J. Korean Soc. Microbiol., Vol. 34, No. 6, 1999

Activation of Urease Apoprotein of *Helicobacter pylori*

Myung-Je Cho, Woo-Kon Lee, Jae-Young Song, Young-Sook An, Sang-Haeng Choi, Yeo-Jeong Choi, Seong-Gyu Park, Mi-Young Choi, Seung-Chul Baik, Byung-Sang Lee and Kwang-Ho Rhee

Department of Microbiology, Gyeongsang National University College of Medicine, 92 Chilam-dong, Chinju, Kyung-Nam 660-280, Republic of Korea

H. pylori produces urease abundantly amounting to 6% of total protein of bacterial mass. Urease genes are composed of a cluster of 9 genes of *ureC, ureD, ureA, ureB, ureI, ureE, ureF, ureG, ureH*. Production of *H. pylori* urease in *E. coli* was studied with genetic cotransformation. Structural genes *ureA* and *ureB* produce urease apoprotein in *E. coli* but the apoprotein has no enzymatic activity. *ureC* and *ureD* do not affect urease production nor enzyme activity *ureF, ureG*, and *ureH* are essential to produce the catalytically active *H. pylori* urease of structural genes (*ureA* and *ureB*) in *E.coli*. The kinetics of activation of *H. pylori* urease apoprotein were examined to understand the production of active *H. pylori* urease. Activation of *H. pylori* urease apoprotein, pH dependency, reversibility of CO_2 binding, irreversibility of CO_2 and Ni^{2+} incorporation, and CO_2 dependency of initial rate of urease activity have been observed *in vitro*. The intrinsic reactivity (*ko*) for carbamylation of urease apoprotein coexpressed with accessory genes was 17-fold greater than that of urease apoprotein expressed without accessory genes. It is concluded that accessory genes function in maximizing the carbamylating deprotonated ε-amino group of Lys 219 of urease B subunit and metallocenter of urease apoprotein is supposed to be assembled by reaction of a deprotonated protein side chain with an activating CO_2 molecule to generate ligands that facilitate productive nickel binding.

Key Words: Urease, *H. pylori*, Apoprotein activation

INTRODUCTION

The habitat of *H. pylori* is highly restricted to the gastric mucus layer of human (11,19). It means that the bacteria has adopted a highly efficient strategy for survival at extremely acidic environment. The massive production of urease with high affinity for urea might be one of the strategies (6, 12).

The *H. pylori* urease is a 550-kDa multimeric enzyme comprising of two distinct subunits of 26.5 kDa and 61.6 kDa (1, 6, 12). Urease genes are located to a single 8.6 kb region of *H. pylori* chromosome (17). Nucleotide sequences of *H. pylori* urease genes were analyzed to be nine open reading frames that were designated *ureC, ureD, ureA, ureB, ureI, ureE, ureF, ureG*, and *ureH* (4, 17). Of nine urease genes, *ureA* and *ureB* are iden-

Received for publication: 2000. 2. 12, Accepted for publication: 2000. 2. 12
Corresponding author: Myung-Je Cho, Tel) 82-591-751-8747, Fax) 82-591-759-1588 e-mail) mjecho@gshp.gsnu.ac.kr, Jae-Young Song, Sang-Haeng Choi, Mi-Young Choi, Seong-Gyu Park, and Byung-Sang Lee are supported by Brain Korea 21 Project of the Ministry of Education. Yeo-Jeong Choi is a recipient of the Internship Program supported form KOSEF.
This work was partially supported by KOSEF (941-0700-037-2).

tified as the two structural peptides of urease (13) and *ureC, ureD, ureI, ureE, ureF, ureG,* and *ureH* are named as regulatory genes and accessory genes of which products were supposed to be involved in generating catalytically active enzyme (4, 1).

In the case of *K. aerogenes*, urease apoprotein could be partially activated *in vitro* by supplementation of dissolved CO_2 in addition to Ni^{2+} ion. Activation test and crystallographic study of *K. aerogenes* urease suggested that CO_2 reacting with ε-amino group of Lys 217 is required prior to nickel binding (7). Transposon mutagenesis in *H. pylori* suggested that accessory genes are essential for production of catalytically active urease (4). The products of accessory genes were supposed to be involved in assembling Ni^{2+}-metallocenter. However, a motif for Ni^{2+} ions could not be found in accessory gene of *H. pylori* urease in contrast to the case of accessory gene *ureE* of *K. aerogenes.*

To understand the role of accessory genes for production of active urease in *H. pylori*, kinetics of activation of urease apoprotein were analyzed in regards to dissolved CO_2 and Ni^{2+} ion incorporation into urease apoprotein,.

MATERIALS AND METHODS

DNA Manipulations and Preparation of *H. pylori* Genomic DNA. Routine DNA manipulations, plasmid DNA preparation, restriction digestion, and ligation were performed according to the standard methods (31). *H. pylori* strain 51 genomic DNA was prepared with lysozyme (3 mg/ml), RNase (50 μg/ml), protease (0.8 mg/ml), proteinase K (0.5 mg/ml), and cetyltrimethyl-ammonium bromide treatment, phenol-CHC13 and CHC13 extraction, and ethanol precipitation as previously described (33).

Preparation of Cosmid Clones Containing Entire Urease Gene Cluster. *H. pylori* strain 51 genomic DNA was partially digested with *Sau*3AI. Digested genomic DNA ranged from 30 to 40 kb was ligated with SuperCos 1 vector DNA (Stratagene) as manufacturer's protocols. The ligated DNA was subjected to *in vitro* packaging by Gigapack II (Stratagene). Packaged recombinant DNAs were amplified by *E. coli* XL1-blue MR and screened by the [^{32}p]dATP-labeled DNA fragment containing *ureC, ureD, ureA*, and *ureB* which had been obtained from the λt11 recombinant clone (λHPR5) (29). The *Pst*I-*Eco*RI fragment of 17 kb, which was confirmed to contain the entire urease cluster, was recovered by electroelution and then ligated with pTZ19R plasmid vector, which had been cleaved by *Pst*I and *Eco*RI and dephosphorylated, to produce pTZ19R/Ure-1. Plasmid clones containing each and parts of the urease genes cluster were constructed by inserting restriction enzyme-digested DNA fragments into plasmid vectors pTZ19R, pBluescript II K/S, pGKV21, and pACYC184 or by unidirectional deletion.

In Vitro Activation of Urease from the Extracts of *E. coli* Transformants. *E. coli* transformants were grown for 24 h at 37℃ in L broth. Harvested bacteria were washed twice in saline and then disrupted by ultrasonication. Cell debris was removed by centrifugation. Protein concentrations were determined by Lowry's method (23). In vitro activation of urease was performed by the method of Park & Hausinger (20, 30).

SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide gel electrophoresis (PAGE) was performed with 12.5% polyacrylamide running gel of 0.5 mm in thickness and 4.5% polyacrylamide stacking gel by using Laemmli method(18). After electrophoresis, the gel was stained with Coomassie brilliant blue R250.

Immunoblot Analysis. The protein bands resolved by SDS-PAGE were electrotransferred at 140 mA for 2 h into a nitrocellulose membrane by using a Hoeffer Transphor electro-

Table 1. Urease activity and immunoblot analysis of the extracts of *E. coli* transformants carrying the plasmid clones inserted with *H. pylori* urease genes that were grown in the LB broth supplemented with 1 mM of nickel chloride

Clones	Urease activity[a]	Immunoblot[b]
pTZ19R/URE-1	2,330±10.4[c]	+
pTZ19R/AB	−	+
pACYC184/AB	−	+
pTZ19R/IEFGH	−	−
pTZ19R/CD	−	−
pTZ19R/ABIEFGH	1,825 (±23.7)	+
pACYC/CD	−	−
pGKV/CD	−	−
pGKV/CD + pTZ19R/AB	−	+
pGKV/CD+pTZ19R/ABIEFGH	1,623 (±35.3)	+
pACYC/CD+pTZ19R/ABIEFGH	1,647 (±37.5)	+
pTZ19R/AB+pACYC/IEFGH	1,980 (±18.5)	+
pACYC184/AB+pTZ19R/IEFGH	2,130 (±10.8)	+
pACYC184/AB+pTZ19R/EFGH	1,720 (±30.1)	+
pACYC184/AB+pTZ19R/FGH	1,113 (±50.1)	+
pACYC184/AB+pTZ19R/(5'△58)FGH	720 (±30.1)	+
pACYC184/AB+pTZ19R/GH	−	+
pACYC184/AB+pTZ19R/IEF	−	+
pACYC184/AB+pTZ19R/IEFG	−	+
pACYC184/AB+pBlue/GH	−	+
pACYC184/AB+pTZ19R/I	−	+
pACYC184/AB+pBlue/E	−	+
pACYC184/AB+pBlue/F	−	+
pACYC184/AB+pBlue/G	−	+
pACYC184/AB+pBlue/H	−	+
pACYC184/AB+pBluescrptII	−	+
pACYC184/AB+pTZ19R	−	+

a, Urease activity is noted as nmole of urea degraded per min and mg of protein. b, Detection of subunit A and B by monoclonal antibodies. c, Standard deviation.

phooresis unit (Hoefer Scientific Instruments, San Francisco, Calif.) as previously described (34). A mixture of monoclonal antibodies HPB 36 (16), which are reactive to urease subunit A and subunit B, respectively, was incubated with the nitrocellulose membrane for 1 hr at 37℃. The membrane was washed three times for 10 min each in a Tris buffered saline-

tween 20 buffer (TBST, 10 mM Tris-HC1 [pH 8.0], 150 mM NaCI, 0.05% Tween 20) and then incubated with anti-mouse immunoglobulin G conjugated with alkaline phosphatase. The membrane was washed three times with TBST and developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate.

RESULTS

Minimal Genes for Catalytically Active *H. Pylori* Urease in *E. coli*. Table 1 and Fig. 1 demonstrate that the *cis* (pTZ19RURE-1 *vs.* pTZ19R/ABIEFGH of Table 1) and trans (pTZ 19r/ABIEFGH *vs.* pGKV/CD+pTZ19R/ABIEFGH or pACYA184/CD+pTZ19R/ABIEFGH) presence of *ureC* and *ureD* did not affect production of urease structural subunits nor the level of urease activity. To define minimal accessory genes for production of active urease in *E. coli, E. coli* cells carrying pACYC184 clone (medium copy number) of urease structural genes were complemented with pBluescript II K/S clones (high copy number) of accessory genes. Table 1 shows that thd minimal accessory genes for production of active urease were *ure*(5'△58) FGH in which 58 bp downstream sequence from the start codon of *ureF* was deleted *E. coli* transformants complemented with *ureGH*, in which 287 bp downstream sequence from the start codon of *ureF* was deleted, did not produce any catalytically active urease.

***In Vitro* Activation of Urease Apoprotein.** Extracts of *E. coli* cells harboring pTZ19R/AB were used as urease apoprotein. $NaHCO_3$ and $NiCl_2$ were essential for activation of urease apoprotein. Optimal concentrations of $NaHCO_3$ and $NiCl_2$ for *in vitro* activation of urease apoprotein were determined as 50 mM and 200 μM, respectively (Fig. 2 & 3). Optimal pH for *in vitro* activation was ranged from 8.0 to 9.0 (Fig. 4).

Pretreatment of urease apoprotein with $NiCl_2$ prior to provision of bicarbonate, reached half of the normal enzyme activity observed when the urease apoprotein were simultaneously treated with bicarbonate and $NiCl_2$ (Table 2). And

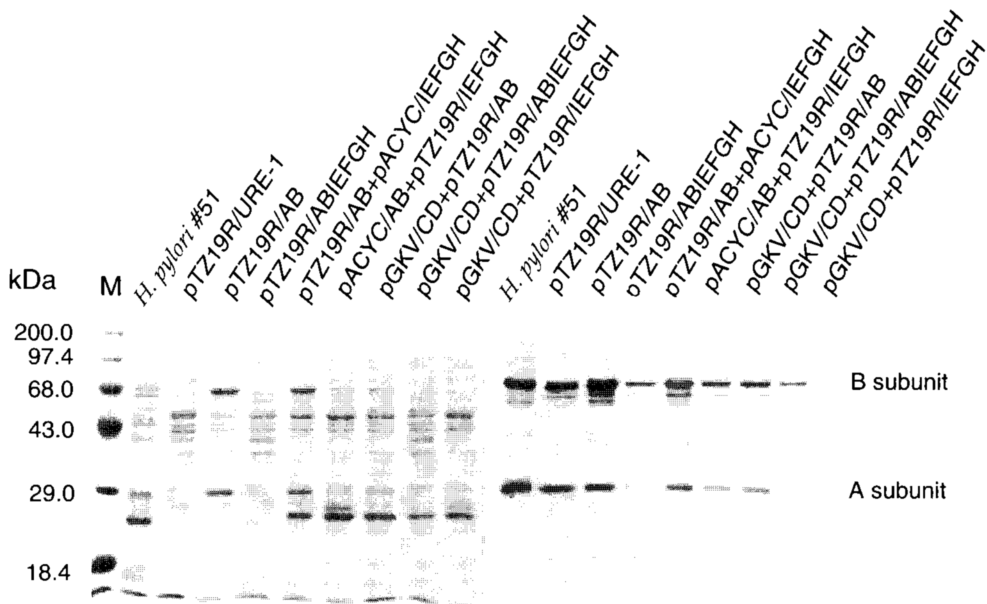


Figure 1. SDS-polyacrylamide gel electrophoresis and immunoblot analysis of *E. coli* transformants which contained plasmid clones containing *H. pylori* genes

Table 2. Effects of carbonic anhydrase and the order in treatment of $NiCl_2$ and $NaHCO_3$ at *in vitro* activation of the extract of *E. coli* cells carrying pTZ19R/AB

Sample	Treatment	Urease activity[a]
Extract	→ $NiCl_2$ and $NaHCO_3$	710±10
	→ $NiCl_2$	0
	→ $NaHCO_3$ → $NiCl_2$	465±4.5
	→ $NiCl_2$ → $NaHCO_3$	362±2.8
	→ $NiCl_2$ and $NaHCO_3$ in the present of carbonic anhydrase	0
Extract	→ $NiCl_2$ and $NaHCO_3$ → dialysisc	700±4.5
	→ $NaHCO_3$ → dialysis → $NiCl_2$	13.5±2.3
	→ $NiCl_2$ → dialysis → $NaHCO_3$	0
	→ $NaHCO_3$ → dialysis → $NiCl_2$ and $NaHCO_3$	705±2.5

a, Urease activity is noted as nmole of urea degraded per min and mg of protein. b, Activation buffer in carbonic anhydrase (0.2 mg/ml). c, The mixtures were dialyzed with 100 mM Hepers buffer (pH 8.3).

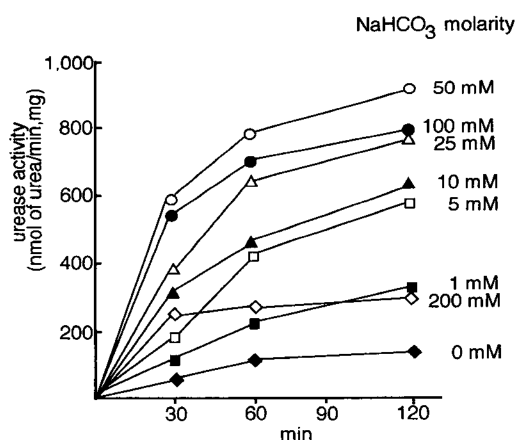


Figure 2. Optimal concentration of $NaHCO_3$ for *in vitro* activation of urease apoprotein

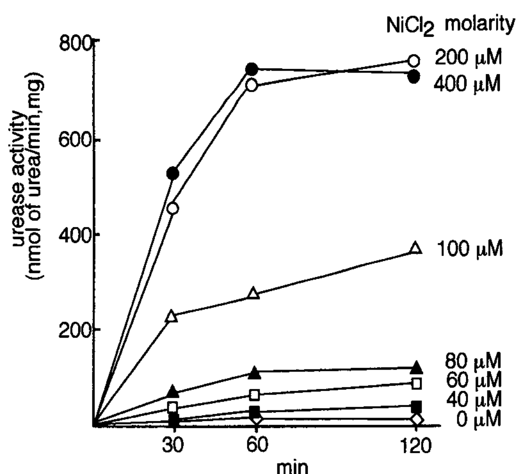


Figure 3. Optimal concentration of $NiCl_2$ for *in vitro* activation of urease apoprotein

pretreatment of bicarbonate activated urease apoprotein better than the pretreatment of $NiCl_2$ did. Addition of carbonic anhydrase into the reaction mixture for *in vitro* activation prevented urease apoprotein from activation. These results indicate that actual activating factor was dissolved CO_2 (Table 2).

Addition of $NiCl_2$ after incubation with bicarbonate and subsequent dialysis to remove remaining bicarbonate and dissolved CO_2 did not activate urease apoprotein, meaning that dissolved CO_2 incorporation to apoprotein is reversible. However, urease apoproteins activated by the incubation of both dissolved CO_2 and Ni^{2+} ion sustained their enzyme activity even though the mixture had been subjected to extensive dialysis for a long time. This result indicates that incorporation of both CO_2 and Ni^{2+} ion generates the stable metallocenter of urease protein (Table 2).

The initial rate of *in vitro* activation of urease apoprotein was examined as a function

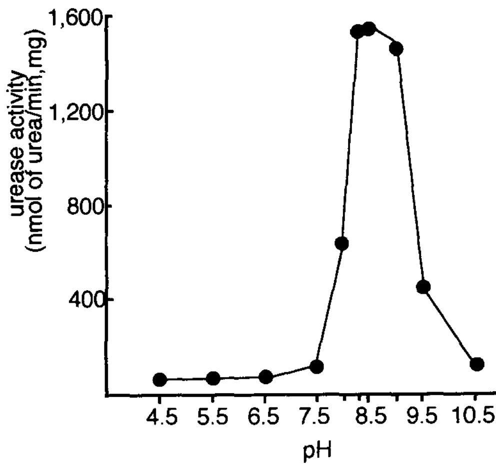


Figure 4. Optimal pH for *in vitro* activation of urease apoprotein

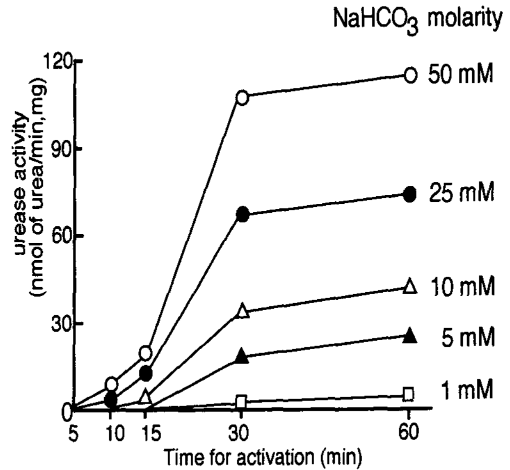


Figure 5. The time course for *in vitro* activation of urease apoprotein by concentration of $NaHCO_3$

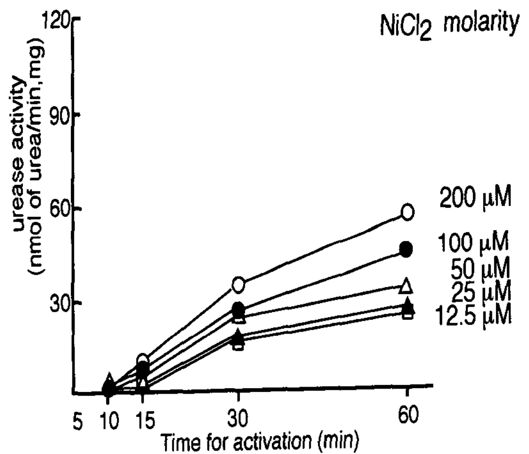
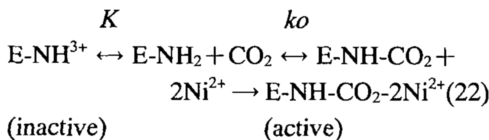


Figure 6. The time course for *in vitro* activation of urease apoprotein by varying concentration of $NiCl_2$

of bicarbonate and $NiCl_2$ concentrations. The results show that the initial rate of activation was proportionally dependent on the bicarbonate concentration more than on $NiCl_2$ concentration as shown in Fig. 5 & 6.

Effect of Accessory Genes on Intrinsic Reactivity (*ko*) for Cabamylating Apoprotein Side Chain. pH dependency, reversibility of CO_2 binding, irreversibility of CO_2 and Ni^{2+} incorporation, and CO_2 dependency of initial rate at *in vitro* activation of *H. pylori* urease apoprotein are postulated to be consistent with the ordered equilibrium mechanism for carbamylating ε-amino group of Lys 219 (corresponding to Lys 217 of *K. aerogenes* urease) in urease B subunit described by:



$$\text{E-NH}^{3+} \overset{K}{\leftrightarrow} \text{E-NH}_2 + CO_2 \overset{ko}{\leftrightarrow} \text{E-NH-}CO_2 + 2Ni^{2+} \rightarrow \text{E-NH-}CO_2\text{-}2Ni^{2+} \quad (22)$$

(inactive) (active)

in which the observed second order rate should be dependent on the concentration of E-NH-CO_2. The formation of E-NH-CO_2 is pH dependent, because deprotonated amino groups are reactive. With the assumption that the rate of carbamylation of the protonated amino groups is negligible compared with the rate of carbamylation of the deprotonated amino groups, the observed second order rate constant k_2(obsd) reflects the fraction of deprotonated amino groups so that the deprotonation cosntant (K) is given by the equation

$$k_2(\text{obsd}) = koK/(K + [H^+])$$

where *ko* is the intrinsic reactivity for carbamylating the deprotonated group (36). This equation could be converted into a linear form

$$1/k_2(\text{obsd}) = [H^+]/Kko + 1/ko$$

A plot of $1/k_2$(obsd) versus $[H^+]$ give a straight line with an ordinate intercept of $1/ko$

Table 3. Intrinsic reactivity (ko) and pka for carbamylating the deprotonated group

Sample	p*Ka*	*ko* (M^{-1}, min^{-1})
pACYC184/AB+pTZ19R/IEFGH	8.7	152
pACYC184/AB	8.4	9

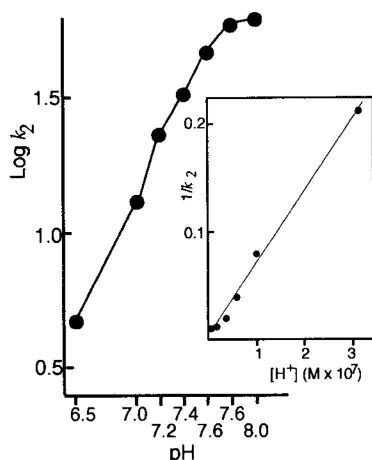


Figure 7. pH dependency of *in vitro* activation of urease apoprotein coexpressed with the entire accessory genes

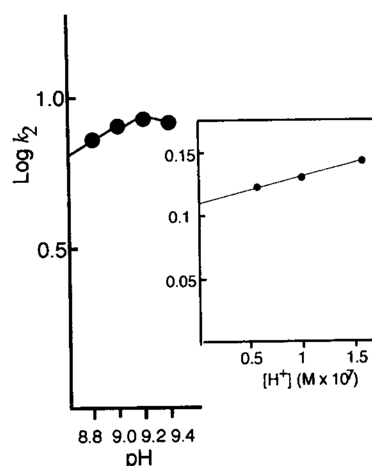


Figure 8. pH dependency of *in vitro* activation of urease apoprotein coexpressed with not accessory genes

and slope of 1/*Kko* which permits determination of *ko* (10). To examine whether products of accessory genes affect intrinsic reactivity (*ko*) for carbamylation of deprotonated urease apoprotein side chain, the extracts of *E. coli* cells cotransformed with both pACYC184/AB and pTZ19R or pBluescript clones containing each or parts of accessory genes were subjected to *in vitro* activation at constant concentration of 50 mM bicarbonate and 200 μM $NiCl_2$ and at varying pH. Fig. 7, 8, and Table 3 show that intrinsic reactivity of urease apoprotein in extracts of *E. coli* cells transformed with pACYC 184/AB and pTZ19R/IEFGH was 152 M^{-1} min^{-1} which was about 17-fold greater than that of urease apoprotein expressed without accessory genes or coexpressed with each or part of accessory genes (Table 3). Deprotonation constant of ε-amino group of Lys 219 in urease B subunit that reacts with dissolved CO_2 (7, 26) was determined as p*Ka* of 8.7 which is acidic when compared with intrinsic p*Ka* values of 10.4-11.1 of ε-amino group of an isolated lysine residue (5).

DISCUSSION

Cussac et al. (17) found that *E. coli* cells transformant of urease genes cluster deleting *ureC* and *ureD* showed greater urease activity than those carrying plasmid with these loci intact. On the contrary, we could not confirm that *ureC* and *ureD* affect the urease activity and urease protein production in *E. coli* cells. Therefore, we conclude that they are not genes for the synthesis and activation of urease apoprotein.

ure(5'△58)FGH, in which 50 bp downstream sequence from the start codon of *ureF* is deleted, was essential for the production of active urease in *E. coli. ureGH*, in which 287 bp downstream sequence from the start codon

of *ureF* is deleted does not produce catalytically active urease in *E. coli*. This is consistent with the previous report that mutant plasmid clones of *H. pylori* urease gene in which *ureF*, *ureG*, or *ureH* had been destroyed by transposon mutagenesis failed to produce the active urease (4). In the sequences of *ureF* that was deleted in the *ureGH*, the motif "FPIGSYTHSFGL" was found to be highly conserved among *ureF*s of various bacterial species like *S. salivarius* (3), *Bacillus* sp. (24), *S. xylosus* (15), *U. urealyticum* (2), and *K. ae-rogenes* (28). The function of this motif deserves to be delineated.

Park and Hausinger (30) observed that dissolved CO_2 was necessary for *in vitro* activation of *K. aerogenes* urease in addition to supplementation of Ni^{2+}-ion. In *K. aerogenes*, UreE was supposed to be a direct Ni^{2+} ion donor to urease apoprotein because it has a histidine-rich motif in its carboxylic terminus (21). On the contrary, Ni^{2+} ion-binding motifs were not found in *H. pylori* urease genes cluster. Nickel binding proteins like heat shock protein (32), NixA (27), P-type ATPases (25), and Hpn (9) of *H. pylori* were reported to be associated with Ni^{2+} ion transport and donation. The presence of nickel binding proteins in *H. pylori* and absence of Ni^{2+} ion-binding motifs in urease genes cluster postulate that polypeptides of *H. pylori* accessory genes were directly associated with incorporation of dissolved CO_2 which could be prerequisite for Ni^{2+} ion binding of urease apoprotein. The initial rate of *in vitro* activation is more dependent on the dissolved CO_2 concentration rather than Ni^{2+} ion concentration as shown in Fig. 6. Urease apoprotein coexpressed with accessory genes is reactive to dissolved CO_2 greater than urease apoprotein coexpressed without accessory genes. Therefore, it is reasonable to propose that products of *H. pylori* accessory genes drive carbamylating reaction of urease apoproteins resulting in converting an inert apoprotein to a competent form for Ni^{2+} ion incorporation.

Lys 219 of *H. pylori UreB*, that corresponds to Lys 217 of *K. aerogenes* UreC, is presumed to react with dissolved CO_2 to produce the carbamate ligand (7, 26). ε-Amino group of Lys 219 should become a deprotonated nucleophile prior to reacting with dissolved CO_2 which is in equilibrium with bicarbonate in solution (8, 37). Therefore, the rate of *in vitro* activation is determined by the concentration of deprotona-ted apoprotein and dissolved CO_2. As $[H^+]$ con-centration in the reaction mixture was increased, the fraction of deprotonated urease apoprotein is inversely decreased whereas dissolved CO_2 concentration is increased (30). Actually, the extract of *E. coli* cells transformed with plasmid clones of urease structural genes did not show urease activity at pH values less than 6.5 and abruptly decreased at pH value of more than 10. The pH of less than 6.5 leads to an extremely low level of deprotonated ε-amino group of Lys 219 under the high dissolved CO_2 concentration and the pH of higher than 10.0 does to an extremely low level of dissolved CO_2 under the high fraction of deprotonated Lys 219.

The fact that intrinsic reactivity (*ko*) for carbamylating the deprotonated amino group of the urease apoprotein coexpressed with the entire accessory genes is higher than otherwise could suggest that products of accessory genes could make urease apoprotein more prone to access of dissolved CO_2.

REFERENCES

1) Austin JW, Doig P, Stewart M, Trust TJ: Macromolecular structure and aggregation states of *Helicobacter pylori* urease. *J Bacteriol* **173:** 5663-5667, 1991.

2) Blanchard A: *Ureaplasma urealyticum* urease gene: use of a UGA tryptophan codon. *Mol Microbiol* **4:** 669-678, 1990.

3) Chen YM, Clancy KA, Burne RA: *Strepto-*

coccus salivarius urease: genetic and biochemical characterization and expression in a dental plaque *Streptococcus. Infect Immun* **64:** 585-592, 1996.

4) Cussac V, Ferrero RL, Labigne A: Expression of *Helicobacter pylori* urease genes in *Escherichia coli* grown under nitrogen-Limiting conditions. *J Bacteriol* **174:** 2466-2473, 1992.
5) Creighton TE: Proteins: structures and molecular properties. 2nd ed. W. H. Freeman and Company. N. Y., PP6, 1993.
6) Dunn BL, Campbell GP, Perez-Perez GI, Blaser MJ: Purification and characterization of urease from *Helicobacter pylori. J Biol Chem* **265:** 9464-9469, 1990.
7) Evelyn J, Carr MB, Hausinger RP: The crystal structure of urease from *Klebsiella aerogenes. Science* **268:** 998-1004, 1995.
8) Fred CH, Milanez S, Eva HL: Ionization constants of two active-site lysyl εamino groups of ribulosephosphate carboxylsase/oxygenase. *J Biol Chem* **260:** 1368-1397, 1985.
9) Gilbert JV, Ramakrishna J, Sunderman FW, Wright A JR, Plaut AG: Protein Hpn: cloning and characterization of a histidine-rich metal-Binding polypeptide in *Helicobacter pylori. Infect Immun* **63:** 2682-2688, 1995.
10) Hartman FC, Milanez S, and Eva HL: Ionization constants of two active-site lysyl εamino groups of ribulosephosphate carboxylase/oxygenase. *J Biol Chem* **260:** 13968-1397, 1985.
11) Hazell SL, Lee A, Brady L, Hennessy W: *Campylobacter pylori* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *J Infect Dis* **153:** 658-663, 1986.
12) Hu LT, Mobley HLT: Purification and N-terminal analysis of urease from *Helicobacter pylori. Infect Immun* **58:** 992-998, 1990.
13) Hu LT, Paul A, Robert R, Mobley HLT: Purification of recombinant *Helicobacter pylori* urease apoenzyme encoded by *ureA* and *ureB. Infect Immun* **60:** 2657-266627, 1992.
14) Hu LT. Mobley HLT: Expression of catalytically active recombinant *Helicobacter pylori* urease at wild-type levels in Escherichia coli. Infect Immun **61:** 2563-2569, 1993.
15) Jose J, Schafer UK, Kaltwasser H: Threonine is present instead of cystein at the active site of urease from *Staphylococcus xylosus. Arch Microbiol* **161:** 384-39246, 1994.
16) Kim JI, Baik SC, Cho MJ, Lee WK, Rhee KH: Purification of the urease of *Helicobacter pylori* and production of monoclonal antibody to the urease of *Helicobacter pylori. J Korean Soc Microbiol* **26:** 531-540, 1991.
17) Labigne A, Cussac V, Courcoux P: Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. *J Bacteriol* **173:** 1920-1931, 1991.
18) Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685, 1970.
19) Lee A, Hazell SL: *Campylobacter pylori* in health and disease: an ecobiolocal perspective. *Microbiol Ecol Health Dis* **1:** 1-6. 1988.
20) Lee MH, Mulrooney SB, Renner MJ, Markowicz Y, Hausinger RP: *Klebsiella aerogenes* urease gene cluster: sequence of *ureD* and demonstration that four accessory genes (*ureD, ureE, ureF, urG*) are involved in nickel metallocenter biosyntesis. *J Bacteriol* **174:** 4324-4330, 1992.
21) Lee MH, Pankratz HS, Wang S, Scott RA, Finnegan MG, Johnson MK, Ippolito JA, Chrisianson DW, Hausinger RP: Purification and characterization of *Klebsiella ae-*

rogenes UreE protein: nickel binding protein that functions in urease metallocenter assembly. *Protein Sci* **2:** 1042-1052, 1993.

22) Lorimer GH, Badger MR, Andrews TJ: The activation of ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetic, a suggested mechanism, and physiological implications. *Biochemistry* **15:** 529-535, 1976.
23) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265-275, 1951.
24) Maeda M, Hidaka M, Nakamura A, Masaki H, Uozumi T: Cloning, sequencing and expression of thermophilic *Bacillius* sp. strain TB-90 urease gee complex in *Escherichia coli. J Bacteriol* **176:** 432-442, 1994.
25) Melchers K, Weitzenegger T, Buhmann A, Steinhilber W, Sachs G, Schafer KP: Cloning and membrane topology of a P type ATPase from *Helicobacter pylori. J Biol Chem* **271:** 446-457, 1996
26) Mobley HLT, Island MD, Hausinger RP: Molecular biology of microbial ureases. *Microbiol Rev* **59:** 451-480, 1995
27) Mobley HLT, Garner RE, Bauerfeind P: *Helicobacter pylori* nickel transport gene *nix*A: Synthesis of catalytically active urease in *E. coli* independent of growth conditions. *Mol Microbiol* **16:** 97-109, 1995.
28) Mulrooney SB, Hausinger RP: Sequence of the *Klebsiella aerogenes* urease genes and evidence for accessory proteins facilitating nickel incorporation. *J Bacteriol* **172:** 5837-5843, 1990.
29) Park CK, Lee W-K, Doh Y-M, Cho M-J, Rhee K-H: Development of diagnostic method of *Helicobacter pylori* infection: I. Molecular cloning and DNA sequencing of urease. *J Korean Soc Microbiol* **26:** 541-552, 1991.
30) Park IS, Hausinger RP: Requirement of carbon dioxide for *in vitro* assembly of the urease nickel metallocenter. *Science* **267:** 1156-1158, 1995.
31) Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: a laboratory manual, 2nd ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
32) Suerbaum S, Thiberge JM, Kansau I, Ferrero R, Labigen A: *Helicobacter pylori hsp*A-*hsp*B heat-shock gene cluster: nucleotide sequence, expression, putative function, and immunogenicity. *Mol Microbiol* **14:** 959-974, 1994.
33) Taylor N S, Fox JG, Akopyants NS, Berg DE, Thomapson N, Shames B, Yan L, Fontham E, Janney F, Hunter FM, Correa P: Long-term colonization with single and multiple strains of *Helicobacter pylori* assessed by DNA fingerprinting. *J Clin Microbiol* **33:** 918-923, 1995.
34) Towbin HT, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76:** 4350-4354, 1979.
35) Weatherburn MW: Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* **39:** 971-974, 1967.
36) Welches WR, Baldwin TO: Active center studies on bacterial luciferase: modification of the enzyme with 2,4-dinitrofluorobenzene. *Biochemistry* **20:** 512-517, 1981.