JOURNAL OF CLINICAL MICROBIOLOGY, May 1988, p. 831–836
0095-1137/88/050831-06$02.00/0

Vol. 26, No. 5

Characterization of Urease from *Campylobacter pylori*

HARRY L. T. MOBLEY,[1]* MANUEL J. CORTESIA,[1] LINDA E. ROSENTHAL,[2] AND BRADLEY D. JONES[1]

Divisions of Infectious Diseases[1] and Gastroenterology,[2] Department of Medicine, School of Medicine, University of Maryland, Baltimore, Maryland 21201

Received 10 November 1987/Accepted 20 January 1988

***Campylobacter pylori*, a suspected agent of gastritis and peptic ulceration, rapidly hydrolyzes urea. Because urease serves as the basis of detection of the organism in gastric biopsies and may represent an important virulence factor, biochemical characteristics of the enzyme were determined. *C. pylori* was isolated from antral biopsies from 10 patients with complaints of abdominal pain or history of peptic ulcer disease. All isolates were urease positive, with an average rate of hydrolysis by cell lysates being 36 ± 28 μmol of NH_3 per min per mg of protein, more than twice that of *Proteus mirabilis* and 10 times that of other urinary tract isolates. The enzyme had an apparent molecular weight of 625,000 ± 15,000 by column chromatography, an isoelectric point of 5.9, a K_m of 0.8 ± 0.1 mM urea, an optimal temperature of 45°C, and an optimal pH of 8.2. Ten isolates tested produced ureases with identical electrophoretic mobilities on nondenaturing 5% polyacrylamide activity gels. Acetohydroxamic acid (100 μg/ml), hydroxyurea (85 μg/ml), flurofamide (0.05 μg/ml), and EDTA (8 mM) inhibited enzyme activity by 50%. Cell lysates retained 50% of initial urease activity after 6 days and 40% activity after 18 days when stored at 4°C in 20 mM sodium phosphate, pH 6.8. At −70°C for 18 days, 1 mM EDTA or 15% glycerol preserved 40 or 34%, respectively, of initial activity. The urease of *C. pylori* appears to be biochemically unique from the enzymes of other common urease-producing species.**

Campylobacter pylori, a spiral-shaped gram-negative microaerophilic rod which rapidly hydrolyzes urea (6, 16, 29), has recently been implicated as an etiologic agent of gastritis and peptic ulceration (1, 4, 12, 20, 21, 27, 34). *C. pylori* has been demonstrated in patients with gastritis by direct culture or histological examination of biopsied tissue (2, 7, 16, 20), by detection of serum antibodies directed against *C. pylori* (5, 15, 28, 30, 33), by urease activity detected by incubation of biopsied tissue in Christensen urea broth (3, 11, 22, 23, 26), or by liberation of $^{13}CO_2$ from patients fed [^{13}C]urea (8). Because a suitable animal model is not available, little is known about the pathophysiology of *C. pylori*. The bacterium produces a variety of enzymes, some of which may have a role in infectivity or tissue damage. In this regard, the urease of *C. pylori* may permit colonization of the stomach by this acid-sensitive organism by generating ammonia to buffer gastric acid. Ammonia itself may cause tissue injury or be toxic to intercellular junctions. Hazell and Lee (13) hypothesized that urea hydrolysis might alkalinize the gastric mucosa, causing back diffusion of hydrogen ions which may also contribute to mucosal injury.

The urease of *C. pylori* has been described as a highly active enzyme that may be associated with virulence (11, 19), yet this enzyme has not been characterized. In this study, the urease of *C. pylori* was found to be a high-molecular-weight enzyme that demonstrates a high affinity for substrate, catalyzes a rapid hydrolysis of urea, and is sensitive to currently available urease inhibitors. Furthermore, the enzyme is distinct from those produced by common urinary tract isolates of *Proteus*, *Providencia*, *Morganella*, and *Klebsiella species*.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and storage. *C. pylori* strains were isolated from gastric biopsies taken by endoscopy from patients with complaints of abdominal pain or history of peptic ulcer disease. Biopsies were cultured on brucella agar containing 10% sheep blood and Skirrow selective supplement (vancomycin, 10 μg/ml; polymixin B sulfate, 2.5 IU/ml; trimethoprim lactate, 5 μg/ml; Prolab Inc., Scarborough, Ontario, Canada) (32). Cultures were incubated for 4 to 5 days at 37°C in an anaerobic jar with palladium catalyst and activated Campypac (BBL Microbiology Systems, Cockeysville, Md.). Gram-negative spiral microaerophilic bacteria from tiny flat translucent nonhemolytic colonies that tested positive for catalase, oxidase, and urease were identified as *C. pylori*. Organisms were stored in Trypticase soy broth (BBL) supplemented with 15% (vol/vol) glycerol.

Isolates of *Proteus*, *Providencia*, *Morganella*, and *Klebsiella* species were cultured from the urine of patients with urinary tract infections as previously described (14, 25).

Urease preparations. Six to ten agar plates (100 by 15 mm) with confluent growth of *C. pylori* were flooded with 2.5 ml of 20 mM sodium phosphate (pH 6.8), and cells were scraped from the surface with a rubber policeman. Suspended cells were collected in an iced 30-ml centrifuge tube, and the procedure was repeated. Cells were collected by centrifugation (8,000 × *g*, 10 min, 4°C) and washed once in 20 mM sodium phosphate, pH 6.8. The pellet was drained well, and cells were suspended in 5 ml of ice-cold 20 mM sodium phosphate (pH 6.8) and ruptured in a precooled French pressure cell (0.375-in. [0.95-cm] piston) at 20,000 lb/in^2. The lysate was centrifuged (8,000 × *g*, 10 min, 4°C), and the supernatant was collected and used for all assays on the same day of preparation unless otherwise indicated. Protein concentrations were determined by the method of Lowry et al. (18) with bovine serum albumin as a standard. Urease preparations from other species were prepared in a similar fashion as previously described (14, 25).

Spectrophotometric urease assay. Urease activity was quantitated spectrophotometrically as previously described (10, 25). Briefly, enzyme preparation (5 to 50 μl) was added to cuvettes (1-cm path length) containing 3 ml of 3 mM sodium phosphate (pH 6.8), 7 μg of phenol red per ml, and

* Corresponding author.

between 0.25 and 200 mM urea. Optical density at 560 nm (OD_{560}) was followed over time. Reactions were carried out at 23°C, and rates were calculated from linear portions of the curves (0.15 to 0.5 OD units). For determination of optimal temperature, cuvettes containing the assay mixture were incubated at 23, 32, 37, 45, 56, and 70°C.

Nondenaturing polyacrylamide gel electrophoresis. Relative mobilities of native ureases from several bacterial species were determined by electrophoresis on nondenaturing polyacrylamide gels by a modification of the method of Senior et al. (31). Equal volumes of cell lysate and tracking dye (50% [wt/vol] sucrose, 0.1% [wt/vol] bromphenol blue) were mixed, and 50-μl samples were loaded onto a 5.5% polyacrylamide (1:32, bisacrylamide-acrylamide) resolving gel (16 by 16 by 0.15 cm) with a 5% stacking gel. Samples were electrophoresed at 100 V for 18 h at 4°C. Gels were removed and equilibrated with 10 to 15 changes of 400 ml of 0.02% cresol red–0.1% EDTA until the entire gel remained yellow. After being well drained, the gel was flooded with a 1.5% (wt/vol) urea solution. Red bands corresponding to areas of migration of urease were recorded with Plus-X Kodak film.

Isoelectric focusing. Cell lysates (25 μl) were loaded onto 5% acrylamide (1:32, bisacrylamide-acrylamide) containing 3% Ampholine carrier ampholytes, pH 3.5 to 9.5 (LKB Instruments, Inc., Rockville, Md.). Proteins were focused on an isoelectric-focusing apparatus (LKB) at 2.5 W for approximately 6 h. Ferritin was used as a control protein for focusing. Urease bands were visualized as described above for nondenaturing polyacrylamide gel electrophoresis.

Optimal pH. Activity of *C. pylori* urease was determined at different pH values by measuring the release of ammonia from urea with an ammonia electrode (Corning Glass Works, Corning, N.Y.). Urease preparations were made in buffers ranging from pH 2 to pH 10.6. Sodium acetate (15 mM) was used for pH 2, 3, and 4; sodium phosphate (20 mM) was used for pH 5, 6, 7, and 8.2; 25 mM glycine–25 mM sodium hydroxide–25 mM sodium chloride was used for pH 9, 10, and 10.6. Reactions (20 ml) were carried out for 15 min at 23°C and stopped by the addition of 0.2 ml of 10 N sodium hydroxide. The electrode was calibrated with ammonium chloride (10^{-6} to 10^{-1} M) according to the instructions of the manufacturer.

Column chromatography. Molecular weights of urease were estimated by Sephacryl S-300 chromatography. Cell lysates (10 mg of protein in 1 ml) were loaded onto a column (90 by 2.5 cm) equilibrated with 20 mM sodium phosphate (pH 6.8)–0.02% sodium azide. Fractions (5 ml) were collected at a rate of 40 ml/h at 23°C. Samples of fractions (100 μl) were added with equal volumes of 0.04% cresol red–0.2% EDTA in 96-well microtiter trays. Active fractions, indicated by a change in color to red over time, were quantitated by the spectrophotometric assay.

Inhibition of urease activity. Rates of urease hydrolysis were measured spectrophotometrically for urease preparations of a *C. pylori* and a *Proteus mirabilis* isolate in the presence of urease inhibitors. Reaction mixtures contained various concentrations of inhibitors (listed in Table 1). The concentration of inhibitor required to diminish enzyme activity by 50% was calculated by plotting percent inhibition against the concentration of inhibitor. One hundred percent activity was determined in the absence of inhibitor. Acetohydroxamic acid and hydroxyurea were purchased from U.S. Biochemical Co. (Cleveland, Ohio). EDTA, L-arginine hydroxamate, L-lysine hydroxamate, and thiourea were obtained from Sigma Chemical Co. (St. Louis, Mo.). Flurofamide was a gift from Norwich-Eaton Pharmaceuticals (Norwich, N.Y.).

Enzyme stability. Urease preparations were made in 20 mM sodium phosphate (pH 6.8) as described above. Samples (1 ml) were transferred to freezer storage tubes, and additions of EDTA, dithiothreitol, glycerol, or phenylmethylsulfonyl fluoride were made according to the concentrations shown in Table 2. Duplicate samples were stored at 4 and −70°C. Activity of the enzyme solution was determined within 1 h of preparation, and this value was used to define 100% activity. Stored preparations were assayed for activity on days 6 and 18 and expressed as percent initial activity.

RESULTS

Urease activity. For 10 strains of *C. pylori*, a mean rate (± standard deviation) of urea hydrolysis of 36 ± 28 μmol of NH_3 per min per mg of protein was found to be significantly higher than those of *Proteus mirabilis* (14.6 ± 11.8 μmol/min per mg of protein, $n = 27$, $P = 0.002$), *Klebsiella pneumoniae* (0.3 ± 0.3, $n = 5$, $P = 0.016$), *Providencia rettgeri* (4.6 ± 1.4, $n = 7$, $P = 0.011$), *Proteus vulgaris* (5.5 ± 4.9, $n = 6$, $P = 0.021$), *Morganella morganii* (5.2 ± 2.6, $n = 7$, $P = 0.012$), and *Providencia stuartii* (5.5 ± 7.7, $n = 8$, $P = 0.009$) when compared by the unpaired t test (Fig. 1). The urease of *C. pylori* was not inducible by growth in the presence of urea.

Rates of urea hydrolysis measured at various concentrations of urea (0.25, 0.5, 0.75, 1, 2, 5, and 10 mM) demonstrated that the enzyme is rapidly saturated in the millimolar range (Fig. 2). A mean K_m of 0.8 ± 0.1 (standard deviation) mM urea was calculated for 10 isolates from the reciprocal plot (Fig. 2, inset).

Optimal urease activity was observed at pH 8.2 and at 45°C (Fig. 3).

Urease characterization. The electrophoretic mobility of the *C. pylori* urease was distinct from the patterns displayed by *Proteus*, *Providencia*, and *Morganella* species and suggested that the enzyme of *C. pylori* was of a high molecular weight (Fig. 4). Urease preparations from nine isolates from nine biopsied patients demonstrated identical electrophoretic mobilities on a nondenaturing activity gel (Fig. 5).

The molecular weight of the enzyme was estimated by fractionation of soluble cell protein with a calibrated Sephacryl S-300 column. The peak of activity corresponded to an apparent molecular weight of 625,000 ± 15,000.

The isoelectric point of *C. pylori* urease was found to be 5.9, generally less acidic than those of isolates of *Proteus mirabilis* (5.9, 5.4, 5.3, and 5.2), *Providencia rettgeri* (5.2), or *Morganella morganii* (5.4).

Urease inhibitors. To determine the usefulness of specific inhibitors in reducing or eliminating enzyme activity, we determined rates of urea hydrolysis in the presence or absence of various concentrations of the compounds listed in Table 1. Inhibition of urease activity by competitive and noncompetitive inhibitors and divalent cation chelation revealed that flurofamide, acetohydroxamic acid, and hydroxyurea were effective inhibitors (Table 1). In addition, chelation of a divalent cation by EDTA inactivated the enzyme.

Acetohydroxamic acid did not inhibit growth of the organism. No zone of growth inhibition was observed on agar plates around paper disks containing 1 or 5 μg of the enzyme inhibitor.

Urease activity of whole cells and cell lysates. Activity and kinetics experiments were done on soluble enzyme preparations. To determine whether intact cells offer a permeability

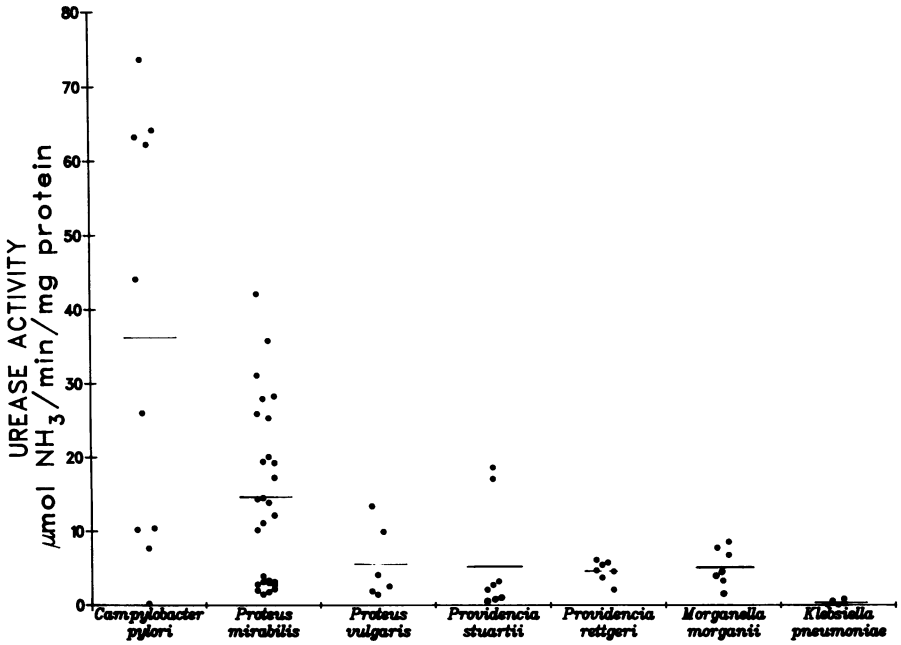

FIG. 1. Urea hydrolysis of *C. pylori* and common urease-producing bacterial species. Cells in suspension were ruptured by passage through a French pressure cell, and insoluble material was removed by centrifugation. Supernatant was assayed for rate of urea hydrolysis by the phenol red spectrophotometric assay with saturating concentrations of urea. Data points represent the means of triplicate determinations. The bar marks the mean urease activity for all isolates within a species.

barrier to urea or to a potentially useful inhibitor, acetohydroxamic acid, we measured urease activity using whole cells and an equivalent amount of cell lysate (Fig. 6). A cell suspension (OD_{550} = 0.5, 1-cm path length) and an identical sample ruptured by two passes in a French pressure cell at 20,000 lb/in^2 were tested for activity. There were no significant differences in rates of hydrolysis between whole and lysed cells, indicating that whole cells do not offer a barrier to the entry of urea. Addition of the inhibitor acetohydroxamic acid at a concentration of 500 μg/ml inhibited activities of both whole cells and cell lysates. Therefore, no cell permeability barrier was offered against either substrate or inhibitor.

To confirm that urease activity is a useful index for the detection of *C. pylori* in the clinical microbiology laboratory, 0.1 ml of suspensions (OD_{660} = 0.2 in 20 mM sodium phosphate, pH 6.8) of whole cells of five *C. pylori* isolates was added to 3 ml of commercial urea broth (Difco Laboratories, Detroit, Mich.) and color change was monitored in the spectrophotometer at 560 nm. Change in absorbance was linear between 0.2 and 0.5 absorbance units. The mean rates for color change were 0.015 ± 0.009, 0.043 ± 0.018, and 0.057 ± 0.030 absorbance units per min at 23, 37, and 43°C,

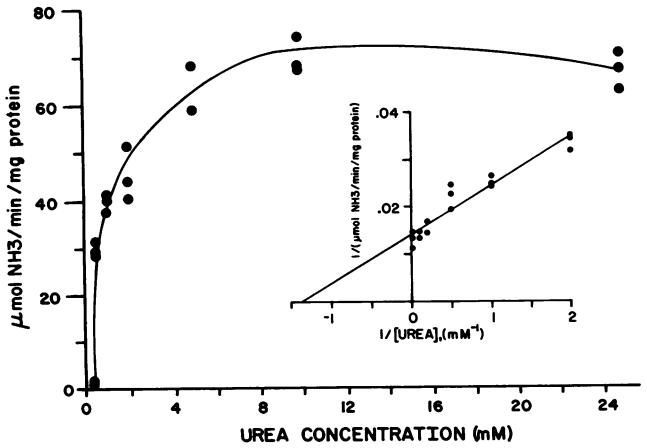

FIG. 2. Kinetics of urea hydrolysis by *C. pylori*. Rates of urea hydrolysis were measured for six concentrations of substrate. Cell lysate (20 μl of 10 mg of protein per ml) derived from agar-grown cultures of *C. pylori* UM41 was assayed by the phenol red spectrophotometric assay in a 3-ml reaction volume at 23°C. Rates were derived from linear portions of the curves. Inset, Reciprocal plot of these data. Data represent means of three assays. Data are typical of all *C. pylori* isolates tested.

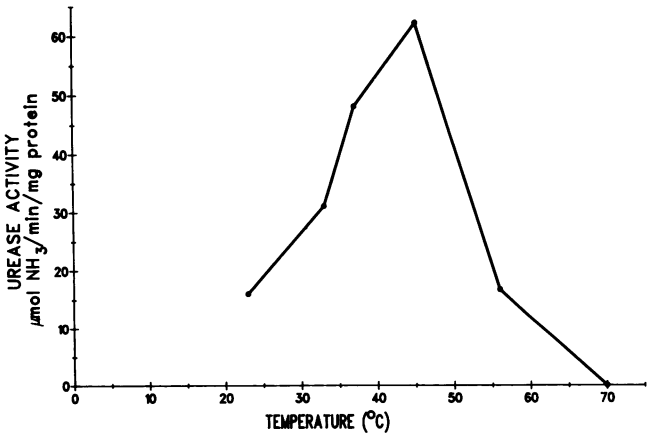

FIG. 3. Effect of temperature on urease activity of *C. pylori*. Cell lysates were assayed for rate of urea hydrolysis by the phenol red spectrophotometric assay. Cuvettes were incubated at the indicated temperatures. Data points represent the mean of triplicate determinations.

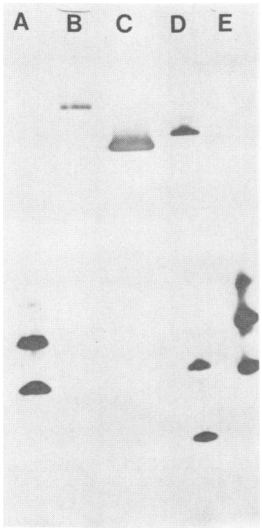

FIG. 4. Urease activity of *C. pylori* and other bacterial species in nondenaturing polyacrylamide gels. Cell lysates were loaded onto a 6% polyacrylamide gel with a 4% stacking gel and electrophoresed at 250 V for 4 h. The gel was equilibrated with 0.02% cresol red–0.1% EDTA and then incubated with 1.5% urea. Color development indicates point of migration of urease. Lanes: A, *Proteus mirabilis* BU7354; B, *C. pylori UM41*; C, *M. morganii* SI4049; D, *Providencia rettgeri* SI5453; and E, *Providencia stuartii* BE2467.

respectively. Rates at 37 and 43°C were 2.8- and 3.8-fold higher, respectively, than those at room temperature. Rates at 43°C were 1.3-fold higher than at 37°C. Inclusion of 0.02% sodium azide in the urea broth did not effect the rate of color change.

Enzyme stability. Conditions for preserving enzyme activity were determined by storing urease preparations at 4 or −70°C with or without a reducing agent, ion chelator, protease inhibitor, or glycerol (Table 2). Cell lysates retained 50% of initial urease activity after 6 days and 40% initial activity after 18 days when stored at 4°C in 20 mM sodium phosphate, pH 6.8. At −70°C for 18 days, 1 mM EDTA or 15% glycerol preserved 40 or 34%, respectively, of initial activity.

DISCUSSION

The rapid hydrolysis of urea by *C. pylori* is catalyzed by a urease that appears to be unique when compared with the enzymes produced by other bacterial species. The high-molecular-weight enzyme demonstrates much higher affinity for substrate and significantly higher activity than ureases of other species tested. The enzyme probably resides intracellularly as cell lysates, centrifuged to remove unbroken cells and membrane, retain most of the activity. This observation is consistent with an optimum pH of 8.2

The kinetic properties of the *C. pylori* urease are distinct from those of the ureases of other species. With a K_m for urea of 0.8 mM, this enzyme recognizes substrate with much higher affinity than the enzymes produced by *Proteus* or *Providencia* species (14, 25). This observation is physiologically consistent with the respective niches of these organisms. *C. pylori*, after making its way to the gastric mucosa, must scavenge urea from serum, and at physiological blood urea concentrations of 10 to 20 mg/dl (1.7 to 3.4 mM), urease is saturated and working at maximum rate. In the urinary tract, urea is plentiful (about 400 mM), so enzymes with lower affinity for urea are nevertheless saturated and working at maximum velocity. Therefore, high affinity for urea may be a selective advantage in the gastric mucosa, but unnecessary in the urinary tract. In addition to higher

TABLE 1. Urease inhibition by specific inhibitors

Inhibitor	IC_{50} (μg/ml)[a] for:	
	C. pylori	*Proteus mirabilis*
Acetohydroxamic acid	100	50
Hydroxyurea	85	89
EDTA	2,903	1,573
Flurofamide	0.05	0.14
L-Arginine hydroxamate	58	24
L-Lysine hydroxamate	84	24
Thiourea	1,000	1,000

[a] Concentration that inhibited enzyme activity by 50%.

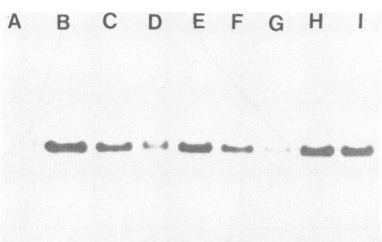

FIG. 5. Urease activity of *C. pylori* isolates in nondenaturing polyacrylamide gel. Cell lysates of *C. pylori* isolates were electrophoresed and urease activity was detected as described in the legend to Fig. 4. *C. pylori* isolates: lane A, UM16; lane B, UM502; lane C, UM14; lane D, UM12; lane E, UM15; lane F, UM18; lane G, UM6; lane H, UM41; and lane I, UM1.

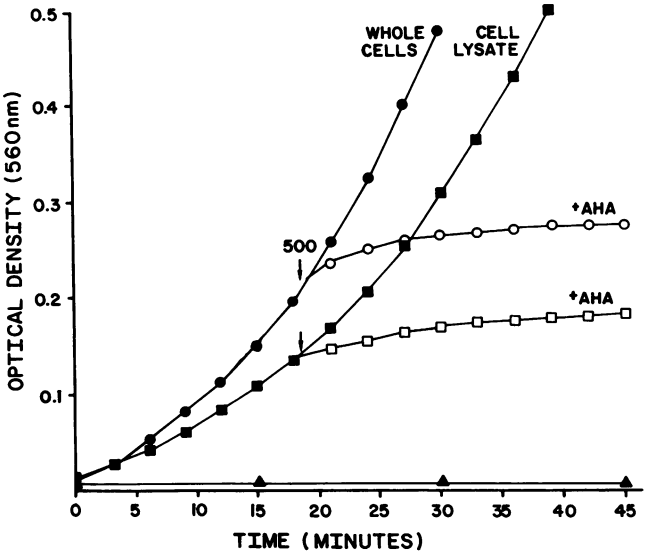

FIG. 6. Inhibition of urease activity in whole and lysed cells of *C. pylori*. Urease activity was measured with whole cells and an equivalent amount of lysed cells by the phenol red spectrophotometric assay. Cell suspension (30 μl of OD_{550} = 0.5) was assayed directly versus an equivalent aliquot of cells ruptured by two passes in a French pressure cell at 20,000 lb/in^2. Acetohydroxamic acid (AHA; 500 μg/ml) was added at the arrow.

TABLE 2. Stability of *C. pylori* urease stored under various conditions

Storage conditions	% Initial activity[a]			
	Day 6		Day 18	
	4°C	−70°C	4°C	−70°C
20 mM sodium phosphate (pH 6.8) plus:	48	8	38	1
1 mM EDTA	36	50	30	40
10 mM dithiothreitol	35	3	36	1
1 mM EDTA + 10 mM dithiothreitol	25	5	32	1
15% glycerol	28	37	32	34
5 mM phenylmethylsulfonyl fluoride	12	2	6	0
Frozen in dry ice-ethanol bath (no supplement)		8		1

[a] 100% activity equalled 44 μmol of NH_3 per min per mg of protein on the day of preparation. Values represent the mean of triplicate assays.

affinity, *C. pylori* isolates hydrolyze urea at more than twice the rate of the very active species *Proteus mirabilis* and about ten times the rate of other common urinary tract organisms. When substrate concentration is low, this high rate of hydrolysis may be necessary to achieve sufficient ammonia concentration to protect the bacteria from the nearby acid environment (C. E. Brady, T. L. Hadfield, J. R. Hyatt, and S. J. V. Utts, Am. J. Gastroenterol. **81**:855, 1986).

An optimum temperature of 43°C for *C. pylori* urease can be estimated from the data presented in Fig. 3. This suggests that much more rapid detection of the organism in gastric biopsies assayed in urea broth or by the *Campylobacter*-like organism test (22) would be achieved if tubes were incubated at this temperature. In addition, because the reaction is catalyzed by preformed enzymes and because *C. pylori* does not grow in this medium, urea broth containing 0.02% sodium azide might be used to prevent the growth of contaminating urease-positive organisms when the tests are held for 24 h or longer.

Since urease inhibitors are useful in the treatment and prevention of renal stones produced by urease-positive organisms in the urinary tract (9, 17, 24, 35) it was important to determine whether the urease produced by *C. pylori* could be inhibited by clinically available compounds. We found that *C. pylori* urease could be inhibited by competitive and noncompetitive inhibitors, as well as by a divalent cation chelator. The concentration of each compound which produces 50% inhibition of the urease activity was determined and was found to be similar for *Proteus mirabilis* and *C. pylori*. Although twice the concentration of acetohydroxamic acid, a commercially available inhibitor, was necessary to inhibit *C. pylori* urease, this value is well within the range of therapeutically achievable concentrations.

Urease activities and kinetic experiments were repeated with intact bacteria and urease inhibitor to determine whether intact cells offer a permeability barrier to substrate or inhibitor. No significant difference in rates of hydrolysis between whole and lysed cells was seen. Addition of acetohydroxamic acid at a concentration of 500 μg/ml inhibited urease activity of both whole cells and cell lysates. Thus, *C. pylori* cell wall and membrane do not present a barrier to the passage of either urea or acetohydroxamic acid into cells, suggesting that acetohydroxamic acid is an effective inhibitor in vivo.

In summary, the urease of *C. pylori* is a high-molecular-weight enzyme with a high affinity for substrate. It is produced in copious amounts when the organism is cultured on solid medium. The enzyme appears to be unique with respect to size, electrophoretic mobility, isoelectric point, and sensitivity to urease inhibitors when compared with the ureases of *Proteus*, *Providencia*, and *Morganella* species and may represent an important virulence factor in the development of gastritis and peptic ulceration.

ACKNOWLEDGMENTS

We thank James Dick and Ben Tall for providing strains.

This work was funded in part by Public Health Service grant R29 AI23328 from the National Institutes of Health.

ADDENDUM IN PROOF

When *C. pylori* cell lysate was fractionated on a Superose 6 (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) molecular sieve column equilibrated with 20 mM sodium phosphate (pH 6.8)–100 mM KCl, fractions with peak activity corresponded to an apparent molecular weight of 510,000.

LITERATURE CITED

1. **Buck, G. E., W. K. Gourley, W. K. Lee, K. Subramanyam, J. M. Latimer, and A. R. DiNuzzo.** 1986. Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer. J. Infect. Dis. **153**:664–669.
2. **Chen, X. G., P. Correa, J. Offerhaus, E. Rodriguez, F. Janney, E. Hoffman, J. Fox, F. Hunter, and S. Diavolitsis.** 1986. Ultrastructure of the gastric mucosa harboring campylobacter-like organisms. Am. J. Clin. Pathol. **86**:575–582.
3. **Christensen, W. B.** 1946. Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from salmonella and shigella. J. Bacteriol. **52**:461–466.
4. **Drumm, B., P. Sherman, E. Cutz, and M. Karmal.** 1987. Association of *Campylobacter pylori* on the gastric mucosa with antral gastritis in children. N. Engl. J. Med. **316**:1557–1561.
5. **Eldridge, J., A. M. Lessells, and D. M. Jones.** 1984. Antibody to spiral organisms on gastric mucosa. Lancet **i**:1237.
6. **Goodwin, C. S., J. A. Armstrong, and B. J. Marshall.** 1986. *Campylobacter pyloridis*, gastritis and peptic ulceration. J. Clin. Pathol. **39**:353–365.
7. **Goodwin, C. S., E. D. Blincow, J. R. Warren, T. E. Waters, C. R. Sanderson, and L. Easton.** 1985. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. J. Clin. Pathol. **38**:1127–1131.
8. **Graham, D. Y., P. D. Klein, D. J. Evans, Jr., D. G. Evans, L. C. Alpert, A. R. Opekun, and T. W. Boutton.** 1987. *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet **i**:1174–1177.
9. **Griffith, D. P., and D. M. Musher.** 1973. Prevention of infected urinary stones by urease inhibition. Invest. Urol. **11**:228–233.
10. **Hamilton-Miller, J. M. T., and R. A. Gargan.** 1979. Rapid screening for urease inhibitors. Invest. Urol. **16**:327–328.
11. **Hazell, S. L., T. J. Borody, and A. Lee.** 1987. *Campylobacter pyloridis* gastritis. I. Detection of urease as a marker of bacterial colonization and gastritis. Am. J. Gastroenterol. **82**:292–296.
12. **Hazell, S. L., W. B. Hennessy, T. J. Borody, J. Carrick, M. Ralston, L. Brady, and A. Lee.** 1987. *Campylobacter pyloridis* gastritis. II. Distribution of bacteria and associated inflammation in the gastroduodenal environment. Am. J. Gastroenterol. **82**:297–301.
13. **Hazell, S. L., and A. Lee.** 1986. *Campylobacter pyloridis*, urease, hydrogen ion back diffusion and gastric ulcers. Lancet **ii**:15–17.
14. **Jones, B. D., and H. L. T. Mobley.** 1987. Genetic and biochemical diversity of ureases of *Proteus*, *Providencia*, and *Morganella* species isolated from urinary tract infection. Infect. Immun. **55**:2198–2203.
15. **Jones, D. M., J. Eldridge, A. J. Fox, P. Sethi, and P. J.**

Whorwell. 1986. Antibody to the gastric campylobacter-like organism (*Campylobacter pyloridis*)—clinical correlations and distribution in the normal population. J. Med. Microbiol. **22**:57–62.

16. **Jones, D. M., A. M. Lessells, and J. Eldridge.** 1984. Campylobacter-like organisms on the gastric mucosa: culture, histological and serological studies. J. Clin. Pathol. **37**:1002–1006.
17. **Kobashi, K., K. Hase, and K. Uehara.** 1962. Specific inhibition of urease by hydroxamic acids. Biochim. Biophys. Acta **65**:380–383.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Marshall, B. J.** 1986. *Campylobacter pyloridis* and gastritis. J. Infect. Dis. **153**:650–657.
20. **Marshall, B. J., D. B. McGechie, P. A. Rogers, and R. J. Glancy.** 1985. Pyloric campylobacter infection and gastroduodenal disease. Med. J. Aust. **142**:439–443.
21. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i**:1311–1315.
22. **Marshall, B. J., J. R. Warren, G. J. Francis, S. R. Langton, C. S. Goodwin, and E. D. Blincow.** 1987. Rapid urease test in the management of *Campylobacter pyloridis*-associated gastritis. Am. J. Gastroenterol. **82**:200–210.
23. **McNulty, C. A. M., and R. Wise.** 1985. Rapid diagnosis of campylobacter-associated gastritis. Lancet **i**:1443.
24. **Millner, O. E., Jr., J. A. Andersen, M. E. Appler, C. E. Benjamin, J. G. Edwards, D. J. Humphrey, and E. M. Shearer.** 1982. Flurofamide: a potent inhibitor of bacterial urease with potential chemical utility in the treatment of infection-induced urinary stones. J. Urol. **127**:346–350.
25. **Mobley, H. L. T., B. D. Jones, and A. E. Jerse.** 1986. Cloning of urease gene sequences from *Providencia stuartii*. Infect. Immun. **54**:161–169.
26. **Morris, A., D. McIntyre, T. Rose, and G. Nicholson.** 1986. Rapid diagnosis of *Campylobacter pyloridis* infection. Lancet **i**:149.
27. **Morris, A., and G. Nicholson.** 1987. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am. J. Gastroenterol. **82**:192–199.
28. **Morris, A., G. Nicholson, G. Lloyd, D. Hiner, A. Roger, and D. Taylor.** 1986. Seroepidemiology of *Campylobacter pyloridis*. N.Z. Med. J. **99**:657–659.
29. **Owen, R. J., S. R. Martin, and P. Borman.** 1985. Rapid urea hydrolysis by gastric campylobacters. Lancet **ii**:111.
30. **Rathbone, B. J., J. I. Wyatt, B. W. Worsley, S. E. Shirer, L. K. Trejdosiewicz, R. V. Heatley, and M. S. Losowsky.** 1986. Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia. Gut **27**:642–647.
31. **Senior, B. W., N. C. Bradford, and D. S. Simpson.** 1980. The ureases of *Proteus* strains in relation to virulence for the urinary tract. J. Med. Microbiol. **13**:507–512.
32. **Skirrow, M. B.** 1977. Campylobacter enteritis: a "new" disease. Br. Med. J. **2**:9–11.
33. **Steer, H. W., and D. G. Newell.** 1985. Immunological identification of *Campylobacter pyloridis* in gastric biopsy tissue. Lancet **ii**:38.
34. **Warren, J. R., and B. Marshall.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **i**:1273–1275.
35. **Williams, J. J., J. S. Rodman, and C. M. Peterson.** 1984. A randomized double-blind study of acetohydroxamic acid in struvite nephrolithiasis. N. Engl. J. Med. **311**:760–764.