Inhibitory Effect of β-Glucosyl-phenolic Hydroxamic Acids against Urease in the Presence of Microfloral β-Glucosidase

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Three glucosyl-phenolic hydroxamates, 4-*O*-(β-D-glucopyranosyl)benzohydroxamic acid, 4-*O*-(β-D-glucopyranosyl)hippuric hydroxamic acid, and 3-[4-*O*-(β-D-glucopyranosyl)phenyl]propionohydroxamic acid (Glc-PPHA), were hydrolyzed to their corresponding aglycones by β-glucosidase of intestinal flora of rat without any major adverse hydrolysis *in vitro*. Inhibitory potency of these glucosyl-hydroxamates on urease was recovered to the same extent as that of the corresponding aglycone hydroxamates by preincubation for 2 h with rat intestinal flora. *p*-Hydroxyphenylpropionohydroxamic acid inhibited noncompetitively jack-bean urease activity and its glucose-ligated form, Glc-PPHA inhibited it competitively. A single oral dose of Glc-PPHA tended to inhibit urease activity in proximal colon contents of rat at 6 h after administration ($p = 0.06$). After ^{14}C-urea was orally administered to rat, $^{14}CO_2$ was collected for to measure the ureolysis *in vivo*. Expired $^{14}CO_2$ was limited to 40% by a single oral dose of Glc-PPHA during 6 h, and 75% of intestinal ureolysis was repressed during the first 1 h in the breath test.

Key words urease; β-glucosidase; hydroxamic acid; rat intestine; pro-drug; hepatic coma

Hydroxamic acid derivatives, highly potent and specific inhibitors of plant and bacterial urease activity,[1,2] are markedly effective *in vitro* against urease from intestinal bacteria.[3,4] These findings together with the modest toxicity of the compound in animal studies,[4] have prompted consideration of hydroxamic acid derivatives as therapeutic agents for hepatic coma,[2,3] especially if complicated by azotemia, when ureolysis may be a major cause of hyperammonemia. The relationship postulated between hepatic coma and hyperammonemia implicates the gastrointestinal tract as a major source of excess ammonia in the peripheral blood of some patients with cirrhosis.[5] Ammonia produced in the colon is generally attributed to the metabolism of urea, glutamine, and other nitrogen-containing compounds by the colonic microflora and intestinal wall.[6,7] The major portion of ammonia which enters the portal circulation is thought to arise in the colon. Fifty-one percent of ammonia released by the colon could be accounted for by the uptake of glutamine (9%) and urea (42%) in the fasting dog. However, it was also reported that ammonia released by the jejunum and ileum could be largely accounted for by the metabolism of glutamine.[8] In severe liver insufficiency, the damaged liver cells are unable to clear ammonia adequately so that this substance can penetrate into the brain; coma may eventually develop through synergistic action with cerebral toxins produced by intestinal bacteria, such as mercaptans and short-chain fatty acids.[9,10] Although the role of ammonia metabolism in the pathogenesis of portal-systemic encephalopathy is not entirely understood, therapeutic measures to reduce the number of urea-hydrolyzing organisms have proven to be effective.[11] Hyperammonemia was also shown to be reduced by administration of acetohydroxamic acid.[2,12]

The role of infection in urinary tracts with urea-splitting organisms in the development of "triple-phosphate" (calcium, magnesium, and ammonium phosphate) stones composed of struvite ($MgNH_4[PO_4]\cdot 6H_2O$) and carbonate apatite ($Ca_{10}[PO_4]_6CO_3$) appears to be well established.[13] The urease inhibitors, acetohydroxamic acid or hydroxyurea have shown to be beneficial postoperative chemotherapeutic agents.[14,15] In the absence of surgery, clinical trials[16–18] demonstrated that oral administration of acetohydroxamic acid or hydroxyurea lowered urinary pH of bacteriuric patients and reduced the size of urinary stones. However, half or more of the patients experienced side effects that required stopping treatment or reducing the size of the dose. As complications, acetohydroxamic acid inhibits complement activity[19] and cause reversible hemolytic anemia and thrombophlebitis. Furthermore, both agents inhibit DNA synthesis, and depress bone marrow biosynthesis at high doses.[20]

Preliminary *in vitro* and *in vivo* results of the effects of glucosyl-hydroxamic acids (Glc-HAs) on urease from intestinal microflora and jack-bean are reported here, together with observations relating to hydrolysis by β-glucosidase of feces. We undertook the present study to elucidate a recovery of urease inhibitory potency of Glc-HAs by the hydrolysis of microfloral β-glucosidase with their sustained inhibitory effect. The kinetics of urease inhibition *in vitro* by three phenolic hydroxamic acids, 3-(4-*O*-phenyl)propionohydroxamic acid (PPHA), *p*-hydroxyhippurohydroxamic acid (HHA), and *p*-hydroxybenzohydroxamic acid (HBHA), is compared with their corresponding glycosides for the purpose of pro-drug.

MATERIALS AND METHODS

Rats Wistar/ST male rats, 6 weeks old with a mean body weight of 150 g, were obtained from SLC, Japan and

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maintained on a daily diet (CE-2, Clea, Japan). Rats were fasted during the experiment from the start of oral administration of urease inhibitors.

Materials ^{14}C-Urea was purchased from Dupont, U.S.A. β-Glucosidase (Almonds, 5.3 unit/mg), D-gluconic acid lactone and nicotino-HA were from Sigma, U.S.A. ACS II scintillation solution was from Amersham, Britain. *Helcobacter pylori* ATCC 43504 was donated by the Research Institute for Microbial Disease, Osaka University. *Proteus mirabilis* E0 5001 was a donation of the Department of Urology, School of Medicine, Kyoto University. All other reagents and solvents were of analytical grade.

Synthesis of Glc-HBHA, Glc-HHA, and Glc-PPHA For synthesizing Glc-HBHA, 4-hydroxybenzoic acid benzyl ester (3.00 g, 13.14 mmol) was conjugated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylbromide (6.49 g, 15.77 mmol) in quinoline (50 ml) and Ag_2O (3.73 g, 15.77 mmol). The conjugated product (4.77 g, 65.0%. *Anal.* Calcd for $C_{28}H_{30}O_{12} \cdot H_2O$: C, 58.53; H, 5.49. Found: C, 58.97; H, 5.59) was hydrogenated in tetrahydrofuran (30 ml) with 10% Pd–C (200 mg) at room temperature for 24 h. After the residue was crystallized from 2-propanol, it (1.45 g, 15.77 mmol, *Anal.* Calcd for $C_{21}H_{24}O_{12}$: C, 53.84; H, 5.16. Found: C, 53.52; H, 5.86) was dissolved in CH_2Cl_2–dimethylformamide (1 : 1) and conjugated with *O*-benzylhydroxyamine (777 mg, 487 mmol) by dicyclohexylcarbodiimide (1.09 g, 5.27 mmol), 1-hydroxybenzotriazol (683 mg, 4.66 mmol), and triethylamine (677 μl, 4.83 mmol) at 0 °C. After stirring overnight, the precipitates were removed by filtration. The filtrate was evaporated and the residue was extracted with CH_2Cl_2 and washed successively with 5% $NaHCO_3$, 1 N aqueous HCl, and saturated aqueous NaCl and concentrated to dryness. The crude product was chromatographed ($CHCl_3$–MeOH) over silica gel to give an oil, which was crystallized from 2-propanol (1.75 g, 75.4%, *Anal.* Calcd for $C_{21}H_{24}NO_{12}$: C, 58.63; H, 5.45; N, 2.44. Found: C, 58.19; H, 5.60; N, 2.61). The residue was hydrogenated in MeOH–THF (1 : 1) with 10% Pd–C (110 mg) at room temperature for 24 h (1.50 g, 98%) and followed by deacetylation using 28% NaOMe (a few drops) in MeOH (10 ml) at 0 °C. The residue was crystallized from MeOH–$CHCl_3$ to give Glc-HBHA (64.2 mg, 35.7%, *Anal.* Calcd for $C_{13}H_{17}NO_8$: C, 49.52; H, 5.44; N, 4.44. Found: C, 48.59; H, 6.14; N, 4.55). Glc-PPHA was also synthesized in the manner described above except that 4-hydroxycinnamic acid benzyl ester (3.00 g, 11.80 mmol) was used instead of 4-hydroxybenzoic acid benzyl ester. In Glc-HHA synthesis, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylbromide (6.4 g, 15.77 mmol) was conjugated with 4-hydroxybenzoic acid benzyl ester (3.00 g, 13.14 mmol) and tosylglycylbenzylester orderly (2.57 g, 7.63 mmol). The other steps were as described above.

Urease Preparation Jack-bean powder (Canavalia ensiformis, 200 g) was stirred for 5 min with five volumes of 31.5% acetone and filtered at 4 °C. The filtrate was adjusted to a final concentration of 40% acetone and centrifugated at 3000 × *g* for 30 min. The pellet was fractionated from 30 to 45% acetone at pH 8.6, adjusted by 2 N NaOH,[21] and dissolved in 20 mM sod. phosphate (pH 7.0), followed by dialysis overnight against the same buffer. The preparation (49 IU/mg protein) was used as jack-bean urease. For the preparation of bacterial ureases, bacteria were cultured at 37 °C for 2—3 d in the following broths : nutrient broth (Difco Lab., U.S.A.) containing 1% urea for *Proteus mirabilis*, and brucella broth (BBL) containing 10% fetal calf serum (FCS) for *Helicobacter pylori*. *H. pylori* was cultured under microaerophilic conditions on a rotary shaker. Cells harvested by centrifugation were suspended in an isotonic buffer and disrupted by ultrasonic vibration for 30 s five times at intervals of 1 min in an ice bath. The debris was removed by centrifugation at 13000 × *g* for 20 min and the supernatant fluid was used as bacterial urease. The specific activity of crude urease of *H. pylori* was 4.2 IU/mg protein. *Proteus mirabilis* urease was partially purified (1700 IU/mg protein) by chromatography on hydoxyapatite and gel filtration on an Ultragel AcA 34 column. Intestinal urease was prepared by washing the intestinal contents of rats with phosphate buffered saline and homogenizing with 20 mM phosphate buffer (pH 7.0), followed by centrifugation at 5000 × *g* for 15 min. The supernatant was used for urease assay. Protein concentrations were determined by the method of Lowry *et al.*, using bovine serum albumin as standard.[22]

Urease Assay and Inhibition Assay Urease activity was quantitated by the indophenol method with minor modification.[23] Briefly, enzyme samples (100 μl) were added to 0.3 ml of urea substrate solution (400 mM urea in 20 mM phosphate buffer, pH 7.0) and incubated at 37 °C for up to 30 min. The reaction mixture was terminated by 0.1 ml of 1 N H_2SO_4, added with phenolnitroprusside reagent and alkaline hypochlorite reagent, and incubated at 65 °C for 20 min. The quantity of ammonia liberated was determined from a standard curve correlating the A_{630} to the ammonium concentration (from $(NH_4)_2SO_4$). In the inhibition assay, enzyme (50 μl) was preincubated with inhibitors (50 μl) or isotonic buffers for various periods. After adding substrate, rates of urea hydrolysis (μmol/min) were measured spectrophotometrically. The concentration of inhibitor required to reduce the enzyme activity by 50% was calculated by plotting inhibition (%). The preincubation period was 15 min without mentioned and lengthened up to 2 h in some experiments.

Inhibition of Microfloral Urease by Glc-PPHA Each of three rats was orally administered Glc-PPHA (74.6 mg/kg), nicotino-HA (30 mg/kg) as an active control of equimolar amounts, and 20 mM phosphate buffered saline, pH 7.0, used as solvent. After 1, 4, and 6 h, rats were anesthetized with i.p. pentobarbital (30 mg/kg), and a segment of intestine was exteriorized through an abdominal incision. Urease activity of intestinal contents was measured at three regions, ileum-caecum, proximal and distal colon.

Breath Test[24] Unless stated otherwise, animals were not fasted before testing. Rats were dosed orally with 1 ml of 0.1 M urea solution containing 44.4 KBq of ^{14}C-urea (0.27 GBq/mmol). Individual animals were transferred immediately to a sealed glass metabolism chamber (approximately 25 cm internal diameter by 15 cm height). Air was drawn through the chambers and expired-breath

was collected over a 6 h period into 99% ethanolamine in two test tubes linked in series. The ethanolamine solution was renewed at intervals of time, 0.5, 1, 1.5, 2, 3, and 4 h, and triplicate 0.2 ml aliquots of the collected solutions were mixed with 6ml of scintillation fluid (ACS II) and counted for radioactivity on an LSC 163 liquid scintillation counter (Aloka, Japan). From the radioactivity profiles obtained, the total recovery percentage of administered radioactivity was calculated.

RESULTS

IC_{50} values of *p*-hydroxyphenolic hydroxamates and their glycosides for urease of various origins are shown in Table I. HBHA and PPHA inhibited plant and bacterial urease in the same order as nicotino-HA, a control inhibitor, though HHA inhibited bacterial urease activity more potently. It is generally accepted that higher concentrations of hydroxamate are necessary to obtain the same inhibition in feces or intestinal contents than in plant and bacterial urease of a strain. However, inhibitory activities of HBHA, HHA and PPHA were markedly decreased by β-glucosylation of their phenolic hydroxyl groups with a 15 min preincubation period; these three glucosyl-hydroxamates inhibited urease activity in rat feces to almost the same extent as jack-bean urease. Inhibitory activity of HHA was increased progressively with the elongation of preincubation period, and there was almost complete loss of activity within 15 min in jack-bean urease. Glc-HHA (Fig. 1-A) showed the same increment as HHA but the inhibitory activity of 10 μM was increased at a constant rate by up to 1 h of preincubation. Inhibitory potency of Glc-HHA (Fig. 1-B) in ileum-caecum contents was also increased with longer preincubation time probably due to β-glucosidase, and eventually this substance showed almost the same pattern of improvement as HHA. HBHA and Glc-HBHA also showed similar patterns as

TABLE I. IC_{50} (μM) of Phenolic Hydroxamates for Urease

	Enzyme sourse			
	Jack-bean	*P. mirabilis*	*H. pylori*	Rat feces
Nicotino-HA	2.9	41	4.1	36
HBHA	1.4	60	7.0	85
HHA	1.6	0.33	1.4	7.0
PPHA	1.8	87	13	20
Glc-HBHA	82	>1000	420	150
Glc-HHA	100	1.1	11	65
Glc-PPHA	>1000	>1000	170	>1000

Values given one means of three observations.

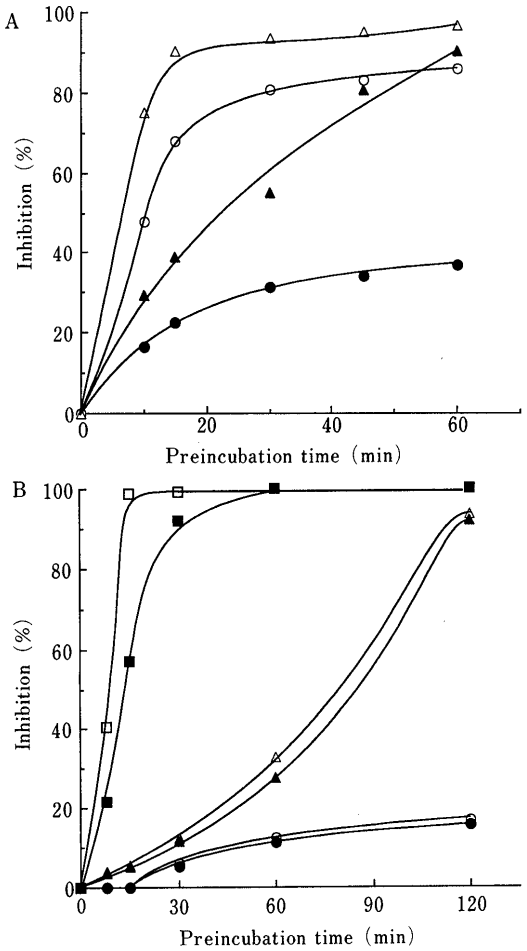

Fig. 1. Increment of Inhibitory Potency of HHA and Glc-HHA with the Increase in Preincubation Time in Jack-bean Urease(A) and Ileum-caecum Content Urease(B)

HHA 100 μM (□) HHA 10 μM (△), HHA 1 μM (○) Glc-HHA 100 μM (■) Glc-HHA 10 μM (▲) Glc-HHA 1 μM (●).

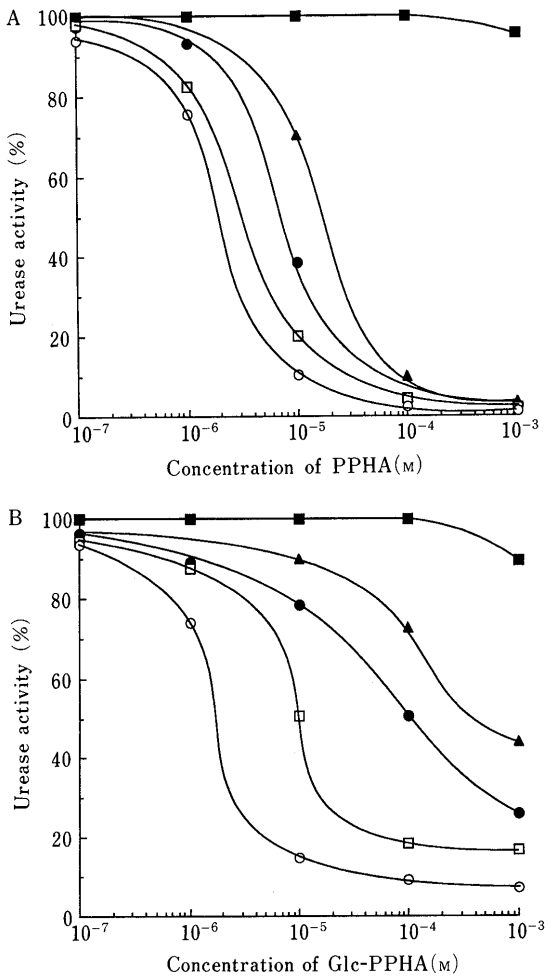

Fig. 2. Inhibition of Rat Fecal Urease by PPHA(A) and Glc-PPHA(B) at Variable Preincubation Times

0 min (■) 15 min (▲), 30 min (●) 60 min (□) 120 min (○).

described above (data not shown). However, the inhibitory potency of Glc-PPHA was increased rapidly with 15 min of preincubation and only slight increase was observed in jack-bean urease even with extention of the preincubation period to 2 h and the application of higher concentrations. Using various orders of concentrations of Glc-PPHA in rat feces (Fig. 2), its inhibitory potency was also increased with longer preincubation than 15 min and showed the same curve as PPHA up to 2 h. At the concentration of 10 μM, Glc-PPHA reached similar inhibition percent to PPHA at 2h preincubation. D-Gluconic acid lactone (Fig. 3), a classical β-glucosidase inhibitor ($IC_{50}=5$ mM for rat feces), was adopted to confirm our finding that Glc-PPHA was converted to PPHA, a more potent inhibitor of urease, by microfloral β-glucosidase of rat feces *in vitro*. Effect of D-gluconic acid lactone on the increase of inhibitory potency of Glc-PPHA was examined with a 2 h preincubation period. Inhibitory potency of the glucoside did not increase further in the rat feces when β-glucosidase was inhibited to 40% by 10 mM D-gluconic acid lactone. But the glycoside inhibited urease to the same extent as aglycone when β-glucosidase was just slightly inhibited to 80% by 1 mM of D-gluconic acid lactone. D-Gluconic acid lactone itself had no effect on urease activity of the rat feces at 10 mM. Investigation of the kinetics of inhibition by PPHA and Glc-PPHA on jack-bean urease using Lineweaver–Burk plots (Fig. 4) showed that PPHA inhibited noncompetitively and Glc-PPHA competitively.

We examined *in vivo* inhibitory effect of a single oral administration of nicotino-HA and Glc-PPHA to Wistar rats on ureolysis of intestinal microflora (Fig. 5). After 1 h, no differences were observed in urease activity of ileum-caecum, proximal colon, or distal colon contents compared with a control group. Although no statistically significant values were detected in ileum-caecum or proximal colon contents by nicotino-HA or Glc-PPHA 4 h after the administration, it was observed that the latter

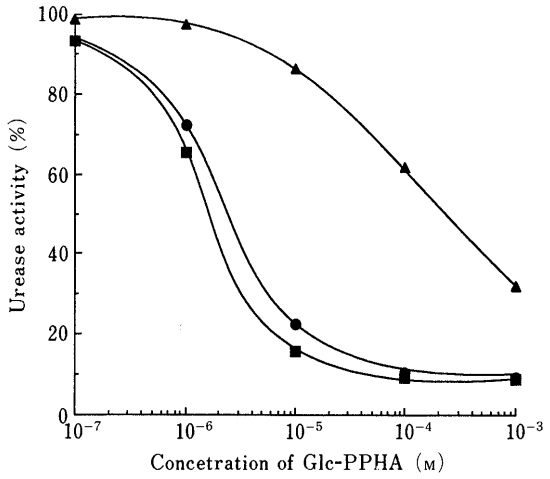

Fig. 3. Inhibitory Effect of Glc-PPHA on Urease of Rat Feces in the Presence of β-Glucosidase Inhibitor

D-Gluconic acid lactone: 0 mM (■) 1 mM (●) 10 mM (▲), preincubated for 2 h.

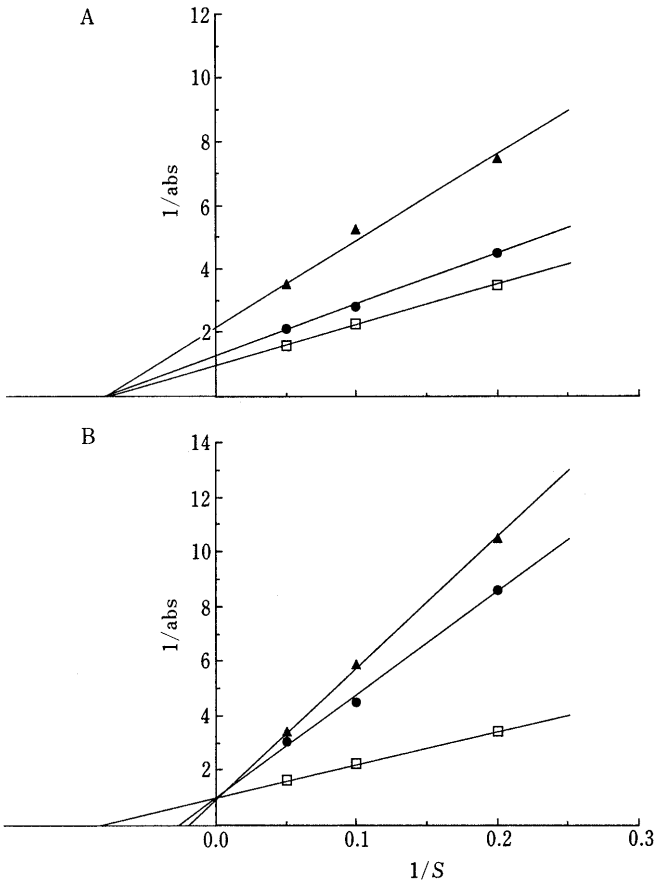

Fig. 4. Lineweaver–Burk Plots for Inhibition of Jack-bean Urease by PPHA(A) and Glc-PPHA(B)

The inhibition pattern was drawn with three concentrations of urea (5, 10, 20 mM) as substrate and 0.5 μM (●) and 1 μM (●) PPHA, 0.5 mM (●) and 1 mM (▲) Glc-PPHA, and without inhibitors (□).

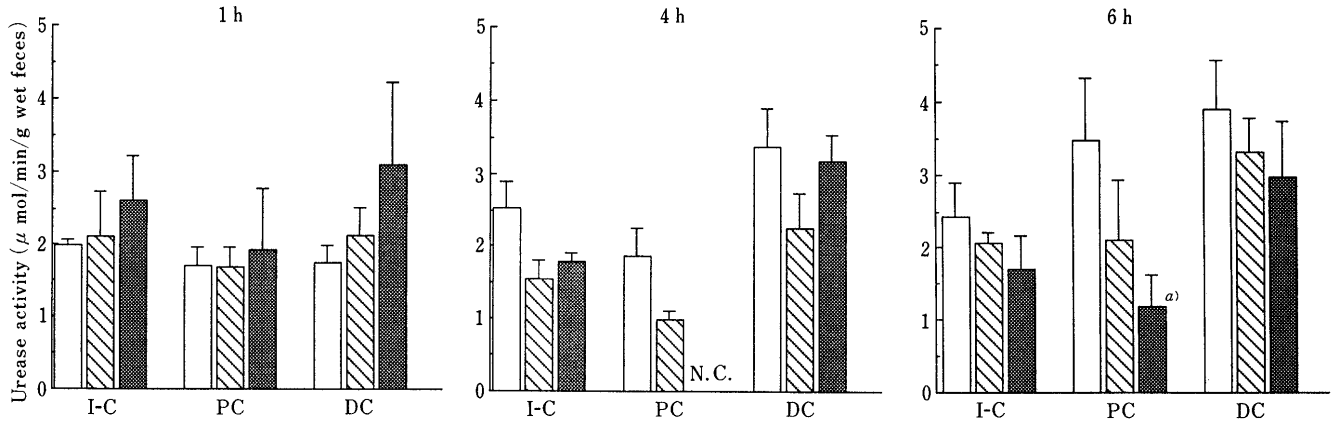

Fig. 5. Urease Activity of Intestinal Contents after the Oral Administration of Nicotino-HA (▨) Glc-PPHA (▩) and Control (□)

I-C ileum-caecum; PC proximal colon; DC distal colon; N. C. No content.

TABLE II. Effect of Glc-PPHA and Nicotino-HA on Ureolysis of Rat *in Vivo*

Time (h)	Expiration % as $^{14}CO_2$					
	Control	Cumulative %	Glc-PPHA	Cumulative %	Nicotino-HA	Cumulative %
0.5	6.63±2.05[a]		0.68±0.11[d]		0.46±0.20[d]	
1.0	3.90±0.68	10.53±2.69[b]	1.96±0.38[c]	2.64±0.38[d]	0.73±0.31[d]	1.18±0.50[d]
1.5	1.52±0.51	12.05±3.2	1.79±0.75	4.43±1.12[c]	0.97±0.35	2.16±0.85[d]
2.0	0.83±0.22	12.88±3.41	1.00±0.37	5.43±1.49[c]	0.93±0.58	3.08±1.41[c]
3.0	0.65±0.2	13.53±3.61	0.78±0.47	6.21±1.95[c]	0.57±0.20	3.65±1.56[c]
4.0	0.36±0.04	13.89±3.64	0.37±0.21	6.58±2.17[c]	0.32±0.07	3.98±1.58[c]
6.0	0.34±0.03	14.23±3.67	0.25±0.12	6.83±2.27[c]	0.21±0.03	4.18±1.56[c]

Ureolysis *in vivo* measurement, ^{14}C-urea (44.4 kBq, 6 mg) was orally administered simultaneously with test hydroxamic acids to rats. The amount of radioactivity expired in breathing air for 6 h was collected and analyzed. Glc-PPHA was orally administered at 74.6 mg/kg and nicotino-HA, 30 mg/kg. *a*) Excretion % as $^{14}CO_2$ in each indicated interval time. *b*) Accumulation of expired $^{14}CO_2$ until the indicated time. *c*) $p<0.05$. *d*) $p<0.01$.

tended to inhibit urease activity in proximal colon contents 6 h after administration ($p=0.06$). The effects of the two urease inhibitors, nicotino-HA and Glc-PPHA, were also evaluated in a breath test (Table II). Fifteen percent of administered ^{14}C-urea was recovered as $^{14}CO_2$ in a control group during 6 h, and this ureolysis was limited to 40 and 28% by Glc-PPHA and nicotino-HA, respectively. Rate of $^{14}CO_2$ release was highest during the first 30 min but even then significantly inhibited to 10 and 7% by Glc-PPHA and nicotino-HA, respectively, compared with the control group.

DISCUSSION

Urea is excreted in the urine of all mammals as a final detoxified product. Human urine contains 25—30 g of urea/d, which results in an annual release of 10 kg of urea per adult. Bacterial urease is implicated in the pathogenesis of many clinical conditions. It is directly associated with the formation of infectious stones and contributes to the pathogenesis of pyelonephritis, ammonia encephalopathy, hepatic coma, urinary catheter encrustation, and peptic ulceration. Schalm and van der May[7] reported that intestinal bacterial toxins are of minor importance in the mechanism of acute hepatic coma of the hepatoectomized rat, and that presumably toxins such as ammonia can be products of nonbacterial metabolism. Their results showed the arterial ammonia to be equally elevated in germ-free rats and in normal rats, although the ammonia concentration in the cecal contents was significantly lower in the germ-free rats. There have been a number of studies on the relation between glutamine metabolism and blood ammonia concentration. When expressed per gram of intestine, the colonic release of ammonia was three- to four-fold greater than jejunum or ileum. In acute hepatic coma, bowel cleansing and nonabsorbable antibiotics treatments have never been demonstrated as effective; however their efficacy in chronic portosystemic encephalopathy is usually effective. Hepatic coma is a complex pathophysiologic state that is multifactorial in origin.

The work described here established the inhibitory effect of β-glucosyl-phenolic hydroxamates on bacterial urease *in vitro* and *in vivo*, and reports the elevation of inhibitory potency of the hydroxamates by hydrolysis of β-glucosyl bond with intestinal flora. Many strains have been reported that produce β-glucosidase with broad substrate specificity from intestinal flora. It was determined that three glucosyl-hydroxamates used in this test were hydrolyzed by β-glucosidase of the rat feces (bacteria were not identified). We also reported that some β-glycosides or β-glucuronides in oriental crude drugs, such as glycyrrhizin, sennoside, and barbaloin, are hydrolyzed to their corresponding active aglycones by β-glycosidase or β-glucuronidase of intestinal bacteria.[25-27] It is therefore assumed that some β-glycosides and β-glucuronides are a kind of pro-drug if they are administered orally and hydrolyzed by microfloral β-glucosidase or β-glucuronidase. We attempted to synthesize a pro-drug, β-glucose ligated form, for a sustained effect with the help of β-glucosidase of intestinal flora. Our results suggest that Glc-PPHA was hydrolyzed to PPHA by β-glucosidase of the rat feces during 2 h preincubation, and it showed the same inhibitory potency as PPHA. We compared the inhibitory effects of three glucosyl-phenolic hydroxamates using jack-bean urease, which has no β-glucosidase activity, to determine the increment of inhibitory effect with increase in preincubation period. Inhibitory potencies of Glc-HBHA and Glc-HHA were continuously increased with the increment of preincubation period to 2 h. However, that of Glc-PPHA was increased for 15 min and increased no further on jack-bean urease even though the preincubation period was lengthened to 2 h ($IC_{50}=160\ \mu M$, data not shown). For the fecal urease, recovery of the inhibitory effect of Glc-PPHA was greatly speeded up during 2 h preincubation and showed the same inhibitory activity as PPHA ($IC_{50}=2\ \mu M$). These results were confirmed by the following study using the β-glucosidase inhibitor, D-gluconic acid lactone. From these results, we concluded that Glc-PPHA is recovered to PPHA during 2 h preincubation with fecal β-glucosidase without any other hydrolysis and showed the same inhibitory activity as PPHA. The inhibition pattern of Glc-PPHA and PPHA using jack-bean urease was also investigated because of its highly conserved homology with bacterial urease. The findings suggested that the binding sites were different between PPHA and Glc-PPHA with jack-bean urease. Microfloral urease was also used to study the inhibitory pattern of Glc-PPHA and PPHA, but no reasonable inhibitory pattern was obtained. A kinetic study using urease of single strain of intestinal flora should be made. Following preparation of Glc-PPHA, a preliminary assessment of the sustained inhibitory effect was made. A

single oral dose of Glc-PPHA tended to inhibit urease of proximal colon contents after 6 h. To define the sustained inhibitory effect of glucosyl-hydroxamates, further kinetic studies should explore the intestinal absorption, distribution, and excretion using radiolabeled hydroxamate. Glc-PPHA inhibition of bacterial urease activity *in vivo* was assessed in adult rats using ^{14}C-urea. Seventy-five percent of intestinal ureolysis was inhibited by Glc-PPHA during the first 1 h, indicating that this substance suppressed ureolysis *in vivo* as effectively as nicotino-HA.

In summation, under the conditions used in these studies, three glucosyl-phenolic hydroxamates were hydrolyzed to their corresponding aglycones by β-glucosidase of intestinal flora and their inhibitory potency was recovered to the same extent as when phenolic hydroxamates were administered. Further investigation is needed of the glucosyl-hydroxamate in the trearment of hepatic coma syndromes, including application of the glucosyl-hydroxamates to infection-induced urinary stones.

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