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Expression of Latent P-Type ATPases and Their Presumptive Roles in Cell Membrane of *Helicobacter pylori*

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Cation motive ATPases on cell membranes of *Helicobacter pylori* were investigated using everted membrane vesicles. Latent ATPases could be ascertained from aggregated vesicle using N,N-dimethylformamide (DMF) and Triton X-100. By contrast, ultrasonication or chloroform treatments caused membranes to be disrupted, resulting in an alteration of sensitivities against azide or vanadate. Considerable amounts of vanadate-sensitive enzymes were identified from vesicle micelles, prepared by the dilution method. These were activated in the presence of either Ni^{2+} or NH_4^+. From studies employing *H. pylori* intact cell systems, we found that ATPase expression of this bacterium was markedly dependent upon air composition. It was interesting that cellular expression of Ni^{2+}- or NH_4^+-motive ATPases was significantly affected by extracellular pH, suggesting that these unique enzymes may physiologically be involved in cellular Ni^2 import and NH_4^+ export, respectively.

In relation to the medical treatment of patients with gastric diseases such as gastritis or peptic ulcers, acid suppression had been used empirically with unproven clinical significance (2). Until late 1980s, therefore, clinical trials in the first-choice modality were virtually completely dependent on antacids, targeted toward gastric parietal histamine-2 receptor and proton pumps (3).

After the discovery of *Helicobacter pylori* in 1982 (20, 21), an enormous number of reports have been made about its etiology in human gastrointestinal tracts. *H. pylori* is currently recognized as a major risk factor for gastric diseases (4, 11). Moreover, its unique ability of being able to survive for long periods in gastric mucosa is thought to contribute to the development of chronisis and recurrence of gastric diseases. *H. pylori* infection is thought to be a prerequisite for duodenal ulcers (12). Furthermore, this bacterium is now accepted as a causative agent for stomach cancer (27). Accordingly, eradication of *H. pylori* is currently a matter of world-wide concern. Unfortunately, however, the administration of antibiotics has not yet been conclusively effective to eradicate *H. pylori* because of its deep colonization and its ambient acidic pH. Antacids incidentally enhance the efficacy of antibiotics to some extent. In any way, complete eradication of *H. pylori* has not been accomplished (6, 13). In fact, because of its infectious nature any human is faced with potential infection by this hostile organism.

H. pylori is a spiral, gram-negative bacterium possessing a bundle of unipolar flagella. This bacterium has been exclusively found in human gastric biopsy specimens (28). Having motility it colonizes the gastric mucosa, and is often found in the tight junctions between surface epithelial cells or even in the secretory canaliculus of the parietal cells (15). *H. pylori* has unique functions being able to adapt against both acidic and neutral pH. At pH 3.0 it can survive for several days *in vitro* (personal observation). Under this condition, *H. pylori* cells exhibit proton-motive force, indicating that the cells extrude excess protons in order to maintain cytoplasmic neutrality (22). This in turn suggests that *H. pylori* has acidophilic potential to combat such ambient acidity *in vivo* (23).

Proton pumps (membrane-bound ATPases) are among the most fundamental enzymes in living systems (25). Cation-dependent P-type ATPases in particular are regarded as being involved in cell turgor maintenance, pH homeostasis or signal transductions (17). These enzymes are widely distributed throughout nature. Bacterial P-type ATPases are trans-membrane proteins, integrated polytopically. Kdp-ATPase was firstly identied from *Escherichia coli* (10). Similar enzymes were subsequently found in bacteria such as *Enterococcus hirae*, implicating roles in H^+ export (1). Recently, bacterial

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Cu^{2+} (34), Cd^{2+}-ATPases (26) were cloned. Also, Melchers *et al.* cloned a gene of unknown ATPase (presummed to be a heavy metal cation transporter) from *H. pylori* (24). Studies on its physiological function by transforming *E. coli* with this gene are being processed in our laboratory.

In our previous report (37) we questioned low ATPase level in cell membranes of *H. pylori* and unusual specific activity. In addition we emphasized the tolerance of the enzyme pool against acidic pH. In this paper, we describe the exhibition of vanadate-sensitive ATPases and their cation motive properties in relation to pH-dependent expression from *H. pylori* intact cells. Also, we will discuss their presumptive role in *H. pylori* physiology.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Strains of *H. pylori* and *E. coli* were generously gifted from Membrane Biology Lab., CURE at WLA/VA Medical Center & UCLA, Los Angeles, California (U.S.A.). Practically, *H. pylori* cells were grown on petri plate at 37°C under limited O_2 concentration (5% O_2, 10% CO_2, 85% N_2) using serum-supplemented brain heart infusion agar medium. If necessary, cultivation was carried out by liquid medium with reciprocal shaking. After incubation, cells were harvested either by scraping out colonies from petri plate or by centrifuging the culture broth at 9,000 g for 10 min. The resulting cell pastes were suspended in 20 mM Tris-HCl buffer (pH 7.4) containing 10% glycerol, and were stored at -80°C before use. For *E. coli*, culture broth was obtained from overnight cultivation in LB medium at 37°C, and centrifuged. Cells harvested were then suspended and stored as above.

Membrane Preparation

Procedures were carried out on ice unless stated otherwise. Frozen cells (*H. pylori* or *E. coli*) were thawed, washed twice by resuspending in buffer A (see below for compositions of buffers: A, B, C). Before disrupting cells, buffer A was replaced by buffer B or buffer C. Cells were passed 3 times through a French Press (16,000 psi; suspended in buffer B at 4°C). In case of ultrasonication, cells were suspended in buffer C and preincubated for 30 min on ice (50 W, 10 min). Following centrifugation of the resulting cell debris (9,000 g, 10 min), the supernatant was successively applied to ultracentrifugation at 190,000 g for 1 h. The sedimented pellet was washed several times with 1 mM HEPES-Tris buffer (pH 7.4) to remove unwanted organic or inorganic molecules. The resultant membrane vesicles were then carefully resuspended in 20 mM Tris-HCl buffer, pH 7.4. The vesicle suspension was either directly used, or was stored at -80°C before use. Individual buffer compositions (33) were as follows; Buffer A: 10 mM $MgCl_2$, 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate (HEPES) with Tris base to be pH 7.4. Buffer B: 10 mM $MgCl_2$, 1 mM 1,4-dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10% glycerol, 50 mM HEPES-Tris buffer, pH 7.4. Buffer C: buffer B plus 10 mM [ethylenedinitrilo]tetraacetate (EDTA), 0.3 M sucrose and 0.1 mg lysozyme/ml.

Intact Cell System (19)

H. pylori cells grown for 2 days by liquid cultivation (logarithmic phase; O.D. 0.2 at 660 nm) were harvested by centrifugation at 9,000 g for 10 min, washed twice by resuspending in 50 mM HEPES-Tris buffer containing 10 mM $MgCl_2$ (pH 7.4) and centrifuged again. Cell pellet obtained was then homogeneously suspended in 50 mM KPO_4 (pH 7.4) containing 0.2 M NaCl and 2% glycerol to give final turbidity of 5.0 (O.D. at 660 nm). Under these conditions, cell motility or ATPase activities were stable for 2 days on ice. Portions of this suspension (0.1 ml) were added into reaction mixtures consisting of 1% glucose and 20 mM $MgCl_2$ with different pHs, adjusted by conc. HCl or NaOH. The intact cell systems were incubated at 37°C for 2.5 h under controlled composition of air as used in *H. pylori* cultivation. Following incubation, cells were disrupted using French Press, and everted membrane vesicles were prepared as described above.

ATPase Assay

Typically, the reaction mixture in a final volume of 0.9 ml was composed of membrane vesicles (100 μg proteins), 2 mM $MgCl_2$, 0.25 M sucrose and 20 mM Tris-HCl, pH 7.4. If necessary, ATPase inhibitors or ionophores were added and preincubated. Following the addition of 0.1 ml of 20 mM ATP on ice, the reaction mixture was transferred to a water bath, and then incubated for 10 min at 37°C. Enzyme reaction was terminated on ice by adding 1 ml of 12% perchloric acid containing 3.6% ammonium molybdate, and placed at room temperature for 5 min to form molybdophosphate adducts. The resulting adducts were then extracted by 2 ml of prechilled *n*-butylacetate with vigorous vortex. The amounts of ATP hydrolyzed were determined spectrophotometrically by measuring the absorbance of adducts in the organic phase at 320 nm (ε_{320}=7,500 $M^{-1}cm^{-1}$) (37).

Protein Determination

Protein concentration was determined according to the method of Lowry *et al.* (18) using bovine serum albumin as a standard.

Chemicals

Sodium azide and ammonium molybdate were obtained from Fisher Scientific. Perchloric acid, sodium orthovanadate were purchased from GMS Chemical (U.S.A.) and Sigma, respectively. All other reagents were of analytical grades.

RESULTS

Determination of Latent ATPase Activity in Vesicle Micelles from *H. pylori*

In our previous report (37) it was noted that the membrane-resident ATPase pool of *H. pylori* was considerably lower in terms of specific activity. We found this was mainly due to the aggregative nature of the membrane vesicles. In fact, once *H. pylori* cells are precipitated, the pellet exhibits a gummy property. Buffered membrane vesicles are readily precipitated, suggesting that the vesicles aggregate each others. An experiment was, therefore, carried out in order to eliminate aggregates as much as possible. The resulting data is shown in Table 1. In the presence of N,N-dimethylformamide (DMF) vesicle aggregates easily changed to micelle forms. As a result ATPase activity was increased by over 50%. There data indicate that DMF perhaps somehow reduces the hydrophobic interaction between membranes. The vesicle micelles could also be obtained simply by diluting the vesicles with a hypertonic solution. By contrast, however, DMF treatment of *E. coli* membranes caused a decrease in enzyme activity.

During this experiment it was found that membrane ATPase activity was apparently increased by standing at 37°C before ATP addition. As can be seen in Fig. 1, ATPase activity of vesicle micelles was gradually increased by increasing the incubation time up to 30 min. Also, the raised enzyme activity was maintained without significant change after prolonged incubation. We presumed this was occasioned by structural randomization of membrane integrity. Accordingly, it was assumed that such conformational looseness would also be achieved by ultrasonication. Table 2 shows the effect of ultrasonication time on enzyme activity. The maximal enzyme activity was observed by treating cell debris for 15 min. Enzyme activity in the supernatant was continuously enhanced with time, suggesting the liberation of the soluble F1-domain of F-type ATPase (5).

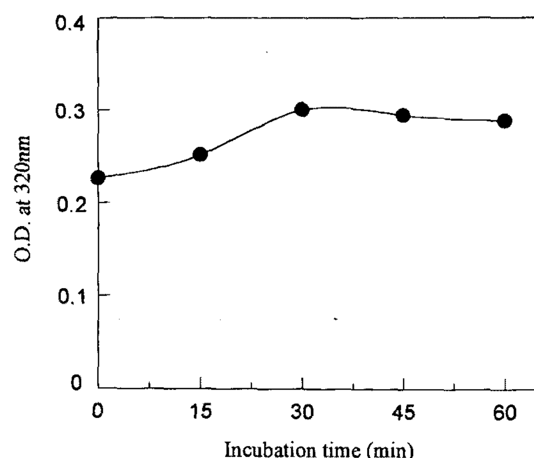

Fig. 1. Effect of preincubation on ATPase activity in everted membrane vesicles from *H. pylori*.

Membrane vesicles (300 μg proteins/ml), suspended homogeneously in 20 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EGTA, were preincubated at 37°C for given periods before the addition of ATP.

Table 1. Effect of N,N-dimethylformamide (DMF) on ATPase activity in membrane aggregates from *H. pylori*.

Buffered membrane vesicles	Amounts of ATPase (U)*			
	H. pylori		*E. coli*	
	(-) DMF	(+) DMF	(-) DMF	(+) DMF
Initial suspension	0.031	0.032	0.295	0.273
1st crop	0.022	0.028	0.026	0.009
2nd crop	0.016	0.025	0	0
3rd crop	0.014	0.024	0	0
4th crop	0.008	0.021	ND	ND
5th crop	0.006	0.018	ND	ND
Sum	0.097	0.148	0.321	0.282

Membrane pellets harvested by ultracentrifugation (190,000 g, 10 min) were homogeneously suspended to give 1 mg of membrane-proteins per ml in 20 mM Tris-HCl buffer containing 0.25 M sucrose (pH 7.4). Most of membrane vesicles of *H. pylori* precipitated significantly after 1 h on ice bath. Individual crops (supernatants) were then consecutively taken by centrifugation at 9,000 g for 10 min with or without 10% DMF (final concentration). The reaction mixture in a final volume of 1 ml containing 0.1 ml of membrane suspension, 0.25 M sucrose, 2 mM $MgCl_2$, 2 mM ATP and 20 mM Tris-HCl (pH 7.4) was incubated at 37°C for 10 min. The enzyme reaction was terminated by the addition of perchloric acid as described in Materals and Methods. *One unit (U) defines 1 μmole of ATP hydrolyzed per minute at 37°C, pH 7.4.

Table 2. Effect of ultrasonic treatment on ATPase activity resided in *H. pylori* cell debris.

Ultrasonic treatment (min)	O.D. at 320 nm		
	sup	ppt*	Sum
0	0.058	0.245	0.303
5	0.079	0.240	0.319
10	0.079	0.319	0.319
15	0.080	0.339	0.419
20	0.097	0.221	0.318
25	0.101	0.153	0.254
Intact cell debris (ppt)[a]	0.037	0.301	0.338
Membrane vesicles (sup)[b]	–	–	0.102

[a] Intact cell debris was obtained by centrifugation (9,000 g × 10 min) after 3 times of passage through French Press (16,000 psi). The precipitated cell debris was suspended in 20 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EGTA. Following centrifugation at 9,000 g for 10 min, the precipitate was then resuspended in 1 ml of buffer B (see Materials and Methods) containing 0.25 M sucrose, 1 mM EGTA and 0.1 mg lysozyme on ice. After 30 min incubation of the suspension, time course ultrasonication (50 W) was carried out. [b] Membrane vesicles (sup) were sufficiently diluted (100 μg/ml in 20 mM Tri-HCl buffer, pH 7.4) in order to prevent aggregation. *The precipitates were taken by bench-top centrifugation (10,000 rpm, 2 min).

Intending to disturb the aggregation of membranes, the effect of Triton X-100 was examined. Data in Fig. 2 illustrate how enzyme activity was accelerated in proportion to the concentration of Triton X-100 up to 0.15%. However, most activity increased by this detergent was in consequence of the solubilization of enzymes, not sedimentable by ultracentrifugation (data not shown). Furthermore, the increased enzyme activity was markedly decreased at higher concentrations of Triton X-100, suggesting the structural derangement of enzyme molecules.

P-type ATPase Assay Using Everted Membrane Vesicles from *H. pylori*

Previously, we reported composite ATPases of *H. pylori* membranes in comparison to gastric parietal (35) and *E. coli* membranes. In order to ascertain the usability of ATPase inhibitors (7) for quantifying activity of particular ATPase, Kdp-ATPase (a P-type ATPase) found in the cell membranes of *E. coli* was examined as an example. The enzyme was motivated by combined use of KCl and nigericin. Progress curves for ATP hydrolysis were taken and are shown in Fig. 3. It shows the alterable enzyme activity either in the presence of 20 mM KCl instead of 2 μg nigericin, or in the presence of both KCl and nigericin. The time-course profiles of ATP hydrolysis were identical within 10 min of enzyme reaction. This observation demonstrates that within a short time of incubation K^+ penetration into vesicles may be enough to activate Kdp-ATPase, provided that KCl is present in sufficient quantities in the medium. The ATPase activated

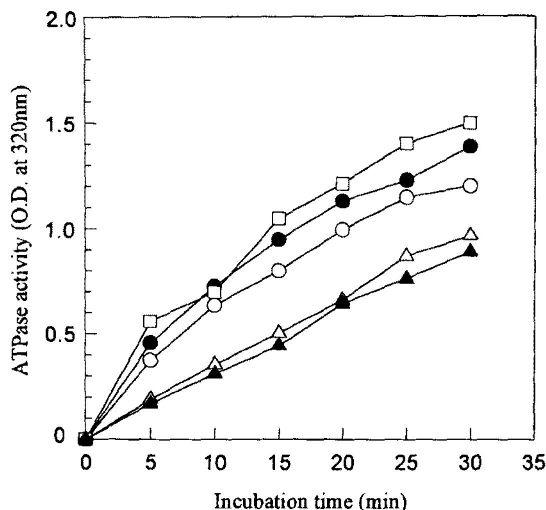

Fig. 3. Determination of Kdp-ATPase on everted membrane vesicles of *E. coli.*

Membrane vesicles (1 ml) were incubated without (○) or with 20 mM KCl (●) and /or extra 2 μg nigericin (□). And the resulted nigericin-containing vesicles were treated with 0.05 mM azide (△) or, in addition, extra 0.1 mM vanadate (▲) for 2 min before the addition of ATP.

by KCl was inhibited by 0.1 mM vanadate. Computing the relative degree of reduction, the corresponding enzyme could be determined as being 1/4 the level of basal P-type ATPases. These data suggest that P-type ATPase can easily be assessed by this method, avoiding the use of radioactive ATP. In this manner the percent content of the P-type ATPase pool in *H. pylori* was measured. As can be seen in Fig. 4, the depressed enzyme pool with azide appeared to give two kinds of inhibitory slopes with vanadate, having

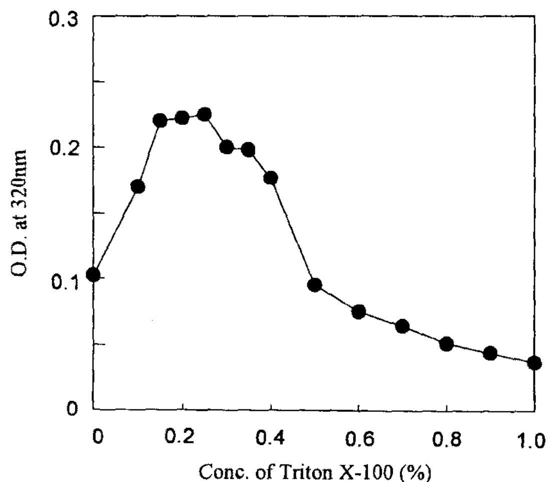

Fig. 2. Effect of Triton X-100 on *H. pylori* membrane ATPase activity.

Vesicle suspensions (200 μg proteins in 200 μl buffer B) with various concentrations of Triton X-100 were placed on ice for 30 min, followed by diluting with same buffer to give final volume of 1 ml (see Materials and Methods for buffer composition). After post incubation for 30 min on ice, portions of the resulted mixtures were examined for ATPase assay.

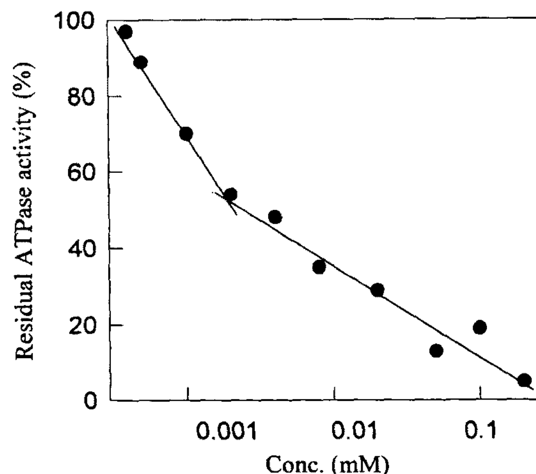

Fig. 4. Vanadate sensitivity of *H. pylori* membrane ATPases.

Vesicles were primarily treated with 0.1 mM azide for 10 min at 37°C before employing this experiment. Vanadate treatment was for 2 min at 37°C.

an intercept at 0.002 mM vanadate. At this concentration of vanadate, more than one-half the amount of the azide-tolerant pool remained. The slope with a gentle gradient taken at higher concentrations of vanadate was perhaps caused by extra ATPases such as V-type ATPase, not inhibitory against the aforementioned inhibitors.

Alterability in Relative Ratio of Composite ATPases of *H. pylori*

By treatment with organic solvents, everted membrane vesicles may release the F1-domain. In consequence, confusion in assessing composite enzymes can occur. It is of importance, therefore, to establish appropriate conditions for particular vesicles. An experiment using chloroform was undertaken and the data is presented in Table 3. After treatment with 10% chloroform, only a few portions of ATPase activity were found in the precipitate with alteration of the inhibitory patterns. This may explain the solubilization of peripheral P-type domains in addition to the F-1 subunit (8) by chloroform. Whereas, considerable amounts of ATPase activity were found in the two phases of the supernatant, fractionated evenly toward both phases. Astonishingly, ATPase activity appeared in the supernatant and became 10-fold more tolerable against vanadate than that of the control. This altered inhibitory specificity of the overall ATPase pool perhaps happened due to conformational disruption of enzyme catalytic sites.

Concentrations of both O_2 and CO_2 are crucial factors for *in vitro* growth of *H. pylori* (31). Previously, we suggested the possibility that ATPase expression of *H. pylori* might be affected by air composition (29). In this study we observed some interesting results as shown in Table 4. In a defined system (see Intact Cell System in Materials and Methods) enzyme sensitivities toward inhibitors were significantly changed with air conditions. Under atmospheric condition containing the highest concentration of oxygen, overall ATPase activities in membrane vesicles were significantly reduced. Moreover, enzyme sensitivities against inhibitors were reversely altered, if it were compared to that found in a controlled composition of air. It was interesting that azide sensitivity was the highest under the condition most suitable for *H. pylori* growth. These observations suggest that membrane ATPases may play an important role in *H. pylori* cells being able to adapt against accidental changes in air conditions.

Table 3. Alteration in inhibitory sensitivity of *H. pylori* membrane ATPases by chloroform treatment.

ATPase found	Relative ATPase activity (%) (O.D. at 320 nm)		
	Azide (0.1 mM)	Vanadate (0.25 mM)	None
Control	0.126 (79%)	0.015 (9%)	0.160
Precipitate	0.008 (100%)	0 (0%)	0.008
Supernatant			
– upper phase	0.038 (82%)	0.042 (91%)	0.046
– lower phase	0.046 (94%)	0.047 (96%)	0.049

Everted membrane vesicles were used. To vesicle suspension (1 mg proteins/ml in 20 mM Tris-HCl buffer, pH 7.4) was added chloroform to become 10% in v/v and vigorously mixed. After 5 min on ice the mixture was centrifuged. For ATPase assay, precipitated pellet was resuspended in 20 mM Tris-HCl buffer, pH 7.4.

Table 4. Effect of air conditions on the expression of membrane ATPase from *H. pylori* intact cells.

Air condition	Relative ATPase activity (O.D. at 320 nm)		
	Control	(+) 0.05 mM Azide	(+) 0.1 mM Vanadate
Atmosphere	0.298(100)	0.268(90)	0.083(28)
N_2 blowing	0.344(100)	0.279(81)	0.107(31)
*Controlled	0.568(100)	0.426(75)	0.210(37)

Cells at logarithmic phase were harvested, suspended in buffer C containing 1% D-glucose. The cell suspension was incubated for 1 h at 37°C under different air conditions before employing to French Press. Everted membrane vesicles (100 μg proteins/ml) were used for ATPase assay. *Air composition; 5% O_2, 10% CO_2, 85% N_2. Values in parentheses present residual enzyme activities.

Presumptive *In vitro* pH Regulation of *H. pylori*

In our previous report it was shown that in the presence of particular cations, i.e., Ni^{2+} and NH_4^+, *H. pylori* ATPases were activated without Mg^{2+}. In addition, the induced activities were completely inhibited by vanadate. This fact might indicate the existence of unique P-type ion-motive ATPases on the cell membranes of *H. pylori*. Based on the data of Table 5, it can be proposed that Ni^{2+} would function not only to correspondingly motivate particular ATPase, but also to form a complex with ATP as an alternative to Mg^{2+}. The latter possibility, however, can not be implicated for NH_4^+-dependent enzyme induction. It was interesting that NH_4^+ was among the most effective cations examined for accelerating ATP hydrolysis by everted vesicles (data not shown).

Table 5. Cation-mediated activation of membrane ATPase of *H. pylori*.

Cation	Concentration (mM)	ATPase acitvity (O.D. at 320 nm)	
		(-) vanadate	(+) vanadate
None	–	0.025	0.023
Mg^{2+}	2	0.130	0.016
Ni^{2+}	0.5	0.170	0.019
NH_4^+	50	0.271	0.021
Mg^{2+}/Ni^{2+}	21/0.5	0.159	0.018

Vesicle suspensions (100 μg proteins/ml in 20 mM Tris-HCl buffer, pH 7.4) were preincubated with 0.05 mM azide and cations for 20 min at 37°C before the addition of ATP (or with 0.1 mM vanadate). For divalent cation experiment, extra 2 μg ionomycin was added.

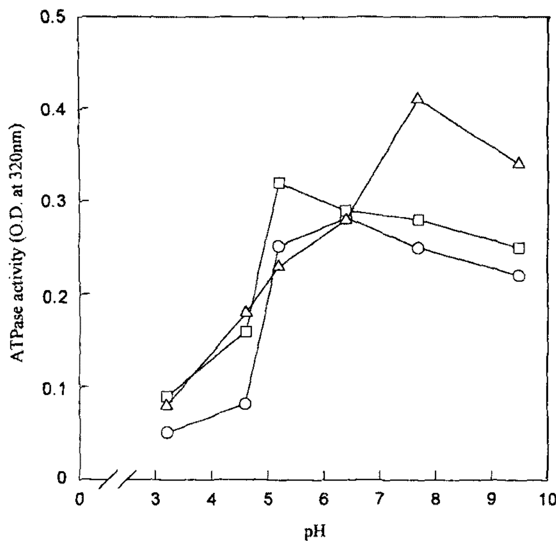

Fig. 5. Effect of pH on the expression of cation motive ATPases in cell-membrane of *H. pylori*.

H. pylori intact cells were incubated at different pHs for 2.5 h under defined system (see Materials and Methods for condition). Following the preparation of everted membrane vesicles, ATPase activity was measured in the absence (○) or presence of Ni^{2+} (□) and NH_4^+ (△).

Evidently, the above cations are important for *H. pylori* life because of their physiological significance. That is, Ni^{2+} is an essential factor for urease maturation, and NH_4^+ is produced by this enzyme. The cells have to control intracellular concentrations of these cations across the cell membranes. Because *H. pylori* occupies gastrointestinal tracts as a unique ecological niche, it was presummed that the ambient pH of *H. pylori* would be closely related to these cations. Accordingly, an experiment employing *H. pylori* intact cells was carried out to clarify how these cations affect related ATPases, or vice versa, with medium pH. Under defined conditions, the basal Mg-ATPase activity was highest at about neutral pH and severely decreased below pH 5.2. Whereas, ATPases motivated by Ni^{2+} or NH_4^+ were shown to exhibit their own pH optimums at pH 5.2 and 7.4, respectively (Fig. 5). Interestingly, the residual activities of these enzymes in an acidic medium (below than pH 4.6) were quite similar, suggesting that the cation-motive enzymes would participate in the cell's physiology against acidic ambience *in vivo*.

DISCUSSION

In the course of our study on ATPases existing in cell membranes of *H. pylori*, we found some unusual properties of everted membrane vesicles, readily precipitable in aqueous solution. This observation suggests that the vesicles prepared would contain certain constituents, causing the vesicle's aggregation. Recent studies on *H. pylori* membranes reveal that the cell membranes contain unique glycolipids, cholesteryl-α-D-glucosides (CGs), as components (14, 32). These CGs, along with lipopolysaccharide, are considered as potential endotoxins. In our opinion large amounts of CGs perhaps help membrane vesicles to become aggregates. Equally, previously observed low ATPase activity from the vesicles might be due to this effect. Intending to quantify the overall ATPase activities in the vesicles, we attempted to perturb their aggregation by using organic solvents or detergents. In Table 1, employing DMF latent ATPase activity was ascertained from vesicles, accompanied by an increase in vesicle micelles. Similarly, Triton X-100 could also be useful to prevent aggregation, resulting in a considerable increase in the whole level of ATPase activities. In this study, however, we did not use these reagents, but we employed the dilution method for further experiments.

ATPase inhibitors are orthodox tools for discriminating between composite enzymes. In this paper data are presented, showing the usability of simple combination of azide and vanadate to determine Kdp-ATPase in *E. coli* membranes (Fig. 3). According to this method *H. pylori* membranes contained at least over 45% of the ATPase pool and were identified as being P-type ATPases. Interpretation based on the slope profile (see Fig. 4) can give rise to a misunderstanding of the data because, in general, ATPases are inhibited nonspecifically under high concentrations of inhibitors.

We found in this study that the cellular expression of vanadate-susceptible ATPases was affected by air composition. This observation suggests that an O_2 concentration is of great importance to the production of P-type ATPases from *H. pylori*. Also, it was notable that most of these were activated by Ni^{2+} and NH_4^+. Interestingly, these cations are unique to *H. pylori*. *H. pylori* cells produce urease copiously, and this enzyme is now believed to have the role of neutralizing ambient acidity of the cells (9). Urease requires 5~6 numbers of Ni^{2+} per molecule for its maturation. But, this metal ion is thought to be among the most rare minerals in dietary sources. Therefore, cells must specifically uptake this metal ion. In this regard Ni^{2+}-motivated ATP hydrolysis seems to be of great interest. Since its motivation was suppressed by vanadate, it was suggested that the corresponding enzyme should be a P-type ATPase. As shown in Fig. 5 this enzyme was most stable at a slightly acidic pH. Since *H. pylori* is exposed to acid in the stomach (30), this observation may be of special significance *in vivo*. On the other hand, the NH_4^+-motive enzyme showed its optimal stability at a neutral pH, suggesting that Ni^{2+} and NH_4^+ would participate in different P-type ATPases, respectively. It was also noteworthy

that the cells maintained such activities at pH 3.2, known to be a borderline pH for *H. pylori* survival (30). At this pH, neither cytoplasmic nor peripheral ATPase activities could be detected. The exhibition of these ion-motive ATPases under such a lethal pH suggests strongly that this bacterium would be protected from intracellular acidification by their possible role of proton pumping.

Under acidic conditions, gastric parietal cells are readily killed by the presence of NH_4Cl *in vitro*, because the cells can't extrude intracellular NH_4^+ (16, 36). In contrast, the NH_4^+-dependent ATPase in *H. pylori* seems to have a function of diminishing the excess amount of H^+ by which the enzyme extrudes NH_4^+ as an alternative to H^+. Although this postulation may be beyond the scope of this study, NH_4^+ export in conjunction with Ni^{2+} import would be of natural significance in *H. pylori*, because production of urease is thought to be one of the cell's adaptive characteristics against gastric acidity.

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