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High-Affinity Binding of the Basement Membrane Proteins Collagen Type IV and Laminin to the Gastric Pathogen *Helicobacter pylori*

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The ability of 16 isolates of the human gastroduodenal pathogen *Helicobacter pylori* to bind ^{125}I-radiolabelled tissue proteins was quantitated by liquid-phase assay. While capable of binding generally low levels of collagen types I and II, vitronectin, and fibronectin (average binding, 8%; highest binding, 23%), the various *H. pylori* isolates were good binders of the basement membrane proteins collagen type IV and laminin (average binding, 27%; highest binding, 60%). *Campylobacter* species tested bound lower levels of collagen type IV and laminin (average binding, 12%; highest binding, 17%). Trypsin and proteinase K treatment of *H. pylori* cells markedly reduced the binding of collagen type IV and laminin, as did heat treatment, suggesting that the binding of basement membrane proteins is mediated by bacterial surface proteins. Binding of both basement membrane proteins was rapid and saturable. ^{125}I-collagen type IV binding to *H. pylori* 915 was inhibited by preincubation with unlabelled collagen type IV but was not inhibited by laminin or a number of other proteins. Once bound, radiolabelled collagen type IV but was not displaced by an excess of unlabelled collagen type IV, indicating that the binding interaction was of high affinity. Binding of laminin was partially reversible, and analysis in a solid-phase nonradiolabel assay showed that the interaction was of high affinity, with a K_d of 7.9 nM. This interaction was affected by salt, indicating the presence of a hydrophobic component in the ability of *H. pylori* to bind laminin.

Since its first isolation by Marshall (46) in 1983 from gastric biopsies from patients with active chronic gastritis, *Helicobacter* (*Campylobacter*) *pylori* has become recognized as one of the most common bacterial pathogens of humans. The natural niche of *H. pylori* is the human stomach, where it colonizes the mucus and adheres to gastric mucosal cells, especially at intercellular junctions (19, 25). Some *H. pylori* organisms also penetrate these intercellular junctions. Colonization by *H. pylori* appears to be essential for many diseases involving the stomach and duodenum. The diseases include gastritis, gastric ulcer, duodenal ulcer, epidemic hypochlorhydria, and nonulcer dyspepsia syndrome (1, 2, 5, 15, 25, 31). The organism may also be associated with carcinoma of the stomach, as a high percentage of patients with this disease are also culture positive for the organism (22, 24).

Although the precise roles of *H. pylori* in stomach and duodenal ulcer disease have yet to be defined, the available evidence supports the hypothesis that the presence of the organism is a prerequisite for ulcer formation (14, 25, 29). Certainly the organism can be found almost universally in the gastric antra of ulcer patients (14, 25, 29) and is associated with the margins of the ulcer itself (22, 35, 40). Moreover, treatment which eradicates *H. pylori* results in restoration to normal mucus content, repair of cell damage, disappearance of inflammatory cells, and much lower relapse rates compared than those of *H. pylori*-positive patients (3, 17, 29, 30, 32).

Few data concerning the properties of *H. pylori* which contribute to its pathogenesis, however, are available. Motility and the organism's spiral shape are probably important for the ability of the organism to colonize the mucus and penetrate to the mucosal surface (19), and recent findings indicate that different strains of *H. pylori* produce hemagglutinins (adhesins) which exhibit different receptor specificities (7, 10, 21, 33). These adhesins appear to be lectins and are likely involved in the initial specific attachment of the pathogen to gastric epithelial cells and/or to mucus. Electron microscopic studies show that following attachment, *H. pylori* exhibits toxicity for the gastric epithelial cells (11). In certain cases, this leads to destruction of the cell, desquamation, and ultimately to the complete erosion of the epithelium, leaving the underlying basement membrane completely bare. This means that in ulcerated tissue the various connective tissue proteins, including those of the basement membrane, become exposed and available to colonizing bacteria. Therefore, the capacity to bind such matrix proteins may be extremely important for the ability of *H. pylori* to colonize and damage the basement membrane before invading the lamina propria in the process of eliciting ulcerative lesions.

We have examined the ability of strains of *H. pylori* to bind to the connective tissue proteins collagen types I, II, and IV and laminin, vitronectin, and fibronectin. Here we report that *H. pylori* strains are generally strong binders of the major basement membrane proteins collagen type IV and laminin. The binding to both extracellular basement membrane proteins appears to be specific, of high affinity, either irreversible or poorly reversible, and mediated by surface-exposed cell components.

MATERIALS AND METHODS

Chemicals. Collagen type I (lot no. 87 7239) was from Collagen Corporation, Palo Alto, Calif. Collagen type II,

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purified by the method of Strawich and Nimmi (41), was graciously provided by P. Speziale (Department of Biochemistry, University of Pavia, Pavia, Italy). CR-collagen type IV (native collagen type IV) purified from basement membrane of the Engelbreth-Holm-Swarm transplantable mouse tumor (23) (lot no. 88-1403 and 88-2218) was purchased from Collaborative Research Inc. (Two Oak Park, Bedford, Mass.). Fibronectin (lot no. FP 108) purified from porcine plasma on gelatin-Sepharose was the generous gift of BioInvent International AB, Lund, Sweden. Laminin purified from Engelbreth-Holm-Swarm transplantable mouse tumor was from E.Y. Laboratories, San Mateo, Calif. (lot no. 060616) and from Boehringer GmbH, Mannheim, Germany (lot no. 14706500). Vitronectin purified as described by Dahlbäck and Podack (4) was a generous gift from B. Dahlbäck, Malmö General Hospital, Malmö, Sweden. Human fibrinogen and gamma globulin were from KABI AB, Stockholm, Sweden.

Bacterial strains. *H. pylori* strains examined in this study were endoscopic biopsy isolates 33, 54, 66, 95, and 165 (Lund, Sweden); 915, 1139, and 12225 (Stockholm, Sweden); CCUG 17874 (Culture Collection of University of Göteborg [same as type strain of species *H. pylori* subsp. *pylori* subsp. nov. NTCC 11637, National Type Culture Collection, Colindale, London, United Kingdom], originally Royal Perth Hospital isolate 13487 [Perth, Australia]); and CCUG 19106 (strain Pylo 10; F. Mégraud, Bordeaux, France). *Campylobacter fetus* VC78 (S-layer deficient [6]), VC119 (S-layer producing [6]); *Campylobacter jejuni* VC74 (26), VC159, and VC164; and *Campylobacter coli* VC167 (18) and VC212 were from the culture collection of T. J. Trust. Stock cultures were maintained at −70°C in 15% (vol/vol) glycerol-Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures of *H. pylori* were grown at 37°C on chocolate-blood agar plates containing 0.001% vancomycin, 0.005% trimethoprim, and 0.00075% colistin in an atmosphere containing 10% carbon dioxide, while cultures of *Campylobacter* strains were grown on chocolate-blood agar.

Radioiodination. Proteins (50 μg) were radiolabelled with 0.2 mCi of ^{125}I (carrier free; Radiochemical Center, Amersham, United Kingdom) to a specific activity of 1×10^6 to 3×10^6 cpm/μg by the chloramine-T method (16).

Binding assay. (i) Liquid-phase assays. Bacteria were washed off plates, suspended in phosphate-buffered saline (PBS), and adjusted to 5% transmission at 540 nm. One hundred microliters of bacterial suspension (5×10^8 cells) in PBS was mixed with 2.5×10^4 cpm (approximately 0.03 μg) of the radiolabelled protein in PBS containing 0.1% (wt/vol) bovine serum albumin (BSA) and incubated at 20°C for 60 min, unless otherwise stated. Two milliliters of ice-cold PBS containing 0.1% (vol/vol) Tween 20 was then added to each tube, and the bacteria were pelleted by centrifugation at $1,350 \times g$ for 25 min. The radioactivity of the pellet was determined in a gamma counter (LKB Wallac Clinigamma, Turku, Finland). High-level binding and nonbinding strains of *Escherichia coli* were included as positive and negative controls (28). Binding experiments were performed in triplicate, and the radioactivity bound was expressed as the percentage radioactivity remaining in the pellet.

To test the ability of proteins to inhibit binding, 100 μl of bacteria was incubated with 100 μl of a 100-μg/ml solution of the inhibitory compound in PBS, pH 7.2, for 60 min at room temperature. Bacteria were then washed with PBS and used for binding experiments. To determine the reversibility of binding, bacteria were first incubated as described above for the binding assay and then incubated at room temperature for 1 h with 100 μl of nonradiolabelled collagen type IV or laminin (100 μg/ml in PBS) or with 100 μl of PBS in the control tubes. The reaction was stopped, and the amount of radioactivity remaining associated with the cells was determined as described previously.

To assess the effects of temperature on the ability of bacteria to bind the basement membrane proteins, suspensions of bacteria in PBS were heated in a water bath either at 80°C for 20 min or at 100°C for 30 min before use in binding assays. The involvement of surface proteins in the binding interactions was assessed by treating cell suspensions with trypsin (2 μg/ml or 50 μg/ml) or proteinase K (2 μg/ml) for 1 h at 37°C. In the case of proteinase K, cells were then washed extensively in PBS, and in the case of trypsin digestions, soy bean trypsin inhibitor (200 μg/ml) was added to stop the digestion.

(ii) Solid-phase assays. Bacterial whole cells, grown as described above, were harvested with 0.1 M carbonate buffer, pH 9.6, washed once with 0.1 M carbonate buffer, pH 9.6, and resuspended to an optical density of 0.4 to 0.6 at 650 nm. To each well of a 96-well enzyme-linked immunosorbent assay plate (Costar, Cambridge, Mass.), 100 μl of cell sample (or carbonate buffer as a blank) was added and incubated at 4°C overnight. Plates were then washed with 0.01 M Tris-buffered saline supplemented with 0.2% (wt/vol) BSA (Sigma) (TBS-BSA) (250 μl per wash) and blocked with 3% BSA in TBS (200 μl per well) for 2 h at room temperature. At this time the wells were washed three times with TBS-BSA. To each well, mouse Engelbreth-Holm-Swarm sarcoma laminin (Boehringer) in TBS (100 μl per well [2 nM]) or TBS itself was added and incubated at room temperature for 2 h. Wells were then washed five times, and rabbit antilaminin immunoglobulin G (E.Y.) was added to each well (100 μl of a 1:3,000 [vol/vol] dilution per well). After the wells were incubated for 2 h at room temperature, they were washed five times. Goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (100 μl of a 1:3,000 [vol/vol] dilution per well) (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) was added to each well. After 2 h, wells were washed five times. A substrate solution consisting of 1.0 M diethanolamine, 0.5 mM magnesium chloride, and 3.8 mM disodium *p*-nitrophenyl phosphate (Sigma), pH 9.5, was added to each well (100 μl per well) and incubated for 30 min. The A_{450} of each well was then determined. Values were corrected for nonspecific binding of laminin to the well (less than 10% of test value).

The time course of laminin binding was determined as follows. To each well, 100 μl of laminin (2 nM) in TBS was added. At various time intervals, the laminin solution was removed from a set of wells and the wells were washed five times. The amount of laminin bound to each well was assessed as described above. The reversibility of laminin binding was determined as follows. Coated, blocked wells were incubated with laminin (2 nM) for 2 h and then washed five times. TBS (100 μl) was then added to each well. This was time zero. At various time intervals, the buffer was removed from a set of wells and the wells were washed three times. The amount of bound laminin was then assessed as described above.

RESULTS

Quantitation of matrix protein binding by liquid-phase assay. Taking a binding value of more than 5% as positive, the majority of *H. pylori* strains tested displayed the ability to bind each of the six extracellular matrix proteins tested in

TABLE 1. Binding of ^{125}I-radiolabelled extracellular matrix proteins to *H. pylori* strains in the liquid-phase assay

Strain no.	Binding (%) of[a]:					
	Collagen			Laminin	Vitronectin	Fibronectin
	Type I	Type II	Type IV			
33	12	10	30	17	7	7
66	4	12	24	—	—	—
54	7	13	25	12	6	7
72	8	7	31	35	9	8
95	9	10	42	32	7	9
114	6	—	32	—	—	—
165	11	23	40	23	7	16
915	9	20	48	23	7	9
1139	11	9	49	53	12	8
5155	3	4	9	16	3	3
5294	8	4	18	28	5	4
5442	3	4	16	26	5	3
9102	9	6	13	13	6	5
12225	8	—	21	—	8	7
17874	6	8	56	60	10	5
19106	8	16	15	8	4	7

[a] Dashes indicate that strains were not tested. Values represent means of from 2 to 14 determinations for each strain.

TABLE 2. Binding of ^{125}I-radiolabelled extracellular matrix proteins to *Campylobacter* strains in the liquid-phase assay

Strain	Binding (%) of[a]:					
	Collagen			Laminin	Vitronectin	Fibronectin
	Type I	Type II	Type IV			
C. fetus						
VC78	3	5	12	17	2	2
VC119	3	5	11	12	1	1
C. jejuni						
VC74	4	5	11	12	1	2
VC159	2	4	8	12	2	3
VC164	2	5	11	12	2	2
C. coli						
VC167	2	4	9	14	1	2
VC212	2	4	10	17	1	1

[a] Values represent means of two determinations for each strain.

liquid-phase assays (Table 1). Thirteen of the 16 strains bound low amounts of collagen type I (average binding, 8%). Eleven of 14 strains bound low levels of collagen type II (average binding, 11%), although strains 165, 915, and 19106, which bound 23, 20, and 16%, respectively, were high-level binders. Twelve of 14 strains tested bound low levels of vitronectin (average binding, 7%), and 11 of 14 strains bound low amounts of fibronectin (average binding, 7%). Binding of the basement membrane proteins collagen type IV and laminin was much stronger. All 16 strains tested bound collagen type IV (average binding, 29%), with 5 strains binding >40% and the highest binding being 56%. Similarly, all 14 strains bound laminin (average binding, 27%), with two strains binding 50% and the highest binding being 60%.

Comparative assays were also performed to determine whether this high-level collagen type IV- and laminin-binding property displayed by *H. pylori* strains was shared with other mucus-colonizing spiral bacteria. The results in Table 2 show that the three strains of *C. jejuni* and two strains each of *C. coli* and *C. fetus* displayed lower binding levels for collagen type IV (average binding, 10%; highest binding, 12%) and laminin (average binding, 14%; highest binding, 17%) than the *H. pylori* strains tested. The campylobacters were unable to bind collagen type I (average binding, 3%), collagen type II (average binding, 5%), vitronectin (average binding, 2%), and fibronectin (average binding, 2%).

On the basis of these results, *H. pylori* 915, which exhibited high binding capacity for collagen type IV and moderate binding of laminin, was selected for more detailed examination of the collagen type IV binding, and *H. pylori* 17874, which exhibited high binding capacity for both basement membrane proteins, was selected for more detailed investigation of laminin binding.

Characteristics of collagen type IV and laminin binding by liquid-phase assay. The *H. pylori* cells bound both basement membrane proteins in a time-dependent manner (Fig. 1). This reaction was rapid, and a plateau of bound collagen type IV and laminin was reached in 15 to 30 min. Continuation of the incubation for up to 3 h did not significantly increase the amount of ^{125}I-radiolabelled protein bound. In terms of cell density in the assay reaction mixture, the binding of collagen type IV by strain 915 and laminin by strain 17874 was essentially saturated at 10 mg (wet weight) of cells per ml (Fig. 2). Binding of collagen type IV by *H. pylori* 915 cells was largely unaffected by pH (Fig. 3), and quantitation of ^{125}I-collagen type IV as a function of increasing concentration of radiolabelled ligand showed that the binding was saturable, yielding a hyperbolic curve (Fig. 4) and indicating that the bacteria contain a limited number of collagen type IV receptors. Scatchard plot analysis (37) of the binding data allowed the calculation of a K_d of 16 nM. Importantly, however, when the reversibility of binding to *H. pylori* 915 cells was tested by the addition of an excess of unlabelled collagen type IV (100 μg), previously bound ^{125}I-collagen type IV was not displaced. Because valid Scatchard analysis requires the binding reaction to be at equilibrium, the above prediction must be regarded as a minimum estimate of the affinity of binding of collagen type IV.

Inhibition experiments to evaluate the specificity of collagen type IV and laminin binding were performed. Preincubation with unlabelled collagen type IV resulted in the inhibition of ^{125}I-collagen type IV binding by *H. pylori* 915, while preincubation with collagen type I, laminin, fibrinogen, fibronectin, or gamma globulin resulted in no inhibition of

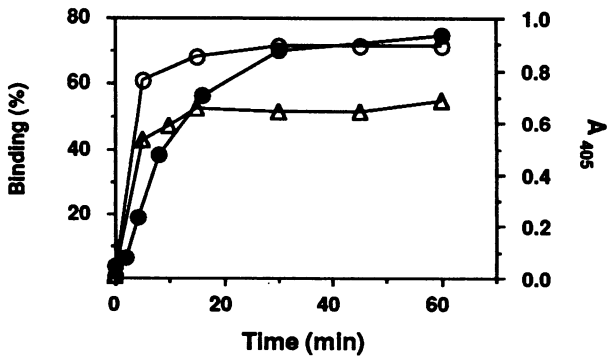

FIG. 1. Kinetics of binding of ^{125}I-radiolabelled collagen type IV to *H. pylori* 915 (○) and ^{125}I-radiolabelled laminin to *H. pylori* 17874 (△) in the liquid-phase assay and unlabelled laminin to *H. pylori* 17874 in the solid-phase assay (●).

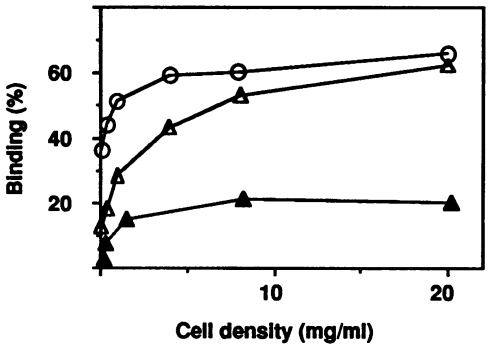

FIG. 2. Effects of bacterial cell density on binding of ^{125}I-radiolabelled collagen type IV to live (△) and boiled (▲) *H. pylori* 915 cells and ^{125}I-radiolabelled laminin to live *H. pylori* 17874 (○) in the liquid-phase assay.

binding. In the case of laminin binding by *H. pylori* 17874, inhibition was obtained only by preincubation with unlabelled laminin. Furthermore, all assays were performed in the presence of 0.05% BSA to minimize any possible contribution of nonspecific protein-protein interactions to the binding quantitation. The receptors involved in this specific binding of collagen type IV and laminin on *H. pylori* appeared to be protein in nature. Binding of both basement membrane proteins was drastically reduced by treatment of the bacterial cells with trypsin or proteinase K and also by subjecting the cells to heat (Table 3).

Solid-phase analysis of laminin binding. The availability of antibodies to laminin afforded us the opportunity to examine the laminin binding interaction by using a solid-phase assay system with nonradiolabelled laminin. Binding of laminin was somewhat slower in this assay system but was still completed by 60 min (Fig. 1). In contrast with collagen type IV binding, binding of laminin was partially reversible, and virtually identical displacement kinetics were obtained with *H. pylori* 17874 and 915 (Fig. 5). These results compared favorably with the 11% displacement of ^{125}I-laminin from *H. pylori* 17874 cells obtained in the liquid-phase assay.

Binding of laminin to cells of strain 17874 exhibited saturation, and Scatchard analysis gave a straight line indic-

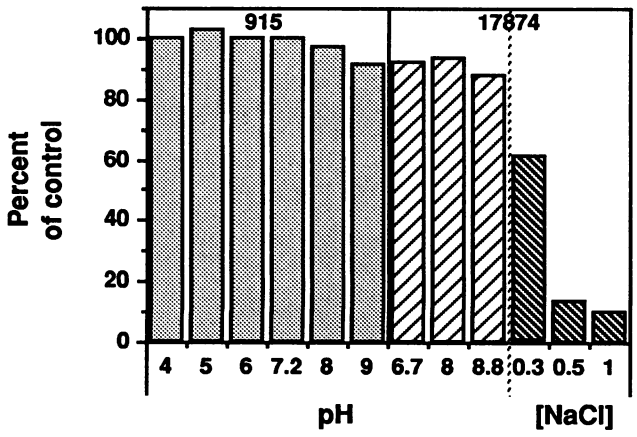

FIG. 3. Effects of pH on binding of ^{125}I-radiolabelled collagen type IV by *H. pylori* 915 in the liquid-phase assay and of pH and sodium chloride concentration on the binding of laminin by *H. pylori* 17874 in the solid-phase assay.

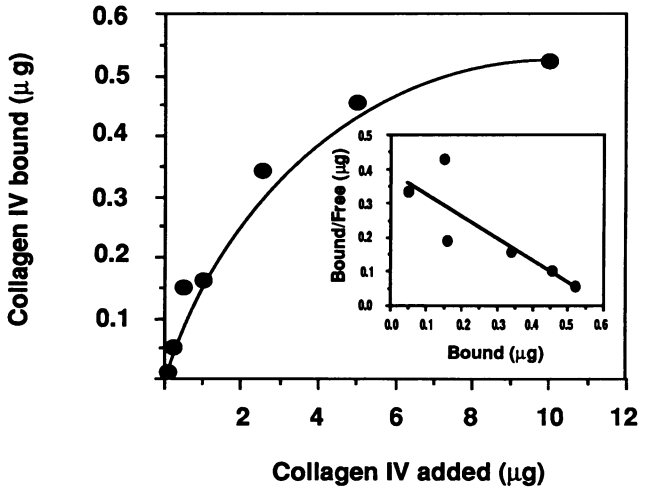

FIG. 4. Analysis of binding of ^{125}I-radiolabelled collagen type IV to *H. pylori* 915 cells in the liquid-phase assay. Inset: Scatchard plot of binding data.

ative of a single class of binding interaction with a binding constant of 7.9 nM (Fig. 6). Changes in the pH of the buffer in which the binding was conducted had no marked effect on laminin binding (Fig. 3), but the concentration of salt in the buffer had a dramatic effect. Figure 3 shows that a 40% decrease in laminin binding was obtained in the presence of 0.3 M NaCl and a 95% decrease was obtained at 1 M NaCl.

DISCUSSION

This study has revealed the ability of the human gastric pathogen *H. pylori* to bind the basement membrane proteins collagen type IV and laminin. The binding of both proteins is insensitive to pH and is specific, rapid, saturable, and of high

TABLE 3. Characteristics of binding of ^{125}I-radiolabelled murine collagen type IV to *H. pylori* 915 and ^{125}I-radiolabelled murine laminin to *H. pylori* 17874 in the liquid-phase assay

Treatment	Collagen type IV binding to strain 915		Laminin binding[a] to strain 17874	
	% Relative binding	% Inhibition	% Relative binding	% Inhibition
None	100	0	100	0
Heat				
80°C for 20 min	46	54	49	51
100°C for 30 min	37	63	32	68
Enzyme				
Trypsin				
2 μg	46	54	52	48
50 μg	29	71	—	—
Proteinase K (2 μg)	5	95	31	69
Inhibitor (10 μg)				
Collagen type I	100	0	100	0
Collagen type IV	50	50	100	0
Fibronectin	100	0	100	0
Fibrinogen	100	0	100	0
Laminin	100	0	53	47
Gamma globulin	100	0	100	0

[a] Dashes indicate that the strain was not tested under this condition.

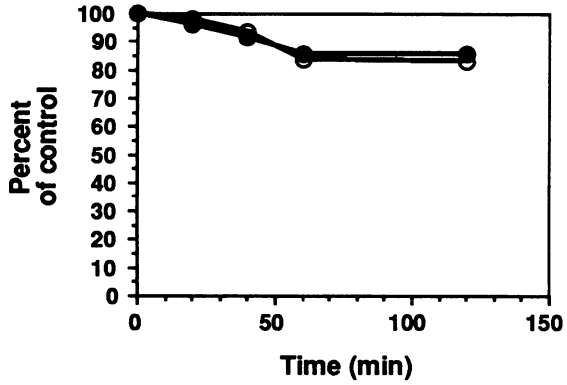

FIG. 5. Displacement of bound laminin in the solid-phase assay. *H. pylori* 915 (●) and 17874 (○).

affinity. Moreover, the binding of collagen type IV appears to be irreversible, while the binding of laminin is only partly reversible. This previously undescribed binding ability of *H. pylori* cells appears to involve two sterically separated receptor sites because despite the large size of the molecules of both basement proteins, neither interfered with the binding of the other. The *H. pylori* receptors appear to be surface proteins because the binding of both collagen type IV and laminin was significantly reduced by boiling the cells and by treating the cells with either trypsin or proteinase K. Indeed, the reduced binding ability exhibited by bacteria treated for 20 min at 80°C, a temperature which will cause perturbation of macromolecular assembled proteins rather than denaturation, suggests that a supramolecular protein structure such as a fimbria might be involved in the binding of the two basement membrane proteins. Certainly *H. pylori* characteristically produces fimbrialike appendages (4a, 10), and fimbriae have been implicated in the binding of other extracellular matrix proteins such as collagen type IV (47) and fibronectin by *E. coli* (34) and collagen type V by *Klebsiella*, *Yersinia*, and *Salmonella* strains (44). Assuming an M_r of 540,000 for collagen type IV, the available number of binding sites for this protein per cell is approximately 3,000. Unfortunately, in the case of laminin, the immunoassay employed here had a limitation for this kind of analysis, and it was not possible to calculate the number of laminin binding sites per bacterial cell.

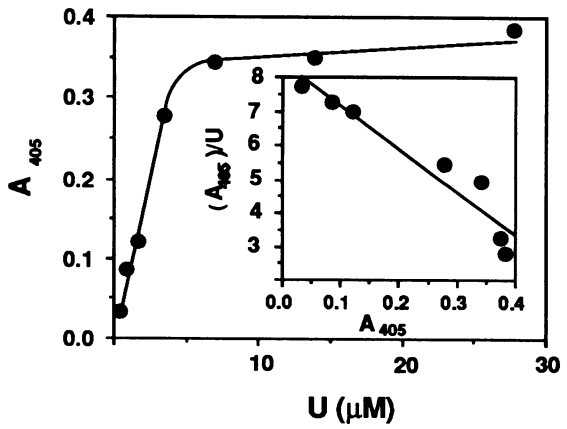

FIG. 6. Analysis of binding of laminin to *H. pylori* 17874 cells in the solid-phase assay. Inset: Scatchard plot of binding data. A_{405}/U represents the relative absorbance divided by the concentration of laminin added.

Regardless of the number of binding sites per cell, the ability to bind high levels of the two major basement membrane proteins appears to be a conserved property of *H. pylori*. This property of high-level binding was not shared with the other strains of mucosa-colonizing spiral bacteria tested here. Moreover, while other bacteria have been shown to bind one or other of collagen type IV and laminin, the ability of individual strains to bind both proteins is not common. For example, *E. coli* cells expressing the O75X adhesin are strong binders of collagen type IV but recognize laminin only weakly (47). Individual strains of *Staphylococcus aureus* have also been reported to bind collagen type IV (45) and/or laminin (27), but the binding of both proteins is not a property shared by all strains. Moreover, in contrast with that of *H. pylori*, the binding spectrum of individual strains of *S. aureus* is not restricted to the basement membrane proteins but is much broader and includes fibronectin (36), collagen type I (20), and collagen type II (39) as well as soluble plasma proteins such as immunoglobulin (12).

The irreversibility of the collagen type IV binding interaction precluded an accurate assessment of binding affinity. However, the binding was clearly of high affinity, probably higher than the estimate of 16 nM obtained here. Irreversibility of binding of extracellular matrix proteins has been reported previously. For example, the binding of collagen type I by *Yersinia enterocolitica* NF-9-4 is irreversible (8), as is the binding of fibronectin by certain strains of *S. aureus* (36). Other than the high affinity of the binding interaction, the reasons for irreversibility have yet to be defined. For example, the property might be an indication that in addition to binding via a receptor, other interactions might be occurring such that the binding obtained is tight and irreversible. Certainly additional classes of binding interactions can occur. As shown by biphasic Scatchard plots, two classes of interaction in the fibronectin binding by *E. coli* (13) and *Vibrio cholerae* (48) have been seen. However, analysis of the Scatchard plots obtained here provides no indication of the involvement of additional binding sites in the binding of either basement membrane protein. The effect of salt on the binding of laminin did indicate that there was a hydrophobic component involved in this interaction. In the case of laminin, binding to the *H. pylori* cells was at saturation and was reversible at the time of assessment, hence in equilibrium. Thus, the criteria for using a modified Scatchard methodology were met. By using this analysis, a 7.9 nM K_d value was obtained. This value is slightly lower than the 2.9 nM laminin binding constant reported for *S. aureus* (27) but significantly higher than the 40 to 80 nM values reported for species of streptococci (42, 43).

This ability to specifically bind both the major components of the basement membrane seems likely to represent an important virulence property for this gastric pathogen. While this binding capability is unlikely to be involved in the initial colonization of gastric epithelial cells in which motility to facilitate access to the specific receptors on the mucosal surface (19) and the presence of primary adhesins which specifically recognize and bind to these receptors appear to be important (7, 9, 10), the property should assume major importance once any initial microlesion in the ulceration process exposes the basement membrane. This exposure may result from *H. pylori* activity. For example, Slomiany et al. (38) have recently shown that *H. pylori* lipopolysaccharide interferes with the binding interaction between the laminin receptor in gastric epithelial membranes and lami-

nin. These workers have proposed that this will lead to disruption of the interaction between the mucosal cells and the underlying extracellular matrix and may account for the loss of mucosal integrity seen in the pathogenesis of *H. pylori* disease. However, even without *H. pylori*-induced disruption to mucosal integrity, feeding by itself produces a massive loss of epithelial cells from the gastric surface, sometimes exposing the lamina propria (49). Certain substances which are consumed are exfoliating, including ethanol, aspirin, and spicy pickles (49). The capacity of *H. pylori* to rapidly, avidly, and largely irreversibly bind both collagen type IV and laminin should facilitate both initial and continued colonization of any basement membrane which becomes exposed as a result of either *H. pylori*-induced disruption of mucosal integrity or the loss of epithelial cells resulting from eating or drinking. Furthermore, the capacity to bind basement membrane proteins at both acid and alkaline pH should promote the continued colonization of any ulcerative lesions formed in either the acid stomach or the alkaline duodenum.

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