $K_m$  characteristic.

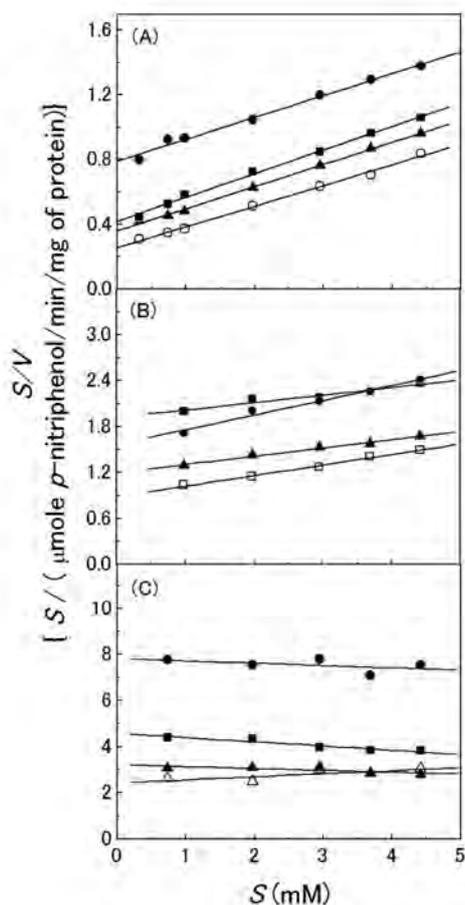


Fig. 4. Effects of Inhibitors on the BXase, BGase, and AFase activities ( $S/V$  versus  $S$  plot).

The activity of 4.2 μg enzyme was measured in 0.33M NaCl-0.133M sodium acetate buffer (pH4.0) with 0.32-4.42mM of substrates for 1.5-25min. The open symbols are controls (no inhibitor). (A), BXase; (B), BGase; (C), AFase; ●, 20 mM mXyl; ■, 30 mM mGlu; ▲, 0.1 mM GluLac.

### 3.6. Effect of sodium chloride

Effect of sodium chloride on the BXase, BGase, and AFase activities was studied in 10mM acetate buffer (pH4.0) to elucidate the effect of the ionic strength, which was not clear in a previous study.<sup>12)</sup> All three

catalytic rates increased exponentially with increase of the sodium chloride concentration from 0 to 3.4M, with almost the same ratio (100% for BXase, 40±1% for BGase, and 25±3% for AFase) for each NaCl concentration. The BXase activity in the absence of NaCl was approximately 47% of the activity in the standard assay, containing 0.33M NaCl.

Dixon plots ( $S$  versus  $V$  plot) with differed NaCl concentrations were investigated to analyze kinetic parameters. As shown in Fig. 5-1, the reaction rates of each BXase, BGase, and AFase activities increased with respect to the additional NaCl concentration however the ratio of the reaction rate of the three activities was changed. Kinetic constants,  $K_m$  and  $k_{cat}$ , of BXase and BGase activities with differed sodium chloride concentration except for AFase activity were analyzed. It was difficult to calculate the kinetic

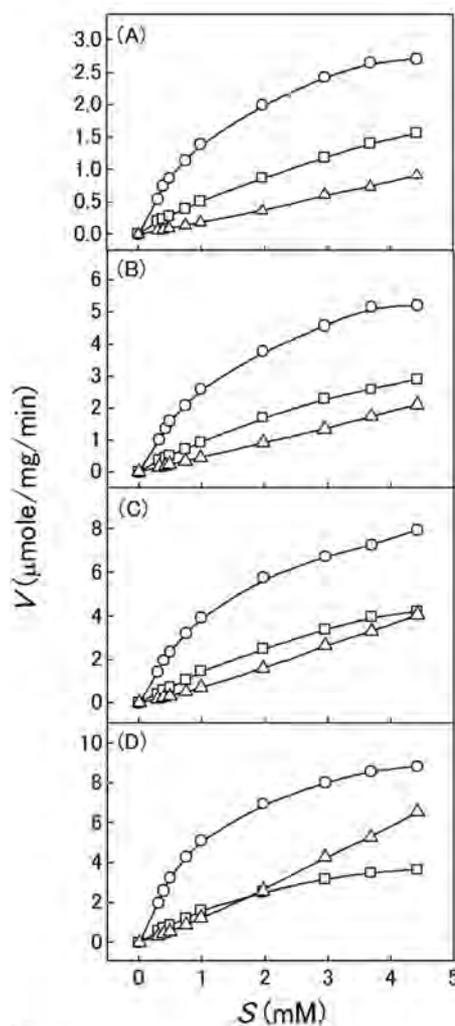


Fig. 5-1. Effects of NaCl on the glycosidase activities ( $S$  versus  $V$  plots)

The activity of 5.1-6.2 μg enzyme was measured in 10mM sodium acetate buffer (pH4.0) with various concentrations of NaCl: 0M (A), 0.33M (B), 1.0M (C), 3.3M (D).

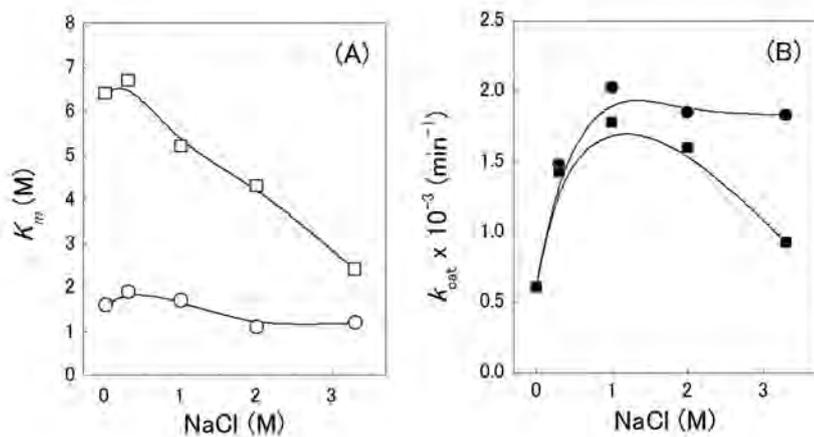


Fig. 5-2. Effects of NaCl on the BXase and BGase Activities.

The open and solid symbols show  $K_m$  (A) and  $k_{cat}$  (B) calculated from the measurement (Fig. 5-1). The circle and square show BXase and BGase, respectively.

constants of AFase because the  $V_{max}$  value was not shown and the  $K_m$  was too high value with the possible range of substrate concentration. As shown in Fig. 5-2, the ratio of  $K_m$  on the BXase and BGase activities changed obviously with increasing of NaCl concentration and the  $k_{cat}$  showed the difference clearly in the presence of 3.3M NaCl, respectively although the catalytic efficiency,  $k_{cat}/K_m$ , of BXase and BGase activities changed almost the same ratio (BXase:BGase= 100: 26  $\pm$  3) with NaCl concentration.

#### 4. Discussion

It is important for enzymology or the applied chemistry to note the glycon specificity of the BXase as trifunctional enzyme, however the enzymatic information of the glycon specificity is limited even with the structural information observed. It is suitable to this study for the simplicity that the *P. canaliculata* hepatopancreatic BXase is constituted from monomer and the activities are detected clearly similar to those of the other multifunctional xylosidases. The glycon specificity of *P. canaliculata* BXase shows the kinetic properties of a trifunctional glycosidase (BXase, BGase, and AFase), of which the three activities derived from the same catalytic site, and shows the conformational state of the sugar binding site is fluctuated with differed temperature or NaCl concentration.

The enzymatic properties of the optimal pH, pH stability and thermo-stability to each of the three activities show almost the same characters, respectively

and also the  $K_m$  values with mixed two substrates are fitting enough to the theoretical values for a single catalytic site. The correlation coefficient ( $r^2$ ) and the standard error (SE) of  $K_m$  difference between the theoretical value and the measurements were  $9.85 \times 10^{-1}$  and 0.11 ( $F_{(BXase/BGase)}$ ), 1.00 and 0.06 ( $F_{(BXase/AFase)}$ ), and  $9.99 \times 10^{-1}$  and 0.74 ( $F_{(BGase/AFase)}$ ), respectively. These results means reasonably that *P. canaliculata* BXase have BGase and AFase as glycon specificity at the same catalytic site. In inhibition experiments as shown in Fig. 3 and 4, GluLac

inhibit competitively each of the three activities with the same dose response. The BXase activity was competitively inhibited by all the three inhibitors, and the BGase activity was competitively inhibited by mXyl. However, the other inhibitory patterns showed that the inhibitory activity was weakened by increasing of the substrate concentration. The hydrophobicity of the solution, caused by the inhibitors methyl group, is assumed to affect the enzyme activity (the ligand binding site) in the order of AFase and BGase. Especially the AFase activity in comparison with the other two activities became relatively higher with increasing NaCl concentration as shown in Fig.5-1. Moreover, the three glycosidase activities show the difference of the activation energy to temperature. The energy difference demonstrates the catalytic surface to the three substrates is not identical one. The inhibition studies suggest further that the binding sites to the glycons, ( $\beta$ -)D-xylopyranose, ( $\beta$ -)D-glucopyranose, and ( $\alpha$ -)L-arabinofuranose, are slightly different. Although effects of the other chlorides or buffers on the BXase, BGase, and AFase activities were not measured, the enzyme will require some ionic strength and/or hydrophobic condition to activate hydrolysis as described below. *Charonia lampas* BXase reported is also shown almost same activation in the presence of sodium chloride<sup>14</sup>. It appears that some conformational change of this enzyme typically may occur.

As described above, *P. canaliculata* BXase has the three glycons specificity and also change the ratio of the catalytic constant in differed temperature or NaCl

concentration. The change maybe modulated by some sub-conformations of the enzyme. It will be suggested that the affinity intensity, i.e. hydrophobicity, hydrogen bonds, and/or the other spatial extent for variable mold (conformational variations in each solvents) of the sugar binding site including the whole protein to the three glycons are ordered as Xyl>Glu>Araf. The substrate binding of the three glycons may occur basically at the C(OH)-1, -2 and -3 positions in each sugar, and depend potently on the configuration of the C(OH)-4 position at Xyl and Glu. The methyl group of Glu and Araf will be a stereochemical hindrance to the glycon-binding site (Fig 6). The chart in Appendix provide an additional explanation. The ligand binding region facing C(OH)-1, -2 and -3 positions in each sugar ring is expected to be a basic structure (as rigid region) regarding in the saccharide binding site, however there is no information of the 3D structure of the BXase. It is also assumed that the binding region facing C(OH)-4, -5 and -6 positions in each sugar give distinctive kinetic properties (as flexible region), in which the stereochemical fluctuation derives from the hydrophobicity of the enzyme, since the activation energies of the three enzyme activities to temperature and the  $k_{cat}$  values in high NaCl

concentration are different obviously. In a previous report at academic meeting, fluorescence spectroscopic analysis of *P. canaliculata* BXase, this enzyme structure was suggested to be shifted to a rigid structure with increasing NaCl concentration. This evidence was indicated by analysis of fluorescence intensity, anisotropy, and quenching with KI<sup>23)</sup>. Bgخال known as a multifunctional  $\beta$ -glucosidase/ $\beta$ -xylosidase/ $\alpha$ -arabinosidase exhibit substrate-dependent responses to changes in ionic strength and act synergistically to substrates in a recent study<sup>27)</sup>. These reports support that *P. canaliculata* BXase exhibit as a multifunctional enzyme accompanied by some conformational changes, "conformational variation", in different glycons and solvents.

Conformational change on several enzyme activity are described in recent studies that there are two different processes, "induced fit" and "conformational selection", for the preliminary step before ES formation<sup>28)</sup>. It is suggested substantially from kinetic properties that *P. canaliculata* BXase includes the conformational fluctuations, "induced fit" and "conformational selection", and under the control of "conformational variation" as shown in Fig. 7. It is surmised that the ligand recognition process of *P. canaliculata* BXase has such a "Pac Man" action type

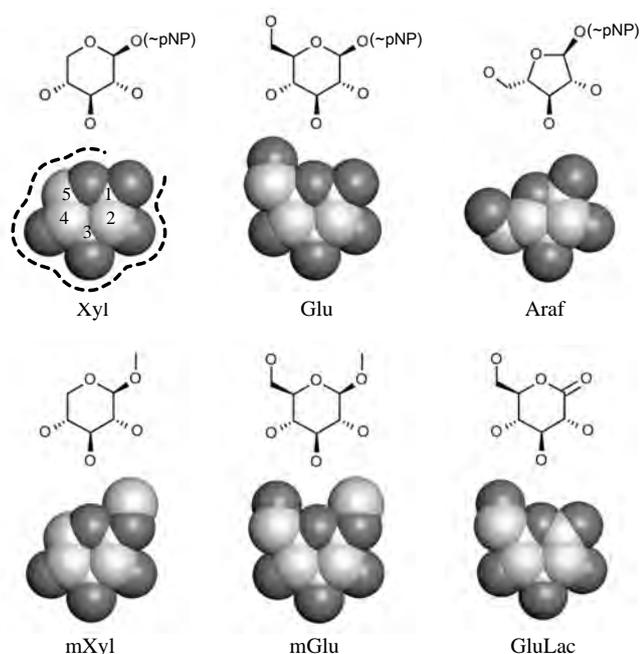


Fig. 6. Chemical structure of ligands.

Ligands are shown 2D and 3D structures in each. Space-filling model show without hydrogen. The drawing figures used Accelrys draw 4.2 (Accelrys Inc.) software. A broken line shows schematic ligand binding site.

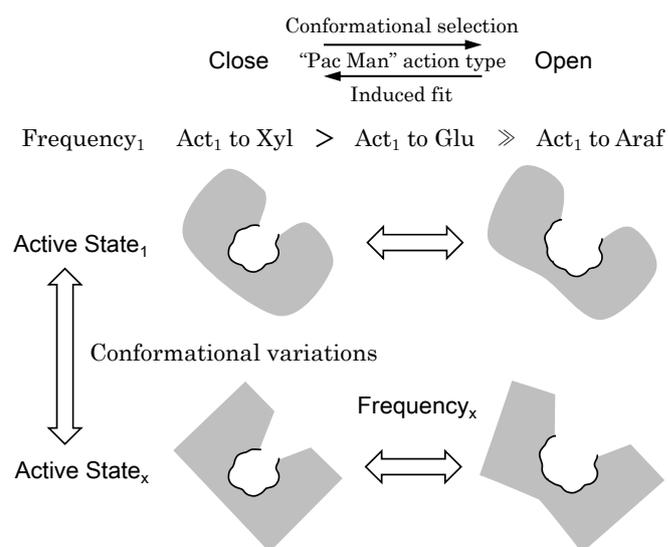


Fig. 7. Schematic simplification of *P. canaliculata* BXase conformational changes of ligand binding.

Vertical arrow shows the fluctuation of the active states (as "conformational variations") in solvent condition. Horizontal arrows show the conformational fluctuations including "conformational selection" and "induced fit". The glycon binding site are shown, especially with including the structural change between C4 to C6.

## References

for the mixed model, “induced fit” and “conformational selection”, as described previously<sup>29</sup>.

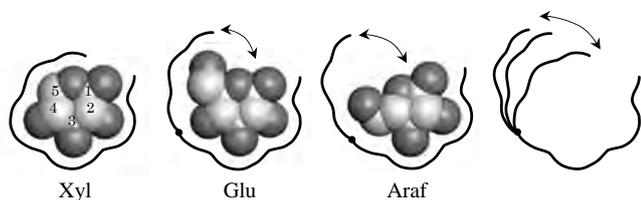
Many BXase have broad substrate (ligand) specificity making variously form of monomer or oligomer. The enzyme with BXase activity have been classified from its amino acid sequence to several glycosidase family (1, 3, 5, 30, 39, 43, 51, 52, 54, 116, and 120) in one side view, on the other hand the family (1, 3, 5, 30 and 116) are with BGase activity, and the family (3, 43, 51 and 54) are with AFase<sup>17</sup>. The partial amino acid sequence (data not shown) from BXase cleavages<sup>30</sup> was homologous highest to glycosyl hydrolases family 3<sup>17,31,32</sup> rather than the family 43<sup>33</sup>. It is difficult to discuss the relation between the glycon specificity and the primary structure, but the glycon specificity of the *P. canaliculata* BXase is similar to that of the *T. ressi* BXLI, which really have another  $\alpha$ -arabinopyranosidase activity almost 25% to the BXase activity. The other BXase known as multifunctional enzyme also may change the ratio of the activities with different conditions. The catalytic property of the BXase will be classified a new type for EC 3.2.1.37 (BXase)/ EC 3.2.1.21 (BGase)/ EC 3.2.1.55 (AFase) as trifunctional enzyme.

## Appendix

Table shows the matching of ligand in comparison with ( $\beta$ )-D-xylopyranose. Lines surrounding the 3D models, shows schematically speculation of the conceivable substrate-binding site. =: same position;  $\approx$ : approximately same;  $\neq$ : not same.

Comparison of 3D structure

Substrate (glycon)			Inhibitor		
Xyl	Glu	Araf	mXyl	mGlu	GluLac
C-1(OH)	=	$\approx$	$\neq$	$\neq$	$\neq$
C-2(OH)	=	$\approx$	=	=	=
C-3(OH)	=	$\approx$	=	=	=
C-4(OH)	=	$\neq$	=	=	=
C-5(OH)	=	$\neq$	=	=	=
(C-6(OH))	$\neq$			$\neq$	$\neq$



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# 軟体動物（スクミリングガイ）多機能性 $\beta$ - キシロシダーゼの反応速度論的特性

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## 概 要

スクミリングガイから精製したエキソ型  $\beta$ -キシロシダーゼは、*p*NP- $\beta$ -D-キシロピラノシド、*p*NP- $\beta$ -D-グルコピラノシド、*p*NP- $\alpha$ -L-アラビノフラノシドに作用する、3 機能性グリコシダーゼである。この  $\beta$ -キシロシダーゼ (BXase)・ $\beta$ -グルコシダーゼ (BGase)・ $\alpha$ -アラビノフラノシダーゼ (AFase) の 3 つの活性は、およそ 100 : 40 : 10 の相対活性を有し、至適 pH(4)、pH と温度の安定性を同じくする。混合基質 (2 つの基質の組合せ) を用いた反応速度論的解析から、3 つの活性は同一の触媒部位から生じていることが示唆された。BXase・BGase・AFase の活性化エネルギーは、それぞれ 68kJ/mole・42kJ/mole・33kJ/mole であった。阻害実験における用量反応曲線からは、D-グルコノ-1,5-ラク톤は 3 つの活性に対して同じ曲線を示したが、メチルキシロースとメチルグルコースは 3 つの活性に対してそれぞれ異なっていた。また阻害実験における反応速度論的解析から、この酵素の基質結合部位はコンフォメーションの動的変化を伴うことが示唆された。NaCl 濃度変化は、BXase と BGase の相対活性比及び kcat を変化させることが分かった。この酵素は同一触媒部位から 3 つの活性を生じ、溶媒条件によって酵素の活性型を変化させることが分かった。

キーワード:  $\beta$ -キシロシダーゼ;  $\beta$ -グルコシダーゼ;  $\alpha$ -アラビノフラノシダーゼ  
; 軟体動物; スクミリングガイ