Infection and Biochemical Diversity of Ureases of Proteus, Providencia, and Morganella Species Isolated from Urinary Tract Infection

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Bacterial urease, particularly from Proteus mirabilis, has been implicated as a contributing factor in the formation of urinary and kidney stones, obstruction of urinary catheters, and pyelonephritis. Weekly urine specimens (n = 1,135) from 32 patients, residing at two chronic-care facilities, with urinary catheters in place for ≥30 days yielded 5,088 phenotypically and serotypically diverse bacterial isolates at >10^5 CFU/ml. A total of 86% of specimens contained at least one urease-positive species, and 46% of 3,939 gram-negative bacilli were urease positive. For investigation of genetic relatedness of urease determinants, whole-cell DNA from 50 urease-positive isolates each of Providencia stuartii, Providencia rettgeri, P. mirabilis, Proteus vulgaris, and Morganella morganii were hybridized with a urease gene probe derived from within the urease operon of Providencia stuartii BE2467. The percentage of strains hybridizing with the gene probe was 98 for Providencia stuartii, 100 for Providencia rettgeri, 70 for P. mirabilis, 2 for M. morganii, and 0 for P. vulgaris. Electrophoretic mobilities of ureases from representative isolates revealed nine different patterns among the five species. The urease gene probe hybridized with fragments of HindIII-digested chromosomal DNA from all isolates except M. morganii. Fragment sizes differed between species. Molecular sizes of the enzymes, determined by Sephacryl S-300 chromatography, were found to be 280 kilodaltons (kDa) (P. mirabilis), 323 to 337 kDa (Providencia stuartii, Providencia rettgeri, P. mirabilis, P. vulgaris), 620 kDa (Providencia rettgeri), and >700 kDa (M. morganii, Providencia rettgeri). K_m's ranged from 0.7 mM urea for M. morganii to 60 mM urea for a P. mirabilis isolate. In general, P. mirabilis ureases demonstrated lower affinities for substrate but hydrolyzed urea at rates 6- to 25-fold faster than did enzymes from other species, which may explain the frequent association of this species with stone formation.

Bacterial urease, an enzyme that catalyzes the hydrolysis of urea, yielding ammonia and carbon dioxide, has been implicated as a factor contributing to pyelonephritis (2, 10, 12, 20), hepatic coma (23), hyperammonemia (21), inactivation of complement (1), and urolithiasis (5, 6, 18; H. L. T. Mobley and J. W. Warren, manuscript submitted for publication). The aged, chronically catheterized patient has been found to be almost universally bacteriuric, usually with more than one species (16, 24, 25). In a recent study by our group, 86% of weekly urine specimens from 32 such patients contained urease-producing organisms (Mobley and Warren, submitted; H. L. T. Mobley, H. L. Muncie, Jr., and J. W. Warren, Abstr. IVth Int. Symp. Pyelonephritis, p. 42, 1986). Overall, 46% of the gram-negative bacilli produced urease. In the long-term catheterized patient, bacterial urease represents a potential virulence factor that is produced by nearly half of the gram-negative bacteria present at ≥10^5 CFU/ml of urine and may therefore represent a common pathogenic factor.

Although urease production is a common phenotype among isolates from urinary tract infection, certain species, such as Proteus mirabilis, are more often associated with struvite and carbonate-apatite stone formation (6) and pyelonephritis (20) than are other urease-positive isolates representing Providencia and Morganella species. These differences in pathogenicity may be caused by additional virulence factors expressed by P. mirabilis or simply caused by different biochemical characteristics (e.g., affinity for substrate and rate of hydrolysis) of the ureases produced by this species.

Only a few reports have appeared comparing the ureases of Proteus, Providencia, and Morganella species. Senior et al. (22) demonstrated that representatives of the genera of the Proteaeae tribe produce ureases of different electrophoretic mobilities, but no molecular weights were calculated. Rosenstein et al. (19) also found that the molecular weights of single representatives of the Proteaeae tribe differ and that Proteus morganii (now Morganella morganii) is more resistant to two urease inhibitors. The high molecular weights (all greater than 560,000) reported here, however, were not consistent with data from experiments described by Senior et al., who observed significant migration of enzymes through 6% polyacrylamide gels. Finally, Guo and Liu (7) found that M. morganii urease is serologically distinct from that of P. mirabilis, Proteus vulgaris, and Providencia rettgeri.

Because the enzymes from different bacterial species seem to possess different properties and because the few existing studies appear to provide conflicting information, we undertook a systematic comparison of the ureases of urinary tract isolates with respect to molecular weight, kinetics of urea hydrolysis, electrophoretic mobilities, isoelectric points, and genetic relatedness of these enzymes. This report describes diversity among ureases from species of Proteus, Providencia, and Morganella and attempts to explain why the ureases of P. mirabilis may be important virulence factors.
MATERIALS AND METHODS

Bacteria. Urine specimens were collected aseptically from 32 elderly patients with urinary catheters in place for 30 days. Bacteria at concentrations of $\geq 10^2$ CFU/ml of urine were identified to the species level by using the Minitek Enterobacteriaceae II System (BBL Microbiology Systems, Cockeysville, Md.) as previously described (16, 25). Isolates were stored at $-70^\circ$C in Trypticase soy broth (BBL) supplemented with 15% (wt/vol) glycerol.

Urease gene probe. A 2.8-kilobase DNA sequence that fell between the sites of two transposon insertions (pMID401 and pMID403) which inactivated the cloned Providencia stuartii urease gene was used as a gene probe (17). Plasmid pMID204 (17) was digested with EcoRV and HindIII, and two adjacent fragments (1.3 and 1.5 kilobases) were electroeluted from an excised gel slice from a preparative 1% agarose gel and extracted with phenol, chloroform, and ether. DNA (0.25 µg) was labeled with [32P]ATP (New England Nuclear Corp., Boston, Mass.; 800 Ci/mmol) by nick translation as described by Maniatis et al. (13).

Dot blot hybridization. Bacteria were grown overnight in 5 ml of nutrient broth (Difco Laboratories, Detroit, Mich.) at 37°C with aeration (200 rpm). Culture was mixed 1:1 with 0.6 M NaCl, 0.2 M NaOH, 0.08% sodium dodecyl sulfate. Cell lysate (15 µl) was spotted onto a gridted nitrocellulose filter (Schleicher & Schuell, Inc., Keene, N.H.) by Maniatis et al. (13). Filters were baked under vacuum for 2 h at $80^\circ$C and hybridized with the 32P-labeled urease gene probe under stringent conditions (50% formamide, 65°C wash) as described by Maniatis et al. (13). Dried blots were placed between acetate sheets and autoradiographed for 18 h at $-70^\circ$C with an intensifying screen in place. Providencia stuartii BE2467, the source of the gene probe, was used as the positive control for each filter.

Chromosomal DNA extraction. Chromosomal DNA was isolated from bacterial cells lysed with sodium dodecyl sulfate and extracted with phenol, chloroform, and ether by the method of Marmur (14).

Urease preparations. Bacteria were grown at 37°C with aeration in 100 ml of Luria broth (15) supplemented with 0.1% (wt/vol) urea after being autoclaved. Cells were harvested by centrifugation at 4°C, washed twice with 20 mM sodium phosphate, pH 6.8, suspended in 5 ml of 20 mM sodium phosphate, pH 6.8-5.5 mM dithiothreitol-1 mM EDTA, and ruptured in a precooled French pressure cell at 20,000 lb/in². The lysate was centrifuged at 27,000 x g for 30 min, and the supernatant was removed with a Pasteur pipette and either used directly or stored in 1-ml portions at $-70^\circ$C. Protein was determined by the method of Lowry et al. (11) by using bovine serum albumin as a standard.

Column chromatography. A urease preparation (1 ml; 10 to 15 mg of protein per ml) was loaded onto a Sephacryl S-300 column (100 by 2.5 cm) equilibrated with 20 mM sodium phosphate-0.02% sodium azide, pH 6.8. Fractions (3 ml) were collected at a flow rate of 30 ml/h. The column was calibrated with standard proteins of known molecular weight (Pharmacia Biotechnology Products, Piscataway, N.J.). Fractions with peak activity were determined by adding samples (100 µl) of each fraction to wells of microtiter dishes containing 100 µl of 0.04% cresol red-100 mM urea. Determinations were made in triplicate.

Nondenaturing polyacrylamide gel electrophoresis. Urease preparations (20 µl) were mixed with 20 µl of 50% sucrose-0.1% bromphenol blue and loaded onto a 5.5% polyacrylamide gel (16 by 16 by 0.15 cm) (1:32 bisacryl-

amide-acrylamide; U.S. Biochemical Co., Cleveland, Ohio) with a 4% stacking gel and electrophoresed for 4 h at 250 V. Gels were equilibrated with 0.02% cresol red-0.1% EDTA (22) and then immersed in 1.5% (wt/vol) urea. The points of migration of ureases were recorded on Kodachrome 64 film (Eastman Kodak Co., Rochester, N.Y.).

Spectrophotometric urease assay. Rates of urea hydrolysis were measured by the spectrophotometric assay of Hamilton-Miller and Gargan (8) and as described previously (17). The assay was calibrated by comparing changes in optical density at 560 nm with ammonia liberation as measured by an ammonia electrode (Corning Glass Works, Corning, N.Y.) connected to a pH meter (model 071; Beckman Instruments, Inc., Fullerton, Calif.), which was calibrated with 10-6 to 10-1 molar ammonium chloride as described by the manufacturer.

Isoelectrofocusing in polyacrylamide gels. Gels were prepared as described for the LKB Ultramodul system (LKB Instruments, Inc., Rockville, Md.). Urease preparations (30 µl) were loaded approximately 2 cm from the cathode onto a 5% polyacrylamide gel (100 by 245 by 0.1 mm) (1:32 bisacrylamide-acrylamide; U.S. Biochemical Co.) with 7.7% ampholytes (pH range, 3.5 to 9.5; LKB). Gels were electrophoresed at a constant power of 3 W for 6 h and cooled with circulating ice water. The pH gradient was measured by using a surface electrode (Beckman), and the gel was equilibrated in 0.02% cresol red-0.1% EDTA. The point of migration of ureases (isoelectric point) was determined by immersion of the gel in 1.5% urea as described above for nondenaturing polyacrylamide gels.

RESULTS

To study genetic and biochemical diversity of ureases, two variables were examined for a large number of strains to select unique enzymes for more detailed analyses. Strains were examined for the ability of whole-cell DNA to hybridize to a urease gene probe derived from a Providencia stuartii strain. On the basis of the degree of hybridization, a series of isolates was used to determine the relative electrophoretic mobilities of the ureases on nondenaturing polyacrylamide activity gels. Isolates producing ureases with unique electrophoretic mobilities were then selected for characterization.

Hybridization with a Providencia stuartii gene probe. Whole-cell DNA from the species listed in Table 1 were used to prepare dot blots. Filters were hybridized with a 32P-labeled Providencia stuartii urease gene probe under stringent conditions. The gene probe recognized homologous sequences with all but one urease-positive strain of Providencia stuartii (Fig. 1). Hybridizations were as strong as with Providencia stuartii BE2467, the strain from which the original recombinant clone was derived. The probe appeared to be specific, showing absolutely no hybridization with urease-negative Providencia stuartii strains (Fig. 1; Table 1) or plasmid vector pBR322. Excellent hybridization (100% positive) also was seen with strains of the closely related species Providencia rettgeri. In addition, 70% of P. mirabilis isolates showed positive hybridization but generally gave a much weaker signal, probably indicating significant homology but to a lesser degree than for isolates of Providencia stuartii or Providencia rettgeri. Little or no hybridization was seen with isolates of P. vulgaris, M. morganii, or Klebsiella pneumoniae by dot blot procedure.

Electrophoretic mobilities of ureases. Ten isolates from each of the species representing each hybridization class
TABLE 1. Dot blot hybridization of bacterial isolates from urinary tract infection with a $^{32}$P-labeledProvidencia stuartiiurease gene probe

<table>
<thead>
<tr>
<th>Bacterial species*</th>
<th>No. of isolates tested</th>
<th>No. of positive hybridizations*</th>
<th>Negative hybridization*</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROVIDENCIA stuartii</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease positive</td>
<td>47</td>
<td>44</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Urease negative</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>PROVIDENCIA rettgeri</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>PROTEUS mirabilis</td>
<td>50</td>
<td>4</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>PROTEUS vulgaris</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>MORGANELLA morganii</td>
<td>51</td>
<td>1</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>KLEBSIELLA pneumoniae</td>
<td>51</td>
<td>3</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>ESCHERICHIA coli</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>CITROBACTER diversus</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>PSEUDOMONAS aeruginosa</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>PSEUDOMONAS sp./</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ALCALIGENES group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACINETOBACTER anitratus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other gram-negative spp.</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
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</table>

* All urease positive except where indicated.
* 50% formamide, 65°C wash.
* X-ray emulsion as dark as control DNA (Providencia stuartii BE2467).
* X-ray emulsion exposed, but not as dark as control DNA.
* X-ray emulsion not exposed.

FIG. 1. Dot blot hybridization of isolates from urinary tract infection with a $^{32}$P-labeled urease gene probe derived fromProvidencia stuartii BE2467. Cells from overnight cultures were lysed with alkaline sodium dodecyl sulfate and spotted on nitrocellulose filters. Filters were hybridized under stringent conditions (13) with a $^{32}$P-labeled gene probe derived fromProvidencia stuartii BE2467, washed, dried, and autoradiographed. Samples are directly below the identifying number.Providencia stuartii BE2467, the source of the gene probe, was used as a positive control (C). Dots: Urease-negativeProvidencia stuartii: 21, 22, 35, 37, 38; urease-positive isolates ofProvidencia stuartii: 1, 2, 7, 8, 17, 18, 20, 28, 43, 44, 49, 55, 56, 60, 66, 73;Providencia rettgeri: 23, 24, 46, 50-53, 57, 59, 62, 63, 67; P. mirabilis: 3, 4, 13, 14, 19, 25, 36, 40, 47, 48, 68, 74, 76, 80; M. morganii: 9-12, 32, 41, 42, 45, 54, 61, 65, 67, 71, 78, 79; P. vulgaris: 30, 31, 58, 69, 75; K. pneumoniae: 5, 6, 15, 16, 34, 39, 64, 70, 72, 77; Pseudomonas aeruginosa: 26; Escherichia coli: 27; andCitrobacter diversus: 29, 33. Weak but significant hybridization withP. mirabilisisolates on the original X-ray film often did not reproduce well in the figure.

FIG. 2. Urease activity in non-denaturing polyacrylamide gels of cell lysates from Proteus, Providencia, and Morganella species. Cells grown in Luria broth supplemented with 0.1% urea were harvested, washed, and ruptured in a French pressure cell at 20,000 lb/in². Insoluble material was removed by centrifugation. Soluble protein was loaded on a 5.5% polyacrylamide gel and electrophoresed for 4 h at 4°C. The gel was equilibrated with 0.02% cresol red-0.1% EDTA and then transferred to a 1.5% urea solution. Bands denote an intense color development at the points of migration of ureases. Lanes: A. Providencia stuartii BE2467; B. P. mirabilis BR2528; C. P. mirabilis H14320; D. P. mirabilis BU517; E. P. mirabilis BU7354; F. P. vulgaris GO1232; G. Providencia rettgeri TA7138; H. Providencia rettgeri SI5453; I. M. morganii TA43; J. (inset) immediate color development ofM. morganii TA43.
representated by two strains of P. mirabilis: 323 to 339 kDa, represented by P. vulgaris, two strains of P. mirabilis, a strain of Providencia rettgeri, and Providencia stuartii; 850 kDa, represented by an isolate of Providencia rettgeri; and 712 kDa from the M. morganii isolate. Providencia rettgeri S15453 expressed two distinct enzymes with molecular sizes estimated to be 315 and 850 kDa.

**Isoelectric points of ureases.** Each of the nine ureases with unique electrophoretic mobility was characterized according to its isoelectric point. The enzymes were electrophoresed in polyacrylamide gels containing ampholytes which formed a pH gradient from 3.5 to 9.5. The urease from each strain revealed a distinct and unique isoelectric point (Table 3). In all cases, a predominant band developed in the gel when the gel was soaked in urea, and the band was followed by the appearance of more slowly developing secondary bands.

With the exception of the high-molecular-weight urease of Providencia rettgeri S15453, which had a pI of 6.8, all species produced enzymes with pIs ranging from 5.1 to 5.9.

**Hybridization with chromosomal DNA digests.** Chromosomal DNA isolated from each of the nine strains was digested with HindIII, electrophoresed, and transferred to nitrocellulose. The DNA was then hybridized with the 32P-labeled Providencia stuartii urease gene probe under stringent conditions. Hybridization was very strong with the positive control Providencia stuartii BE2467 as well as with P. mirabilis BR2528, Providencia rettgeri TA1738, and Providencia rettgeri S15453. Weaker hybridization was also seen with P. mirabilis H4320 and P. mirabilis BU517. It was surprising that very weak hybridization was also observed for P. mirabilis BU7354 and P. vulgaris GO1232, because they had not demonstrated homology to the probe by dot blot hybridization. No hybridization was seen with M. morganii DNA, even at reduced stringency. The urease gene probe hybridized strongly with HindIII chromosomal DNA restriction fragments of P. mirabilis BR2528 and Providencia rettgeri S15453 with the same electrophoretic mobility of the fragment derived from Providencia stuartii BE2467. All four P. mirabilis strains, including strain BR2528, possessed a smaller HindIII fragment of equal size that shared some homology with the gene probe. In addition, Providencia rettgeri TA1738 and P. vulgaris GO1232 possessed fragments different from those of all other isolates that hybridized with the gene probe.

**Hybridization with plasmid DNA.** Plasmid DNA was isolated from each of the nine strains, electrophoresed, transferred to nitrocellulose, and hybridized with the 32P-labeled Providencia stuartii urease gene probe under stringent conditions. Although six of nine isolates carried plasmids of various mobilities, the probe hybridized only with the high-molecular-weight plasmid of Providencia stuartii BE2467 (16.17) from which the probe was derived.

### Table 3. Characteristics of ureases of unique electrophoretic mobility from representative isolates of *Providencia, Proteus,* and *Morganella* species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Hybridization with urease gene probea</th>
<th>$K_u$ (mM urea)$^b$</th>
<th>Maximum hydrolysis by induced lysate (µmol of NH₃/min per mg of protein)$^c$</th>
<th>Molecular size (kDa)$^d$</th>
<th>Isoelectric point (pH)$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Providencia stuartii</em></td>
<td>BE2467</td>
<td>+ (Control)</td>
<td>12$^f$</td>
<td>4</td>
<td>337 ± 18</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em></td>
<td>TA1738</td>
<td>+ W</td>
<td>11</td>
<td>6</td>
<td>335 ± 13</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>S15453</td>
<td>+</td>
<td>ND</td>
<td>9</td>
<td>315 ± 11, 850 ± 18</td>
<td>5.1, 6.8</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>H4320</td>
<td>+ W</td>
<td>39</td>
<td>51</td>
<td>281 ± 8</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>BR2528</td>
<td>+</td>
<td>22</td>
<td>40</td>
<td>338 ± 15</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>BU517</td>
<td>+ W</td>
<td>24</td>
<td>35</td>
<td>282 ± 12</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>BU7354</td>
<td>+</td>
<td>60</td>
<td>55</td>
<td>322 ± 11</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>GO1232</td>
<td>–</td>
<td>10</td>
<td>3</td>
<td>323 ± 12</td>
<td>5.7</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>TA43</td>
<td>–</td>
<td>0.7</td>
<td>0.4</td>
<td>712 ± 32</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Jack bean</em></td>
<td>ND</td>
<td>10</td>
<td>0.7</td>
<td>0.4</td>
<td>574</td>
<td>5.1</td>
</tr>
</tbody>
</table>

$^a$ +, Strong hybridization; + W, weak hybridization; −, no hybridization by dot blot hybridization (Table 1); ND, not determined.

$^b$ Values represent means of three determinations.

$^c$ Values represent means ± standard deviations for three determinations.

$^d$ Values represent means of two determinations.

$^e$ Two distinct ureases were produced by this isolate.
**DISCUSSION**

Proteus, Providencia, and Morganella species are distinguished from other genera of the Enterobacteriaceae by the production of urease. This positive phenotype characterized by the hydrolysis of urea is indeed represented by a highly diverse group of proteins which differ within and between species with respect to size, charge, affinity for substrate, and DNA homology of the genes which encode these enzymes.

In previous studies comparing ureases of Proteus, Providencia, and Morganella species, certain aspects of diversity were noted. In a serological study, cross-precipitation of ureases by antisera directed against urease preparations of P. vulgaris, (four strains), P. mirabilis, (three strains), M. morganii (three strains), and Providencia rettgeri (three strains) was reported. Guo and Liu (7) observed that the ureases of P. vulgaris, P. mirabilis, and Providencia rettgeri were immunologically cross-reactive but were distinct from the enzyme produced by M. morganii. That the Morganella urease is distinct from that of Proteus and Providencia species is also supported by our data. No significant hybridization of the Providencia gene probe was observed with 51 Morganella isolates. In addition, the electrophoretic mobility and molecular size of 712 kDa, which makes it one of the largest enzymes observed, were distinct from those of the other enzymes studied, and this urease demonstrated the highest affinity (lowest $K_m$) for substrate. Although we saw genetic cross-reactivity of P. mirabilis, Providencia rettgeri, and Providencia stuartii, the 15 isolates of P. vulgaris did not hybridize on dot blots, indicating a significant lack of homology with the gene probe. However, a HindIII restriction fragment of P. vulgaris chromosomal DNA did hybridize weakly with the gene probe, whereas no such hybridization was seen for M. morganii even at reduced stringency.

In a study of electrophoretic mobilities of ureases for Proteus, Providencia, and Morganella species, Senior et al. (22) previously noted differences in rates of migration through nondenaturing polyacrylamide gels. However, no molecular weights were reported. As we report, the greatest variability in mobilities within species occur with P. mirabilis and Providencia rettgeri as ureases showed similar patterns within species for Providencia stuartii, P. vulgaris, and M. morganii.

Rosenstein et al. (19) examined single isolates of P. mirabilis, P. vulgaris, Providencia rettgeri, and M. morganii for a number of biochemical parameters. Molecular sizes (from a Sephadex G-200 column) were reported to be from 560 to 800 kDa and, with the exception of M. morganii, are not in agreement with values reported here. For $K_m$s, although our values are somewhat different from those reported by Rosenstein et al. (19), the general trend that M. morganii exhibits a higher affinity for substrate than other species is in agreement with our data.

Indeed, the most significant observation in this report may relate to the relative affinities of the ureases for substrate and the maximum rates of urea hydrolysis by lysates of induced strains. The habitat of these isolates is urine, which has been reported to contain 400 to 500 mM urea (6). At this concentration, undoubtably, ureases from any species would be saturated with substrate and would be working at the $V_{\text{max}}$. Species producing high-affinity ureases, such as M. morganii TA43, would have no selective advantage over other strains listed in Table 2 with lower affinity enzymes because of the plentiful substrate in urine. All four P. mirabilis strains had ureases with significantly higher $K_m$ values (and thus lower affinity) than the enzymes from all other species ($P < 0.023$ but, importantly, demonstrated rates of urea hydrolysis that were six- to thirty-fold higher than for any other isolate. This observation alone may explain why P. mirabilis is so often linked with urinary and kidney stone formation (6), obstruction of urinary catheters (Moley and Warren, submitted), and pyelonephritis (2, 12, 20), whereas such reports for other Proteus, Providencia, and Morganella species are seen much less often.

Genetic relatedness of urease genes as determined by hybridization of whole-cell DNA and chromosomal DNA restriction fragments with a Providencia stuartii urease gene probe demonstrated that the genes encoding the enzyme of Providencia rettgeri are very homologous. Strong and weak hybridization was seen for 44 and 56% of the Providencia rettgeri isolates, respectively. One isolate that initially did not hybridize produced a high-molecular-weight, high affinity ($K_m = 2$ mM urea) enzyme and was later reidentified as M. morganii. Isolates of P. mirabilis also showed significant hybridization with the gene probe, but strong hybridization occurred with only 8% of isolates; 62% hybridized weakly. Three of four strongly hybridizing strains produced a urease of molecular weight not significantly different from that of Providencia stuartii BE2467. The other strongly hybridizing isolate produced a smaller enzyme of 280 kDa. Surprisingly, none of the 15 isolates of P. vulgaris tested hybridized on dot blots of whole-cell DNA, indicating that, although the molecular size of the enzyme may be similar to that of both P. mirabilis and Providencia stuartii isolates, the genes are not as closely related. In addition, M. morganii urease was also genetically unrelated and was different from all other ureases characterized in every category tested.

Certain observations can be explained on the basis of hybridizations with restriction enzyme-digested chromosomal DNA. P. mirabilis BR2528 (Fig. 3B) and Providencia rettgeri SI5453 (Fig. 3G) demonstrated strongly hybridizing HindIII fragments of the same size as the parent, Providencia stuartii BE2467. Both of these strains show evidence for two distinct ureases (stepladder formation in Fig. 2B and...
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high and low patterns in Fig. 2H). Molecular weights of one of the P. mirabilis BR2528 ureases may overlap with a Providencia stuartii enzyme-like enzyme produced by that strain, and the smaller Providencia rettgeri enzyme has a molecular weight similar to that of Providencia stuartii. We can postulate that these secondary ureases may have been acquired at some point by genetic exchange with a Providencia stuartii plasmid-encoded urease (4, 16). This theory is supported by the fact that identical copies of the Providencia stuartii urease gene appear to reside both on a conjugal plasmid and on chromosomal sequences in Providencia stuartii BE2467.

The data presented here summarize a systematic analysis of urease diversity in Proteus, Providencia, and Morganella isolates cultured from the urine of patients with urinary catheters in place for ≥30 days. The analysis of these enzymes gives clues to why the ureases of P. mirabilis may be more important virulence factors in urinary tract infection than the ureases of other common urease-producing species. Furthermore, the diversity observed among these three closely related genera suggests that the substrate specificity for urea may be one of the few similarities for an otherwise dissimilar group of proteins.

ACKNOWLEDGMENTS

We thank Linda Horne for expert manuscript preparation, Robert Hausinger for informative discussions, and Edward Gaskin for expert technical assistance.

This research was supported by Public Health Service grants R23AI23328 and PO1AG04393 from the National Institutes of Health.

LITERATURE CITED