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Title	Inhibition of β -Glucuronidase by Purine Nucleotides
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Description	Short Paper
Citation	Fisheries Science, 65(4), 667-668 https://doi.org/10.2331/fishsci.65.667
Issue Date	1999
Doc URL	https://hdl.handle.net/2115/49563
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Type	journal article
File Information	FS65_667.pdf



Inhibition of β -Glucuronidase by Purine NucleotidesHiroyuki Tohda, Hideyuki Kurihara,[†] Masashi Hosokawa, and Koretaro Takahashi

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(Received September 7, 1998)

Key words: β -glucuronidase, inhibition, purine nucleotides, IMP, AMP, ATP

Beta-glucuronidase (EC 3.2.1.31), widely distributed in animals, plants, and bacteria, catalyzes hydrolysis of β -glucuronides.¹⁾ In the large intestine, β -glucuronidase produced by intestinal bacteria hydrolyzes detoxified glucuronide conjugates of xenobiotics. The toxic aglycons released are occasionally re-absorbed and accumulated,²⁾ thus leading to colon cancer^{3,4)} and drug side effects.^{2,5,6)} Therefore, β -glucuronidase inhibitors can prevent exposure of toxic compounds in the large intestine.²⁾ For example, Takeda *et al.*⁷⁾ demonstrated that administration of a β -glucuronidase inhibitor effectively decreased a risk of azoxymethane-induced colon cancer in rats. Furthermore, Takasuna *et al.*⁶⁾ reported that β -glucuronidase inhibitors might prevent side effects caused by certain antitumor agent.

Recently, we have screened constituents of marine organisms and sea foods for inhibitory activities against glycosidases.^{8–10)} In this study, we disclosed β -glucuronidase-inhibitory activity of inosine 5'-monophosphate (IMP), an *umami* constituent of dried bonito, and other nucleotides.

Inhibition assay for β -glucuronidase was carried out using a colorimetric method with *p*-nitrophenyl β -D-glucuronide as the substrate. The reaction mixture (total volume: 2.5 ml) consisted of 10 mM phosphate buffer (pH 7.0), 10% ethanol, 0.125–1.0 mM *p*-nitrophenyl β -D-glucuronide, aliquots of the compound tested, and 2.0 Fishman unit/ml β -glucuronidase (from *Escherichia coli*, Sigma Chemical). The compounds tested were as follows. Adenosine 5'-triphosphate (ATP), IMP, guanosine 5'-monophosphate (GMP), cytidine 5'-monophosphate (CMP), inosine, guanosine, cytidine, hypoxanthine, guanine, cytosine, and D-ribose were purchased from Wako Pure Chemical, while adenosine 5'-monophosphate (AMP), adenosine, and adenine were purchased from Sigma Chemical. D-Glucaro-1,4-lactone¹⁾ (Nacalai Tesque) was used as positive control. The reaction was carried out at 37°C for seven minutes, then terminated by adding 0.25 M Na₂CO₃ (1.5 ml). The *p*-nitrophenol liberated was determined by measuring absorbance at 400 nm.

From the results of the inhibition assay (Table 1), IMP, AMP, and ATP inhibited β -glucuronidase activity, while the others showed no inhibition. Lineweaver-Burk plot analyses of IMP, AMP, and ATP demonstrated that these nucleotides inhibited β -glucuronidase activity competitively (Fig. 1). The inhibitor constants (K_i) of these nucleotides, evaluated by Dixon plot analyses, were between 2.00

Table 1. Inhibition of β -glucuronidase by nucleotides

Nucleotides* ¹⁾	IC ₅₀ (mM)	K _i (mM)	Type of Inhibition
IMP	1.67	2.00	Competitive
AMP	1.30	2.28	Competitive
GMP	—* ²⁾	—	—
CMP	—	—	—
ATP	1.23	2.38	Competitive
D-Glucaro-1,4-lactone* ³⁾	0.14	0.01	Competitive

*¹⁾ Abbreviations as follows: IMP, inosine 5'-monophosphate; AMP, adenosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; CMP, cytidine 5'-monophosphate; ATP, adenosine 5'-triphosphate.

*²⁾ No inhibition.

*³⁾ A known β -glucuronidase inhibitor.¹⁾

and 2.38 mM. A K_i value of D-glucaro-1,4-lactone, a well known β -glucuronidase inhibitor,¹⁾ was 0.0114 mM.

IMP, AMP, and ATP are purine nucleotides which bear phosphoryl group at the 5'-position of ribose moiety. We suggested that 5'-phosphorylated D-ribose moiety should be required for β -glucuronidase inhibition. Among the purine nucleotides assayed, however, GMP showed no inhibitory activity. In addition, CMP, a pyrimidine nucleotide, also showed no inhibitory activity. From these results, β -glucuronidase inhibition also requires a purine base without a substituent at the 2-position. As inhibition of β -glucuronidase by IMP, AMP, and ATP was competitive, these nucleotides might act as a substrate mimic toward β -glucuronidase. Many competitive inhibitors toward β -glucuronidase characterized as β -glucuronide mimics have a quasi uronic acid structure.^{11–13)} We considered that 5'-phosphorylated D-ribose might be spatially similar to β -glucuronic acid in the position of hetero atoms and negative charge. However, their structural similarity of 5'-phosphorylated D-ribose to glucuronide must be lower than the known inhibitors since the K_i values of these nucleotides are higher than those of the known inhibitors.^{11–13)}

Parish¹⁴⁾ reported that purine nucleosides inhibited β -glucosidase (EC 3.2.1.22) reaction. In the present study, purine nucleotide inhibited β -glucuronidase reaction. In the same way, D-glucono-1,4-lactone inhibits β -glucosidase,¹⁵⁾ while D-glucaro-1,4-lactone inhibits β -glucuronidase.¹⁾ From this evidence, a certain β -glucosidase inhibitor might be converted into a β -glucuronidase inhibitor by way of converting the hydroxymethyl group with sugar moiety of the β -glucosidase inhibitor into negatively

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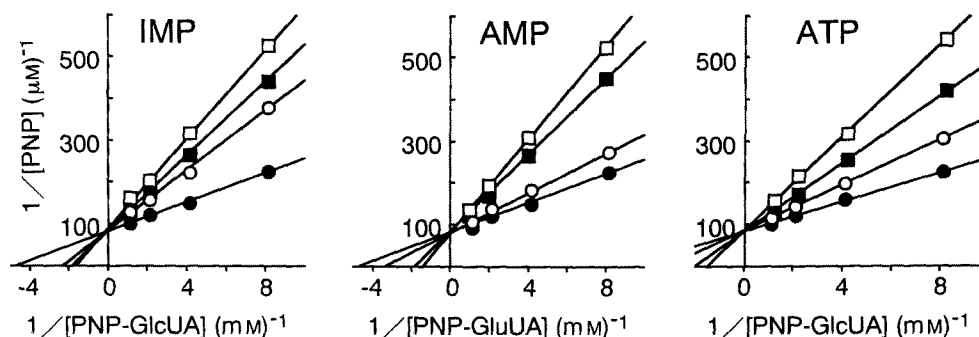


Fig. 1. Lineweaver-Burk plot analyses of inhibition against β -glucuronidase by nucleotides.

Abbreviations are as follows: PNP, *p*-nitrophenol; PNP-GlcUA, *p*-nitrophenyl- β -D-glucuronide. ●, 0 mM; ○, 1 mM; ■, 3 mM; □, 5 mM.

charged groups such as the carboxyl or phosphoryl group. Although inhibitory potencies of IMP, AMP, and ATP were lower compared with known inhibitors, a continuous intake of them would prevent undesirable side effects because they are food components. D-Xylose, a food additive, moderately inhibited sucrase activity ($K_i = 1.1$ mM), however, comparatively high administration of D-xylose will be expected to prevent hyperglycemia for diabetics.¹⁶⁾ In the same way, IMP, AMP, and ATP as ordinarily ingestible food components could have desirable effects due to inhibition of β -glucuronidase activity.

Acknowledgments This work was supported partly by Grants-in-Aid from the Akiyama Foundation and the Ministry of Education, Science, Sports, and Culture of Japan.

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