Stereoselective Hydrolysis of *p*-Nitrophenyl Glycoside by Boronic Acid

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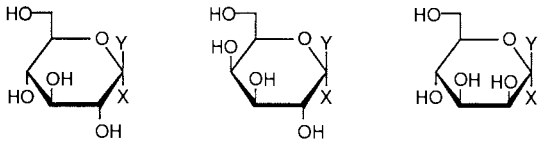
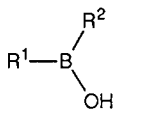
The alkaline hydrolysis of *p*-nitrophenyl α-D-glucoside, α-D-galactoside, and β-D-mannoside was selectively accelerated by addition of methyl boronic acid, as compared to that of the corresponding β-D-glucoside, β-D-galactoside, and α-D-mannoside. In the case of *p*-nitrophenyl α (or β)-D-glucoside, the α/β selectivity was increased up to 110 when four molar equivalents of methyl- or phenylboronic acid were added.

In recent years, numerous studies on enzyme models have been carried out in the field of biomimetic chemistry, and the development of novel artificial systems for understanding the functions of enzyme at the molecular level has attracted much attention. Among them, crown ethers,[1] cyclodextrins,[2] micelles,[3] and water soluble polymers[4] are included as the hydrolase models. However, there is no report on the model of a glycosidase which discriminates between two configurations at the C-1 position of glycoside in hydrolysis, that is, one which recognizes α- and β-glycosidic bonds. Meanwhile, it is well known that boronic acid such as phenylboronic acid (PBA) reacts with a saccharide molecule to form a cyclic boronate ester by condensation with two particular hydroxyl groups.[5] Recently, by utilizing this unique property, a variety of studies on recognition of saccharides such as selective transport of saccharides through a liquid membrane by boronic acid carriers[6] and spectroscopic sensing of saccharides by boronic acid probes,[7] have been reported. Here, we report that the selective rate enhancement of alkaline hydrolysis of *p*-nitrophenyl α-D-glucoside, α-D-galactoside, and β-D-mannoside, compared to that of the corresponding β-D-glucoside, β-D-galactoside, and α-D-mannoside, respectively, was accomplished by addition of boronic acid.



α-D-Glc : X=PNP, Y=H α-D-Gal : X=PNP, Y=H α-D-Man : X=PNP, Y=H
β-D-Glc : X=H, Y=PNP β-D-Gal : X=H, Y=PNP β-D-Man : X=H, Y=PNP

PNP = -O-C6H4-NO2



BA : R^1 = OH, R^2 = OH
MBA : R^1 =Me, R^2 = OH
BBA : R^1 = *n*-Bu, R^2 = OH
PBA : R^1 = Ph, R^2 = OH
DPBA : R^1 = Ph, R^2 =Ph

The hydrolysis of *p*-nitrophenyl α- and β-D-glycoside was carried out in 100 mM phosphate buffer (pH 11.0) at 25 ℃. In order to determine the reaction rate, the hydrolysis was spectrophotometrically followed by measuring the absorbance of the released *p*-nitrophenolate anion at 400 nm. The rate constants (k_α and k_β) were calculated using the data obtained in the restricted region where the rates obey the first-order kinetics. Each experiment was repeated at least three times to ensure reproducibility (± 10%).

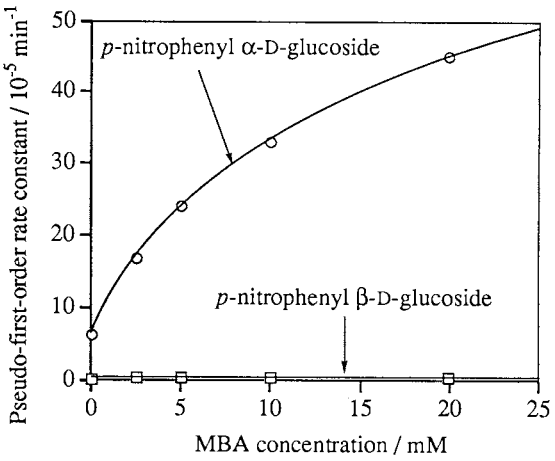


Figure 1. Plots of the observed pseudo-first-order rate constants *vs.* methylboronic acid (MBA) concentration in 100 mM phosphate buffer (pH 11.0) at 25 °C. [*p*-nitrophenyl α (or β)-D-glucoside] = 5.0 mM.

Figure 1 shows the plots of the observed pseudo-first-order rate constant for hydrolysis of *p*-nitrophenyl α- or β-D-glucoside *vs.* methylboronic acid (MBA) concentration. As reported previously,[8] *p*-nitrophenyl α-D-glucoside was hydrolyzed faster than the corresponding β-D-glucoside under

Table 1. Kinetic constants for hydrolysis of *p*-nitrophenyl α- and β-D-glucoside in the presence of boric, boronic, or borinic acid

Additive[a]	Conc. /mM	k_α[b] /10^{-5} min^{-1}	k_β[b] /10^{-5} min^{-1}	k_α / k_β
none	0	6.1	0.27	23
MBA	5.0	24	0.42	57
MBA	20	45	0.42	110
BBA	20	42	0.45	93
PBA	20	42	0.39	110
BA	20	14	0.32	44
DPBA	5.0	7.3	0.27	27

[a]MBA: methylboronic acid, BBA: butylboronic acid, PBA: phenylboronic acid BA: boric acid, DPBA: diphenyborinic acid. [b]Observed in 100 mM phosphate buffer (pH 11.0) at 25 °C, [*p*-nitrophenyl α (or β)-D-glucoside] = 5.0 mM.

alkaline conditions. But the selectivity of hydrolysis was moderate in the absence of MBA (k_α/k_β = 23). The rate constant for *p*-nitrophenyl α-D-glucoside increased remarkably with the increase of MBA concentration, following the saturation kinetics. On the other hand, the rate constant for *p*-nitrophenyl β-D-glucoside was little affected by the change in MBA concentration. The α/β selectivity increased up to 110 by addition of four molar equivalents of MBA (20 mM).

The kinetic constants and the α/β selectivity for the hydrolysis of these α- and β-D-glucosyl ethers in the presence of three types of boronic acids, boric acid (BA), or diphenylborinic acid (DPBA) are summarized in Table 1. The hydrolysis of the α-D-glucosyl ether was selectively accelerated in preference to that of the β-D-glucoside by addition of any of the boronic acids examined in this work. In particular, when MBA or PBA was added to the hydrolytic system, the highest α/β selectivity was observed. On the other hand, BA effected to a lesser extent the acceleration of hydrolysis of both the α-glucosyl and the β-glucosyl ethers. DPBA only slightly accelerated the hydrolysis of these glucosides. The reason for the difference in the acceleration effect among the boronic, boric, and borinic acids is not clear at the present time. Contrary to our expectation, no information on complexation between *p*-nitrophenyl glucosides and these hydroxyboron compounds in alkaline aqueous solution was obtained in ^{1}H-NMR spectra.

We also investigated the effect of MBA on the hydrolysis rates and α/β selectivity of other types of *p*-nitrophenyl α- and β-D-glycosides, such as *p*-nitrophenyl α- and β-D-galactoside and *p*-nitrophenyl α- and β-D-mannoside (Table 2). By addition of MBA, the hydrolysis of *p*-nitrophenyl α-D-galactoside was selectively accelerated in comparison with that of the corresponding β-D-galactoside, similarly to the case of the glucosyl ether. Interestingly, in contrast to these cases, *p*-nitrophenyl β-D-mannoside was hydrolyzed more rapidly than the corresponding α-D-mannoside by addition of MBA. These results indicate that the hydrolysis rates of the *p*-nitropheny glycosides, in which the hydroxyl group at C-2 is *cis* with respect to the *p*-nitrophenoxy group, were more accelerated by addition of MBA, whereas the hydrolysis rates of the other *p*-nitropheny glycosides, in which the configuration of the hydroxyl group at C-2 and the *p*-nitrophenoxy group is *trans* with each other, were little affected. Thus, the acceleration of hydrolysis of glycosides by MBA is evidently dependent on the stereochemistry of the hydroxyl group of glycoside.

Table 2. Kinetic constants for hydrolysis of *p*-nitrophenyl α- and β-D-glycoside in the presence of methylboronic acid

Substrate[a]	[MBA] /mM	k_α[b] /10^{-5} min^{-1}	k_β[b] /10^{-5} min^{-1}	k_α / k_β
D-Glc	0	6.1	0.27	23
	20	45	0.42	110
D-Gal	0	6.9	0.28	25
	20	19	0.44	43
D-Man	0	0.83	7.6	9.2[c]
	20	1.3	31	24[c]

[a]D-Glc: *p*-nitrophenyl α (or β)-D-glucoside, D-Gal: *p*-nitrophenyl α (or β)-D-galactoside, D-Man: *p*-nitrophenyl α (or β)-D-mannoside, [b]Observed in 100 mM phosphate buffer (pH 11.0) at 25 °C, [*p*-nitrophenyl α (or β)-D-glycoside] = 5.0 mM. [c]k_β / k_α.

In conclusion, boronic acid selectively accelerated the hydrolysis of either *p*-nitrophenyl α- or β-glycoside. This selectivity is related to the stereochemical relationship between the hydroxyl group at C-2 and the *p*-nitrophenoxy group. Further work on the mechanism of the selective hydrolysis of these glycosides by boronic acid is now in progress in our laboratory.

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