INFECTION AND IMMUNITY, Jan. 1989, p. 110–116
0019-9567/89/010110-07$02.00/0

Vol. 57, No. 1

# *Staphylococcus saprophyticus* Urease: Characterization and Contribution to Uropathogenicity in Unobstructed Urinary Tract Infection of Rats

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Received 28 July 1988/Accepted 4 October 1988

**We studied the biochemical properties of the urease of *Staphylococcus saprophyticus* and the possible role of the urease in experimental urinary tract infections. For this purpose, the nonhemagglutinating and nonadherent strain 9325, which was isolated from a case of symptomatic urinary tract infection, was used. The urease was shown to have a $K_m$ of 6.64 mM urea and a $V_{max}$ of 4.59 μmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$. The enzyme was inhibited by acetohydroxamic acid in a noncompetitive manner. By means of Sephacryl S-300 column chromatography, we determined a mean molecular weight (± standard error of the mean) of 420,000 ± 16,000. To assess the contribution of *S. saprophyticus* urease to uropathogenicity, a urease-negative mutant was constructed by nitrosoguanidine mutagenesis. In the rat model of ascending unobstructed urinary tract infection, higher numbers of CFU · gram of tissue$^{-1}$ and more-severe lesions were detected with the parent strain. Moreover, bladder stones were found in animals infected with the urease-positive strain only. Interestingly, the difference in mean bacterial counts of the bladders was found to be significant by the Wilcoxon two-sample test ($P < 0.05$), whereas that between the kidney bacterial counts was not. Immunoblot studies revealed a faint antibody response in rats infected with the mutant strain, although bacteria could still be detected in the kidneys after 7 days. Sera of animals challenged with the parent strain reacted strongly with many antigens of *S. saprophyticus*. Our data indicate that urease is a major factor for invasiveness of *S. saprophyticus*, especially in the tissue of the bladder, whereas persistence in the urinary tract and nephropathogenicity of this organism are governed by factors other than urease.**

The ureases of several bacteria, especially those of the tribe *Proteeae* of the family *Enterobacteriaceae*, have been recognized to be virulence factors because they lead to alkalization of the urine and thus may induce the formation of kidney and bladder stones (11). The high incidence of urinary tract infections (UTIs) caused by these bacteria has prompted intensive studies of this enzyme (11, 17, 21, 24–26). The ureases of these urinary pathogens have been reported to have high molecular weights, ranging from 280,000 to >700,000 (17). The properties of the enzymes were found to differ markedly between species (17, 27). The enzymes hydrolyze urea, yielding carbon dioxide and ammonia, resulting in a rise of pH. This, in turn, may lead to precipitation of struvite ($MgNH_4PO_4 \cdot 6H_2O$) or carbonate-apatite [$Ca_{10}(PO_4CO_3OH)_6(OH)_2$] (11).

Previous animal studies with urease-negative mutants of *Proteus mirabilis* (21) or urease inhibitors (26) have shown that *P. mirabilis* urease contributes to virulence in experimental infections.

*Staphylococcus saprophyticus*, which also produces urease (18), is a frequent cause of UTIs in young female outpatients (32), but the organism has also been found to cause bacteremia (10, 20) and, rarely, UTIs in elderly males with obstructive disorders (14). Thus, infections occur predominantly in patients without abnormalities of the urinary tract. Moreover, *S. saprophyticus* has also been found in urinary calculi (16). In previous studies, the urease of one strain was found to have a $K_m$ of 7.36 mM urea and an $M_r$ of about 250,000 (9). Since there are no data on the possible role of the urease of *S. saprophyticus* in UTIs, we assessed some biochemical properties of the enzyme and studied its contribution to uropathogenicity in experimental urinary tract infections of rats by using a pair of strains differing in urease production only.

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## MATERIALS AND METHODS

**Bacterial strains.** *S. saprophyticus* 9325 was isolated from the urine of a female patient with symptomatic UTI. This strain did not hemagglutinate sheep erythrocytes (15) and did not adhere to HEp-2 or rat uroepithelial cells, as tested by methods described previously (12, 22). The identification was based on resistance to novobiocin and on biochemical tests (APIStaph; API-bioMerieux, Nürtingen, Federal Republic of Germany; Kloos and Schleifer [18]). Strain GJ1187 was a urease-negative mutant of strain 9325 constructed by nitrosoguanidine mutagenesis as described below.

**Mutagenesis.** An overnight broth culture (P broth; 10 g of Bacto-Peptone (Difco Laboratories, Detroit, Mich.) · $liter^{-1}$, 5 g of yeast extract (Difco) · $liter^{-1}$, 1 g of $Na_2HPO_4 \cdot liter^{-1}$, 1 g of glucose · $liter^{-1}$ [pH 7.3]) of strain 9325 was washed in 0.1 M citrate buffer (pH 5.0) and resuspended in this buffer to give $10^8$ cells · $ml^{-1}$. A solution of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Sigma; Munich, Federal Republic of Germany) (2 mg · $ml^{-1}$ in citrate buffer) was added to give a concentration of 200 μg · $ml^{-1}$. The mixture was incubated in a water bath with gentle shaking for 1 h. At 10-min intervals, two 10-μl samples were removed. One was transferred to 5 ml of prewarmed brain heart infusion broth and incubated overnight (18 to 20 h) without shaking, and the other was diluted and plated for determination of viable counts. For screening for mutants, we chose a culture which had been inoculated at a time when

viable counts had dropped to half of the initial value. Appropriate dilutions of this culture were plated on urea-containing agar (Christensens urea; Oxoid Ltd., Basingstoke, England) and incubated for 36 to 48 h. By this time, urease-positive clones had developed a small red ring around the colonies.

From 3,000 colonies screened, we were able to isolate one mutant which was confirmed to be urease negative. This strain (GJ1187) showed the same biochemical reactions as the parent strain, except for urease production. Tested reactions were utilization of arabinose, fructose, glucose, inositol, lactose, maltose, mannitol, mannose, melizitose, *N*-acetylglucosamine, rhamnose, raffinose, sucrose, sorbitol, trehalose, xylitol, and xylose; production of alkaline phosphatase and acetoin; presence of arginine dihydrolase, DNase, catalase, β-galactosidase, and β-glucosidase; and reduction of nitrate to nitrite. We also tested resistance to novobiocin and arsenate, as well as growth on agar containing 10% NaCl and pigmentation of colonies. For both strains, generation times in P broth (without urea) and rat urine were also tested. We found mean (standard error of the mean) generation times in P broth of 34 (2.6) min for strain 9325 and 32 (4.5) min for strain GJ1187. In rat urine, the figures were 58 (4.5) min for 9325 and 50 (1.5) min for GJ1187 in triplicate determinations. After 18 h of incubation in P broth containing urea (0.2%), the pHs were 8.55 for strain 9325 and 5.40 for strain GJ1187. In rat urine, the pH rose from 6.94 to 7.07 for 9325 and dropped to 6.80 for GJ1187 within 6 h of observation.

When urease activity in bacterial lysates was analyzed, activities of 4 to 10 μmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ were found for strain 9325, whereas no activity could be detected in lysates of strain GJ1187, even with a prolonged incubation time of 20 min. The detection limit was approximately 30 nmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ (methods described below).

**Preparation of cell lysates.** Bacteria were grown in P broth (1,000 ml), with or without urea, overnight (16 h, 37°C) with constant agitation. Cells were harvested by centrifugation (3,000 × *g*, 4°C), washed with 0.01 M cold sodium phosphate buffer (pH 6.8), and suspended in 10 ml of this buffer. Ten grams of glass beads (size, 75 to 150 μm; Sigma) and 100 μl of a solution of phenylmethylsulfonyl fluoride (Sigma) (0.1 M in ethanol) were added. The cells were disrupted in a Braun homogenizer (B. Braun, Melsungen, Federal Republic of Germany) with constant carbon dioxide cooling for 90 s. The supernatant was centrifuged once at 2,000 × *g* (4°C) to remove intact cells and glass beads, transferred to a new tube, and spun at 20,000 × *g* (4°C). This resulted in clear solutions containing 8 to 10 mg of protein as determined by the method of Bradford (5) by using a commercially available reagent (Bio-Rad Protein Dye Concentrate; Bio-Rad, Munich, Federal Republic of Germany) with bovine serum albumin as the standard.

**Urease assay.** Ten microliters of bacterial lysate containing 18 to 25 μg of protein was added to 1 ml of urease substrate buffer (60 mM urea, 45 mM sodium phosphate buffer [pH 6.8]) and incubated for 10 min at 22°C. A 0.4-ml sample was removed and mixed with 1.4 ml of sodium phosphate buffer (0.01 M, pH 6.8) and 0.2 ml of a commercially available ammonia reagent (Sigma Ammonia Color Reagent). The ammonia assay had been calibrated with various concentrations of $NH_4Cl$ ranging from 0.01 to 5 mM. The development of ammonia had been found to be linear by time by using various concentrations of jack bean urease (EC 3.5.1.5; 20,000 $U \cdot g^{-1}$; lot no. 96F-7275; Sigma). In experiments assessing urease kinetics, seven concentrations of urea, ranging from 1.25 to 80 mM, were used. In some experiments, the effect of acetohydroxamic acid (Sigma) was also tested.

The urease activity of cell lysates was expressed as micromoles $NH_3$ liberated · $minute^{-1}$ · milligram of $protein^{-1}$ in lysate.

**Column chromatography.** A Sephacryl S-300 column (1.5 by 74 cm; Pharmacia, Freiburg, Federal Republic of Germany) was prepared according to the specifications of the manufacturer. A sample of lysate containing 8 mg of protein was applied to the column and eluted at 25 ml · $h^{-1}$. Eluent buffer was 0.02 M sodium phosphate buffer (pH 6.8) containing 0.05% $NaN_3$. The column was calibrated with dextran blue for void volume determination and thyreoglobulin (669,000 daltons [Da]), ferritin (440,000 Da), catalase (232,000 Da), and aldolase (158,000 Da) (all purchased from Pharmacia). The fractions (2 ml each) were screened for the presence of urease activity by using a screening test. A sample of the solution to be tested (100 μl) was added to 100 μl of substrate (100 mM urea, 20 μg of phenol red · $ml^{-1}$, 10 mM sodium phosphate buffer [pH 6.8]) and incubated until color developed.

Fractions that were positive as indicated by color development within 45 min of incubation at 22°C were further analyzed by the quantitative urease assay.

**SDS-PAGE.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 0.15-cm-thick slab gel with a 6% stacking gel and an 11% resolving gel was conducted as described by Laemmli (19). Samples of lysates containing about 40 μg of protein were heated for 5 min (90°C) in sample buffer (62.5 mM Tris hydrochloride [pH 6.8] containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromphenol blue) and applied to one lane. Samples were electrophoresed for 4 h at 10 V · $cm^{-1}$. Gels were stained with Coomassie blue (Serva, Heidelberg, Federal Republic of Germany) by standard procedures.

**Native PAGE and detection of urease.** Nondenaturing PAGE and detection of urease in gel was done as described by Mobley et al. (25). Briefly, 30-μl samples were mixed with equal volumes of 40% sucrose–0.05% bromphenol blue and electrophoresed at 10 V · $cm^{-1}$ in a slab gel (14 by 11 by 0.15 cm) with a 7% running gel and a 4% stacking gel (1:32, bisacrylamide:acrylamide; Sigma). After electrophoresis, the gels were immersed in 0.1% EDTA–0.02% cresol red (Fluka, Neu-Ulm, Federal Republic of Germany) until the gels remained yellow. The gels were then transferred to a 1.5% solution of urea. Development of localized red bands could be observed within a few minutes.

For preparation of urease by elution from native gels, the visualized bands were carefully cut out of the gel, cut into small pieces, and put into siliconized glass tubes. Two milliliters of 10 mM Tris hydrochloride (pH 6.8), 2 mM EDTA, 5 mM 2-mercaptoethanol, and 0.5% SDS were added, and the tubes were agitated vigorously for 16 h at room temperature (22°C). The gel was removed by centrifugation, and the supernatant was transferred to a new tube and precipitated with 4 volumes of ice-cold acetone. The pellet was dried, suspended in 10 mM Tris–2 mM EDTA (pH 6.8), and subjected to SDS-PAGE.

**Immunoblot.** Electrophoretic transfer of proteins to nitrocellulose (NC) membranes (BA85; 0.45-μm pore size; Schleicher & Schuell, Dassel, Federal Republic of Germany) was done with a semidry blot apparatus (Pharmacia-LKB, Freiburg, Federal Republic of Germany). NC was blocked with phosphate-buffered saline (10 mM $Na_2HPO_4 \cdot NaH_2PO_4$, 0.14 M NaCl [pH 7.2]) containing 0.5% Tween 20

(PBST). Rat sera were diluted 1:100 in PBST and incubated with NC overnight at room temperature (about 22°C). The NC was washed three times for 10 min in PBST. Incubation with alkaline phosphatase-conjugated anti-rat immunoglobulin G (Sigma) diluted 1:1,000 in PBST was done for 3 h at room temperature. The blots were washed again as described above, followed by one wash in diethanolamine buffer (10% [vol/vol] diethanolamine, pH 9.8) prior to the enzyme reaction. The alkaline phosphatase color reaction was performed as described by Blake et al. (4), one minor change being the substitution of diethanolamine buffer for the Veronal-acetate buffer of the original procedure.

**Animal experiments.** Experiments with female albino WISTAR rats (strain Han:WIST) were conducted as previously described (23), with slight modifications. Bacterial suspensions containing $10^9$ CFU · $ml^{-1}$ were prepared in phosphate-buffered saline (pH 7.0) from bacteria grown on Mueller-Hinton agar overnight (37°C). The animals were infected transurethrally with 1.5 ml of the bacterial suspension. This technique leads to a vesicoureteral reflex resulting in renal colonization and the appearance of histological signs of pyelonephritis, especially near the fornix of the kidney. In addition, leukocyturia, erythrocyturia, and bacteriuria can be detected (23). Nineteen animals were infected with strain 9325, and fourteen animals were infected with strain GJ1187.

On postinfection day 7, 10 animals of each group were placed singly into metabolic cages, and urine was collected over a period of 2 h for determination of bacteriuria and leukocyturia. The rats were sacrificed on the same day, blood was obtained by cardiac puncture, and the macroscopic appearance of the kidneys and bladders was recorded. If urinary calculi were found, these were collected and subjected to infrared absorption spectroscopy (analyses were kindly conducted by C. Pietschmann, Medizinische Universität zu Lübeck, Lübeck, Federal Republic of Germany). The spleens, kidneys, and bladders were removed and homogenized, and viable counts were performed on CLED agar (cystine-lactose-electrolyte-deficient agar). Results were expressed as log CFU · gram of $tissue^{-1}$. For five rats of each group, parts of the bladders were examined histologically.

**Confirmation of strains isolated from rats.** The identity of bacteria isolated from tissue homogenates was confirmed by standard laboratory tests (presence of catalase, resistance to novobiocin, APIStaph). Strains isolated from rats infected with the urease-negative mutant (GJ1187) were grown on urease agar to confirm this phenotype.

**Statistical evaluations.** We tested the hypothesis that strain GJ1187 was less virulent than strain 9325 by using the Wilcoxon two-sample test (3).

## RESULTS

**Urease contributes to uropathogenicity.** Leukocyturia and bacteriuria were found to differ markedly between the two strains. Rats infected with strain 9325 shed 7,710 leukocytes · $min^{-1}$ and $1.95 \times 10^4$ *S. saprophyticus* cells · $ml^{-1}$, whereas in the other group only 1,484 leukocytes · $min^{-1}$ and $8.71 \times 10^2$ bacteria · $ml^{-1}$ were found. Both differences were statistically significant. Cultures of the spleens yielded *S. saprophyticus* in three animals infected with strain 9325 and in none of the group infected with the mutant. Although this difference was not significant, a significant difference was found between the mean weights of the spleens (0.78 versus 0.64 g). On macroscopical examination, the size and appearance of the kidneys of the two groups did not differ markedly. This situation is also reflected by the mean kidney weights, which were 0.84 g in strain 9325-infected rats and 0.72 g in strain GJ1187-infected animals. This difference was not significant. In contrast, the bladders of the group which received the urease-positive strain were enlarged, and the bladder walls were thickened. Correspondingly, the mean bladder weights were 0.52 g for strain 9325-infected animals and 0.14 g for strain GJ1187-infected animals. This difference was significant as tested by the Wilcoxon two-sample test ($P < 0.05$). In 3 of 19 animals challenged with the urease-positive strain, bladder stones were found, whereas no concretions were found in the 14 rats infected with strain GJ1187. Infrared absorption spectroscopy revealed the calculi to be struvite. The stones were usually rather small, so that it cannot be excluded that some may have escaped detection. In the renal tissue, 51 CFU · $g^{-1}$ was found with strain 9325 and 19 CFU · $g^{-1}$ was found with strain GJ1187 (means of the log-transformed values). Of 38 kidneys, 18 were colonized with strain 9235, and 12 of 28 kidneys yielded strain GJ1187. These differences were not found to be statistically significant. In contrast, strains differed markedly in colonization of the bladders. Of 19 bladders, 10 were colonized with strain 9325, whereas the mutant strain could be detected in only 2 of 14 bladders. The mean bacterial counts in bladder tissue were 105 CFU · $g^{-1}$ for strain 9325 and 2.2 CFU · $g^{-1}$ for strain GJ1187 (Fig. 1). This difference was found to be significant by the Wilcoxon two-sample test ($P < 0.05$). On histological examination, severe destruction of the bladder tissue leading to removal of the uroepithelium and formation of abscesses was noted with the parent strain. With the mutant, only a mild acute cystitis was seen (Fig. 2).

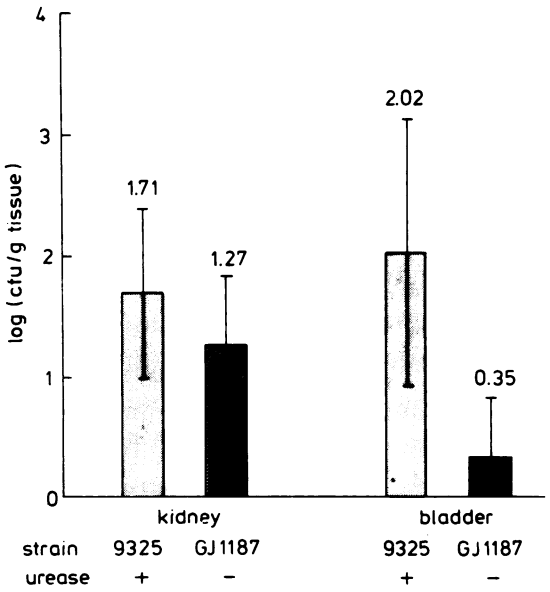


FIG. 1. Results of experimental infections. Means and 95% confidence limits are shown. Nineteen rats were infected with strain 9325, and fourteen rats were infected with strain GJ1187. The differences between the bacterial counts in the bladders were found to be statistically significant by the Wilcoxon two-sample test ($P < 0.05$).

Bacteria isolated from the animals always showed the same phenotype as the infecting strain.

**Characterization of the enzyme.** When urease activity in lysates was assessed by using various concentrations of

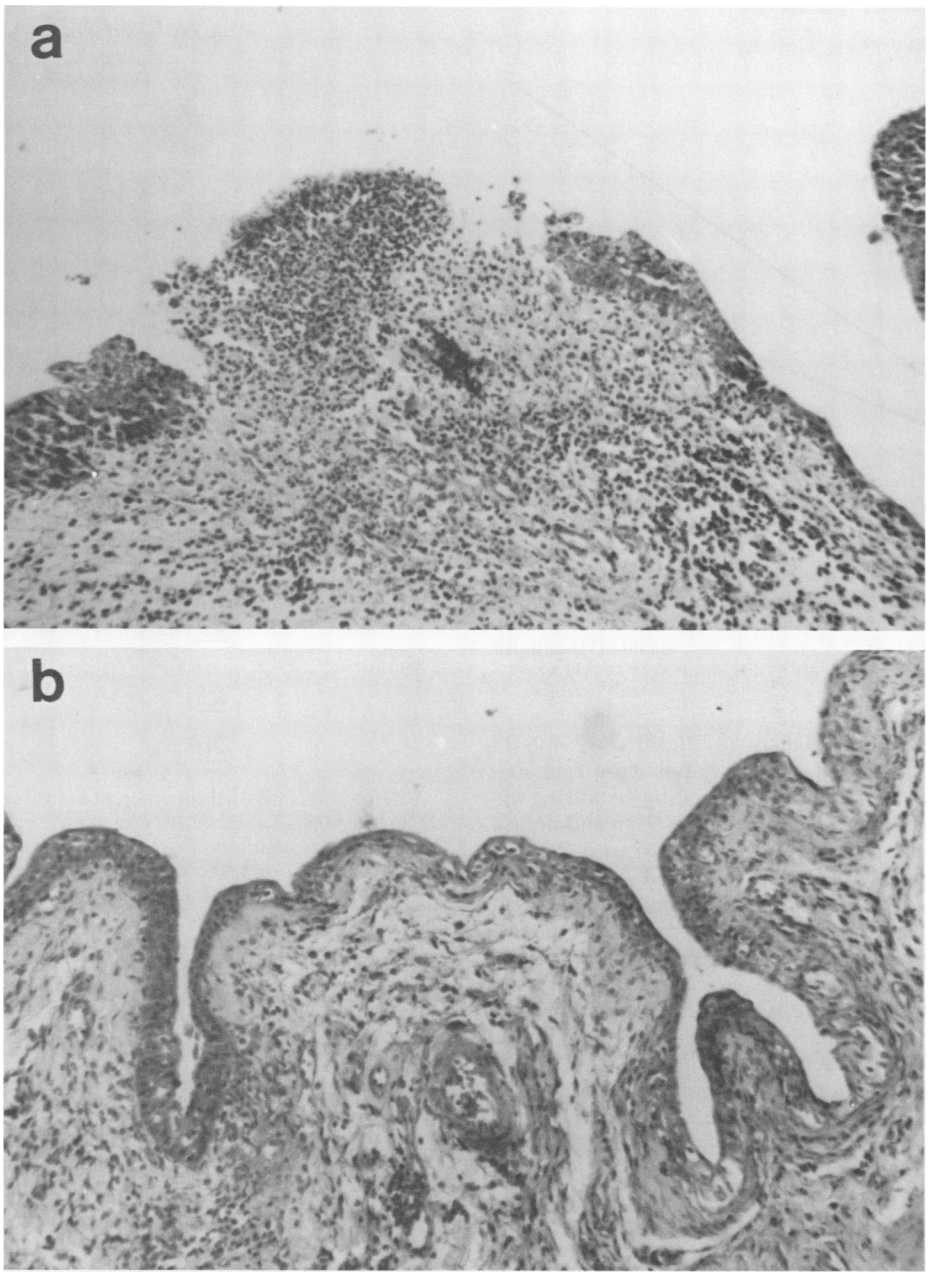


FIG. 2. Histological sections of bladders. Periodic acid-Schiff stain. Magnification, ×100. (a) Bladder of animal infected with urease-positive strain 9325. Severe, destructive cystitis. Infiltrations of leukocytes into the bladder wall leading to abscesses can be seen. The uroepithelium is partially dissected by the infection. Structures of the bladder wall are barely identifiable. (b) Bladder of animal infected with the mutant. Mild acute cystitis with scanty subepithelial infiltrations of leukocytes. The wall and the epithelium are not destroyed.

urea, a $K_m$ of 6.64 (0.42) mM and a $V_{max}$ of 4.59 (0.91) μmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ were found (means [standard error of the mean] of eight independent determinations). In quadruplicate experiments on the action of acetohydroxamic acid, it was shown that the compound reduced the $V_{max}$ to approximately 50% at a concentration of 3 mM, leaving the $K_m$ unaffected, thus acting as a noncompetitive inhibitor (Fig. 3).

Preparations from cells grown in the presence of urea (0.2%) showed an enzyme activity of 5.5 (0.46) μmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$, whereas the activity of bacteria grown without urea was 5.7 (0.51) μmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ (means [standard error of the mean] of four determinations). The apparent molecular weight as determined by Sephacryl S-300 column chromatography was 420,000 ± 16,000 (mean of four independent determinations ± standard error of the mean).

Electrophoresis of different urease preparations on native gels always yielded two bands showing enzyme activity (Fig. 4a). No activity was found in preparations of strain GJ1187. When the two bands exhibiting urease activity were eluted separately from the native gel and subjected to SDS-PAGE, the large band (fraction [F1]) was found to consist of two major polypeptides with molecular weights of 70,000 and 63,000. In contrast, the smaller urease-positive band (fraction 2 [F2]) produced only one major band in SDS-PAGE,

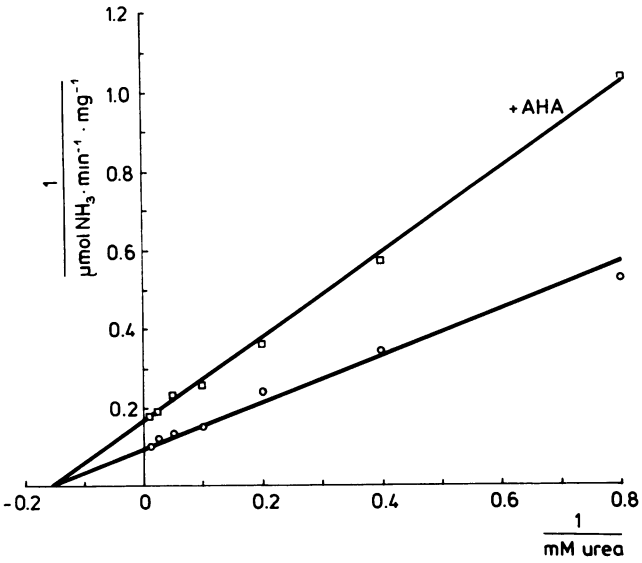


FIG. 3. Effect of acetohydroxamic acid on urease activity. Lysates (10 µl) containing 9 mg of protein · $ml^{-1}$ were appropriately diluted and incubated with 1 ml of substrate buffer containing seven concentrations of urea (1.25, 2.5, 5, 10, 20, 40, and 80 mM) and, for inhibition experiments, 3 mM acetohydroxamic acid (+AHA).

with an apparent molecular weight of 70,000 (Fig. 4b). In both fractions, some additional minor bands were visible.

**Antibody response to *S. saprophyticus* antigens during experimental infection.** When lysates of strain 9325 and urease fractions eluted from native gels were blotted to NC membranes and developed with antisera derived from rats infected with strain 9325, an antibody response to a great number of *S. saprophyticus* antigens was evident (Fig. 5a). Antibodies of these rats also reacted with many antigens of strain GJ1187. The responses to lysates of strains 9325 and GJ1187 did not differ markedly. On careful examination of the antibody responses to the eluted urease fractions, it was found that the upper band seen in F1 really consisted of two antigens with only little differences in molecular weights. In the fraction eluted from the faster-migrating urease band (F2), only the smaller antigen of this double band was apparent. Interestingly, the 63-kDa band of F1 was not recognized.

On immunoblots developed with sera derived from rats infected with strain GJ1187 (Fig. 5b), no reactivities to antigens of F2 were observed, but sera recognized the upper band of the double band of F1. Only a faint response to most of the antigens present in lysates of strain 9325 and GJ1187 could be recorded. However, sera clearly reacted with an approximately 24-kDa antigen of the lysates.

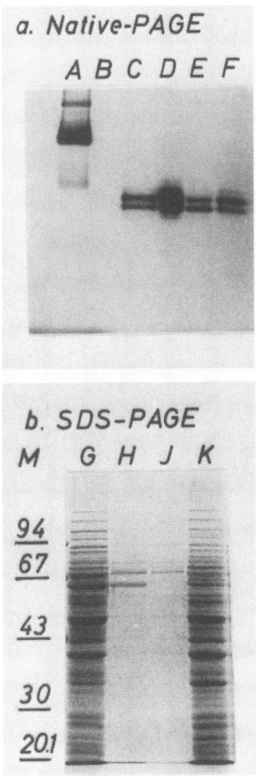


FIG. 4. (a) Native gels of different urease preparations stained by the urease stain. Lanes: A, jack bean urease; B, strain GJ1187; C to F, different preparations of strain 9325 grown in the presence of urea (lanes C and E) or without urea (lanes D and F). No differences between the preparations are visible. (b) SDS-PAGE of lysates and material eluted from native gels. Lanes: G, strain 9325; H, material eluted from the slower-migrating band (F1); J, F2, the faster-migrating band; K, strain GJ1187; M, positions of molecular size markers. Markers were phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), and soybean trypsin inhibitor (20.1 kDa) (all from Pharmacia).

## DISCUSSION

In this study, we showed for the first time that *S. saprophyticus* urease is a virulence factor of this organism. Previous studies using animal infection or in vitro experiments usually focused on the relevance of surface-associated structures (2, 7, 13, 31). Here, we used an *S. saprophyticus* isolate derived from a patient with symptomatic UTI. This strain was devoid of hemagglutinating and cell adherence properties. To assess the contribution of the enzyme urease to experimental infection, we compared the virulence properties of the urease-positive wild strain with those of a mutant strain derived by nitrosoguanidine mutagenesis. Although chemical mutagenesis involves the risk of double mutations, it has been used widely for organisms that could not be mutagenized otherwise (28, 30). Moreover, the frequency of a second phenotype generated by nitrosoguanidine has been estimated to be less than 8% (1, 29). Our derivative exhibited the same biochemical reactions as the parent strain, except for urease production (29 reactions tested), and did not differ substantially in generation times, as tested in P broth or rat urine. Several physiological and biochemical tests showed that strain GJ1187 was truly urease negative.

Urinary calculi were found in the rats infected with the urease-positive parent strain but not in the animals challenged with strain GJ1187. We therefore concluded that urease is functionally expressed during infection and is the primary cause of urinary stones in this model. Previous in vivo studies which showed the relevance of the *Proteus* urease for virulence (21, 26) included the implantation of a foreign body as a carrier for the organism or used the intravenous route for infection. Interestingly, stone formation was not reported in studies of ascending experimental UTI, but it was reported in a study that used the intravenous route (6). In our model, the infection was established by instillation of bacteria via the urethra, the natural route of UTI. The fact that concretions developed under these con-

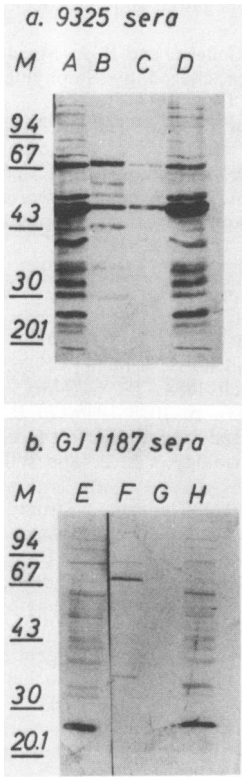


FIG. 5. Immunoblots of lysates and eluted material developed with sera of both groups. Lanes: A and E, lysate of strain 9325; B and F, eluted F1; C and G, eluted F2; D and H, strain GJ1187; M, positions of molecular size markers (see legend to Fig. 4). (a) Blots were developed with sera from rats infected with strain 9325. The upper band of F1 consisted of two antigens with very little difference in molecular weights. The lower (faster-migrating) antigen corresponds to the upper band of F2. (b) Blots developed with sera from strain GJ1187-infected rats. Only a very faint staining could be achieved for most antigens. Clear reactivity was found with the upper band of F1 but not with any other antigen of both fractions eluted from native gels.

ditions with the urease-positive strain but not with the mutant during an observation time of 7 days further stresses the relevance of *S. saprophyticus* urease for UTI. Additional evidence for the expression of the enzyme under conditions in vivo was given by our immunoblot studies. Antibodies of rats infected with strain 9325 recognized many antigens of both strains and of the two urease fractions eluted from native gels. In contrast, sera of rats challenged with the mutant strain exhibited a rather faint response to most of the antigens present in cell lysates, and antibodies did not bind to any antigen of urease F2. The comparatively weak immune response of this group suggests that the urease-negative strain possesses a lower invasive potential than the parent strain. This may be a result of the less severe tissue lesions induced by the mutant, which are reflected by the significant differences in bladder weights. The observation that only spleens of animals infected with the parent strain yielded the infecting organism and that the mean weight of the spleens was significantly higher than that of the group infected with the mutant further supports the hypothesis of a lower invasive potential in strain GJ1187. Moreover, histological examination of the bladder tissue revealed severe and destructive lesions in bladders infected with strain 9325 only. In the experiments of Braude and Siemienski (6) on the pathogenetic relevance of *Proteus* urease, it was shown that the enzyme is capable of inducing tissue lesions in the kidneys. Since our experiments did not reveal differences in kidney weights or differences in renal bacterial counts, we conclude that the enzyme urease is a major factor for cystopathogenicity, whereas additional factors may contribute to nephropathogenicity.

Interestingly, hemorrhagic cystitis is a common symptom during the clinical course of *S. saprophyticus* UTI (32). This indicates that severe bladder lesions also occur in human infections. But typical symptoms also include flank pain, a sign for the involvement of the upper urinary tract (16). In previous studies, a possible role for the hemagglutinin of *S. saprophyticus* in the involvement of the kidneys was suggested (13). Our data indicate that the hemagglutinin, the cell adhesin(s), and the urease are not prerequisites for colonization of the kidneys, since our strains were non adherent and nonhemagglutinating but had the ability to persist in the kidneys regardless of their action on urea. For *P. mirabilis*, it has been shown that invasive properties of the organism are governed by the urease, whereas the ability to establish infection and to persist in the urinary tract are dependent on other factors (26).

Nondenaturing gel electrophoresis followed by urease-specific staining always yielded two bands exhibiting urease activity. The phenomenon of isoenzymes leading to more than one band showing enzyme activity is a common observation with bacterial ureases (17, 24, 25). Differential elution of these two bands yielded two major polypeptides in the case of the slower-migrating band, whereas while the other band consisted of one major protein only. Immunoblot studies with sera of rats of both groups revealed the presence of additional antigens in these mixtures. The immune response of strain 9325-infected rats was mainly directed against a 48-kDa antigen and one of the major bands, a 70-kDa antigen. These antigens were recognized in both eluted fractions. By examining the 70-kDa band in the fraction obtained from the slower-migrating enzyme, it was found that it consisted really of two antigens with only slightly different molecular sizes. The immunoblot with sera from rats infected with the mutant strain supports this interpretation, since these sera recognized a 70-kDa antigen in the fraction containing the slower-migrating urease band but did not react with any antigen of the other fraction (F2). Interestingly, antibodies did not bind to the other (48-kDa) antigen either. Our findings are consistent with the interpretation that the native urease is composed of the 70-kDa and, possibly, the 48- and 63-kDa polypeptides. A complex structure has been shown to occur in the urease of *Providencia stuartii*, which includes 73- and 25-kDa subunits (24). The size of the enzyme found in this study was about twice as great as the previously reported value of approximately 250 kDa (9). We do not know whether this difference is a result of the preparation methods used or must be attributed to variability between strains. Other biochemical parameters evaluated for the urease of strain 9325 were within the range reported for other bacterial ureases (17, 24, 25, 27). In addition, the $K_m$ found here did not differ markedly from the previously published value (9).

The urease inhibitor acetohydroxamic acid, which has been found to modulate the virulence of *Proteus* strains in experimental infection (26) and which has also been used in the therapy of urinary calculi in humans (33), inhibited the urease of strain 9325 in a noncompetitive manner. This type of interaction has also been found with other ureases of bacterial (27) and plant (8) origin. For jack bean urease, it

was shown that the inhibition is the result of an interaction of the compound with the nickel ion at the active site of the enzyme (8). Concentrations needed for 50% inhibition of *S. saprophyticus* urease were similar to those found for other bacterial ureases (17, 27).

Our work showed that in *S. saprophyticus* a gram-positive urinary pathogen urease also represents a major virulence factor for uropathogenicity. Expression of the urease contributes mainly to invasiveness of the organism into the bladder tissue, whereas persistence in the kidney is guided by other factors. Further genetic studies are needed to elucidate the role of proposed virulence factors, such as the hemagglutinin and the adhesin(s) alone and in combination with urease, to the pathogenicity of this organism.

## ACKNOWLEDGMENTS

We are greatly indebted to K. Sack for conducting the histological examinations.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG Ga352/1-1 and Ma864/2-2).

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