Helicobacter pylori Survival in Gastric Mucosa by Generation of a pH Gradient

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ABSTRACT *Helicobacter pylori* has been established as the major causative agent of human active gastritis and is an essential factor in peptic ulcer disease and gastric cancer. The mechanism that has been proposed for *H. pylori* to control its inhospitable microenvironment happens to coincide with the pH control technique developed by us. This technique was developed to separate an acidic environment from a basic environment for a sequential enzymatic reaction by the hydrolysis of urea within a thin layer of immobilized urease. In this paper, a mathematical model is presented to consider how *H. pylori* survives the gastric acidity. The computed results explain well the experimental data available involving *H. pylori*.

INTRODUCTION

It is well known that the human stomach prevents the passage of ingested, potential pathogens into the gut. The parietal cells secret copious amounts of hydrochloric acid and maintain the stomach with a pH < 2 (Fox, 1991). Most of the bacteria that enter the stomach are killed because of this high acidity (Giannella et al., 1972). Therefore, for a very long time, the human stomach has been considered an inhospitable environment for bacterial growth. With the isolation of *Helicobacter pylori* (formerly *Campylobacter pylori*) from human gastric biopsies, however, this point of view has been revised (Cover and Blaser, 1995). *H. pylori* is a Gram-negative microaerophilic spiral bacterium that lives in the human stomach and duodenum. Its high mobility and spiral morphology promote its persistence (Blaser, 1993). Its presence in gastric mucosa has been shown to be closely associated with histologic gastritis and gastroduodenal ulcer disease (Buck, 1990; Buck et al., 1986; Marshall and Warren, 1984; Marshall et al., 1985; Rathbone et al., 1986). Once acquired, *H. pylori* can inhabit the human stomach for years, for decades, or possibly for life. Such a long-term *H. pylori* infection may also be a risk factor for the development of adenocarcinoma of the stomach and gastric lymphomas (Forman et al., 1991; Nomura et al., 1991; The Eurogast study group, 1993; Asaka et al., 1994). Understanding how *H. pylori* can survive transient gastric acidity could lead to the development of improved treatment strategies for the diseases of the upper gastrointestinal tract of humans.

H. pylori produces extraordinary amounts of urease (Mobley et al., 1988). Its ability to produce large amounts of urease is believed to be critical for *H. pylori* colonization of the human gastric mucosa. *H. pylori* survival in the gastric mucosa appears to be due to the generation of a pH gradient across its cell envelope. A conceptual hypothesis has been proposed by Marshall et al. (1990) to explain how *H. pylori* is able to control its pH microenvironment in the extremely acidic stomach. In their hypothesis, the urease, which is located in the cell wall, provides ammonium and bicarbonate by hydrolyzing the urea diffusing in from the gastric juice, promoting *H. pylori* survival and initial mucosal colonization. In support of this hypothesis experimental studies have shown that the urease of *H. pylori* is always exposed on the surface (Dunn et al., 1990; Hawtin et al., 1990). With the small amounts of urea present in the stomach, the urease produced by *H. pylori* is able to hydrolyze urea and produce ammonia. The ammonia forms a thin acid-neutralizing layer, or "cloud" around the bacterium, thus protecting *H. pylori* from being destroyed by the acid (Lee and Mitchell, 1994). Kinetic studies have revealed that, compared with the urease of other bacterial species, the urease of *H. pylori* has a low Michaelis constant (K_m) with urea as the substrate (0.17–0.8 mM) (Mobley et al., 1988; Dunn et al., 1990; Hu and Mobley, 1990; Evans et al., 1991; Ferrero and Lee, 1991), and a high specific activity (V_{max}) from 1100 to 1700 μmol of urea hydrolyzed per minute per mg of protein (Dunn et al., 1990; Hu and Mobley, 1990). With a lower K_m value, the enzyme binds urea with much higher affinity than those produced by other bacteria, whereas the higher rate of the hydrolysis of urea provides sufficient amounts of ammonia to protect *H. pylori* from the nearby gastric acid environment. Such kinetic properties of *H. pylori* urease are consistent with its physiological niche (Mobley and Foxall, 1994).

The conceptual hypothesis proposed by Marshall et al. (1990) to explain how *H. pylori* can survive in the extremely acidic environment of the stomach is similar to the optimal pH control technique developed by us (Byers et al., 1992, 1993; Chen et al., 1996; Fournier et al., 1996) for sequential enzyme reactions. We have experimentally shown that urease from Jack beans immobilized in a flat glutaraldehyde-activated membrane (diameter = 47 mm, thickness = 0.55 mm, pore size = 1 μm) is capable of separating an acidic (pH ~4.5) environment from a basic (pH ~7.5) environ-

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ment. This very thin, highly permeable urease membrane was able to control and maintain this large difference of three or more pH units when only small amounts of urea (~10 mM) were maintained in the acidic environment (Byers et al., 1993). The urease-catalyzed decomposition of urea generates ammonia, which consumes the hydrogen ions diffusing into the membrane from the acidic bulk solution. Furthermore, we have shown that, by depositing a thin layer of urease on the external surface of commercial immobilized glucose isomerase pellets, these pellets can have near their optimal pH activity (pH 7.5) when immersed in a bulk solution whose pH is much less than the optimal pH for glucose isomerase (pH 4.6) (Chen et al., 1996; Fournier et al., 1996). We have also developed a mathematical model of pH control by the hydrolysis of urea (Byers et al., 1992). This model successfully explained our experimental data (Byers et al., 1993). In this paper, we adapt this mathematical model of pH control (Byers et al., 1992) to the case of *H. pylori*. The model predicts under what conditions *H. pylori* can survive in the gastric acidity of the stomach. The predictions of the model are then compared with the available experimental data for *H. pylori* survival at various pH levels.

MODEL DEVELOPMENT

H. pylori is 3–5 μm in length and 0.5–0.6 μm wide, with unipolar flagella (Lee and O'Rourke, 1993). The cell envelope of a Gram-negative bacteria such as *H. pylori* consists of the outer membrane and the cytoplasmic membrane that are separated by a periplasmic space containing a thin network of peptidoglycan. Experimentally, it has been shown that nearly 80% of the *H. pylori* urease can be removed by water extraction without affecting bacterial viability (Dunn et al., 1990). This suggests that the majority of the urease is associated with the periplasm and the outer membrane of the bacteria, referred to as the cell wall.

We assume that urease is uniformly distributed in the cell wall. The radius of the bacteria is much larger than the thickness of the cell wall, which is generally 10–15 nm for Gram-negative bacteria (Pelczar et al., 1986). Therefore, to simplify the problem, we neglect curvature and treat the cell wall as a one-dimensional flat plate in the development of the mathematical model. Fig. 1 provides an illustration of the region within the cell wall where the following reactions occur:

$$(NH_2)_2CO + 2H_2O \xrightarrow{\text{urease}} 2NH_3 + H_2CO_3 \quad (1)$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad K_2 = 1.79 \times 10^9\ M^{-1} \quad (2)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad K_3 = 4.30 \times 10^{-7}\ M \quad (3)$$

$$WH \leftrightarrow W^- + H^+ \quad (4)$$

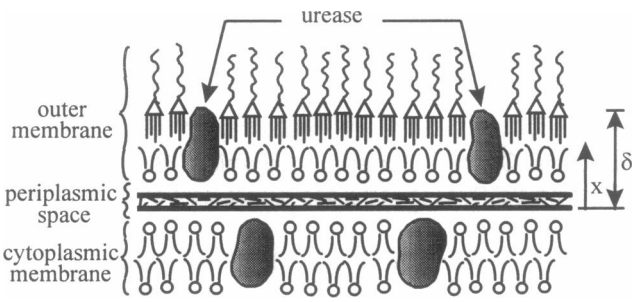

FIGURE 1 Illustration of the cell wall for a Gram-negative bacterium such as *H. pylori*.

The urease in the cell wall catalyzes the hydrolysis of urea that is present in the stomach and diffusing into the bacterium to yield ammonia and carbonic acid. With one mole of urea being consumed, two mole of ammonia and a single mole of carbonic acid are produced. At physiological pH, the carbonic acid dissociates again into HCO_3^-. The ammonia combines with the hydrogen ions freed in the above dissociation reaction and those diffusing in from the stomach, thus resulting in the generation of a pH gradient across the cell wall of the bacterium. An association and dissociation reaction for a weak acid (WH) or buffer is included in the model reactions for the completion of the model development, but we assumed that its total concentration is zero. That is, if the buffering capacity is not considered in the following calculations, the total buffer concentration and its equilibrium dissociation constant for Eq. 4, K_4, should be set to zero. We do not account for the role of buffers on the transport of hydrogen ions because we suspect that at the very low pH in the gastric environment most substances do not exhibit any significant buffering capacity.

If we let $x = 0$ define the interface between the cell wall and the cytoplasmic membrane of the bacterium, and δ the thickness of the cell wall, the reaction and diffusion phenomena of the chemical species of interest in this region can be described by the following set of coupled differential equations derived from Fick's law.

Urea
$$D_u \frac{d^2C_u}{dx^2} = \nu_1 \quad (5)$$

H^+
$$D_{H^+} \frac{d^2C_{H^+}}{dx^2} = \nu_2 - \nu_3 - \nu_4 \quad (6)$$

NH_3
$$D_{NH_3} \frac{d^2C_{NH_3}}{dx^2} = -2\nu_1 + \nu_2 \quad (7)$$

NH_4^+
$$D_{NH_4^+} \frac{d^2C_{NH_4^+}}{dx^2} = -\nu_2 \quad (8)$$

H_2CO_3
$$D_{H_2CO_3} \frac{d^2C_{H_2CO_3}}{dx^2} = -\nu_1 + \nu_3 \quad (9)$$

HCO_3^-
$$D_{HCO_3^-}\frac{d^2C_{HCO_3^-}}{dx^2} = -\nu_3 \tag{10}$$

WH
$$D_{WH}\frac{d^2C_{WH}}{dx^2} = \nu_4 \tag{11}$$

W^-
$$D_{W^-}\frac{d^2C_{W^-}}{dx^2} = -\nu_4 \tag{12}$$

The subscripts of the reaction rate terms (ν_i) in the above equations correspond to the numbers previously assigned to chemical reactions 1–4.

Equations 5–12 must satisfy the following boundary conditions:

$$@\ x = 0, \qquad \frac{dC_i}{dx} = 0 \tag{13}$$

and

$$@\ x = \delta, \qquad C_i = C_i^b \tag{14}$$

where the superscript b denotes the species concentration outside the cell wall of the bacterium. The concentrations of solutes and ions at $x = 0$ are assumed to be at equilibrium with the cytoplasm. Therefore, the fluxes of all of these components from the outer membrane into the cytoplasm will be zero; this is reflected by the boundary condition given in Eq. 13.

Ordinarily, all of the eight equations would be solved simultaneously with expressions for the rates of each of the reactions together with their boundary conditions. This would be a difficult task. By introducing some reasonable assumptions, however, we have previously shown that these original eight differential equations can be greatly simplified, resulting in the following two equations (Byers et al., 1992).

$$D\frac{d^2C_u}{dx^2} = \nu_1 \tag{15}$$

$$\frac{d}{dx}\left(D_{app}\frac{dC_{H^+}}{dx}\right) = \nu_1. \tag{16}$$

The diffusivity, D, is assumed to be the same for all chemical species due to the development of a diffusional potential gradient (Ruckenstein and Varanasi, 1984), which enhances the transport of the slower ions, retards the transport of the faster ions, and thus reduces the differences in the Fickian diffusivities of the individual ions. For the sake of simplicity, the diffusivities of the uncharged species involved in the reactions have also been taken equal to D. D^{app} is the apparent diffusivity defined for hydrogen ions and is given by (Byers et al., 1992):

$$-\frac{D_{app}}{D} = \frac{1 + \dfrac{K_4C^b_{T,WH}}{(K_4 + C_{H^+})^2}}{\dfrac{K_3}{K_3 + C_{H^+}} - \dfrac{2K_2C_{H^+}}{K_2C_{H^+} + 1}} + \left\{(C_{H^+} - C^b_{H^+})\left[1 + \frac{K_4C^b_{T,WH}}{(K_4 + C_{H^+})(K_4 + C^b_{H^+})}\right] + C^b_{T,H_2CO_3}\left(\frac{K_3}{K_3 + C^b_{H^+}} - \frac{2K_2C^b_{H^+}}{K_2C^b_{H^+} + 1}\right)\right\} \times \left[\frac{\dfrac{K_3}{(K_3 + C_{H^+})^2} + \dfrac{2K_2}{(K_2C_{H^+} + 1)^2}}{\left(\dfrac{K_3}{K_3 + C_{H^+}} - \dfrac{2K_2C_{H^+}}{K_2C_{H^-} + 1}\right)^2}\right]. \tag{17}$$

K_2, K_3, and K_4 are equilibrium constants for reaction 2, 3, and 4, respectively. C^b_{T,H_2CO_3} and $C^b_{T,WH}$ in Eq. 17 are the composite, or "total" concentrations for H_2CO_3 and WH outside the cell wall of the bacterium, which are defined below:

$$C_{T,H_2CO_3} = C_{H_2CO_3} + C_{HCO_3^-} \tag{18}$$

$$C_{T,WH} = C_{WH} + C_{W^-} \tag{19}$$

we have previously shown that $C_{T,WH} = C^b_{T,WH}$ for all values of x (Byers et al., 1992).

Equations 15 and 16 can be equated and further simplified to express the concentration of urea in terms of the local hydrogen ion concentration (Byers et al., 1992) as follows:

$$C_u = C^b_u - (C_{H^+} - C^b_{H^+})\frac{1 + \dfrac{K_4C^b_{T,WH}}{(K_4 + C_{H^+})(K_4 + C^b_{H^+})}}{\dfrac{K_3}{K_3 + C_{H^+}} - \dfrac{2K_2C_{H^+}}{K_2C_{H^+} + 1}} + C^b_{T,H_2CO_3}\left[1 - \frac{\dfrac{K_3}{K_3 + C^b_{H^+}} - \dfrac{2K_2C^b_{H^+}}{K_2C^b_{H^+} + 1}}{\dfrac{K_3}{K_3 + C_{H^+}} - \dfrac{2K_2C_{H^+}}{K_2C_{H^+} + 1}}\right]. \tag{20}$$

It should be pointed out that the reaction rate, ν_1, which couples Eqs. 15 and 16, is dependent on the concentrations of both hydrogen ions (that is, pH) and urea. With Eqs. 20 and 17, Eq. 16 permits an independent solution for the hydrogen ion concentration in the region of the outer membrane of the bacterium if the rate expression, ν_1, is known for the hydrolysis of urea by the urease of *H. pylori*. Dixon et al. (1980) have carried out an extensive study of the hydrolysis of urea by the urease from Jack beans. Their studies indicate that the hydrolysis of urea by the urease from Jack beans follows Michaelis-Menton kinetics over the wide pH range (3.4–7.8) they studied, with the maximum velocity, V_{max}, and the Michaelis constant, K_m, being

functions of pH. Although Cesareo and Langton (1992) have pointed out that the urease of *H. pylori* has different kinetic properties from the urease of Jack beans, the mechanism for the action of the *H. pylori* urease on urea is unknown. As an approximation, therefore, we assume here that the hydrolysis of urea by the urease of *H. pylori* also follows Michaelis-Menton kinetics, and that the maximum velocity of the reaction catalyzed by *H. pylori* urease has the same pH dependence as that catalyzed by the urease from Jack beans. The rate expression, v_1, can then be written as

$$v_1 = \frac{V_{max} C_u}{K_m + C_u} \tag{21}$$

where K_m is the Michaelis constant for the urease of *H. pylori*. In the following calculations, K_m is assumed to be constant since there are no experimental data available to determine its pH dependence. The expression for the maximum velocity and its dependence on pH is given by (Byers et al., 1992):

$$V_{max} = \frac{k_1 e_0}{C_{H^+}/K_{SEH_3} + 1 + K_{SEH_2}/C_{H^+} + K_{SEH_2} K_{SEH}/C_{H^+}^2} + \frac{k_2 e_0}{C_{H^+}^2/K_{SEH_3} K_{SEH_2} + C_{H^+}/K_{SEH_2} + 1 + K_{SEH}/C_{H^+}} \tag{22}$$

where k_1 and k_2 are the rate constants for the dissociation of the urea-urease enzyme substrate complex into product. The value e_0 is the total concentration of urease contained in the outer membrane of the bacterium. K_{SEH_3}, K_{SEH_2}, and K_{SEH} are the dissociation constants for the different forms of the enzyme substrate complex. The pK and rate constant values used in the calculation for the urease of *H. pylori* are assumed to be the same as those for the urease from Jack beans, which were given by Dixon et al. (1980): $pK_{SEH_3} = 3.0$, $pK_{SEH_2} = 6.25$, $pK_{SEH} = 9.0$, $k_1 = 3600\ s^{-1}$, and $k_2 = 6350\ s^{-1}$.

By introducing the following dimensionless parameters,

$$\bar{C}_{H^+} = \frac{C_{H^+}}{K_{SEH_2}}, \quad \bar{C}_u = \frac{C_u}{K_m}, \quad \chi_1 = \frac{K_{SEH_2}}{K_{SEH_3}}, \quad \chi_2 = \frac{K_{SEH}}{K_{SEH_2}},$$

$$\eta = \frac{\chi}{\delta}, \quad V^* = \frac{k_2}{k_1}, \quad \bar{D}_{app} = \frac{D_{app}}{D}, \quad \lambda = \frac{K_m}{K_{SEH_2}}.$$

and substituting the rate expression v_1 given by Eq. 21, together with Eq. 22, into Eq. 16, the following dimensionless differential equation is obtained,

$$\frac{d}{d\eta}\left(\bar{D}_{app}\frac{d\bar{C}_{H^+}}{d\eta}\right) = \Phi^2\lambda \frac{\bar{C}_{H^+}^2 + V^*\bar{C}_{H^+}}{\chi_1\bar{C}_{H^+}^3 + \bar{C}_{H^+}^2 + \bar{C}_{H^+} + \chi_2} \times \frac{\bar{C}_u}{\bar{C}_u + 1}. \tag{23}$$

The quantity Φ in the above equation is the Thiele modulus, which is defined as:

$$\Phi^2 = \frac{\delta^2 k_1 e_0}{D K_m} \tag{24}$$

The dimensionless boundary conditions are:

$$@\ \eta = 0, \qquad \frac{d\bar{C}_{H^+}}{d\eta} = 0 \tag{25}$$

and

$$@\ \eta = 1, \qquad \bar{C}_{H^+} = \bar{C}^b_{H^+}. \tag{26}$$

Multiplying both sides of Eq. 23 by $2\bar{D}_{app}(d\bar{C}_{H^+}/d\eta)$ and rearranging, the resulting equation is obtained:

$$\frac{d}{d\eta}\left[\left(\bar{D}_{app}\frac{d\bar{C}_{H^+}}{d\eta}\right)^2\right] = 2\bar{D}_{app}\Phi^2\lambda f(\bar{C}_{H^+})\frac{d\bar{C}_{H^+}}{d\eta} \tag{27}$$

where

$$f(\bar{C}_{H^+}) = \frac{\bar{C}_{H^+}^2 + V^*\bar{C}_{H^+}}{\chi_1\bar{C}_{H^+}^3 + \bar{C}_{H^+}^2 + \bar{C}_{H^+} + \chi_2} \times \frac{\bar{C}_u}{1 + \bar{C}_u}. \tag{28}$$

Equation 27 is now integrated subject to the boundary condition given by Eq. 25 to obtain

$$\bar{D}_{app}\frac{d\bar{C}_{H^+}}{d\eta} = \Phi\sqrt{2\lambda}\left[\int_{\bar{C}^0_{H^+}}^{\bar{C}_{H^+}} \bar{D}_{app} f(\bar{C}_{H^+}) d\bar{C}_{H^+}\right]^{1/2} \tag{29}$$

$\bar{C}^0_{H^+}$ is the dimensionless hydrogen ion concentration at $\eta = 0$. Equation 29 can now be integrated again using the boundary condition given by Eq. 26, and rearranged as

$$\Phi = \frac{1}{\sqrt{2\lambda}}\int_{\bar{C}^0_{H^+}}^{\bar{C}^b_{H^+}} \frac{\bar{D}_{app}\, d\bar{C}_{H^+}}{\left[\int_{\bar{C}^0_{H^+}}^{\bar{C}_{H^+}} \bar{D}_{app} f(\bar{C}_{H^+})\, d\bar{C}_{H^+}\right]^{1/2}} \tag{30}$$

where $\bar{C}^b_{H^+}$ represents the dimensionless hydrogen ion concentration outside the cell wall of the bacterium.

By noting that the integral in the denominator of Eq. 30 is equal to zero when $\bar{C}_{H^+} = \bar{C}^0_{H^+}$, Eq. 30 becomes divergent at this point. However, the following integral equation can be derived by integrating Eq. 30 by parts.

$$\Phi = \sqrt{\frac{2}{\lambda}}\left\{\frac{\sqrt{U(\bar{C}^b_{H^+})}}{f(\bar{C}^b_{H^+})} - \int_{\bar{C}^0_{H^+}}^{\bar{C}^b_{H^+}} \sqrt{U(\bar{C}_{H^+})}\frac{d}{d\bar{C}_{H^+}}\left(\frac{1}{f(\bar{C}_{H^+})}\right) d\bar{C}_{H^+}\right\} \tag{31}$$

where

$$U(\bar{C}_{H^+}) = \int_{\bar{C}^0_{H^+}}^{\bar{C}_{H^+}} \bar{D}_{app} f(\bar{C}_{H^+})\, d\bar{C}_{H^+}. \tag{32}$$

This integral equation can then be used to compute the value of Φ required to achieve a given pH at the interface between

the cell wall and the bacterial cytoplasm ($C^0_{H^+}$) for a given bulk pH ($C^b_{H^+}$).

The dimensionless urea concentration, $\bar{C}_u$, in Eq. 28 can be obtained by rewriting Eq. 20 as

$$\bar{C}_u = \bar{C}^b_u - \frac{\theta}{\lambda\alpha}(\bar{C}_{H^+} - \bar{C}^b_{H^+}) + \frac{\bar{C}^b_{T,H_2CO_3}}{\lambda}\left(1 - \frac{\alpha^b}{\alpha}\right) \quad (33)$$

with the following additional dimensionless groups:

$$\theta = 1 + \frac{K_4 C^b_{T,WH}}{(K_4 + C_{H^+})(K_4 + C^b_{H^+})},$$

$$\alpha = \frac{K_3}{K_3 + C_{H^+}} - \frac{2K_2 C_{H^+}}{1 + K_2 C_{H^+}},$$

$$\bar{C}_{T,H_2CO_3} = \frac{C_{T,H_2CO_3}}{K_{SEH_2}}.$$

If the value of $\bar{C}^0_{H^+}$, that is, the pH value at the interface of the cytoplasmic membrane and the cell wall, is known, the integrals contained in Eqs. 31 and 32 can be easily computed by using simple numerical integration schemes such as Simpson's method and the Newton-Cotes' integration method. After evaluating these integrals, the value of Φ required to achieve this pH at the interface can be obtained. On the other hand, if we know the Thiele modulus for the urease of *H. pylori* in the cell wall of the bacterium, we can find the maximum pH value *H. pylori* could have in the cytoplasm for a given pH outside the cell wall of the bacterium. These calculations would allow us to determine the conditions under which *H. pylori* could survive in the stomach.

RESULTS AND DISCUSSION

The Thiele modulus for *H. pylori* can be estimated from Eq. 24. In this equation, we take the thickness of the cell wall of *H. pylori* as the average value of the thickness for a Gram-negative bacterium, δ = 12 nm. The diffusivity, D, was obtained from the relationship between the thickness of the cell wall of *H. pylori* and the urea permeability coefficient, P, where $D = P\delta$. Tien (1974) gives permeability coefficient values that lie between 10^{-7} and 4.2×10^{-6} cm/s for urea transport in bilayer lipid membranes. If we take the permeability coefficient for urea as the average value given by Tien (1974), 2×10^{-6} cm/s, we obtain a $D = 2.4 \times 10^{-12}$ cm^2/s. The kinetic data for urease required in Eq. 24, K_m and $k_1 e_0$, were obtained from the work of Dunn et al. (1990). They showed that the purified urease from human gastric *H. pylori* exhibited a K_m of 0.3 mM and a V_{max} of 1100 μmol of urea hydrolyzed per minute per mg of protein at 22°C in 31 mM Tris-HCl buffer, pH 8.0. At a pH = 8.0, Eq. 22 reduces to $V_{max} = k_2 e_0$, and $k_1 e_0$ in Eq. 24 can be calculated as $(k_1/k_2)V_{max}$. The additional quantities used for unit conversion are that the protein content in *H. pylori* is assumed to be 55% of the dry weight of the cell, which is the average value of bacterial protein content (40–70%) (Shuler and Kargi, 1992); and that urease represents 6% of the total *H. pylori* proteins (Mobley and Foxall, 1994). By substituting these values into Eq. 24, the Thiele modulus for the urease of *H. pylori* is calculated to be 26. A Thiele modulus of this magnitude indicates that the hydrolysis of urea is diffusion-controlled.

The urea concentration used in our calculations is 2.5 mM, which is the average value reported for the urea concentration in the stomach (~1.5–4 mM) (Marshall and Langton, 1986). The results of our calculations are summarized in Figs. 2 and 3. Fig. 2 shows the pH at the inner surface of the cell wall, pH_0, as a function of the Thiele modulus, Φ, for a range of values of the bulk pH, pH_b. Fig. 3 shows these same results with pH_0 shown as a function of the bulk pH for a range of values of the Thiele modulus.

The experimental evidence of Clyne et al. (1995) showed that in the absence of urea, *H. pylori* survives only in solutions whose final pH ranges from ~ 5.0 to 7.2. In addition, they have also shown that urease-negative *H. pylori* are able to survive in a similar pH range in the absence or presence of urea (Clyne et al., 1995). A number of authors also showed in the absence of urea that *H. pylori* growth is restricted to a pH range of 6.5 to 7.5 (Goodwin et al., 1986; Goodwin and Armstrong, 1990) with an optimum pH at ~7 (Morgan et al., 1987). Based on these results, the

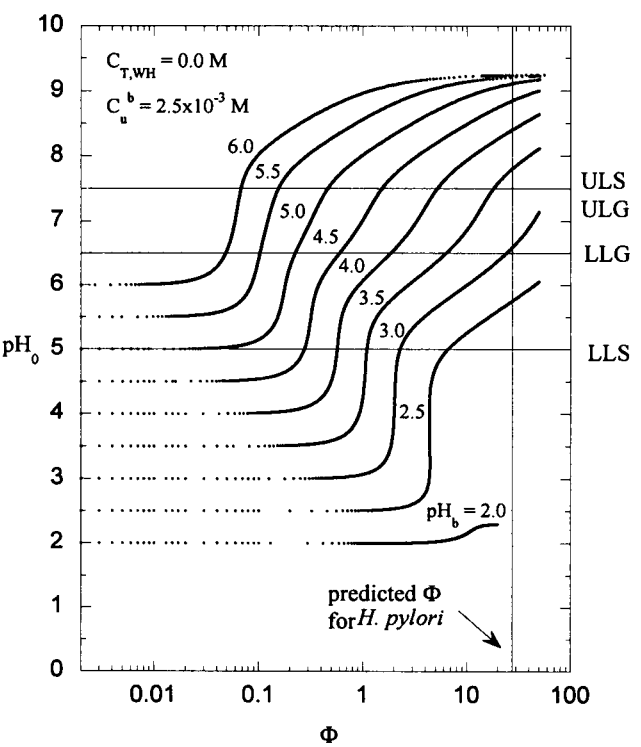

FIGURE 2 Model predictions for pH control by *H. pylori* showing the dependence of the pH at the inner surface of the cell wall, pH_0, as a function of the Thiele modulus, Φ, with the bulk pH as a parameter. (ULG: upper limit for growth, ULS: upper limit for survival, LLG: lower limit for growth, LLS: lower limit for survival).

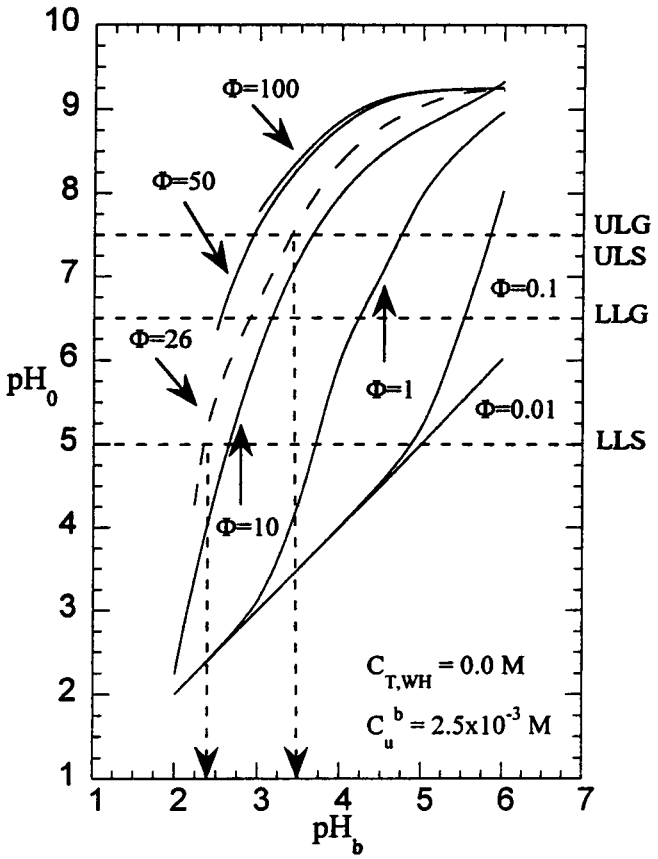

FIGURE 3 Model predictions for pH control by *H. pylori* showing the dependence of the pH at the inner surface of the cell wall, pH_0, as a function of the bulk pH, with the Thiele modulus as a parameter. (ULG: upper limit for growth, ULS: upper limit for survival, LLG: lower limit for growth, LLS: lower limit for survival).

lowest intracellular pH level for survival, the lowest value for growth, and the highest values for growth and survival (Clyne et al., 1995, Goodwin et al., 1986; Goodwin and Armstrong, 1990) have been labeled on Figs. 2 and 3. The model predicts identical internal pH for *H. pylori* in the absence of urea and for urease-negative *H. pylori* ($\Phi = 0$). When the Thiele modulus is small, for example for the case of $\Phi = 0.01$ in Fig. 2, the values of pH_0 are almost the same as those of pH_b. This is due to the fact that in this case the amount of ammonia produced by urease is so small that the desired pH gradient cannot be generated. With an increase in the Φ value for a given bulk pH, the protective effect of urease on *H. pylori* is evident, as shown in Fig. 2.

A significant feature of the calculations shown in Fig. 3 is that the model predicts that for extracellular pH values below ~2.3, *H. pylori* will be unable to maintain a $pH_0 >$ 5.0. Thus, for survival, *H. pylori* has to migrate from the stomach to a region in the mucosa where the pH is not <2.5. This is in close agreement with the conclusion of Mobley and Foxall (1994), who pointed out that, with a pH of 3 or less, the initial colonization of *H. pylori* in the stomach is difficult unless the bacterium is protected from exposure to acid. Such a result is also consistent with the experimental data of Marshall et al. (1990), who observed that when pH_b is < 2.5, *H. pylori* does not increase the pH of its immediate environment even in the presence of 5 mM urea. Although their results showed that urea protects *H. pylori* colonies in solution when the pH is < 2.5, this protection could be temporary based on our model predictions.

The urease activity of *H. pylori* enhances its chances for survival in the gastric acidic environment, but it also contributes significantly to *H. pylori*'s damage. It has been shown in vitro that the ammonia liberated by *H. pylori* urease is directly cytotoxic to gastric epithelial cells (Smoot et al., 1990) and Vero cells (Xu et al., 1990), and that such a cytotoxic effect of urease can be reduced by adding the urease inhibitor acetohydroxamic acid. Similar cytotoxic effects of ammonia produced by *H. pylori* have been shown by using chemical mutants (Segal et al., 1992) and spontaneously arising urease-negative mutants (Pérez-Pérez et al., 1992). By noting that the amounts of ammonia generated by *H. pylori* were identical under acidic and neutral conditions, however, Clyne et al. (1995) revealed that the ammonium ion per se is not toxic; the cytotoxicity results from the hydroxide ions generated by the equilibrium of ammonia with water (Mobley and Foxall, 1994; Mobley et al., 1995). They further found that *H. pylori* does not survive above a bulk pH of 4.0 in the presence of urea due to the generation of alkaline pH conditions within the bacterium. Such experimental results can be easily interpreted according to Figs. 2 and 3. For the case of $pH_b = 4.0$, the interface pH is ~8.3 at $\Phi = 26$, which is evidently toxic to the bacterium. The damage to *H. pylori* would be more severe for pH > 4.0 in the stomach, since they lead to even higher pH values within the bacterium. Indeed, the self-destruction of the bacterium has been observed at pH 6 with the presence of urea (Greig et al., 1991). A recent paper (Sjöström and Larsson, 1996) further demonstrated that urease is an essential survival and virulence factor of *H. pylori*, depending on the initial pH. The authors found that supplementation with urea increased *H. pylori*'s survival at pH 3 but reduced its growth and cell viability at pH 7 and 9.

The mucosal layer of the stomach seems to be the natural habitat of *H. pylori*. By close examination of stomach biopsy specimens using light and electron microscopy, Hazell et al. (1986) found that *H. pylori* is located within the gastric mucus and in close proximity to the surface of gastric epithelial cells. Since *H. pylori* is quite sensitive to low pH (Hazell and Lee, 1985), it evades the acidic internal peripheral layer of mucus to congregate in greater amounts around the intercellular junctions (Hazell et al., 1986), where the pH is approximately neutral (Allen and Garner, 1984). In fact, the thick mucous layer in the human stomach, which covers the gastrointestinal tract, provides a pH gradient that ensures a neutral pH at the luminal domain of the surface epithelium (Sarosiek et al., 1994). During the period when *H. pylori* faces the acidic environment of the stomach lumen, we would expect that its survival depends on significant production of urease. However, once *H. pylori* passes through the mucous layer and begins to experience a

less acidic environment, it is expected that urease production would be down-regulated, allowing *H. pylori* to colonize the intercellular junctions between the gastric mucosal cells.

In summary, the mathematical model presented here explains well the phenomena observed in a variety of experiments involving *H. pylori*. The model predictions are in good agreement with the experimental data available, suggesting that the assumptions made in the development of the model are reasonable. Such a model is useful for providing increased understanding of diseases in the upper gastrointestinal tract of humans and may also be of value for designing treatment methods.

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