Journal of Bacteriology and Virology 2011. Vol. 41, No. 4 p.255 – 265
http://dx.doi.org/10.4167/jbv.2011.41.4.255

Original Article

Purification and Characterization of *Helicobacter pylori* γ-Glutamyltranspeptidase

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Gamma-glutamyltranspeptidase (GGT) was purified to electrophoretic homogeneity from the cell extract of *H. pylori*. The purified enzyme consisted of heavy and light subunits with molecular weights of 38 kDa and 21 kDa, respectively. N-terminal amino acid sequence of heavy and light subunits revealed that *H. pylori* GGT was processed into 3 parts for a signal peptide of 27 amino acid residues, a heavy subunit of 352 residues, and a light subunit of 188 residues during translation. The reaction rate for hydrolysis of γ-GpNA was 84.4 μmol/min per milligram of protein, and that for the γ-glutamyl transfer from γ-GpNA to gly-gly was 23.8 μmol/min per milligram of protein. The apparent *Km* values of *H. pylori* GGT for γ-glutamyl compounds were on the order of 10^{-3} to 10^{-4} M and those for acceptor peptides and amino acids were on the order of 10^{-1} to 10^{-2} M. The GGT protein kept approximately 80% of the initial enzymatic activity on incubation at 60℃ for 15 min. The optimum temperature and pH for reactions of both hydrolysis and transpeptidation were 40℃ and 9.0, respectively. The transpeptidation and hydrolysis reactions catalyzed by *H. pylori* GGT were strongly inhibited by L-Gln and moderately inhibited by L-Ala, L-Ser, β-chloro-L-Ala, and L-Glu. These results demonstrated that the biochemical properties of *H. pylori* GGT are different from those of other bacterial GGTs. Further, *H. pylori* GGT might degrade glutathione in the gastric mucous layer of humans if the enzyme could be secreted in the bacterial niches.

Key Words: *H. pylori*, γ-glutamyltranspeptidase, Glutathione

INTRODUCTION

Helicobacter pylori (*H. pylori*) was first identified and isolated from the human gastric mucosa in 1982 by Warren and Marshall (1). *H. pylori* infects and colonizes the human gastric mucosa and areas of gastric metaplasia in the duodenum, exhibiting a specific tropism for gastric epithelium of humans (2, 3).

H. pylori infection causes accumulation of the inflammatory cells in the lamina propria of gastric mucosa, leading to the development of the active and chronic inflammation (2, 4). The inflammatory cells produce oxygen free radicals that can damage the DNA of adjacent cells resulting in mutagenic and carcinogenic events in the gastric mucosa, which is thought to be the main pathogenic mechanism connecting from *H. pylori* infection to gastric disorders including gastritis and gastric cancers (5~7).

The gastric mucosa is destined to be continuously ex-

Received: September 20, 2011/ Revised: November 29, 2011
Accepted: December 2, 2011
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**This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (521-2007-1-E00035).

posed to luminal oxidants generated endogenously and exogenously so that defense systems against such oxidants should present in the epithelium of stomach. Reduced glutathione (GSH) was reported to be an important cellular protectant against reactive oxygen metabolites in gastric mucous and chief cells (8~10). In addition, extracellular GSH protects against peroxidant toxicity in gastric epithelial cell by accelerating intracellular GSH synthesis and by eliminating extracellular oxidants (11~16).

Gamma-glutamyltranspeptidase (GGT) catalyzes the hydrolysis of γ-glutamyl compounds and the transfer of their γ-glutamyl moiety to amino acids and peptides in mammalian and plant tissues (10, 17~23). Also, GGT activity is widely found in the bacterial species (24~28) and its enzymatic properties were investigated with a cell-free preparation of *Proteus mirabilis* and *Escherichia coli* (27, 29). Bacterial GGTs have been confirmed to be important in glutathione metabolism, consistent with the mammalian enzymes (10, 25, 29, 30).

H. pylori has been reported to produce a total of 31 enzymes in normal culture conditions (31). The enzymes include urease, oxidase, and catalase that are critical features for identification of *H. pylori*. Additionally, another salient feature was the presence of GGT. The presence of *H. pylori* GGT may be detrimental to the intact gastric mucosa since the organism colonizes the gastric mucous layer in which glutathione is one of the major factors for neutralizing the luminal oxidants (13~15). *H. pylori* GGT can also compete with gastric epithelial GGT in enzymatic reactions using glutathione of gastric mucous layer. *H. pylori* GGT could degrade the mucosal glutathione leading to the depletion of oxidant scavengers in the gastric mucosa. Therefore, the presence of *H. pylori* results in damaging defense systems against reactive oxygen in the gastric mucosa. Taken together, *H. pylori* GGT might be one of the candidate factors for disrupting the gastric mucosal defense mechanism against oxidants.

The *H. pylori* GGT gene was cloned and its nucleotide sequence was determined (32). It was controversial that *H. pylori* GGT is essential for colonization of the gastric mucosa of mice (32, 33). *H. pylori* GGT was reported to up-regulate COX-2 and EFG-related peptide expression in human gastric cells (34). In addition, *H. pylori* GGT induces an apoptosis through mitochondria pathway and cell cycle arrest at G1-S phase transition (35, 36). *H. pylori* GGT was partly characterize its enzymatic properties using recombinant proteins (37~39). However, detailed biochemical and enzymatic properties of *H. pylori* GGT have to be investigated for the understanding of GGT's roles in the pathogenesis of *H. pylori* infections. In this report, we present the purification and characterization of *H. pylori* GGT. *H. pylori* GGT was characterized by catalytic properties, substrate specificity, effect of inhibitors, and N-terminal amino acid sequence determination of subunits.

MATERIALS AND METHODS

Reagents

L-γ-glutamyl-*p*-nitroanilide (γ-GpNA), glycylglycine (gly-gly), reduced glutathione (GSH), oxidized glutathione (GSSG), S-methyl GSH, 6-diazo-5-oxo-norleucine, azaserine, borate, iodoacetic acid, $HgCl_2$, L-serine, L-alanine, L-α-amino-*n*-butyrate, γ-amino-*n*-butyrate, D-arginine, L-arginine, L-asparagine, L-aspartic acid, benzoyl-L-tyrosine ethyl ester, S-carboxymethyl-L-cysteine, β-chloro-L-alanine, D-cysteine, L-cysteine, L-cysteic acid, L-dihydroxy-phenylamine (L-DOPA), L-glutamine, L-glutamic acid, glycylglycylglycine, glycyl-L-leucine, D-histidine, L-histidine, L-homoserine, L-isoleucine, L-leucine, L-leucyl-glycine, D-lysine, L-lysine, L-methionine, 3-methoxy-L-tyrosine, S-methyl-L-cysteine, *O*-methyl-L-tyrosine, L-phenylalanine, L-proline, L-threonine, L-tryptophane, L-tryptophyl-L-glycine, D-tyrosine, L-tyrosine, L-valine, Sephacryl S-200, acrylamide, Coomassie brilliant blue R-250 and Polybuffer 96 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bisacrylamide and sodium dodecyl sulfate (SDS) were from Amresco (Solon, CA, USA). Other chemicals were purchased from commercial sources.

Bacterial strain and culture

Frozen *H. pylori* strain 51 (40) was retrieved from a liquid nitrogen tank and rapidly thawed in a 37℃ water bath.

The bacteria was grown on a brucella agar supplemented with 10% bovine serum overnight at 37℃, 10% CO_2, and 100% relative humidity. The grown cells were harvested in a 50 ml tube. Approximately 38 g (wet weight) of cells were obtained from 250 petri dishes (87 mm in diameter). The packed cells were stored at -20℃ until purification of the enzyme.

Enzyme assay for GGT activity

GGT activity was determined by a spectrophotometric method and amino acid analysis depending on the purpose of the assay as described previously (27). The assay solution contained 2.5 mM of L-γ-GpNA, 60 mM of gly-gly, 50 mM of Tris-HCl [pH 8.0], 750 mM of NaCl, and the enzymatic sample in a final volume of 0.5 ml. After incubation at 37℃, reaction was stopped by the addition of 1 ml of 3.5 N acetic acid. The mixture was centrifuged at 12,000 rpm for 5 min at 4℃. The difference in A_{410} between reaction mixtures with and without gly-gly was used to calculate the transferase activity. Hydrolyase activity of γ-glutamyl compound was measured without a γ-glutamyl acceptor under the same conditions. One unit of enzyme activity was defined as the amount of the enzyme which transferred 1 μmol of γ-glutamyl moiety per min. Specific activity was noted in units per milligram of protein.

When γ-glutamyl compounds other than γ-GpNA were used, each reaction was terminated by adding 1 ml of 0.1 N HCl, and then the mixtures were injected into the amino acid analyzer (LKB, Sweden) equipped with PEEK Sodium High Resolution Column for Biochrom 20 (Biochrom Ltd, St. Albans, UK). γ-Glutamyl-glycylglycine was measured to calculate the transferase activity and glutamic acid was measured to calculate the hydrolyase activity.

Protein determination

The protein concentration was spectrometrically determined by the method of Lowry *et al*. (41) using bovine serum albumin as a standard.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was performed with 12.5% polyacrylamide running gel (0.75 mm thick) and 3% polyacrylamide stacking gel by the Laemmli method (42). After electrophoresis, a portion of the gel was stained with Coomassie brilliant blue R250. The subunit molecular weight was estimated by SDS-PAGE. High range protein molecular weight standard marker (200, 97.4, 68, 43, 29, 18.4, and 14.3 kDa) from GibcoBRL, Life Technologies, Inc (Rockville, NY, USA) was used as a standard.

Purification of GGT from *H. pylori*

Purification of *H. pylori* GGT was performed by a modification of previously described methods (29). Frozen cell paste (wet weight, 38 g) was suspended in 80 ml of 50 mM Tris-HCl buffer [pH 8.0]. The cells were disrupted with a ultrasonicator (Sonics & Materials Inc. Danbury, CA, USA) and the supernatant solution was obtained by centrifugation. Solid ammonium sulfate was added to the cell extract to 40% saturation, and the mixture was stirred on ice for 2 hr. The precipitate was removed by centrifugation at 16,900 g for 1 hr at 4℃, and the supernatant was adjusted to 60% saturation and stirred on ice for 2 hr. The precipitate was collected by centrifugation as described above and dissolved in 20 ml of 50 mM Tris-HCl [pH 8.0]. This solution was twice dialyzed against 5 ℓ of the same buffer for 24 hr at 4℃. The dialyzed enzyme solution was applied to a Sephacryl S-200 column (1.6 by 100 cm) equilibrated with 50 mM Tris-HCl [pH 8.0], and eluted with the same buffer at a flow rate of 2 ml/10 min in 2 ml fractions. The active fractions were combined and concentrated by the addition of ammonium sulfate to 60% saturation. The precipitate was collected by centrifugation and then dissolved in 50 mM citrate-phosphate [pH 5.0]. This solution was dialyzed against the same buffer. The dialyzed enzyme solution was applied to a FPLC Resource S column (Pharmacia, Upsala, Sweden) equilibrated with 50 mM citric acid-sodium phosphate dibasic buffer [pH 5.0] for ion exchange chromatography and eluted with 25 ml of the same buffer containing a linear gradient of 0~1 M NaCl at a flow rate of 1 ml/min in 1 ml fractions. The active fractions that eluted at 0.2 M NaCl were combined and dialyzed against 1 mM sodium phosphate [pH 7.5]. The dialyzed enzyme solution was

Table 1. Summary of purification of GGT from *H. pylori* 51

Steps	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Yield (%)
Whole cell lysate	2,050	123.0	0.06	100
Ammonium sulfate (40~60%)	475.95	76.15	0.16	23.22
Sephacryl S-200	273.24	68.31	0.25	13.33
Resource S	8.81	46.87	5.32	0.43
Hydroxyapatite	2.29	43.56	19.02	0.11
Chromatofocusing	1.13	26.91	23.82	0.06

applied to a hydroxyapatite column (Econo-pac CHT-II, BIO-RAD, Hercules, CA, USA) equilibrated with 1 mM sodium phosphate [pH 7.5] and eluted by a linear gradient of 1 mM to 0.5 M sodium phosphate at a flow rate of 0.5 m1/min. The active fractions eluted at 0.33 M sodium phosphate were combined and dialyzed against 25 mM ethanolamine-acetic acid [pH 9.4]. The dialyzed mixture was applied on a Mono P column (1 by 5 cm, Pharmacia, Upsala, Sweden) equilibrated with 25 mM ethanolamine-acetic acid [pH 9.4], and eluted with 12 bed volumes of polybuffer 96-acetic acid (tenfold dilution [pH 6.0]) to form a descending linear gradient of pH 9 to 6.

N-terminal amino acid sequencing

Purified GGT was subjected to SDS-PAGE and was transferred electrically to a polyvinylidene difluoride membrane (BIO-RAD) by a modification of the previously described method (43). After transfer, the membranes were washed with distilled water and followed by staining with Ponceau S solution (0.5% W/V Ponceau S in 7.5% W/V trichloroacetic acid in water). Stained subunits were excised and subjected to determination of N-terminal amino acid sequences with a Procise® Protein Sequencing System (Applied Biosystems, Foster, CA, USA).

RESULTS

Enzyme purification

The purification scheme (Table 1) using ammonium sulfate fraction, three sequential column chromatographic fractionations, and chromatofocusing, gave a 416-fold purification with a recovery of 0.06%. The final step of chromatofocusing gave one protein peak showing GGT activity (Fig. 1). SDS-PAGE of purified *H. pylori* GGT gave heavy and light subunits with apparent molecular weights of 38 and 21 kDa, respectively (Fig. 2).

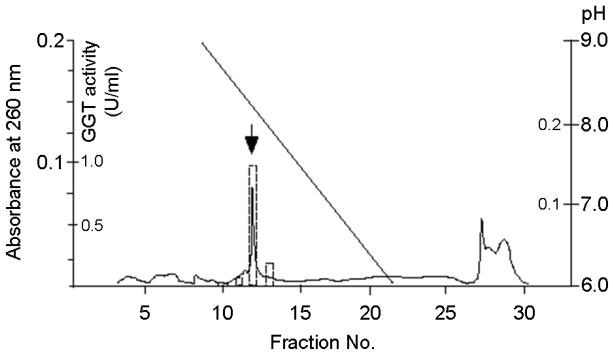

Figure 1. Chromatofocusing pattern of *H. pylori* GGT. Enzyme fractions collected from a hydroxyapatite column were applied on a Mono P column (1 by 5 cm, Pharmacia) equilibrated with 25 mM ethanolamine-acetic acid buffer (pH 9.4) and eluted with 12 bed volumes of polybuffer 96-acetic acid (tenfold dilution [pH 6.0]) to form a descending linear gradient of pH 9 to 6 in the column. Dashed rectangle bars represented the enzyme activity (unit/m1) of fractions. The active fractions (arrow) eluted at pH 8.2 were pooled.

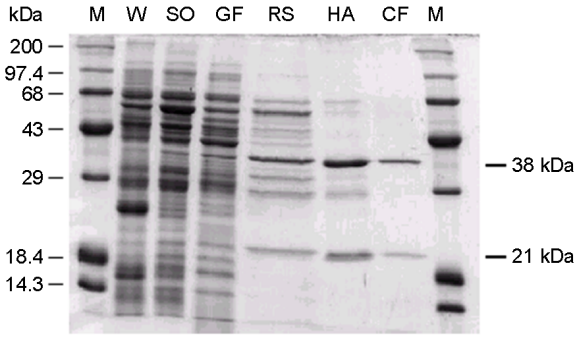

Figure 2. SDS-polyacrylamide gel electrophoresis of *H. pylori* GGT in the purification steps. Approximately 8 μg of each fraction was applied. SDS-PAGE was performed with 12.5% polyacrylamide running gel (0.75 mm thick) and 3% polyacrylamide stacking gel at 15 mA for 2 hrs. After electrophoresis, a portion of the gel was stained with Coomassie brilliant blue R250. The subunit molecular weight was estimated by SDS-PAGE. Protein molecular weight standard, high range marker (200, 97.4, 68, 43, 29, 18.4, and 14.3 kDa) was used as a standard. M, marker; W, cell extract; SO, salt precipitation; GF, gel-filtration fraction; RS, Resource S fraction; HA, hydroxyapatite fraction; CF, chromatofocusing fraction.

Table 2. Substrate specificity of *H. pylori* GGT for γ-glutamyl donors

Substrates	Relative activity[a] (%)	
	Transpeptidation	Hydrolysis
γ-GpNA	100[b]	100
GSH	152.3	108.1
GSSG	229.3	127.8
S-methyl GSH	27.8	73.2
γ-L-Glu-L-Tyr	123.1	100.6
γ-L-Glu-L-His	147.9	110.3
γ-L-Glu-L-Phe	149.8	108.2
γ-L-Glu-L-Leu	147.7	136.8
L-Gln	137.8	110.1
D-Gln	389.7	105.5
α-L-Glu-L-Ala	0	0

[a] Both activities were measured with an amino acid analyzer as described in Materials and Methods. Concentrations of donors and gly-gly were 2.5 and 60 mM, respectively. Activity is denoted relative to that found with γ-GpNA.
[b] The indicated values are the mean of two independent experiments.

Table 3. Substrate specificity of *H. pylori* GGT for γ-glutamyl acceptors

Substrates	Conc. (mM)	Relative rate (%)		
		H. pylori	*P. mirabilis*[a]	*E. coli*[b]
Gly-gly	60	100	100	100
Gly-gly	20	62.4	–[c]	29.4
L-Ala	60	0	4.0	0
L-α-Amino-*n*-butyrate	60	0	5.5	12.7
γ-Amino-*n*-butyrate	60	54.0	16.3	22.3
D-Arg	60	37.2	−	18.1
L-Arg	60	79.9	20.7	141
L-Asn	60	38.7	−	31.8
L-Asp	60	0	−	4.2
Benzoyl-L-Tyr ethyl ester	20	0	−	0
S-Carboxymethyl L-Cys	20	0	−	1.9
β-chloro-L-Alanine	60	0	0	−
D-Cys	20	0	−	0
L-Cys	20	0	15.7	13.5
L-Cysteic acid	20	17.7	−	0
L-DOPA	20	0	−	63.9
L-Gln	60	0	2.5	−
L-Glu	60	0	0	10.2
Gly	60	0	16.6	20.8
Gly-gly-gly	60	29.7	−	14.2
Gly-Leu	60	0	−	12.5
D-His	60	0	−	−
L-His	60	0	36.9	49.5
L-Hse	60	0	6.7	0
L-Ile	60	19.8	25.2	13.8
L-Leu	20	17.1	20.7	2.5
Leu-Gly	60	0	−	12.5
D-Lys	60	55.0	−	16.5
L-Lys	60	46.6	16.0	121
L-Met	60	46.8	46.7	91.8
3-Methoxy-L-Tyr	20	0	−	−
S-Methyl-L-Cys	20	0	−	13.5
O-Methyl-L-Tyr	20	0	−	5.6
L-Phe	60	52.6	51.7	42.9
L-Pro	60	50.0	13.2	2.4
L-Ser	60	0	6.2	9.6
L-Thr	60	0	15.6	22.9
L-Trp	20	30.7	36.9	29.8
Trp-Gly	20	0	−	23.6
D-Tyr	20	0	−	1.1
L-Tyr	20	20.4	6.6	2.8
L-Val	60	0	12.6	10.8

[a] Nakayama et al. (29)
[b] Suzuki et al. (27)
[c] Data were not available.

N-terminal amino acid sequence of subunits

Of subunits of *H. pylori* GGT, N-terminal amino acid sequence of the heavy subunit was determined to be SYPPIKNTKVGL and that of light subunit was TTHYSVADRW, completely corresponding to the deduced amino acid sequence of the reported *H. pylori* GGT gene

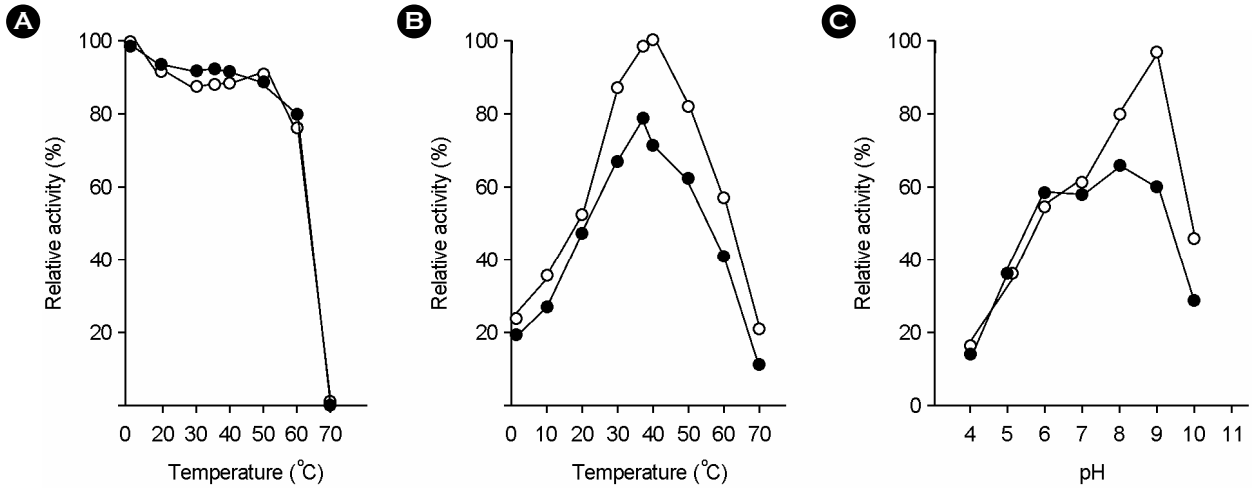

Figure 3. Temperature stability and reactivity of *H. pylori* GGT at the various conditions of temperature and pH. (A) reaction mixtures were incubated at various temperatures for 15 min in 20 mM Tris-HCl (pH 8.0), and then GGT activities were measured; (B) GGT activities were measured at various temperature on the standard conditions described in Materials and Methods; (C) the pH of the reaction mixture containing the enzyme was adjusted with phosphate buffer for pH 4.0~6.0 or Tris-HCl buffer for pH 7.0~11.0 (open circles, transpeptidation activity; closed circles, hydrolysis activities). The indicated value is the mean of two independent experiments.

(32). This result revealed that *H. pylori* GGT is processed into 3 parts for a signal peptide of 27 amino acid, a heavy subunit of 352 residues, and a light subunit of 188 residues during translation.

Catalytic properties of *H. pylori* GGT

The purified enzyme catalyzed the reactions of both hydrolysis and γ-glutamyl transpeptidation of γ-glutamyl compounds (Table 2 & 3). The reaction rate for hydrolysis of γ-GpNA was 84.4 μmol/min per milligram of protein, and that for the γ-glutamyl transfer from γ-GpNA to gly-gly was 23.8 μmol/min per milligram of protein under the standard reaction conditions. The GGT protein kept approximately 80% of the initial enzymatic activity on incubation at 60℃ for 15 min and lost most of the activity on incubation at 70℃ (Fig. 3). The optimum temperature and pH for reactions of both hydrolysis and transpeptidation were 40℃ and 9.0 under the standard conditions, respectively (Fig. 3).

Substrate specificity

The enzyme activities of the purified GGT for hydrolysis and γ-glutamyl transfer of γ-glutamyl compounds to gly-gly were measured at pH 9.0 (Table 2). Various γ-glutamyl compounds acted as a donor of γ-glutamyl moiety except for α-L-Glutamyl-L-alanine. D-Gln was the best γ-glutamyl donor for transpeptidation. The enzyme used various amino acids as γ-glutamyl acceptors (Table 3). L-Arg, D-Lys, L-Lys, L-Met, L-Phe, L-Pro, and γ-amino-*n*-butyrate were good acceptors and other amino acids and peptides were poor or inactive as γ-glutamyl acceptors. The apparent *Km* values for γ-GpNA and GSH as γ-glutamyl donors in the transpeptidation and hydrolysis reactions were on the order

Table 4. *Km* values[a]

Parameters	Substrate	mM
Transferase activity		
Donor[b]	γ-GpNA	0.66±0.03
	GSH	1.95±1.04
Acceptor[c]	Gly-gly	32±6
	L-Arg	107±6
Hydrolase activity		
	γ-GpNA	1.64±0.06
	GSH	1.06±0.27

[a] Calculated by Lineweaver-Burk plotting. The indicated values are the mean of two independent experiments ± the standard deviation of the mean.
[b] 60 mM gly-gly was used as an acceptor.
[c] 2.5 mM γ-GpNA was used as a donor.

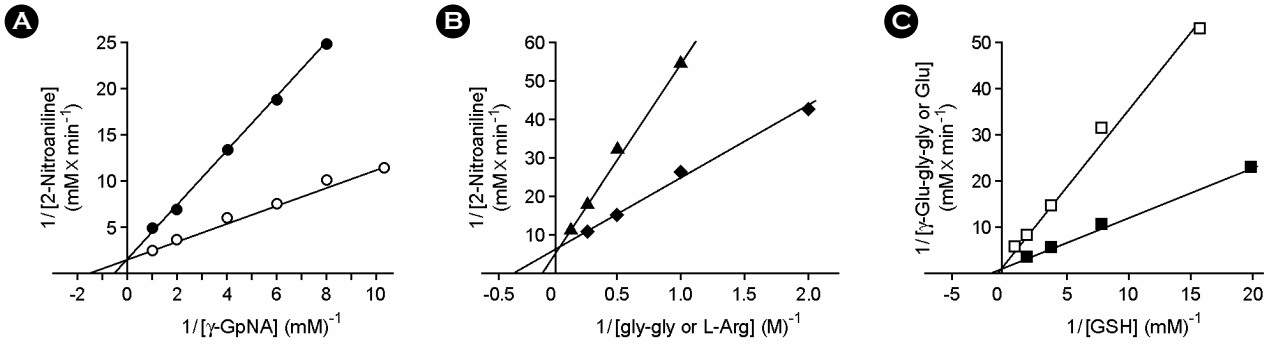

Figure 4. Lineweaver-Burk plot of transpeptidation and/or hydrolysis of γ-GpNA, gly-gly, L-Arg, and GSH as catalyzed by purified *H. pylori* GGT. The enzyme activities were measured on the conditions described in Materials and Methods except for the change of the pH 9.0. (A) donor activities of γ-glutamyl moiety of γ-GpNA in transpeptidation reaction (○) and in hydrolysis reaction (●); (B) acceptor activities of gly-gly (◇) and L-Arg (△) in transpeptidation reaction; C, donor activities of glutamyl moiety of GSH in transpeptidation reaction (□) and in hydrolysis reaction (■). The reciprocals of the rates of 2-nitroaniline production (A, B) or γ-glutamyl-glycylglycine and glutamic acid production (C) were plotted against those of different concentrations of γ-glutamyl moiety donors or acceptors. The indicated values are the mean of two independent experiments.

Table 5. Inhibition of *H. pylori* GGT by amino acids

Amino acids	Inhibition (%) of		
	Conc. (mM)	Transpeptidation	Hydrolysis
None		0[a]	0
L-Ala	10	56.1	54.8
L-Ser	10	48.3	48.4
L-Hse	10	36.3	18.9
L-α-Ab	10	16.1	20.9
Gly	10	22.9	25.8
D-Cys	10	72.1	12.4
L-Cysteic acid	10	72.8	3.9
L-Asp	10	22.3	12.0
β-chloro-L-Ala	10	57.0	42.3
L-Cys	10	61.3	11.2
L-Gln	10	91.7	89.8
L-Glu	10	72.7	46.5

[a] The indicated values are the mean of two independent experiments.

of 10^{-3}~10^{-4} M (Table 4 & Fig. 4). The *Km* values for gly-gly and L-Arg as γ-glutamyl donors in the transpeptidation were on the order of 10^{-1}~10^{-2} M (Table 4 & Fig. 4).

Inhibition by amino acids and other inhibitors

The transpeptidation and hydrolysis reactions catalyzed by *H. pylori* GGT were strongly inhibited by L-Gln and moderately inhibited by L-Ala, L-Ser, D-Cys, L-Cysteic acid, β-chloro-L-Ala, L-Cys and L-Glu (Table 5). Inhibition by affinity labeling reagents such as DON and azaserine and other inhibitors was also investigated (Table 6).

Table 6. Inhibition of *H. pylori* GGT by various inhibitors

Inhibitors	Final Conc. (mM)	Inhibition (%) of	
		Transpeptidation	Hydrolysis
None		0.0[b]	0.0
DON[a]	1	100.0	97.4
Azaserine	1	100.0	95.4
L-Ser + borate	1	100.0	11.9
	10	100.0	83.9
Iodoacetic acid	1	41.6	5.6
$HgCl_2$	1	25.8	0.0

[a] 6-diazo-5-oxo-norleucine
[b] The indicated values are the mean of two independent experiments.

DISCUSSION

A number of mammalian cellular GGTs have now been purified and their properties and functions have been well characterized (22, 23, 42, 44, 45). In addition, several bacterial GGTs have been identified and investigated up to date (24, 25, 27~29, 46).

Cell-free extracts of *P. mirabilis* and *E. coli* were purified

to give 2 homogenous proteins showing GGT activity, a major peak, fraction A and a minor peak, fraction B (27, 29). Fraction B was thought to be a protein modified by proteolysis (27, 29). In the case of *H. pylori*, a final step of purification gave only one protein peak (Fig. 1).

Mammalian GGT generally consisted of light subunit (ca. 22 kDa) and heavy subunit (ca. 45~64 kDa) (22). *P. mirabilis* and *E. coli* GGTs have subunits of 47 and 28 kDa, and of 39 and 22 kDa, respectively (27, 29). The light subunit of *H. pylori* GGT has a molecular weight of 21 kDa, which is the same as those of mammalian and *E. coli* GGTs in molecular size, while the heavy subunit has a molecular weight of 38 kDa, which is smaller than those of mammalian and other bacterial GGTs in molecular size.

Nucleotide sequence of the *H. pylori* GGT gene was reported to span 1,704 bp encoding a polypeptide of 567 amino acid residues (32). The data for the nucleotide sequence and N-terminal amino acid sequences of subunits of *H. pylori* GGT revealed that it was processed into 3 parts for a signal peptide composed of 27 amino acid, a heavy subunit of 352 residues, and a light subunit of 188 residues post or co-translationally, which is similar to the features of other bacterial GGTs (27, 29). The N-terminal amino acid residue of light subunit has previously been reported to be the 27^{th} residue (Ala) (32, 39), however, which has been confirmed as the 28^{th} residue (Ser) in this study.

The *H. pylori* GGT catalyzed hydrolysis and transpeptidation of γ-glutamyl compounds, including the oxidized and reduced forms of glutathione and γ-glutamyl compounds of *p*-nitroaniline as reported for mammalian and other bacterial GGTs (Table 2). *H. pylori* GGT prefers L-Arg, D-Lys, L-Lys, L-Met, L-Phe, L-Pro, and γ-amino-*n*-butyrate as acceptors of the γ-glutamyl moiety in the transpeptidation reaction, while other amino acids and peptides were poor or inactive (Table 3). Basic amino acids and aromatic amino acids were relatively good acceptors for *H. pylori* GGT (Table 3), which is consistent with other bacterial GGTs (27, 29). Neutral amino acids such as L-Cys, L-Glu, and L-Ser, which were good acceptors for mammalian GGTs, were inactive as acceptors for *H. pylori* GGT. D-Arg, D-Lys and L-Pro, which are poor acceptors for mammalian GGTs, were relatively good acceptors for *H. pylori* GGT.

In the case of other bacterial GGTs, the transferase activity was reported to be higher than or similar to the hydrolase activity (27, 29). On the contrary, the reaction rate (84.4 μmol/min per mg of protein) of *H. pylori* GGT protein for hydrolysis was more than 3.5 times higher than that (23.8 μmol/min per milligram of protein) for γ-glutamyl transfer when used γ-GpNA as a donor of γ-glutamyl moiety and gly-gly as an acceptor. The relative activities between γ-GpNA and D-Gln for hydrolase and transpeptidase showed that both enzymatic activities may be similar when used D-Gln as a donor (Table 2).

The thermal stability of *H. pylori* GGT was better than that of other species' GGT because it kept approximately 80% of the initial enzymatic activity on incubation at 60℃ for 15 min. The optimum temperatures of hydrolysis and transpeptidation of *H. pylori* GGT were near body temperature (Fig. 3). The optimum pH was more alkaline than other bacterial GGTs (Fig. 3). Therefore, the activity of *H. pylori* GGT in the gastric mucosa may be maximized in concert with its urease activity.

In the contrary to recombinant GGT (32-7), the apparent *Km* values of *H. pylori* GGT for γ-glutamyl donors and acceptors in the transpeptidation reaction and that of donor in hydrolysis reaction were ordinary when compared with those of mammalian and other bacterial GGTs (Table 4 & Fig. 4). Like other bacterial GGTs, transpeptidation is not thought to be the major function because its *Km* values for γ-glutamyl donors are low while those for γ-glutamyl acceptors are high (27, 29).

Several amino acids inhibited *H. pylori* GGT activity as other bacterial GGTs (Table 5) (27, 29). *H. pylori* GGT activity was strongly inhibited by L-Gln and was weakly inhibited by other amino acids when compared with *P. mirabilis* GGT. DON, azaserine, and L-Ser with borate that were known as inhibitors of *E. coli* GGT strongly inhibited *H. pylori* GGT (Table 6).

There is little evidence that GSH is important to the maintenance of the redox balance in *H. pylori*. On the basis of genome analyses, *H. pylori* does not appear to contain homologs of the genes encoding for the synthesis of glu-

tathione (γ-glutamylcysteine synthetase) and the reduction of oxidized glutathione (glutathione reductase). It was reported that reduced glutathione level of gastric mucosa ranges from 0.3 to 1.7 mM in chronic gastritis (47), supporting that *H. pylori* GGT is sufficient to degrade the glutathione in gastric mucous layer. Since the localization of *H. pylori* GGT remains to be determined, we couldn't conclude that the presence of *H. pylori* leads to bacterial breakdown of glutathione in the gastric mucous layer. This problem has to be resolved in the future investigation.

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