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Characteristics of *Helicobacter pylori* Variants Selected for Urease Deficiency

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The urease of *Helicobacter pylori* is suspected to play a role in the pathogenesis of gastritis. Although all clinical isolates of *H. pylori* are urease positive (U^+), we have selected and characterized several spontaneously arising urease-negative (U^-) variants from wild-type strain 60190. Urease-negative variants were identified by growth in medium containing 60 mM urea and arose at a frequency of 10^{-5} to 10^{-6}. The urease activity of the wild-type strain inhibited growth of this strain in the presence of 60 mM urea. U^- variants retained the U^- phenotype for more than 100 passages on medium with or without urea. The urease activities of the original U^+ and derived U^- cells were 9.55 to 16.7 and 0.01 to 0.17 U/mg of protein, respectively. Colonial growth and other biochemical characteristics were identical for the strains. U^- variants showed three classes of whole-cell sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles: (i) identical to U^+; (ii) change in the migration of the 61-kDa urease subunit; and (iii) lack of 61- and 30-kDa subunits. These differences were confirmed by immunoblotting and by protein separation using fast protein liquid chromatography. The U^+ strain but not U^- variants tolerated exposure to pH 4.0 for 60 min in the presence of urea. Supernatants of the U^+ strain and U^- variants contained vacuolating cytotoxin activity for HeLa cells in similar titers. By enzyme-linked immunosorbent assay, human serum samples recognized water extract from the U^+ strain significantly better than extract from a U^- variant lacking urease subunits. In conclusion, this study demonstrates that U^- *H. pylori* variants may arise spontaneously, that urease activity enhances survival at acid pH, and that urease and cytotoxin activities are disparate phenotypes.

Helicobacter pylori is now recognized as being involved in the etiology of active chronic gastritis and peptic ulceration (2, 3, 11), and there is accumulating evidence that it is associated with adenocarcinoma of the stomach (18, 30). *H. pylori* produces large amounts of urease, which hydrolyzes urea to ammonia and carbon dioxide (15, 37). All wild-type isolates of *H. pylori* from humans are urease positive, suggesting that this enzyme plays an important role in the maintenance of infection. In vitro studies suggest that urease activity enhances *H. pylori* survival at acidic pH when substrate urea is present (25, 26), and a chemically mutagenized *H. pylori* strain that lacked urease activity was not able to colonize gnotobiotic piglets (15). In addition, both urease per se (22, 23) and the ammonia produced (24) may play roles in tissue inflammation and injury. Recently, McLaren et al. reported a technique to select urease-negative (U^-) variants from a population of urease-positive (U^+) *H. pylori* strains (26).

The goals of the present study were (i) to attempt to reproduce the method of McLaren et al. (26) to select U^- variants; (ii) to characterize spontaneously arising urease-negative variants of *H. pylori*; (iii) to determine the effect of urease activity on the survival of *H. pylori* at acidic pH; and (iv) to study the relation between cytotoxin and urease activities. We found that the U^- phenotype occurs naturally, that this phenotype is associated with several different structural variants, and that the vacuolating cytotoxin activity is independent of the urease activity.

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MATERIALS AND METHODS

Bacterial strains. *H. pylori* 60190 (ATCC 49503) was used for the selection of urease-negative variants. This strain has been used in the evaluation and purification of the vacuolating cytotoxin of *H. pylori* (6–8). *H. pylori* U2-1, 4, and 5 were the U^- variants selected. As a control in the cytotoxin experiments, Tox^- strain Tx30a was used (7). All the bacteria used in this study were identified as *H. pylori* by Gram stain morphology and by oxidase and catalase tests (27). The organisms were maintained frozen at −70°C in brucella broth containing 15% glycerol.

Variant isolation. Urease-negative variants were selected by the method of McLaren et al. (26). In brief, we made serial 10-fold dilutions of a bacterial suspension (10^8 CFU/ml) harvested from a 48-h culture of *H. pylori* 60190 on blood agar and plated them onto Columbia agar plates (Oxoid) containing 5% heat-denatured sheep blood with 60 mM urea and 25 mM sodium citrate in 50 mM Na_2HPO_4 buffer (pH 7.0) or onto blood agar plates without additives for comparison (BBL Microbiology Systems, Cockeysville, Md.). The plates were incubated for 72 h at 37°C in a CO_2 incubator. Colonies isolated on the selective medium were initially determined to be U^- by incubation of cell suspensions in urea broth (BBL). Colonies that did not turn the broth pink within 5 min were streaked onto blood agar plates containing 60 mM urea for further analysis.

Stability assays. To determine the stability of U^- variants, we serially subcultured strains on Columbia blood agar plates with 60, 30, 10, 5, 2, 1, or 0 mM urea, as well as on Trypticase soy–5% sheep blood–agar plates (BBL) without urea.

Analytical procedures. Urease activity was assayed by

using a coupled-enzyme assay as previously described (13). Urease activity of all strains was expressed as specific activity (enzyme units per milligram of protein). Protein concentrations of whole cells and extracts were measured by using the BCA protein assay (Pierce, Rockford, Ill.) with bovine serum albumin as the standard, as described previously (36). Protein profiles were determined in a modified Laemmli sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system as described by Ames (1). Proteins were resolved in gels by using the silver stain of Oakley et al. (31) as we previously described (4).

Serological methods. To compare the antigenicity of the wild-type U^+ strain and the U^- variants, we used a panel of serum samples from 40 adults, from the State University of New York Health Science Center, Syracuse, in an enzyme-linked immunosorbent assay (ELISA) as described previously (13, 33). The 40 adults were classified as *H. pylori* infected or uninfected on the basis of culture and histologic examination of gastric biopsy specimens and by serologic testing as described previously (12, 33). To sensitize the ELISA plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, VA), we added 0.5 μg of the water-extracted proteins (13) from each of three *H. pylori* strains (one U^+ strain and two U^- variants) to microtiter plate wells and processed them as previously described (33).

Immunoblot procedures. Immunoblotting was performed as previously described (34). Proteins from U^- variants and U^+ *H. pylori* were transferred from acrylamide gels to nitrocellulose paper by electroblotting for 30 min at 1,000 mA. The nitrocellulose paper was incubated at 25°C for 1 h with a 1:100 dilution of the test serum samples. The rabbit serum samples used were raised to (i) whole cells of *H. pylori* 60190, (ii) the complete urease molecule from *H. pylori* 84-183 excised from a nondenaturing acrylamide gel, or (iii) the 61-kDa subunit of urease from strain 84-183 as previously described (14). After being washed, the nitrocellulose paper was incubated for 1 h with a 1:2,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit or anti-human immunoglobulin (Boehringer Mannheim), and the paper was then washed and color developed.

FPLC. Size-exclusion fast protein liquid chromatography (FPLC) of water extract preparations of U^+ strain 60190 and U^- strains U2-1 and 4 with similar protein concentrations was performed at a flow rate of 0.30 ml/min on a Superose-12 HR 10/30 column (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.) with buffer containing 20 mM phosphate (pH 7.4) as previously described (13). Individual fractions were examined by SDS-PAGE for comparison of protein profiles.

Vacuolating cytotoxic activity. To assess production of the vacuolating cytotoxin, we assayed supernatants from U^+ and U^- *H. pylori* strains as previously described (8). In brief, concentrated supernatant from 48-h broth cultures was added to adherent HeLa cells and incubated for 18 h at 37°C, and then the cells were stained for 4 min with 0.05% neutral red. Intracellular neutral red was quantitated spectrophotometrically and provided a measurement of cell vacuolation (8).

Survival in acidic conditions. To evaluate the survival of U^+ and U^- strains under different pH conditions, cell suspensions from 48-h cultures were incubated at 37°C for 1 h with or without 10 mM urea in glycine-HCl buffer (pH 2.0), McIlvain's buffer (0.2 M Na_2HPO_4, 0.1 M citric acid [pH 4.0], or 0.1 M phosphate buffer (pH 7.0). After incubation, serial 10-fold dilutions of the cell suspensions in 150 mM NaCl were made, plated onto blood agar plates, and incubated for 48 to 72 h at 37°C to determine the number of CFU per milliliter.

TABLE 1. Stability of urease activity of the wild-type strain and U^- variant strains of *H. pylori* 60190

No. of passages[a]	Sp act for *H. pylori* strain[b]:			
	60190	U2-1	4	5
1	12.81 ± 4.0	0.026 ± 0.03	0.41 ± 0.04	0.36 ± 0.07
10	13.78 ± 3.7	0.17 ± 0.07	0.01 ± 0.02	0.02 ± 0.05
20	9.55 ± 3.3	0.06 ± 0.01	0.02 ± 0.04	0.02 ± 0.04
33	16.70 ± 3.2	0.01 ± 0.01	0.03 ± 0.03	0.02 ± 0.02
>100	10.92 ± 4.1	0.01 ± 0.01	ND[c]	ND

[a] Number of times strain was passaged on blood-agar medium before being tested for urease activity.
[b] Urease specific activity is expressed as units per milligram of protein. Results shown are the means of four determinations ± standard deviations (see Materials and Methods).
[c] ND, not determined.

Statistical methods. Distribution of optical density ratios were compared by Student's *t* test (two-tailed) for independent and dependent variables.

RESULTS

Selection and stability of *H. pylori* urease-negative variants. On the basis of the self-destruction of urease-positive (U^+) strains of *H. pylori* in the presence of high concentrations of urea (60 mM) (26), we were able to select spontaneously arising U^- variants of wild-type *H. pylori* 60190. No differences were observed in the colony morphology between U^+ and U^- colonies or between U^- variants (data not shown). Of the original 27 colonies surviving incubation with urea, all were urease negative as tested by a rapid urea-phenol red assay. However, after transfer to blood agar plates, low urease activity was detected in 20 of these strains. From the number of true U^- colonies identified, we calculated that U^- variants arose at a frequency of 10^{-5} to 10^{-6}. Since we do not know the generation time for *H. pylori* under these growth conditions, we cannot calculate a rate per cell per generation. The urease activity of the U^- variants was 70- to 500-fold lower than that of the wild-type strain (Table 1). The U^- variant strain U2-1 retained the U^- phenotype for more than 100 passages on blood-agar plates whether or not urea was present.

Comparison of the U^+ strain and U^- *H. pylori* variants. The U^+ strain and U^- variants demonstrated identical colonial morphologies and similar catalase and oxidase activities (data not shown). In contrast, SDS-PAGE profiles of these variants revealed differences (Fig. 1). U^- variant strain 4 lacked a band at 61 kDa which corresponds to the large subunit of *H. pylori* urease (lane 3). That the 30-kDa subunit is also not present can be better appreciated in Fig. 3, lane 3. For U^- variant U2-1, we observed that the 61-kDa band was absent but that a new band migrating at about 59 kDa was present (Fig. 1, lane 1). For a third type of U^- variant (represented by strain 5), the SDS-PAGE profile was no different from that of the wild-type strain (Fig. 1, lanes 2 and 4). This was the most common variant (25 of 27 strains studied including all 20 with low urease activity). These differences in protein profile were clearly demonstrated by immunoblotting (Fig. 2). The immunoblot analysis did not reveal any antigenic differences other than those relating to the large (61-kDa) urease subunit as described above. Recognition of the 30-kDa band by anti-urease serum was very

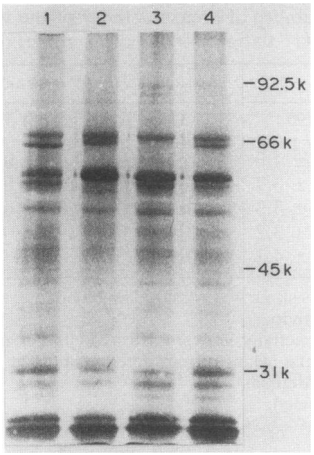

FIG. 1. SDS-PAGE profiles of whole-cell preparations of the U^+ strain and U^- variants of *H. pylori*. Lanes: 1, U^- strain U2-1; 2, U^- strain 5; 3, U^- strain 4; 4, U^+ wild-type strain 60190. Preparations were electrophoresed in 10% acrylamide, and bands were resolved by silver staining. Molecular mass markers (in kilodaltons) are shown at the right.

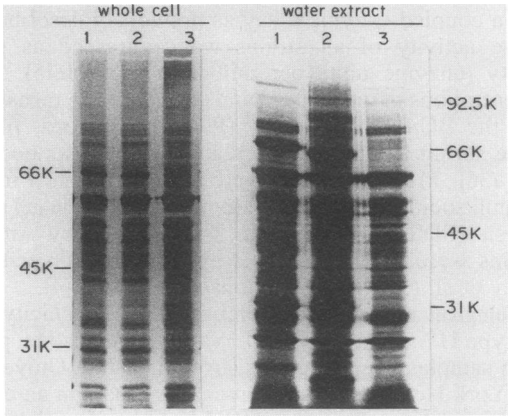

FIG. 3. SDS-PAGE profiles (10% acrylamide) of whole-cell and water extract preparations of the U^+ strain and U^- variants of *H. pylori*. Lanes: 1, U^+ wild-type strain 60190; 2, U^- strain U2-1; 3, U^- strain 4.

weak on Western immunoblots. We have observed this in multiple experiments, as previously described (14). Whether this is because the 30-kDa subunit is poorly antigenic or whether it loses its antigenicity when denatured by SDS-PAGE is not clear. Thus, failure to resolve the band by Western blot (Fig. 2) but its visualization by silver stain (Fig. 3; see also Fig. 5) is not inconsistent. Of particular interest is that the 59-kDa band observed in strain U2-1 was recognized by the anti-urease serum (Fig. 2, lane 1), indicating that it represents a truncated version of the wild-type 61-kDa urease subunit with a different electrophoretic mobility.

Effect of urease on the acid resistance of *H. pylori* cells. To compare the ability of the U^+ strain and U^- variants to resist acidic pH, the U^+ strain and U^- variants were incubated for 60 min at 37°C in different buffered solutions at pH 2, 4, and 7 or in 0.15 M saline as a control. We were concerned that the same buffer may not be reliable over a broad pH range (2.0 to 8.0) and thus chose three different buffers. All strains survived incubation at pH 7 in the presence or absence of urea (Table 2). However, survival of the wild-type U^+ strain at pH 7 was 1 $\log_{10}$ unit lower in the presence of urea than in its absence, a difference associated with an increase in pH at 60 min to 7.92, presumably reflecting ammonia production. Conversely, no change in pH occurred with the U^- strains. At pH 2, there was no detectable survival ($<10^{0.5}$ CFU/ml) for any strain with or without urea. The pH observed after a 60-min incubation was 2.13 to 2.65. At pH 4, in the presence of urea, the wild-type U^+ strain survived to the same extent as at pH 7.0; after 60 min the pH had risen to 6.41 (mean of two experiments). In the absence of urea, the pH remained at 4.36 and there was almost no survival (>5 $\log_{10}$ unit difference). The survival of U^- variants (strains U2-1 and 4) at pH 4 was significantly decreased compared with that at pH 7 ($P < 0.01$), regardless of whether urea was present. These studies indicate the importance of urease activity in buffering acidic pH. An interesting observation was that both U^- variants showed marked killing at pH 4 ($>10^{-4}$) in the presence of urea compared with that of the wild-type strain. In all three experiments, strain 4 was more acid resistant than strain U2-1 (and more resistant than the wild-type strain in the absence of urea).

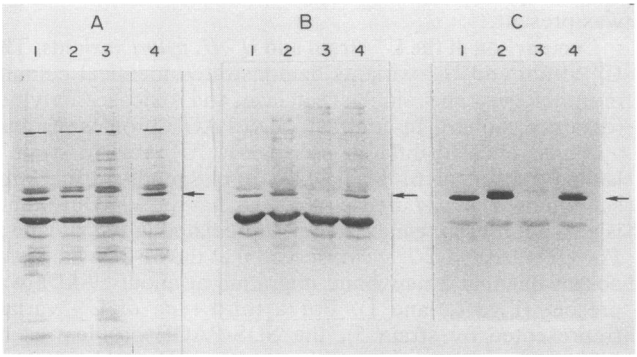

FIG. 2. Immunoblots from SDS-PAGE (10% acrylamide) of whole-cell preparations of the U^+ strain and U^- variants of *H. pylori*. Lanes: 1, U^- strain U2-1; 2, U^- strain 5; 3, U^- strain 4; 4, U^+ strain 60190. The panels represent the immunoblots produced by rabbit antiserum to whole cells of strain 60190 (A), rabbit anti-urease from strain 84-183 (B), and rabbit antiserum to the 61-kDa subunit of urease from strain 84-183 (C). In each case the arrow points to the 61-kDa urease subunit in the U^+ strain.

Water extraction of proteins from U^+ and U^- variants of *H. pylori*. Previous studies have shown that water extraction of whole *H. pylori* cells yields a urease-enriched preparation (13). We compared the protein profiles of whole cells and

TABLE 2. Survival of U^+ and U^- *H. pylori* strains after incubation in solutions of different pH

Strain	$\log_{10}$ survival after incubation in buffer at[a]:					
	pH 7.0		pH 4.0		pH 2.0	
	+ Urea[b]	− Urea	+ Urea	− Urea	+ Urea	− Urea
60190 (U^+)	5.8	6.8	6.9	1.2	<0.5	<0.5
U2-1 (U^-)	7.8	6.8	<0.5	1.5	<0.5	<0.5
4 (U^-)	8.1	6.8	2.4	2.7	<0.5	<0.5

[a] Survival after incubation of $10^{8.8}$ CFU of inocula per ml in buffered solutions of known pH for 60 min (see Materials and Methods). Results shown are representative of three replicate experiments.

[b] Urea (10 mM) had been added to the buffer.

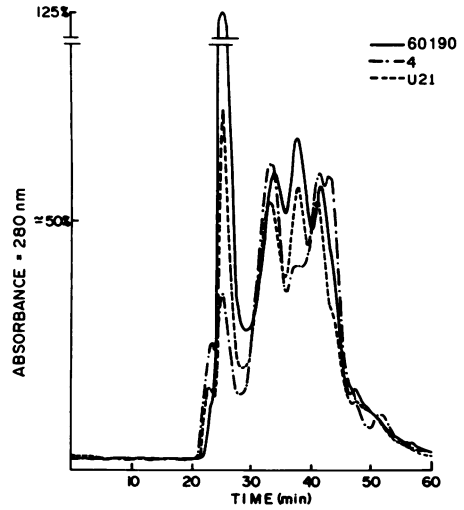

FIG. 4. Gel filtration chromatography of water extracts of urease variants of *H. pylori*. The strains are 60190 (U^+), 4 (U^-), and U2-1 (U^-).

water extract preparations of the U^+ strain and U^- variants (Fig. 3). As expected, the whole-cell profiles were as observed in Fig. 1. However, the water extract of strain U2-1 differed significantly from that of the wild-type strain in the regions above 66 kDa and below 25 kDa. The water extract profile of strain 4 was similar to that of the wild-type strain, with the exception of the missing 61- and 30-kDa bands, representing urease subunits. The water extract profile of strain 5 was the same as that of the wild-type strain, as expected (data not shown). The water extract data suggest that for variant U2-1 the U^- phenotype is associated with the late elution of other surface-exposed proteins in *H. pylori*.

Partial purification of surface proteins extracted from the U^+ wild-type strain and U^- *H. pylori* variants. To further characterize this phenomenon, we separated water extract preparations of the U^+ strain and U^- variants by gel filtration chromatography. Chromatographic tracings showed a large peak for the wild-type U^+ strain in fractions eluting between 24 and 27 min, which corresponds to urease (13) (Fig. 4). In contrast, the peak for strain U2-1 was much smaller and that for strain 4 was nearly absent. Similarly, a peak at 38 min was much diminished for strain 4. To determine the composition of each peak, we pooled chromatographic fractions and subjected them to SDS-PAGE (Fig. 5). For the earliest elution time studied (24 to 27 min), the bands present for U2-1 are essentially the same as for the wild-type strain but less protein is present and a band migrating at 59 instead of 61 kDa is observed. In contrast, for strain 4, both the 61- and 30-kDa bands are absent. For strain U2-1, a band at about 25 kDa elutes in much later fractions (36 to 39 min) than for either the wild-type strain or strain 4. Thus, in strain U2-1 the U^- phenotype is associated with the late elution of nonurease proteins as mentioned above.

Antigenicity of water extracts to humans. Since naturally infected humans may show different antibody responses to *H. pylori* than do hyperimmunized rabbits, and since several serological tests have been based on urease as the major antigen (10, 16), we assessed the antigenicity of the water extracts from the wild-type and two variant organisms. By ELISA, human serological recognition of the water extract from the strain with the aberrant 59-kDa protein was nearly

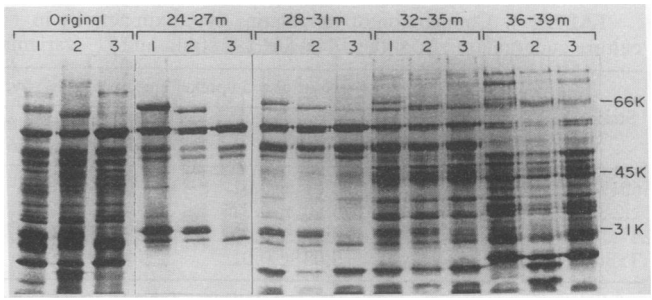

FIG. 5. SDS-PAGE profiles (10% acrylamide) of water extracts and Superose-12 fractions collected for *H. pylori* 60190 (lanes 1) and U^- variants U2-1 (lanes 2) and 4 (lanes 3). "Original" refers to the water extract before chromatography, and the other headings refer to the elution time (in minutes) of the Superose-12 fractions from each of the three strains.

identical to that for the wild-type strain (Table 3). In contrast, there was a significantly lower response among *H. pylori*-infected persons to the water extract from strain 4, which lacks the urease subunits. This study provides further evidence for the importance of urease as an *H. pylori* antigen but also indicates that other antigens invoke an immune response.

Effect of urease activity on vacuolating cytotoxin activity. Culture supernatants from some but not all wild-type *H. pylori* strains induce vacuolation in eukaryotic cells (21). Although others have suggested that vacuolation is due to urease activity (37), we have previously shown that inhibition of urease activity by acetohydroxamic acid (AHA) fails to abolish vacuolating activity (8). To study this phenomenon further, we now compared the known Tox^+ wild-type U^+ strain (strain 60190), a known Tox^- wild-type U^+ strain (Tx30a), and two of the selected U^- strains (Table 4). As expected, as quantitated in the neutral red uptake assay (8), supernatant from the Tox^+ strain induced significantly greater vacuolation than the Tox^- strain did, an effect that was not blocked by AHA treatment. Supernatant from both U^- variants induced neutral red uptake similar to that from the U^+ parental strain, again without inhibition by AHA. This study provides further evidence that the vacuolating activity in *H. pylori* supernatants is mediated by a cytotoxin distinct from urease.

DISCUSSION

Urease activity appears to be essential to *H. pylori* in that all clinical isolates are urease positive (17). However, this

TABLE 3. Human serological recognition of water extracts from *H. pylori* 60190 and derived U^- strains

H. pylori strain[a]	Optical density values[b]:	
	H. pylori infected (n = 20)	*H. pylori* uninfected (n = 20)
60190 (U^+)	0.71 ± 0.08[c]	0.05 ± 0.01[e]
U2-1 (U^-)	0.81 ± 0.10[d]	0.08 ± 0.02[e]
4 (U^-)	0.44 ± 0.06	0.02 ± 0.01[e]

[a] Strain used as the source of antigen.
[b] Optical density ratios (means ± standard errors of the means) in ELISA with water extract from specified strain as the antigen. For each patient, the serum was assayed in duplicate wells for each antigen.
[c] Compared with results for strain 4 (P = 0.005).
[d] Compared with results for strain 4 (P = 0.002).
[e] Compared with results for infected persons (P < 0.001).

TABLE 4. Comparison of vacuolating cytotoxin activity in culture supernatants from the *H. pylori* U$^+$ strain and U$^-$ variants

Strain	Urease[a]	Neutral red uptake[b] (mean ± SEM) by:	
		Supernatant alone	Supernatant with AHA (100 μg/ml)
60190	+	0.61 ± 0.01	0.54 ± 0.03
U2-1	−	0.50 ± 0.02	0.52 ± 0.02
4	−	0.57 ± 0.02	0.55 ± 0.02
Tx30a	+	0.08 ± 0.01	0.10 ± 0.03

[a] Relative production of urease by the test strain (see Table 1). Strain Tx30a is a wild-type U$^+$ strain known to be Tox$^-$ (7).

[b] Net neutral red uptake of HeLa cells produced by 1:20 dilutions of concentrated culture supernatants, as described previously (8).

study has confirmed the observation that it is possible to select variants with essentially absent urease activity from a population of wild-type strains (26). This method of selection, by providing a high concentration of substrate for urease, leads to the destruction of urease-producing *H. pylori* cells in the population as a result of ammonia production and alkalinization of the microenvironment, as previously suggested (29). This phenomenon further illustrates that *H. pylori* is adapted for survival in acidic milieus (5) and suggests that urease activity is not significantly down-regulated by alkaline pH.

Marshall and colleagues showed that a U$^+$ *H. pylori* strain but not *Campylobacter jejuni* (which lacks urease activity) may survive incubation under acidic conditions in the presence of urea (25). We have confirmed and extended that observation by showing that it is urease and not another property associated with *H. pylori* that confers this marked acid resistance. A chemically mutagenized *H. pylori* strain that had only minimal urease activity was unable to colonize gnotobiotic piglets, suggesting that urease activity is important in vivo in initiation or maintenance of colonization (15). Since urease is so useful to *H. pylori*, the selection of spontaneous mutants without appreciable activity at a frequency of 10^{-5} to 10^{-6} is of considerable interest. Why a population of *H. pylori* may contain a U$^-$ subpopulation is not obvious, but perhaps such cells may provide a survival advantage for the population in a nonacidic environment. Alternatively, the selection of U$^-$ strains may be entirely an in vitro phenomenon. Characterization of the genetic bases for this phenotype may permit elucidation of whether it exists in vivo.

Despite a greater than 500-fold difference in urease activity, the vacuolating cytotoxic activities of the urease-positive strain and urease-negative variants were essentially identical. A similar conclusion was reached in a previous study in which a chemically derived U$^-$ mutant was tested (8). The failure of AHA to inhibit cytotoxin activity confirms previous results (8) and provides further evidence that a vacuolating cytotoxin, which is not dependent on urease activity, is present. Although we concluded that vacuolating cytotoxin and urease activities are disparate phenotypes, Segal et al. (35) recently produced U$^-$ mutants by chemical mutagenesis and concluded that *H. pylori* urease may act as a cytotoxin. The method of Segal et al. is different from ours, since they (i) used whole cells rather than culture supernatants, (ii) added urea to the medium to induce vacuolation, and (iii) used an assay in which the end point was cell death rather than vacuolation. It is indeed likely that urease-mediated ammonia production contributes to cytolethal toxicity and also potentiates vacuolating cytotoxin activity (8).

That the U$^-$ phenotype is associated with three different SDS-PAGE patterns indicates that the physiology of urease activity and its genetics must be complex in *H. pylori*. Since nine genes appear necessary for expression of urease activity in *H. pylori* (9), determining the genetic basis for each of the three variants observed will be of great interest. Although the U$^-$ phenotype, once established, is stable in vitro, in vivo studies should confirm whether urease is necessary for pathogenicity and whether reversion occurs.

On a functional level, the lack of the two urease subunits is sufficient to explain the lack of activity. However, most of the variants contained the subunits with little or no activity. Since nickel is critical for urease activity (20, 28), lack of its insertion into the protein is one mechanism for the presence of inactive subunits. It is interesting to speculate that the 59-kDa urease protein of strain U2-1 may have undergone posttranslational modification such as truncation. The presence of phase variants has been described for *Campylobacter coli* (flagellar proteins) (19) and *Campylobacter fetus* (S-layer proteins) (32), two organisms related to *H. pylori*. For the *H. pylori* variant U2-1 described in this study, it is possible that there is a defect in the assembly of macromolecules or transport of these molecules to the cell surface (Fig. 5). Whether this is of sufficient magnitude to explain the nearly complete loss of urease activity remains to be determined.

Finally, the U$^-$ variant (strain 4), which does not have the subunits present, is immunologically different from the wild-type strain. This study reaffirms the importance of the urease subunits as antigens for detection of human infection but indicates that other antigens also play a role in diagnostic serological testing.

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