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Single-Step Purification of *Proteus mirabilis* Urease Accessory Protein UreE, a Protein with a Naturally Occurring Histidine Tail, by Nickel Chelate Affinity Chromatography

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***Proteus mirabilis* urease, a nickel metalloenzyme, is essential for the virulence of this species in the urinary tract. *Escherichia coli* containing cloned structural genes *ureA*, *ureB*, and *ureC* and accessory genes *ureD*, *ureE*, *ureF*, and *ureG* displays urease activity when cultured in M9 minimal medium. To study the involvement of one of these accessory genes in the synthesis of active urease, deletion mutations were constructed. Cultures of a *ureE* deletion mutant did not produce an active urease in minimal medium. Urease activity, however, was partially restored by the addition of 5 μM $NiCl_2$ to the medium. The predicted amino acid sequence of UreE, which concludes with seven histidine residues among the last eight C-terminal residues (His-His-His-His-Asp-His-His-His), suggested that UreE may act as a Ni^{2+} chelator for the urease operon. To exploit this potential metal-binding motif, we attempted to purify UreE from cytoplasmic extracts of *E. coli* containing cloned urease genes. Soluble protein was loaded onto a nickel-nitrilotriacetic acid column, a metal chelate resin with high affinity for polyhistidine tails, and bound protein was eluted with a 0 to 0.5 M imidazole gradient. A single polypeptide of 20-kDa apparent molecular size, as shown by sodium dodecyl sulfate–10 to 20% polyacrylamide gel electrophoresis, was eluted between 0.25 and 0.4 M imidazole. The N-terminal 10 amino acids of the eluted polypeptide exactly matched the deduced amino acid sequence of *P. mirabilis* UreE. The molecular size of the native protein was estimated on a Superdex 75 column to be 36 kDa, suggesting that the protein is a dimer. These data suggest that UreE is a Ni^{2+}-binding protein that is necessary for synthesis of a catalytically active urease at low Ni^{2+} concentrations.**

Proteus mirabilis is not a common cause of urinary tract infection in the healthy host (31). This organism does, however, infect a high proportion of patients with complicated urinary tracts, that is, those with functional or structural abnormalities or with chronic catheterization (31, 34). In these patients, bladder and renal stone formation is a hallmark of infection with this species and is due to the expression of urease (5). The enzyme hydrolyzes urea to CO_2 and NH_3, often resulting in elevation of urinary pH (24). Alkalinization of the urine leads to precipitation of Ca^{2+}, Mg^{2+}, and other ions to form carbonate-apatite or struvite stones (5). Urease-negative mutants, constructed by allelic exchange, are unable to form stones in transurethrally infected mice and are significantly less virulent than the urease-positive parent strains (12, 13).

Urease is one of only four classes of nickel metalloenzymes which also include hydrogenase, methyl coenzyme M reductase, and carbon monoxide dehydrogenase (8). These divalent cations are required for synthesis of catalytically active urease in bacteria and plants (8). It has been observed that the apourease, synthesized in the absence of nickel, is difficult to activate in vivo by the addition of nickel chloride when protein biosynthesis is inhibited (18, 30). This suggests that insertion of nickel ions (Ni^{2+}) into the metallocenter takes place primarily at the time of synthesis of the enzyme subunits, although there is some evidence to the contrary (1). Little is known, however, about the mechanism by which nickel is inserted into the urease protein.

Urease genes of *P. mirabilis* have been cloned (15) and sequenced (16, 28, 32), and deletion mutations have been constructed (10, 32). In the course of these studies, we observed that clones lacking *ureE*, *ureF*, or *ureG* (accessory genes in the *P. mirabilis* urease gene cluster) do not express catalytically active urease in unsupplemented medium. However, the enzymatic activity of a clone lacking only *ureE* may be partially restored when the medium is supplemented with $NiCl_2$. The predicted amino acid sequence of UreE (16) revealed a polyhistidine tail that was a potential metal-binding motif (3) at the C terminus. This led us to hypothesize that UreE may serve as the cytoplasmic protein that binds nickel ions and, in turn, provides the ions to the urease apoenzyme.

We present evidence that UreE is necessary for normal synthesis of active urease and that the protein can be isolated in a single step by Ni^{2+} chelate chromatography. Our results are compared with those of Lee et al. (20), who purified UreE of *Klebsiella aerogenes* by conventional chromatography and subjected the protein to rigorous biochemical analysis.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Escherichia coli* SE5000 [*araD139* Δ(*argF*$^-$ *lac*)*U169 rpsL150* (Str^r) *relA1 flbB5301 deoC1 pts F25 rbsR recA56*] was used as a host for recombinant plasmids. *P. mirabilis* HI4320 was originally isolated from an elderly (>65-year-old) woman with urinary catheter-associated bacteriuria (14, 34) and produces a urea-inducible urease (14). Strains were maintained on Luria agar.

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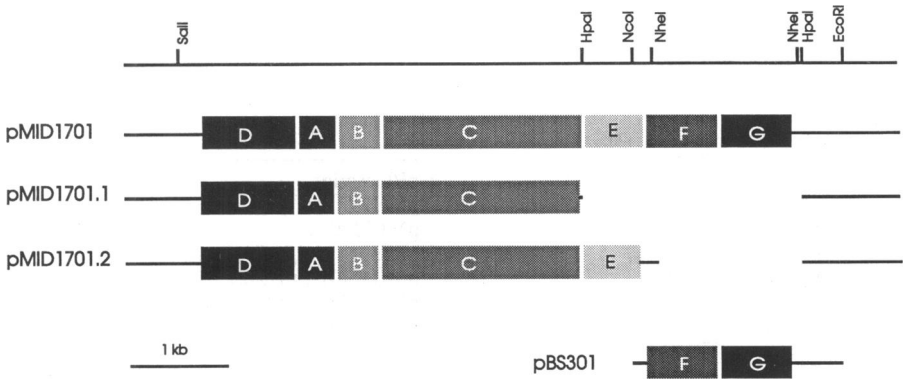

FIG. 1. *P. mirabilis* urease constructs used in this study. Plasmid pMID1701 represents urease genes isolated originally from *P. mirabilis* HI4320. A *Sal*I-*Eco*RI fragment carrying *ureDABCEFG* (as shown) was cloned into the *Sal*I-*Eco*RI site of pBluescript (vector not shown [33]). Plasmids pMID1701.1 and pMID1701.2 are deletion mutants lacking *ureEFG* and *ureFG*, respectively, and were constructed as described in Results. Plasmid pBS301 was constructed by cloning a 2.2-kb *Eco*RI-*Nco*I fragment from pMID1701 into pACYC184 also cut with *Eco*RI-*Nco*I.

For long-term storage, bacterial suspensions were stored at −70°C in Trypticase soy broth (BBL) supplemented with 20% (vol/vol) glycerol.

Plasmids. Phagemid pMID1701 carries *P. mirabilis* urease genes *ureDABCEFG*, is not subject to regulation by urea or *ureR*, and was constructed from pJMK7 as described previously (33). pBluescript KSII/− (Amp^r) was used as a vector control. Additional constructs are shown in Fig. 1.

Urease assays. Rates of urea hydrolysis by soluble protein in cell lysates were measured by the phenol red assay of Hamilton-Miller and Gargan (7), as adapted for quantitative measurement of urease activity (25). Protein concentrations were determined by the BCA (bicinchoninic acid) protein assay (Pierce) as directed by the manufacturer, with bovine serum albumin as a standard.

Nickel incorporation. *E. coli* SE5000 containing plasmids pMID1701.1 and pBS301, pMID1701.2 and pBS301, or pBluescript KSII/− were cultured for 18 h at 37°C with aeration in 20 ml of M9 minimal medium (22) containing 0.025 μM $^{63}NiCl_2$ (specific activity, 6.35 mCi/ml for 0.46 mg of Ni per ml; Amersham). Bacterial cells were harvested by centrifugation (10,000 × *g* for 10 min at 4°C), resuspended in 20 mM sodium phosphate, pH 6.8, and ruptured in a precooled French pressure cell at 20,000 lb/in^2. The cell lysate was centrifuged (10,000 × *g* for 10 min at 4°C) to remove cell debris, and then the supernatant was centrifuged (100,000 × *g* for 90 min at 4°C) to remove any membrane. The supernatant was concentrated by centrifugation (2,000 × *g* for 10 min at 4°C) in a Centrisart I (Sartorius; 10,000 molecular weight cutoff) and loaded onto a Superose 12 column (HR 10/30) (Pharmacia); the running buffer was 20 mM sodium phosphate, pH 6.8, containing 150 mM KCl, 1 mM β-mercaptoethanol, and 1 mM EDTA. The amount of $^{63}Ni^{2+}$ in each 0.5-ml fraction was estimated by counting a 100-μl sample by liquid scintillation.

Maxicell analysis. Plasmid-encoded polypeptides were labelled with [^{35}S]methionine (specific activity, 800 to 1,000 Ci/mmol; Amersham) and by using UV-treated *E. coli* SE5000 containing various plasmids by the method of Gherardini et al. (4). After labelling, cells were ruptured in a precooled French pressure cell at 20,000 lb/in^2. The cell lysate was centrifuged (100,000 × *g* for 90 min at 4°C) to remove cell debris and membrane. The supernatant was concentrated as described above and loaded onto the Superose 12 column. Radioactivity was determined in a 100-μl sample of each 0.5-ml fraction by liquid scintillation.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Soluble protein from whole-cell French press lysates and from column fractions was denatured in SDS-gel sample buffer (17) and electrophoresed on SDS–10 to 20% polyacrylamide gradient gels (6).

Column chromatography. The molecular weight of purified UreE was estimated on a Superdex 75 HR 10/30 column (Pharmacia) in 20 mM sodium phosphate, pH 6.8, containing 150 mM KCl, 1 mM EDTA, and 1 mM β-mercaptoethanol. The molecular weights of albumin (67,000), ovalbumin (43,000), chymotrypsinogen (25,000), and ribonuclease A (13,700) were used as molecular standards (2 mg of total protein representing the four standards was loaded onto the column).

N-terminal analysis. A modification of the procedure of Moos et al. (26) was used for determination of the N-terminal amino acid sequence. Purified UreE (30 μg), denatured in SDS-gel sample buffer for 5 min at 100°C, was electrophoresed on a 10 to 20% polyacrylamide gradient gel (6). Polypeptides were transferred to polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.) with a Hoeffer Transphore Power Lid (model TE 50) at 100 V 500 mA for 4 h, and stained with 0.1% Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif.) in 50% methanol. Membranes were destained with 10% acetic acid–50% methanol. Stained protein bands were excised, and N-terminal amino acid sequences were determined with a pulsed liquid-phase sequencer (model 477A; Applied Biosystems).

Purification of UreE. Bacterial cultures (100 to 500 ml) of *E. coli* SE5000 containing pMID1701, pMID1701.2, or pBluescript KSII/− and *P. mirabilis* HI4320, induced with 50 mM urea, were grown in Luria broth at 37°C with aeration for 18 h and harvested by centrifugation (10,000 × *g* for 10 min at 4°C). The cells were washed twice and resuspended in 50 mM sodium phosphate–300 mM NaCl, pH 8.0, and lysed by passage through a precooled French pressure cell at 20,000 lb/in^2. Unbroken cells were removed by centrifugation (10,000 × *g* for 10 min at 4°C). The membrane was removed by further centrifugation (100,000 × *g* for 90 min at 4°C). The supernatant was loaded onto a nickel-nitrilotriacetic acid (Ni-NTA) column (Qiagen) previously equilibrated with 50 mM sodium phosphate–300 mM NaCl, pH 8.0. Protein was eluted with 0.05 M steps (1 ml per step) of imidazole from 0 to 0.5 M in 50 mM sodium phosphate–300 mM NaCl–10% glycerol–10 mM

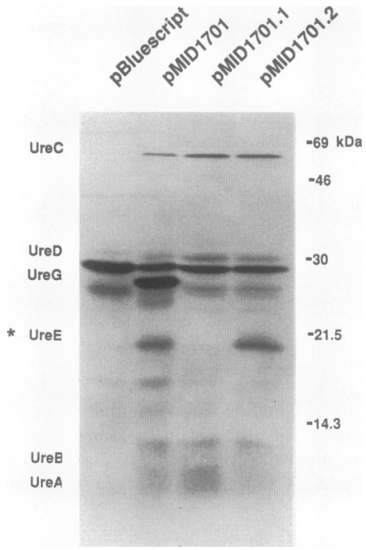

FIG. 2. Maxicell analysis of urease constructs. Plasmid-encoded polypeptides were labelled with [^{35}S]methionine in maxicell preparations of *E. coli* SE5000 transformed with the plasmids listed at the top of the figure. Soluble protein was electrophoresed on an SDS–10 to 20% polyacrylamide gel which was dried and autoradiographed. Designations for urease polypeptides are shown on the left at their predicted molecular masses (16, 32). The points of migration of molecular weight standards are shown on the right side. The asterisk denotes the predicted electrophoretic mobility of UreE, which is not made by pMID1701.1 or the pBluescript vector control.

β-mercaptoethanol, pH 6.0. Fractions were collected and analyzed by SDS-PAGE.

RESULTS

Construction of plasmids for complementation analysis. Phagemid pMID1701, which encodes an active urease produced at constitutive levels, was used to construct a series of deletion mutations within the urease operon (Fig. 1). pMID1701.1 (*ureEFG* deletion), constructed by deletion of a 2.3-kb *Hpa*I fragment downstream of *ureC* from pMID1701, is phenotypically urease negative. pMID1701.2 (*ureFG* deletion), constructed by deletion of a 1.6-kb *Nhe*I fragment downstream of *ureE*, is also urease negative on modified urea segregation agar (9) and was used for complementation experiments. Plasmid pBS301 carrying *ureFG* was constructed by cloning a 2.2-kb *Eco*RI-*Nco*I fragment from pMID1701 into pACYC184 also cut with *Eco*RI-*Nco*I.

To verify the synthesis or lack of synthesis of urease polypeptides, plasmid-encoded polypeptides were labelled for each construct with [^{35}S]methionine in maxicells. Proteins were denatured by direct treatment in SDS-gel sample buffer, and electrophoresed on an SDS-polyacrylamide gel. An autoradiograph of pMID1701 (Fig. 2) revealed polypeptides encoded by the urease gene cluster with apparent molecular sizes of 10.5 (UreA), 12.5 (UreB), 65 (UreC), 33 (UreD), 20 (UreE), and 26 kDa (UreG). UreF, however, either was synthesized at undetectable levels or comigrated with UreG (M_rs of UreF and UreG are predicted to be 23,000 and 22,400, respectively [16, 32]). The *ureEFG* mutant lacks bands corresponding to UreEFG, and the *ureFG* mutant also expressed no band corresponding to UreF and UreG but clearly synthesized UreE. Plasmid pBS301 (data not shown), which carries *ureFG*, yielded a band at an M_r of 26,000, corresponding to the predicted sizes of UreF and UreG.

TABLE 1. Urease activities of recombinant clones cultured in medium supplemented with $NiCl_2$

Plasmid[a]	Urease sp act in M9 minimal medium at μM $NiCl_2$ concn[b]				
	0	0.05	0.5	5	50
pBluescript	<0.01	<0.01	<0.01	<0.01	<0.01
pMID1701.1	<0.01	<0.01	<0.01	<0.01	<0.01
pMID1701.2	<0.01	<0.01	<0.01	<0.01	<0.01
pBS301	<0.01	<0.01	<0.01	<0.01	<0.01
pMID1701.1/pBS301	<0.01	<0.01	<0.01	8.3	37.5
pMID1701.2/pBS301	76.5	118.1	125.8	274.0	283.4
pMID1701	24.3	30.5	95.1	197.1	193.9

[a] Transformed into *E. coli* SE5000.

[b] Specific activity values are in micromoles of NH_3 per minute per milligram of protein and are averages of two determinations, except for pMID1701, which represents the mean of four determinations.

Complementation analysis. Plasmid pBS301 was transformed into *E. coli* SE5000(pMID1701.1) and *E. coli* SE5000 (pMID1701.2). Recombinant clones were selected on Luria agar containing ampicillin (200 μg/ml) and tetracycline (12 μg/ml). Clones containing pMID1701.1 and pBS301 were qualitatively urease negative on modified urea segregation agar, whereas clones containing pMID1701.2 and pBS301 were qualitatively urease positive, demonstrating that cloned *ureFG* could functionally complement a construct containing a *ureFG* deletion.

Urease activity of constructs. The urease activity of each construct was measured for cells grown in M9 minimal medium with or without supplemental $NiCl_2$ (Table 1). No urease activity was detected for pMID1701.1 and pMID1701.2 at any concentration of $NiCl_2$. *E. coli* SE5000(pMID1701.2/pBS301) expressed high levels of urease activity and also showed significant increases in activity as $NiCl_2$ concentration increased, a phenomenon previously observed for wild-type *P. mirabilis* (30). In contrast, *E. coli* SE5000(pMID1701.1/pBS301) produced undetectable levels of urease activity with 0, 0.05, and 0.5 μM $NiCl_2$; however, urease activities rose to 4.2 and 19.3% of that produced by pMID1701 with 5 and 50 μM $NiCl_2$, respectively. This suggested that exogenously added nickel ions could partially restore the urease activity of the UreE deletion mutant and that UreE may play a role in providing nickel ions to the newly synthesized urease apoenzyme, even at a low nickel ion concentration.

Purification of UreE. The DNA sequence of *ureE* predicts a 17.9-kDa polypeptide ending with the sequence His-His-His-His-Asp-His-His-His (16). This sequence was recognized as a potential metal-binding motif that could likely be exploited to purify UreE. Indeed, using soluble protein (Fig. 3, left-hand lane A) derived from cell lysates of *E. coli* SE5000(pMID1701) which had been cultured in the absence of supplemental $NiCl_2$, we were able to purify UreE in a single step on an Ni-NTA column. Numerous proteins were eluted following the addition of imidazole at concentrations of 0.05, 0.10, and 0.15 M (data not shown). The purified protein was eluted following addition of the 0.25 M imidazole step through the 0.4 M step, peaking at 0.35 M (Fig. 3, left-hand lane C). Fractions, electrophoresed on an SDS–10 to 20% polyacrylamide gradient gel, revealed a single polypeptide on Coomassie blue-stained gels, with an apparent molecular size of 20 kDa, near the predicted size (17.9 kDa) of UreE (16). Protein that passed through the column (Fig. 3, left-hand lane B) appeared to lose the 20-kDa

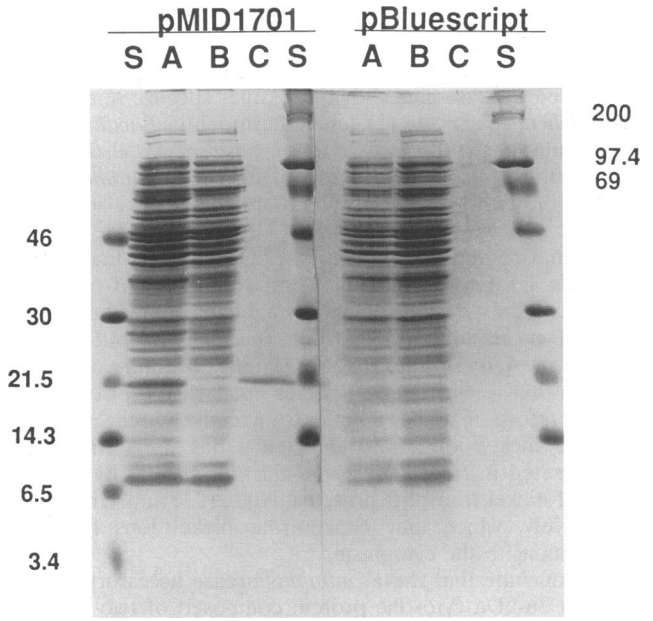

FIG. 3. SDS-PAGE of purified UreE. Soluble protein derived from *E. coli* SE5000 transformed with pMID1701 (left lanes) or pBluescript (right lanes) was passaged through an Ni-NTA column, washed, and eluted with an imidazole step gradient. Samples were denatured in SDS-gel sample buffer and electrophoresed on an SDS–10 to 20% polyacrylamide gel. Lanes S, low- or high-molecular-mass markers (sizes shown in kilodaltons); lanes A, soluble protein from whole-cell lysates prior to passage through the Ni-NTA column; lanes B, soluble protein after passage through the Ni-NTA column; lanes C, protein eluted from the Ni-NTA column with 0.35 M imidazole. All of the samples shown were run on the same gel. Duplicate samples were spliced from the photograph.

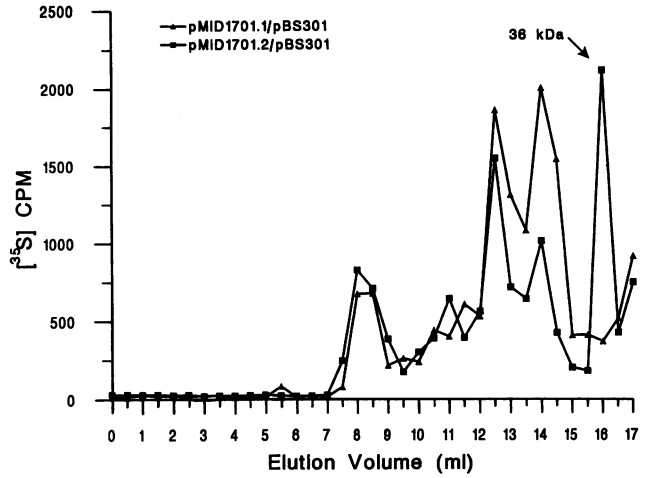

FIG. 4. Superose 12 chromatography of soluble protein derived from urease clones labelled with [^{35}S]methionine or $^{63}NiCl_2$. Plasmid-encoded polypeptides encoded by *E. coli* SE5000 cotransformed with pMID1701.1/pBS301 or with pMID1701.2/pBS301 were labelled in maxicell preparations with [^{35}S]methionine. Soluble protein was fractionated on a Superose 12 column, and counts per minute were determined for each fraction. The arrow points to a peak corresponding to a molecular size of 36 kDa that is present in the UreE-producing clone but not in the clone that does not synthesize UreE.

polypeptide that appears to be present in the unfractionated cytosolic extract (Fig. 3, left-hand lane A). A similar purification was successfully carried out for *E. coli* SE5000 (pMID1701.2) (data not shown). No polypeptide synthesized by *E. coli* SE5000(pBluescript) was eluted from the column with a concentration of imidazole of 0.25 M or higher (Fig. 3, right-hand panel).

When the purification protocol was carried out with soluble protein from urease-positive recombinant clones cultured in medium supplemented with 1 mM $NiCl_2$, no UreE could be recovered from the column. The inability to purify the protein under these circumstances may reflect the possibility that UreE was saturated with Ni^{2+} ions and thus would not bind to the column. We also attempted to purify the polypeptide, using soluble protein from wild-type *P. mirabilis* HI4320 induced with 50 mM urea. No band was visible on Coomassie blue-stained polyacrylamide gels when Ni-NTA column fractions that were eluted with 0.35 M imidazole were electrophoresed.

N-terminal analysis. To determine that this polypeptide was UreE, the purified protein was subjected to N-terminal amino acid analysis. Comparison of the first 10 amino acids of the purified protein (Met-Lys-Lys-Phe-Thr-Gln-Ile-Ile-Asp-Gln) with that of the amino acid sequence of *P. mirabilis* UreE predicted from the nucleotide sequence (16) revealed an exact match. This demonstrated that the protein which was purified from the Ni-NTA column was indeed UreE.

Native size of UreE. To determine the native molecular size, purified undenatured UreE protein, eluted from the Ni-NTA column, was loaded onto a Superdex 75 column (molecular weight range, 3,000 to 70,000). The protein eluted at 22 min as measured by the relative A_{280}, corresponding to an apparent molecular size of 36 kDa. This suggested that the protein is a dimer in its native state.

To determine whether UreE could be identified among soluble proteins and whether the native protein is capable of binding nickel ions in vivo, maxicells were prepared for *E. coli* SE5000 containing (i) pMID1701.1/pBS301 (lacking *ureE*) and (ii) pMID1701.2/pBS301 (urease-positive-complemented deletion mutant). Plasmid-encoded polypeptides were labelled with [^{35}S]methionine. Cells were lysed in a French pressure cell, and soluble protein was fractionated on the Superose 12 column (Fig. 4). In the clone carrying *ureE* (pMID1701.2/pBS301), a peak which corresponded to an apparent molecular size of 36 kDa was noted at 16 ml. This peak was clearly absent in the *ureE* deletion mutant (pMID1701.1/pBS301).

In parallel, a duplicate set of the *E. coli* SE5000 transformants were labelled for 18 hours with $^{63}NiCl_2$. Soluble protein derived from French press lysates was fractionated on the Superose 12 column. Counts per minute of $^{63}Ni^{2+}$ were determined for each column fraction. The fraction corresponding to an elution volume of 16 ml contained 375 cpm of $^{63}Ni^{2+}$ for the *ureE*-containing clone and 234 cpm for the clone lacking *ureE*. These low counts are insufficient to establish a meaningful estimate of the number of Ni^{2+} ions bound per UreE molecule. These data, however, further support the suggestion that the native molecular size of UreE is 36 kDa and suggest that this protein may carry Ni^{2+} ions in vivo.

DISCUSSION

The urease gene cluster of *P. mirabilis* contains eight genes, including the urea-dependent transcriptional activator *ureR*, the enzyme structural genes *ureABC*, and the four accessory genes *ureDEFG* (16, 28, 32). Mutations in structural or accessory genes usually result in a complete loss of catalytic activity. Mutations in *ureE* also result in dramatic reduction in enzyme activity;

however, the urease activity of this mutant (pMID1701.1/pBS301) can be partially restored by supplementing the growth medium with 5 or 50 μM $NiCl_2$. This observation indicates that this gene product is not essential for the production of a catalytically active urease but, when present, significantly facilitates the synthesis of active enzyme. The function of UreE appears to be most critical at low nickel concentrations.

The predicted primary structure of UreE, deduced from the nucleotide sequence (16), suggests a role for nickel binding in the cytoplasm where native urease resides (15). The hydropathy profile of the predicted amino acid sequence is clearly that of a soluble protein, and the lack of a traditional signal sequence suggests that the protein does not escape the cytoplasm (16). In addition, the predicted C-terminal amino acid sequence (His-His-His-His-Asp-His-His-His) has the characteristics of a metal-binding motif (3) and previous work has demonstrated a role for multiple histidine residues in the specific coordination of Ni^{2+} ions in the urease itself (29, 33), albeit by a distinct pattern of Ni^{2+} coordination. The experimental data support these predictions. Soluble protein derived from *E. coli* SE5000 overexpressing *P. mirabilis* urease from pMID1701 was used to purify UreE in a single step on an Ni-NTA column. The purified protein, eluted with imidazole, was composed of a single polypeptide whose apparent molecular size of 20 kDa is consistent with the deduced size of 17.9 kDa. In addition, the experimentally determined N-terminal sequence of the first 10 amino acids was found to be identical to that predicted from the nucleotide sequence.

Interestingly, the method we chose for single-step purification of UreE is one that is currently used for isolation of proteins that have been modified by the intentional addition of a histidine tail (11) (Qiagen). To purify proteins by using an Ni-NTA, a metal chelate resin with high affinity for polyhistidine tags, DNA sequences encoding polyhistidine are added at the 5′ or 3′ end of the gene of interest. UreE of *P. mirabilis*, however, contains a naturally occurring histidine tail at the C terminus, and therefore we were able to purify the protein in a single-step process using this column. To our knowledge, this is the first protein that bears a naturally occurring histidine tail to be isolated by using the Ni-NTA column. Another protein, WHP, produced by an *E. coli* strain, however, has a potential metal-binding domain which includes histidine (one His in the last 15 residues), cysteine, and acidic amino acids and was previously purified by this method (35). Although the predicted amino acid sequence of *K. aerogenes* UreE reveals a similar metal-binding motif at the C terminus, Lee et al. (20) reported that UreE of this species did not bind to an iminodiacetic acid metal ion affinity resin (another metal-binding column). This difference may be due to the primary amino acid sequence (i.e., His-Gly-His-His-His-Ala-His-His-Asp-His-His-Ala-His-Ser-His) for *K. aerogenes* UreE, which has no more than three His residues in a row, compared with a stretch of four histidine residues in a row for *P. mirabilis* UreE.

The native configuration of UreE appears to be a dimer. By molecular sieve chromatography, the UreE protein purified on the Ni-NTA column eluted in fractions that corresponded to an apparent molecular size of 36 kDa, a size twice that of the predicted UreE polypeptide (16). In addition, when plasmid-encoded polypeptides were labelled in maxicells and proteins from cell lysates were fractionated on Superose 12, a peak of radioactivity was also noted in the fraction that corresponded to 36 kDa for the clone carrying *ureE* but not in clones lacking this gene. These finding are consistent with those of Lee et al. (20), who reported that the purified UreE of *K. aerogenes* eluted from a Superose 12 column as an apparent dimer (M_r, 35,000). The purified UreE was estimated to bind approximately six Ni^{2+} ions per molecule.

UreE homologs have been predicted from the nucleotide sequence of urease genes for three other species: *K. aerogenes* (27), *Helicobacter pylori* (2), and a thermophilic *Bacillus* species (21). Mutations in the *ureE* genes of these species also result in a reduction in activity, as observed for the *P. mirabilis* gene. Among these, however, only the *Klebsiella* UreE also bears a histidine tail with 9 of the last 13 (and 10 of the last 15) C-terminal residues being histidine. Despite this feature, the *Klebsiella* UreE did not bind to an Ni^{2+}-charged metal ion affinity column (20) and thus was unable to be purified on the basis of nickel ion affinity. For *Helicobacter* and *Bacillus* species, the UreE proteins share significant amino acid similarity to that of the *Proteus* species but lack the histidine tail and therefore may be unable to effectively scavenge free cytosolic nickel ions. This deficiency has apparently been compensated in *Helicobacter* and *Bacillus* species by the synthesis of nickel transport proteins, NixA (23) and UreH (21), respectively, which may concentrate nickel ions from the environment to the cytoplasm.

We conclude that the *P. mirabilis* urease accessory protein UreE, a 36-kDa cytosolic protein composed of two identical copies of a 17.9-kDa polypeptide, functions by binding free Ni^{2+} ions. Data for *P. mirabilis* presented here, in conjunction with previous observations with cloned *K. aerogenes* and *Bacillus* sp. urease gene clusters (19–21), allow us to speculate that UreE, in turn, delivers bound Ni^{2+} ions by an as yet undetermined mechanism to newly synthesized apoenzyme. While the protein is not required for production of catalytically active protein, its presence allows synthesis of active enzyme under conditions of low nickel concentration.

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