The urease enzymes of *Campylobacter pylori* and a related bacterium

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Summary. The urease enzyme of *Campylobacter pylori* was studied and compared with that of a related spiral-shaped bacterium, St1, isolated from the rodent ileum. Both bacteria possessed constitutive urease enzymes with activities up to 20–70 times that of *Proteus vulgaris*. This activity was retained on SDS-polyacrylamide gels. A major catalytic subunit of mol. wt 300 000 was located for all (six) strains of *C. pylori* subjected to SDS-PAGE whereas St1 had two active forms of mol. wts 140 000 and 150 000. Western-blot analysis indicated the presence of anti-urease antibodies in the sera of patients with *C. pylori*-associated gastritis. The response to *C. pylori* urease was not strain-specific but no cross-reactivity was detected between the *C. pylori* enzyme and that of St1. The very high urease activity of these bacteria is likely to be important in colonisation of the host. Possession of glutamate dehydrogenase activity by both organisms suggests that one role of the urease may be to assimilate the available urea nitrogen. Modification of the local environment to facilitate long-term colonisation is another possible function. Protection from acid is unlikely to be a primary role as the natural habitat of the organism St1 is the non-acid-secreting tissue of the small intestine.

Introduction

The enzyme urease (E.C.3.5.1.5.), originally isolated from jack beans (Sumner, 1926), catalyses the hydrolysis of urea to ammonia and carbon dioxide. Urease enzymes have since been isolated and purified from many species of bacteria, in which they play an important role in nitrogen metabolism. The ability to hydrolyse urea has several consequences for mammalian hosts colonised by urease-positive micro-organisms, including: regulation of transfer of endogenous urea across the rumen wall (Cheng and Wallace, 1979); an effect upon the nitrogen metabolism of ruminants (Wozny *et al.*, 1977); production of urinary stones (Griffith and Musher, 1973); and contribution to the pathogenesis of pyelonephritis (Braude and Siemienski, 1960; Musher *et al.*, 1975).

Campylobacter pylori, an organism which is an aetiological agent of active chronic gastritis and a possible contributor to peptic ulceration (Marshall and Warren, 1984; McNulty and Watson, 1984; Rollason *et al.*, 1984), possesses an extremely high level of urease activity. Moreover this property provides a useful basis for the diagnosis of *C. pylori*-

associated gastritis by the detection of urease directly from gastric biopsy tissue (McNulty and Wise, 1985; Hazell *et al.*, 1987). Hazell and Lee (1986) hypothesised that the high urease activity of *C. pylori* caused hydrogen ion back-diffusion in the gastric epithelium and that this was a predisposing factor in gastric ulcer formation. Others have proposed that, given the sensitivity to acid of *C. pylori*, a function of the urease may be to create an insulating neutral-to-alkaline blanket around the organism to protect it from the lethal effects of stomach acid (Goodwin *et al.*, 1986).

An appreciation of the role of any given phenotypic property of an organism within its environmental niche may be enhanced by comparative studies of organisms that preferentially colonise similar environments. In this regard, comparisons between a urease-positive spiral-shaped bacterium isolated from the ileal crypts of rodents (Lee and Phillips, 1978) and *C. pylori* may provide useful information. This rodent bacterium, St1, is an autochthonous member of the rodent gastrointestinal tract and, like *C. pylori*, is micro-aerophilic, possesses sheathed polar flagella and has adapted to a mucus environment.

The similarities between these spiral bacteria may permit comparisons of the effect of urease on

mucosal colonisation in acid-secreting and non-acid-secreting environments. The first consideration in any such study is to ascertain the comparative levels of urease activity of each bacterial type and to determine whether the activity of the enzyme can be regulated by the presence of nitrogen or ammonia. Second, there is a need to isolate and purify the enzyme. This paper describes studies on the urease enzymes of *C. pylori* and St1 which are an essential pre-requisite to an investigation of the role of these enzymes in the colonisation of intestinal mucosae.

Materials and methods

Bacterial strains

C. pylori strains P2, P3, P16, P17, P21, 5298, G1, D1 and NCTC 11639 were clinical isolates. The culture of St1 was described previously (Lee and Phillips, 1978). *Proteus vulgaris* was provided by the Culture Collection of the School of Microbiology, University of New South Wales. Bacteria were grown on Blood Agar Base No. 2 (Oxoid) supplemented with lysed horse blood 5% v/v and amphotericin B (E. R. Squibb and Sons, Inc., Princeton, USA) 0.5% w/v. Plates inoculated with *C. pylori* and St1 were incubated at 37°C in a micro-aerophilic atmosphere for 3–4 days. *P. vulgaris* was grown on the same medium and incubated at 37°C, aerobically for 18 h.

Urease assay

Bacteria were washed from culture plates with freshly prepared saline, and the cells were centrifuged at 10 000 *g* for 3 min. After resuspending in fresh saline, the optical density of the suspension was adjusted to a value of 1.0 at a wavelength of 600 nm. Before the assay, cells were further diluted with an equal volume of saline.

Urease activity of whole bacteria was measured by a modification of the method of Kaltwasser and Schlegel (1966). The reagents used were obtained from the Urea/Ammonia test system (Boehringer Mannheim GmbH, Mannheim, Germany). Reagents per 3 ml quartz cuvette were: NADH 0.4 mg and 2-oxoglutarate 2.2 mg in 1.0 ml of triethanolamine buffer (pH 8); 0.1 ml of a urea solution (140 mg/ml); 20 µl of glutamate dehydrogenase (c. 830 U/ml); and 1.9 ml of distilled water. For *C. pylori* and St1, the reaction was initiated by the addition of 20 µl of a bacterial suspension. Comparatively, the rate of urea hydrolysis by *P. vulgaris* was so low that a 1.0-ml suspension of this organism had to be added to the reaction mixture. Oxidation of NADH was read at a wavelength of 340 nm with a distilled water blank, at 35 ± 1°C, in a Pye Unicam SP 1800 double beam spectrophotometer (Unicam Instruments Ltd, Cambridge). The reaction was recorded on a Pye Unicam AR25 linear change recorder calibrated to read in the 0–

2 optical density range at a chart speed of 1 cm/min, and the reaction rates were determined. A minimum of eight assays/medium/passage (where appropriate)/strain were done with pooled cells from 2–5 plates. Each culture was tested on at least three separate occasions. All data were included in the calculation of means and 95% confidence intervals and were compared by a one-way analysis of variance, determined with the STATCALC programme (Lee, McInerney and Mullins, Auckland, New Zealand) on an Apple IIe computer.

Controls were reaction mixture minus urea, and reaction mixture minus bacteria. The concentration of bacterial protein was determined for each cell suspension, and the rate of urea hydrolysis calculated as the rate of urea hydrolysed/min/mg of bacterial protein.

Glutamate dehydrogenase determination

This was modified from the method of Smith *et al.* (1981). Bacteria were washed twice in cold (4°C) KCl 1% w/v. The resuspended cells were sonicated with a Sonifier Model B-30 Cell Disruptor (Branson Sonic Power Co., Danbury, CT, USA) at 14 kHz for 1 min/ml of washed cells. The sonicate was centrifuged at 10 000 *g* for 5 min to remove the cell debris and the supernate was stored at 4°C until assayed.

A test solution consisting of NH₄Cl 32 mg, 2-oxoglutarate 2.2 mg, NADH or NADPH 0.2 mg, and KCl 6.5 mg was prepared in 1 ml of triethanolamine-HCl buffer (pH 7.5). The assay was run at 35 ± 1°C and the oxidation of NADH or NADPH was read at a wavelength of 340 nm. The reaction was initiated by adding 1 ml of crude bacterial extract to 2 ml of test solution in the spectrophotometer. The rate of NADH or NADPH oxidation in a control solution (the test solution without NH₄Cl) was subtracted from that initiated by the bacterial extract. The rate of NH₄⁺ utilisation was calculated in terms of µg of NH₄⁺ consumed/h/mg of bacterial protein extract.

Protein determination

Protein estimations were made with the BCA protein assay reagent (Pierce Chemical Co., IL, USA). Bovine serum albumin was the protein standard.

Preparation of samples for SDS-PAGE

The cells were harvested from the blood-agar plates and washed twice in distilled water, at 4°C. After resuspending the pellet in a small volume of solubilisation buffer, the cells were sonicated at 8 kHz, 50% cycle for 1 min. The solubilisation buffer contained 0.625M Tris-HCl (pH 6.8), sodium dodecylsulphate (SDS) 3% w/v, 2-mercaptoethanol 5% w/v, glycerol 10% v/v and bromophenol blue 0.01% w/v. Cell debris was removed from the sonicate by centrifugation at 13 000 *g* and the cell-free extract was stored in small volumes at –20°C.

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Extracts containing urease were analysed by SDS-PAGE (Laemmli, 1970) in a modification of the method of Senior *et al.* (1980). The cell-free extracts were applied to a slab gel comprising a stacking gel of acrylamide 4.5% w/v and a resolving gel of acrylamide either 5 or 6% w/v. The samples were stacked at 10 mA and separated at 20 mA constant current. The gels were then washed in several changes of a filtered solution of disodium ethylenediaminetetraacetic acid (EDTA) 0.1% w/v and cresol red indicator 0.02% w/v until the gel turned yellow, indicating neutral or slightly acidic conditions. Gels were developed by discarding the washing solution and immersing them in a small volume of urea 1.5% w/v. Areas of urease activity appeared as cherry-red bands against the yellow background due to the local production of alkali as a result of enzymic degradation of urea.

The developed gels could be kept conveniently as a permanent record by attaching the gel (through capillary action) to a piece of white paper, which was then photocopied.

Isolation of crude urease

To isolate urease, a 1.5 mm-thick gel was loaded with only one type of whole-cell extract and run as described above. Once developed, the bands of urease activity were cut from the gel and placed in 0.1M sodium phosphate buffer (pH 7.0) containing 0.01M disodium EDTA and 0.01M 2-mercaptoethanol (stabilisation buffer). The gel slices were macerated with a tissue homogeniser and the preparations were concentrated with Amicon CF25 centriflo ultrafiltration membrane cones (Amicon, Danvers, USA), centrifuged at 850 *g* for 10 min at 4°C. The retentate was removed from the cones with 0.2 ml of fresh stabilisation buffer and stored in small volumes at -20°C.

Western blotting

Both the whole-cell extracts of bacteria and the crude urease preparations were run on SDS-polyacrylamide gels and then transferred electrophoretically to nitrocellulose paper by the method of Towbin *et al.* (1979). The transfer step was done in a Trans-Blot Electrophoretic Transfer Cell (Bio-rad, Richmond, CA, USA) for 4 h at 70V with cooling.

The nitrocellulose membrane was blocked with casein 5% w/v in PBS at room temperature, for 2 h. Human sera from diagnosed cases of *C. pylori*-associated gastritis were diluted 1 in 100 in casein 1% w/v in PBS and allowed to react at 4°C overnight. All preparations were agitated constantly on a platform shaker. The membrane was washed three times for 5 min each time with Tween 20 0.05% v/v in PBS, then once with PBS. The membrane was incubated in a solution of biotin-anti-human (total) immunoglobulin (Kirkegaard and Perry Lab., Inc., MD, USA) diluted 1 in 1000 in casein 1% w/v in PBS at room

temperature for 1 h. After washing as described above, the nitrocellulose was allowed to react with avidin-peroxidase (KPL) diluted 1 in 2000 in casein 1% w/v in PBS for a further hour. The membrane was washed and the bound protein detected by adding a substrate solution containing 4-chloro-1-naphthol 0.3% w/v. The reaction was terminated by bathing the nitrocellulose in distilled water.

Results

Urease activity

Preliminary investigations with *C. pylori* (NCTC 11639) revealed that its urease activity was elevated up to two and a half times in cultures incubated for 96 ± 2 h compared with cultures incubated for 72 ± 2 h, whether it was grown on lysed horse-blood agar or lysed horse-blood agar containing ammonium chloride 0.2% w/v. However, those cultures incubated for 120 ± 2 h showed a dramatic decrease in urease activity, probably as a result of cell death and autolysis. Despite the relatively lower urease activity of the 72-h cultures compared with the 96-h cultures, the shorter incubation period was chosen for the remainder of the investigation because the margin between viable and non-viable cells could not be judged easily in the older cultures. Similarly, cultures of *P. vulgaris* were harvested well in advance of the late stationary phase (i.e., after 18 h).

The urease activities of washed cells of *C. pylori*, the rodent spiral bacterium, St1, and *P. vulgaris* were compared (fig. 1). The urease activities of the *C. pylori* strains were in the range 200–700 μ g of urea hydrolysed/min/mg of bacterial protein. There was no significant difference ($p > 0.05$) between the urease activities of St1 and *C. pylori* NCTC 11639. Moreover, strain NCTC 11639 exhibited an activity which was, within one order of magnitude, similar to that of the two freshly isolated strains of *C. pylori*, 5298 and P2. Conversely, *P. vulgaris*, an archetypal ureolytic bacterium, exhibited a markedly lower urease activity of 9.4–10.6 μ g of urea hydrolysed/min/mg of bacterial protein.

To test the effect of ammonia on the urease of *C. pylori*, ammonium chloride 0.2% w/v was added to the lysed-blood media (fig. 1). The two fresh clinical isolates exhibited an initial depression in urease activity when plated on to the ammonium-enriched medium but this was neither significant nor, in the case of the one strain tested further, sustained. Strain NCTC 11639 did not show this initial depression of urease activity. The results suggested that urease is a constitutive enzyme in *C. pylori* and

Fig. 1. Urease activity of the rodent spiral bacterium (St1), three strains of *C. pylori* and *P. vulgaris* after growth on lysed-horse-blood agar (■). Columns 1, 2 and 3 indicate the activities of cultures passaged 1, 2 and 3 times respectively, on medium supplemented with NH_4Cl 0.2% w/v (▨). The results represent the mean activity with 95% confidence intervals.

St1. Furthermore, the level of urease activity of *C. pylori* is independent of either the effects of a rich nitrogen source, or the presence of ammonia.

Glutamate dehydrogenase activity

Three fresh isolates (strains P16, P17 and P21) of *C. pylori* were tested and found to have NADPH-dependent glutamate dehydrogenase (GDH) activities of 28–84 µg of ammonia consumed/h/mg of bacterial protein. St1 expressed an NADPH-dependent GDH activity of 15 µg ammonia consumed/h/mg of bacterial protein. This indicated that *C. pylori* and St1 have the ability to assimilate ammonia and may use the ammonia released during ureolysis to synthesise important amino acids.

Identification of urease by SDS-PAGE

The urease enzymes of *C. pylori* and St1 retain their enzymic activity on SDS-polyacrylamide gels, thus simplifying enzyme identification and isolation. Fig. 2a is a photocopy of a gel on which whole-cell extracts of St1 and five strains of *C. pylori* were run. The banded areas represent zones of intense red colouration where the pH of the gel has changed due to the hydrolysis of urea at that site. All six strains of *C. pylori* tested had a 300 000-mol.wt major active subunit. Occasionally a band of higher mol.wt appeared but this was not restricted to any one strain or preparation, nor was it a consistent finding. This phenomenon was thought to be due to an aggregation of the urease molecule, possibly with a non-active subunit. In contrast, St1 had two major subunits of mol. wts 140 000 and 150 000.

Anti-urease antibodies

Western blot analysis of a crude urease preparation from *C. pylori* NCTC 11639 with human *C. pylori* antiserum is presented in fig. 2b. The recognition of two proteins at mol.wt c. 300 000 correspond to the major subunit of *C. pylori* urease described above. These results suggested that the complete urease enzyme may exist in a dimeric form, but whether both molecules are enzymically active is not known. The protein of mol.wt 120 000 is probably a degradation product or a non-catalytic isoenzyme (Fishbein *et al.*, 1969; Delisle, 1977).

The urease enzymes from three strains of *C. pylori* were isolated and the responses to these with three samples of human anti-*C. pylori* serum (collected as part of another study) were tested. It was highly unlikely that any of the sera could have been from persons infected with a strain of *C. pylori* that was homologous to those used for the enzyme isolation, yet each of the three sera reacted with the urease enzymes of one or more of the *C. pylori* strains. Thus the 300 000- and 120 000-mol.wt proteins of *C. pylori* were not strain specific, nor were they recognised by rabbit antiserum raised against saline-washed whole cells of St1 although this antiserum reacted with both St1 gel bands. Similarly, anti-*C. pylori* human antisera did not react with a crude preparation of St1 urease.

Discussion

Although there has been much speculation about the role of the urease of *C. pylori* (Goodwin *et al.*, 1986; Hazell and Lee, 1986; Rathbone *et al.*, 1986), no detailed work on the enzyme has been published until now. *C. pylori* is a member of a large group of spiral bacteria which associate with the mucus lining the mammalian gastrointestinal tract. Another spiral bacterium, St1, isolated from the ileal crypts of rodents by workers in our laboratory (Lee and Phillips, 1978) shares several properties with *C. pylori*. Both bacteria are micro-aerophilic, have multiple sheathed flagella, have adapted to a mucus environment, and possess an extremely high level of urease activity. *C. pylori* and St1 preferentially colonise different areas of the gastrointestinal tract; we postulate that these micro-organisms have adapted to their particular niches by developing characteristics that give them a selective advantage in the colonisation of the mucus that lines the gastrointestinal tract and that urease is an important colonisation factor for both bacteria.

Savage (1983) suggested that urea may be a valuable nutrient for organisms colonising the

Fig. 2. (a) SDS-PAGE of whole-cell extracts of St1 and five *C. pylori* strains on a resolving gel of acrylamide 6% w/v. The bands correspond to areas of urease activity. Samples were from (lanes): 1, *C. pylori* strain D1; 2, *C. pylori* NCTC 11639; 3, *C. pylori* P21; 4, *C. pylori* G1; 5, *C. pylori* P3; 6, St1. The numbers on the left indicate mol. wts 10^3 . *C. pylori* urease was extracted from a gel, subjected to SDS-PAGE and transferred to nitrocellulose paper. (b) The electroblotted proteins from a crude urease preparation were allowed to react with human serum from a case of *C. pylori*-associated gastritis. Bands labelled a and b represent two urease subunits of mol. wt c. 300 000. Band c represents a 120 000-mol. wt. protein and label d indicates the level of the protein front.

mucosal surfaces of the gut. Evidence for this suggestion is the consistent finding of bacterial urease activity in the stomach and rumen of animals (Fitzgerald and Murphy, 1950; Von Korff and Glick, 1951; Von Korff *et al.*, 1951; Kornberg *et al.*, 1954*a,b*; Wallace *et al.*, 1979). Hazell *et al.* (1986) described the localisation of *C. pylori* on the human gastric mucosa close to the intercellular junctions. Nutrients diffusing across the mucosa via the paracellular route would thus be readily available to the organism; one such solute is urea which primarily traverses the mucosa by this pathway (Powell, 1981). Urea is an available nitrogen source that represents up to 50% or more of the non-protein nitrogen of human serum.

The prime function of urease in many species of bacteria is to supply urea nitrogen to cells in a form that can be assimilated. Ammonia, a product of urea hydrolysis, has been shown to indirectly repress the urease activity of *Selenomonas ruminantium* and *Klebsiella aerogenes* (Friedrich and Magasanik,

1977; Smith *et al.*, 1981). In some species of bacteria, urease can only be detected following nitrogen starvation (Kaltwasser *et al.*, 1972) whereas others (e.g., *P. vulgaris* and *Sporosarcina ureae*) are unaffected by the external ammonia concentration, and appear to synthesise urease constitutively (Friedrich and Magasanik, 1977).

The urease activity of *C. pylori* does not require nitrogen starvation for detection and is not repressed by an elevated concentration of ammonium ions (fig. 1); therefore the enzyme in this species is constitutive. The finding of a lower level of urease activity in strain NCTC 11639, compared with that of the two freshly isolated strains, may be due to the number of subcultures that it had undergone, both before and after its arrival at our laboratory, or may reflect a true variation within the species.

Kaltwasser *et al.* (1972) suggested that as long as the product of urea hydrolysis did not accumulate, repression of urease may not occur. Utilisation of ammonia is accomplished via glutamate dehydro-

genase (GDH) or glutamine synthetase (GS), the former operating in environments rich in ammonia (Kleiner *et al.*, 1981). The gastrointestinal tract has a high concentration of ammonia, thus GDH would be the most important ammonia-assimilating enzyme in such an environment (Hespell and Smith, 1983). Both *C. pylori* and the rat ileal isolate St1 possess an NADPH-dependent GDH and would be able to assimilate the large amounts of ammonia, released during urea hydrolysis, for the synthesis of glutamate from α -ketoglutarate. These substances are the preferred metabolites for transaminase reactions, which play an important role in the degradation and synthesis of amino acids. Therefore, an important function of urease in these bacteria may be the supply of urea nitrogen, in the form of ammonia, to the cell.

Ammonia is the most abundant nitrogen compound in the rumen and arises from the breakdown of dietary proteins or urea. Most ruminal bacteria are able to use ammonia as their sole source of nitrogen and, generally, do not require amino acids for growth (Hespell and Smith, 1983). Similarly, major species isolated from the human colon have a preference for ammonia (Bryant, 1974). Many of these bacteria produce urease, the activity of which is regulated by the availability of nitrogen for growth.

C. pylori is more fastidious in its growth requirements than either the ruminal or colonic bacteria. The gastric campylobacter grows on enriched media containing complex organic constituents such as blood, peptones and yeast extract. *C. pylori* seems to depend upon nitrogen compounds, particularly amino acids, for its growth.

If, as we assert, *C. pylori* utilises ammonia released during ureolysis for the *de novo* synthesis of amino acids, why should the organism also require the uptake of amino acids for growth? The low affinity of GDH for NH_4^+ (Smith *et al.*, 1981) and the large quantities of ammonia liberated by the urease enzyme suggest that it is unlikely that all of the ammonia could be utilised for the synthesis of amino acids. Moreover it would be metabolically inefficient to do so as amino acids and peptides are freely available in the stomach as a result of the digestion of dietary protein. The nutritional requirement of *C. pylori* for particular amino acids may be necessary for the conversion to other metabolically useful substrates.

A salient observation is that *C. pylori* can grow on media which is essentially free of carbohydrates. McNulty and Dent (1987) tested between 25 and 129 isolates of *C. pylori* for each sugar commonly used in sugar fermentation tests and found that

none of the isolates were able to metabolise sugars. The rodent isolate St1 has a similar requirement for nitrogen compounds and also cannot metabolise sugars (Phillips and Lee, 1983). The inability to oxidise or ferment sugars is a character shared by all members of the genus *Campylobacter* and most of the bacteria assigned to the aerobic or micro-aerophilic, motile, helical or vibrioid gram-negative taxa described in Bergey's Manual of systematic bacteriology (1984). How *C. pylori* derives its carbon and energy needs is not known. It is possible that this organism fulfills these needs by the metabolism of nitrogen compounds via TCA cycle intermediates, conversion of amino acids or uptake of the carbon dioxide released during ureolysis.

Goodwin *et al.* (1986) suggested that the prime function of the urease of *C. pylori* is the defence of the organism against acid damage, by surrounding the bacterium with an alkaline cloud. If the urease activity of *C. pylori* plays any role in the defence of the bacterium against acid, it is important to consider whether this is a primary or secondary function.

C. pylori is generally found in close proximity to the epithelial cells or within the gastric mucus; the former is an essentially neutral environment and the latter a component of a system which limits acid diffusion (Williams and Turnberg, 1980, 1981; Smith *et al.*, 1985). Furthermore, the spiral bacterium St1 has a level of urease activity similar to that of the gastric campylobacter (fig. 1), and yet preferentially colonises the non-acid-secreting mucosae of the rodent ileum. It seems unlikely that an organism would devote a significant proportion of its anabolic activity to the synthesis of an enzyme that depends on an external supply of substrate as a protective mechanism useful only transiently while the bacterium passes from the gastric lumen into the mucus layer. A more logical strategy would be the development of acid tolerance, as seen with the lactobacilli present in the acid environments of the mouse stomach and the human vagina.

Rather, as previously suggested (Hazell and Lee, 1986), the rapid hydrolysis of urea by *C. pylori* localised at the gastric intercellular junctions would induce a net flux of urea across the mucosa, thus maintaining a near constant supply of urea nitrogen. Because of the effect this constant urea hydrolysis would have on the local pH, the bacterium would become adapted to an alkaline environment, thus rendering *C. pylori* to some degree acid-sensitive. In this circumstance, the presence of ammonia may, as a secondary consequence, confer some protection from gastric acidity. Whereas we do not consider the function of urease to be primarily an

acid-protective mechanism, the extremely high level of activity of this enzyme appears to be greater than would be needed if its sole purpose was to capture urea nitrogen. Thus the change in environment caused by the breakdown of urea to ammonia (and carbon dioxide) is likely to be of further benefit to the organism. The nature of this further ecological advantage provided by the enzyme is unknown.

Investigation of the role of urease as a colonisation factor for *C. pylori* and St1 requires the identification and characterisation of the enzymes from these two bacteria. Thus the proteins in whole-cell extracts of *C. pylori* and St1 were separated by SDS-PAGE and the urease enzymes, which retained their activity on SDS-polyacrylamide gels, were identified. In the reducing, denaturing conditions used in our method, a catalytically-active band of mol. wt 300 000 was isolated from all strains of *C. pylori* tested (fig. 2a). This was thought to be a subunit of the complete enzyme structure. The urease profile of St1 differed appreciably from that

of *C. pylori* with two subunits present at mol. wts 140 000 and 150 000. Different urease profiles do not discount the possibility that the two organisms are closely related, given that even within the genus *Proteus* there is diversity in isoenzyme patterns (Senior *et al.*, 1980).

Regardless of whether the urease of *C. pylori* plays a role in the pathogenesis of gastric ulcer, is required for the nutrition of the organism, or is protective against acid, one would expect antibodies to be produced against the enzyme. Such antibodies were found in diagnosed cases of *C. pylori*-associated gastritis. Demonstration of a homogeneous urease molecule in *C. pylori* strains, which is not strain-specific and which does not cross-react with that of a closely related bacterium, may be of practical value in the diagnosis of *C. pylori*-associated gastritis by immunoassay.

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