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Identification and Molecular Cloning of a Unique Hyaluronan Synthase from *Pasteurella multocida**

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Type A *Pasteurella multocida*, a prevalent animal pathogen, employs a hyaluronan [HA] polysaccharide capsule to avoid host defenses. We utilized transposon insertional mutagenesis to identify the *P. multocida* HA synthase, the enzyme that polymerizes HA. A DNA fragment from a wild-type genomic library could direct HA production *in vivo* in *Escherichia coli*, a bacterium that normally does not produce HA. Analysis of truncated plasmids derived from the original clone indicated that an open reading frame encoding a 972-residue protein was responsible for HA polymerization. This identification was confirmed by expression cloning in *E. coli*; we observed HA capsule formation *in vivo* and detected activity in membrane preparations *in vitro*. The polypeptide size was verified by photoaffinity labeling of the native *P. multocida* HA synthase with azido-UDP sugar analogs. Overall, the *P. multocida* sequence is not very similar to the other known HA synthases from streptococci, PBCV-1 virus, or vertebrates. Instead, a portion of the central region of the new enzyme is more homologous to the amino termini of other bacterial glycosyltransferases that produce different capsular polysaccharides or lipopolysaccharides. In summary, we have discovered a unique HA synthase that differs in sequence and predicted topology from the other known enzymes.

Certain bacterial pathogens, namely Gram-negative Carter type A *Pasteurella multocida* and Gram-positive Groups A and C streptococci, produce extracellular capsules composed of HA[1] (1, 2). HA, a linear polysaccharide consisting of alternating β1,4-GlcA and β1,3-GlcNAc, is also a component of many vertebrate tissues (3). The HA capsules of these bacteria are virulence factors that allow the microbes to be more successful pathogens by rendering host defenses, including complement-mediated killing and phagocytosis, less effective (4, 5). Furthermore, HA is not immunogenic because the polymer is a normal component of the host body (6). The capsules of other bacteria that are composed of different polysaccharides, however, are usually major targets of the immune response. The antibodies generated against capsular polymers are often responsible for clearance of microorganisms and long term immunity. Therefore, the HA capsule serves *P. multocida* and Groups A and C streptococci as molecular camouflage.

Various HAS enzymes have been described in the last 5 years (reviewed in Ref. 7). HasA from *Streptococcus pyogenes* was the first HAS to be definitively identified (8). This integral membrane protein utilizes intracellular UDP-GlcA and UDP-GlcNAc as substrates. The nascent HA chain is extruded through the membrane to form the extracellular capsule. DG42, a *Xenopus* protein with a previously unknown function that had similarity to HasA (9), was shown to be a HA synthase (10, 11). Several groups identified human and murine homologs of DG42, named HAS1, HAS2, and HAS3, that directed HA synthesis (7); these mammalian enzymes are similar at the amino acid level, but they reside on different chromosomes.

Recently a viral HAS, an ORF called A98R, with 28–30% identity to the streptococcal and vertebrate enzymes was discovered (12). PBCV-1 (*Paramecium bursaria Chlorella* virus) produces authentic HA polysaccharide shortly after infection of its *Chlorella*-like green algae host (12). A98R is the first virally encoded enzyme reported to produce a carbohydrate polymer. This finding is also the initial discovery of HA outside of animals and their bacterial pathogens.

Carter type A *P. multocida*, the causative agent of fowl cholera, is responsible for great economic losses in the U. S. poultry industry. A capsule mutant did not thrive in the bloodstream of turkeys after intravenous injection in comparison with the encapsulated parental strain (13). This spontaneously arising mutant strain was also 10^5-fold less virulent than wild type, but the nature of the genetic defect was not known (13). Wild-type cells treated with hyaluronidase became complement-sensitive (14) and were more readily phagocytosed (4) in comparison with untreated microbes. Here we report the identification of a unique HAS from *P. multocida* with a primary structure that does not strongly resemble the enzymes from *Streptococcus*, PBCV-1 virus, or higher animals.

EXPERIMENTAL PROCEDURES

Tn916 Insertional Mutagenesis and Probe Generation—Tn*916* was used to disrupt and to tag the HA biosynthesis locus. The Tn element on a nonreplicating plasmid, pAM150 (provided by D. B. Clewell), was introduced into the wild-type encapsulated *P. multocida* strain P-1059 (ATCC number 15742) by electroporation (15). Altered colony morphology was initially screened by visual examination with oblique lighting. Wild-type P-1059 forms large mucoid ("wet" appearance) colonies that appear iridescent (red and green coloration). Smaller, "drier" colonies

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF036004.

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[1] The abbreviations used are: HA, hyaluronan, hyaluronate or hyaluronic acid; HAS, HA synthase; Tn, transposon; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Glc, glucose; GalNAc, *N*-acetylgalactosamine; ORF, open reading frame; kb, kilobase(s); PCR, polymerase chain reaction.

lacking iridescence were chosen and streaked out. India ink staining and light microscopy were used as a secondary screen to assess the state of encapsulation. The position of the Tn elements in the mutant chromosome was mapped by Southern analysis (15, 16).

The DNA sequences at the Tn-disrupted sites from several independently selected mutants were obtained by direct dideoxy sequence analysis of tagged chromosomal DNA (15). Briefly, a chimeric DNA fragment consisting of a 12-kb portion of the Tn*916* element and a short region of the *P. multocida* DNA generated by *Hha*I digestion of mutant chromosomal DNA was purified by agarose gel electrophoresis (all of the wild-type P-1059 *Hha*I genomic fragments are ≤ 7 kb). The chimeric fragment served as the template in cycle sequencing reactions using ^{33}P terminators (Thermosequenase® system, Amersham) and a Tn*916* right arm terminus primer (5′-GACCTTGATAAAGTGTGATAAGTCC-3′). The sequence data were used to design PCR primers. Gel-purified PCR products were labeled with digoxigenin utilizing the High Prime system (Boehringer Mannheim).

Isolation of a Functional HAS Locus—A λ library of *Sau*3A partially digested P-1059 DNA was made using *Bam*HI-cleaved λZap Express® vector system (Stratagene). The plaque lifts were screened by hybridization (16) with digoxigenin-labeled PCR product. *Escherichia coli* XLI-Blue MRF′ was co-infected with individual purified positive λ clones and ExAssist helper phage to yield phagemids. The resulting phagemids were transfected into *E. coli* XLOLR cells to recover the plasmids.

The plasmids were transformed into a host more suitable for HA polysaccharide production, *E. coli* K5 (strain Bi8337–41; provided by I. Orskov and F. Orskov). This strain produces UDP-GlcA, a required substrate for HA biosynthesis that is *not* found at significant levels in most laboratory strains. Additionally, K5 possesses many other genes essential for capsular polysaccharide transport in *E. coli* (17). Another host employed for expression studies was *E. coli* EV5 (provided by R. P. Silver; Ref. 18), an acapsular derivative of a K1 strain (which produces a polysialic acid capsule) that possesses all the same general capsular polysaccharide transport machinery as K5 but not high levels of UDP-Glc dehydrogenase.

Cultures of the *E. coli* transformants with the candidate plasmids grown in completely defined medium were tested for HA polysaccharide production as described previously (19) except that the cell pellets were extracted with 8 M urea, 0.01% SDS at 95 °C for 2 min. The HA test assay (Pharmacia Biotech Inc.) employs a specific HA-binding protein to detect HA at concentrations of ≥0.1 μg/ml (20). Multiple determinations of HA levels were averaged. The HA concentration in bacterial cultures was normalized for differences in cell number by measuring the A_{600} value and presenting the data as μg HA/ml /A_{600} of bacteria. One plasmid, pPm7A, with a 5.8-kb insert conferred *E. coli* K5 with the ability to produce HA; no HA was produced by cells with vector plasmid alone. A truncated derivative of pPm7A containing a ~3.3-kb insert, called pPmΔ6e, could direct the biosynthesis of HA when transformed into *E. coli* K5. Therefore, the sequence of both strands of the pPm7A plasmid corresponding to the pPmΔ6e DNA insert was determined. A single complete 972-residue ORF, which we called PmHAS, was found.

Expression of Recombinant P. multocida HAS—The PmHAS ORF in the pPm7A insert was amplified by 13 cycles of PCR (16) with *Taq* polymerase (Fisher) and primers corresponding to the sequence near the deduced amino and carboxyl termini (codons in capital letters: sense, 5′-gcgaattcaaaggacagaaa**ATG**AAcACATTATCACAAG-3′, and antisense, 5′-gggaattctgcag**tta**TAGAGTTATACTATTAATAATGAAC-3′; start and stop codons, respectively, in bold). Codon 2 (T → C) was altered (italic lowercase letter) to increase protein production in *E. coli*. The primers also contained *Eco*RI and *Pst*I restriction sites (underlined letters) to facilitate cloning into the expression plasmid pKK223-3 (*tac* promoter; Pharmacia). The resulting recombinant construct, pPmHAS, was transformed into *E. coli* SURE cells (Stratagene), and this strain was used as the source of membrane preparations for *in vitro* HAS assays (21). Log phase cultures (LB broth, 30 °C) were induced with 0.5 mM isopropylthiogalactoside for 3 h before harvest. The plasmid was also transformed into *E. coli* K5; the resulting strain was examined for the presence of capsule by light microscopy and buoyant density centrifugation (19). The K5 bacterial cultures were not induced routinely because isopropylthiogalactopyranoside addition did not increase HA levels in LB or defined medium significantly.

Photoaffinity Labeling of Native P. multocida HAS—The radiolabeled UDP sugar analogs, [^{32}P]azido-UDP-GlcA (3 mCi/μMol) and [^{32}P]azido-UDP-GlcNAc (2.5 mCi/μMol), were prepared and purified as described (Refs. 22 and 23, respectively). Membrane preparations from *P. multocida* P-1059 (24) in 50 mM Tris, 20 mM $MgCl_2$, pH 7, were incubated with either probe (final concentration, 20 μM) for 30 s on ice before irradiation with ultraviolet light (254 nm, 90 s). The proteins were precipitated with 5% trichloroacetic acid before SDS-polyacrylamide gel electrophoresis analysis (16, 22). No radiolabel was incorporated if the irradiation step was omitted. As a specificity control, 10-fold molar excess of the normal UDP sugar was co-incubated with the probe and membranes. [^{32}P]Azido-UDP-Glc (3 mCi/μMol; Ref. 22) was also used as another control.

RESULTS

Production and Characterization of HA Capsule Mutants—About 8 × 10^4 Tn-containing transformants produced by several rounds of mutagenesis were screened for differences in colony morphology. By light microscopy with India Ink, the cells from small noniridescent colonies (n = 4) possessed no detectable capsule (acapsular), whereas the cells from medium-sized iridescent colonies (n = 8) appeared to have a capsule of about 10–25% of the diameter of the wild type (microcapsular). Interestingly, two of the acapsular mutants, named H and L, which had Tn elements that mapped to the same *Hin*dIII or *Bst*XI genomic fragments (data not shown), reverted to wild-type colony morphology at rates of ~10^{-3}. The Tn element in each revertant had excised from the original position and reinserted at different, new locations as judged by Southern analysis; on the other hand, all acapsular subclones retained the Tn element at the original location (data not shown). No significant HAS activity was detected in membrane preparations from mutant H cells, whereas substantial HAS activity was obtained from wild-type P-1059 cells (≤0.7 *versus* 120 pmol transfer GlcA/mg protein/h, respectively). These findings suggested that the Tn elements in mutants H and L were indeed responsible for disrupting the HA biosynthesis locus.

Molecular Cloning of the P. multocida HAS Locus—To bridge the gap between the Tn insertion sites of two acapsular mutants, PCR using the mutant L chromosomal DNA template was performed with a primer derived from sequence at the mutant H disruption site, PmHF (5′-CTCCAGCTGTAAATTA-GAGATAAAG-3′), and a primer corresponding to the left terminus of Tn*916*, TnL2 (5′-GCACATAGAATAAGGCTTTAC-GAGC-3′). A specific ~1-kb PCR product was obtained; on the other hand, *no* product was formed if PmHR (inverse complement of PmHF) or the Tn*916* right arm primer were substituted. The PCR product was used as a hybridization probe to obtain a functional copy of the *P. multocida* HAS. Six positively hybridizing plaques were found after screening ~10^4 plaques, and these phage were converted into plasmids. One plasmid, pPm7A, was found that could direct *E. coli* K5 to produce HA *in vivo* (20 μg HA/ml/A_{600} of bacteria). *E. coli* K5 with control plasmids did *not* produce HA (≤0.05 μg HA/ml/A_{600}). *E. coli* XLOLR or *E. coli* EV5 cells (which lack UDP-Glc dehydrogenase activity) do *not* produce HA even if they contain the pPm7A plasmid (≤0.05 μg HA/ml/A_{600}). This genetic evidence implied that the insert of pPm7A does not encode a functional UDP-Glc dehydrogenase enzyme.

A truncated derivative of the pPm7A plasmid with the smallest insert capable of directing HA biosynthesis (85 μg HA/ml/A_{600} of K5 bacteria), pPmΔ6e, contained a single complete ORF encoding a 972 residue protein (Fig. 1). Smaller plasmids with partial deletions of this ORF lost the ability to direct HA polymer production in *E. coli* K5. We hypothesized that this protein was the HA synthase and called it PmHAS. The predicted M_r is 111,923, and the calculated isoelectric point is 6.84.

We used PmHAS as the query in BLASTP (25) searches of the protein sequence data base. The central portion of PmHAS (residues 436–536) is most homologous to bacterial glycosyltransferases from a wide variety of genera, including *Streptococcus*, *Vibrio*, *Neisseria*, and *Staphylococcus*, that form exopolysaccharides or the carbohydrate portions of lipopolysac-

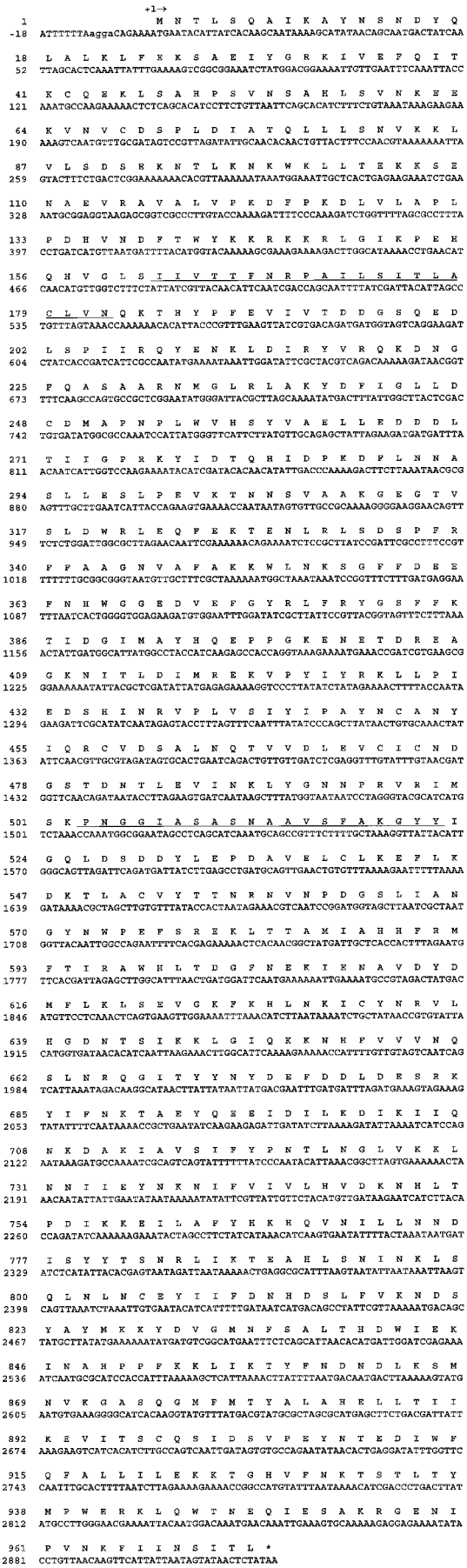

FIG. 1. **Sequence of the *P. multocida* HA synthase.** The nucleotide sequence of the gene (GenBank℠ accession number AF036004) responsible for HA production and its deduced translation is depicted. No obvious promoter is present, but there is a predicted ribosome-binding site (*lowercase*). The two putative transmembrane regions predicted by TMPRED (29) are *underlined*.

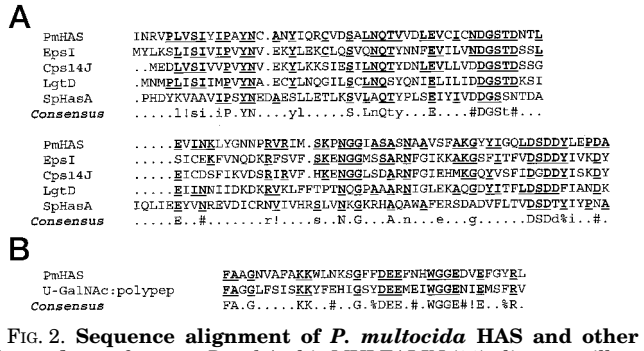

FIG. 2. **Sequence alignment of *P. multocida* HAS and other glycosyltransferases.** *Panel A*, this MULTALIN (31) alignment illustrates that the central region of the new HAS (residues 436–536) is most similar to the amino-terminal portions of various enzymes that produce other exopolysaccharides (*Streptococcus thermophilus* EpsI; Type 14 *S. pneumoniae* Cps14J) or the carbohydrate moiety of lipopolysaccharides (*H. influenzae* LgtD homolog). Only a few examples are shown. *S. pyogenes* HasA (residues 61–168) has limited similarity to this depicted region of PmHAS. *Panel B*, in this alignment, residues 342–383 of PmHAS are most similar to residues 362–404 of the mammalian UDP-GalNAc:polypeptide GalNAc-transferase. Identical residues are *bold* and *underlined*. The consensus symbols are: !, either I or V; #, any one of N, D, E, or Q; %, either F or Y. Clusters of acidic residues are well conserved.

charides (smallest sum probabilities, 10^{-22}–10^{-10}; Fig. 2*A*). The most notable sequence similarities are the DGSTD and D*X*DD motifs. Unexpectedly, there was no significant overall similarity of PmHAS to the streptococcal, viral, or vertebrate HASs (HasA smallest sum probability 0.33). Only one short region of streptococcal HasA aligns with PmHAS in a convincing fashion (Fig. 2*A*). A few segments of the first half of PmHAS are also similar to portions of the mammalian UDP-GalNAc: polypeptide GalNAc-transferase, an enzyme that initiates *O*-glycosylation of mucin-type proteins (smallest sum probability, $\sim 10^{-3}$; Fig. 2*B*).

The partial ORF (27 residues) downstream of PmHAS is very similar to the amino terminus of several UDP-Glc dehydrogenases from bacteria including *E. coli*, *Salmonella typhimurium*, and *Streptococcus pneumoniae* (67–74% identity). The severe truncation in the original pPm7A clone would be expected to result in complete loss of activity. The other ORF (623 residues) upstream of PmHAS is very homologous to the *E. coli* K5 KfaA protein (smallest sum probability, 10^{-52}), a protein putatively involved in the transport of capsular polysaccharide out of the cell.

Photoaffinity Labeling of P. multocida Membranes—The predicted size of 972 residues (112 kDa) for PmHAS was confirmed by photoaffinity labeling of membrane preparations from *P. multocida* P-1059. Both [^{32}P]azido-UDP-GlcA and [^{32}P]azido-UDP-GlcNAc probes photoincorporated into a ~110-kDa protein in an UV-dependent manner (Fig. 3*A*). Competition with the corresponding unlabeled natural UDP sugar precursors lowered the extent of probe photoincorporation. In parallel experiments, [^{32}P]azido-UDP-Glc, an analog of the normal HA precursors, did *not* label this 110-kDa protein (data not shown). Furthermore, membranes derived from Tn mutants had either no or very low amounts of azido-UDP-GlcA photoincorporation into this protein (Fig. 3*B*).

Heterologous Expression of P. multocida HAS—Membranes derived from *E. coli* SURE cells containing the pPmHAS plasmid, but *not* samples from cells with the vector pKK223-3 alone, synthesized HA *in vitro* when supplied with both UDP-

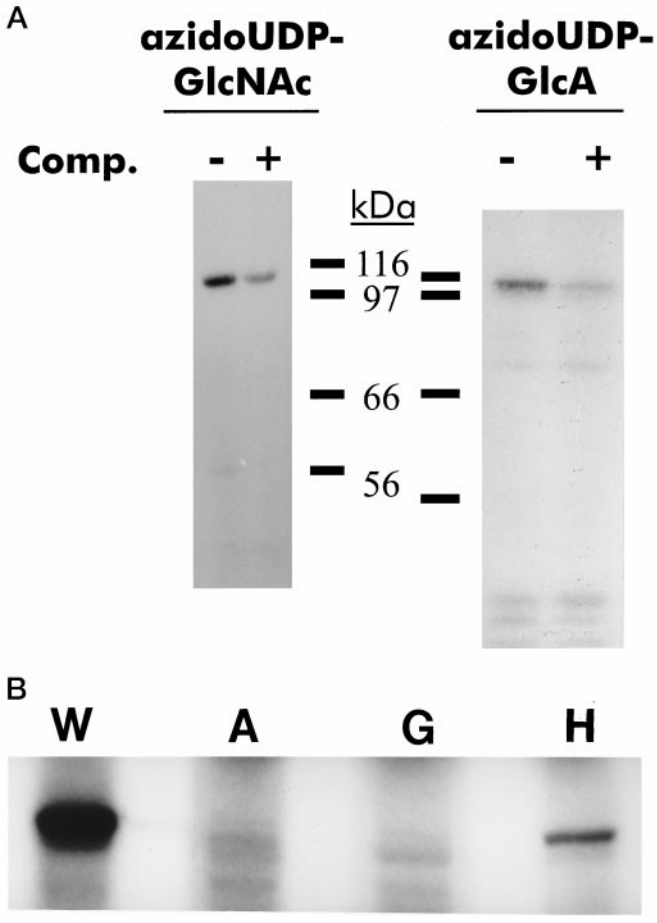

FIG. 3. **Photoaffinity labeling with UDP sugar analogs.** *Panel A*, [^{32}P]azido-UDP-GlcNAc or [^{32}P]azido-UDP-GlcA were incubated with membrane preparations (45 μg of protein) isolated from wild-type *P. multocida* and irradiated with UV light. Autoradiograms (5-day exposures) of 10% gels are shown. Both probes photolabel a ~110-kDa protein in an UV-dependent manner (*− lanes*). To assess the specificity of photoincorporation, a parallel sample was treated identically except that the reaction mixtures included a 10-fold excess of unlabeled competitor (UDP-GlcNAc or UDP-GlcA, respectively; *+ lane*); the band intensities are reduced in comparison with *− lanes*. Standards are marked in kDa. *Panel B*, Membrane preparations (60 μg of protein) from wild-type (*W*) or various acapsular Tn mutants (*A*, *G*, or *H*) were photolabeled with [^{32}P]azido-UDP-GlcA. The region of the autoradiogram in the vicinity of the ~110-kDa protein is shown. No photoincorporation is seen in the A and G samples. The small extent of photolabeling in the H sample is due to the low rate of reversion observed with this particular mutant. The size of the photoaffinity labeled protein corresponds well to the predicted M_r of the cloned PmHAS ORF.

GlcA and UDP-GlcNAc (25 *versus* ≤1.5 pMol GlcA transfer/mg protein/h, respectively). No incorporation of [^{14}C]GlcA was observed if UDP-GlcNAc was omitted or if divalent metal ions were chelated with EDTA. The HAS activity derived from recombinant HAS was similar to the enzyme obtained from wild-type *P. multocida* membranes because Mn^{2+} stimulated at least 10-fold more activity than Mg^{2+} (24).

Cultures of recombinant *E. coli* were also tested for the presence of HA polysaccharide with a radiometric assay utilizing labeled HA-binding protein. *E. coli* K5 with pPmHAS produced 460 μg HA/ml/A_{600}. K5 cells with pKK223-3 vector alone did *not* produce HA (≤0.01 μg HA/ml/A_{600}). For comparison, wild-type *P. multocida* P-1059 grown in the same media produced 1,100 μg HA/ml/A_{600}. *E. coli* K5 with pPmHAS produced such high levels of HA that the cells became encapsulated (Fig. 4*A*). The radius of the capsule of the recombinant strain was

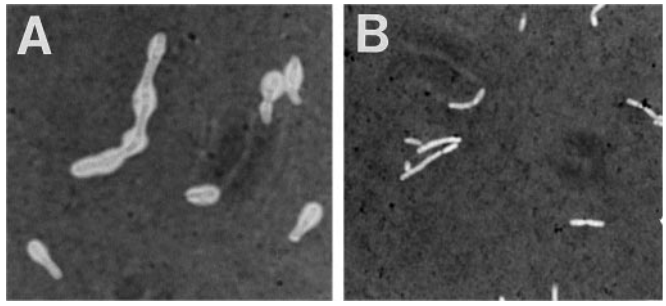

FIG. 4. **Photomicrographs of recombinant *E. coli*.** *Panel A*, India ink staining (1,000× magnification) reveals that *E. coli* K5 cells with pPmHAS produce a substantial capsule that appears as a *white halo* around the cells. *Panel B*, the capsular material could be removed from the *E. coli* K5(pPmHAS) cells by brief treatment with *Streptomyces* HA lyase. PmHAS directs polymerization of the HA polysaccharide.

~0.2–0.5 μm (assuming a bacterial cell width of 0.5 μm). This capsule could be removed by treatment with either ovine testicular hyaluronidase or *Streptomyces* HA lyase (Fig. 4*B*). Neither the native K5 host strain nor transformants containing pKK223-3 vector possessed a readily observable capsule as determined by light microscopy. K5 cells with pPmHAS were also deemed encapsulated by buoyant density centrifugation. The recombinant cells floated on top of the 58% Percoll cushion, whereas the vector control cells or hyaluronidase-treated recombinant cells pelleted through the Percoll cushion (not shown).

DISCUSSION

We found that the PmHAS protein polymerizes authentic HA polysaccharide. The carbohydrate from recombinant *E. coli* was recognized by the cartilage HA-binding protein and was sensitive to HA lyase digestion; these reagents are regarded as specific for HA polysaccharide (20, 26). Both UDP-GlcA and UDP-GlcNAc were required for HA synthesis *in vitro*. Furthermore, azido-UDP-GlcA and azido-UDP-GlcNAc but *not* azido-UDP-Glc specifically photoincorporated into PmHAS. As in the case of streptococcal HasA (21) and *Xenopus* DG42 (11), it appears that one polypeptide species, PmHAS, transfers two distinct sugar groups to the nascent HA chain.

Many encapsulated Gram-negative bacteria, including *E. coli*, *Neisseria meningitidis*, and *Hemophilus influenzae*, possess clusters of genes responsible for capsule biosynthesis organized in operons (17, 18, 27). These operons often contain genes encoding (i) enzymes required for sugar nucleotide precursor synthesis, (ii) glycosyltransferases for polymerizing the exopolysaccharide, and (iii) proteins implicated in polysaccharide export. Thus far we have found that the Type A *P. multocida* HA capsule operon contains: a KfaA analog, a HA synthase, and a putative UDP-Glc dehydrogenase. The Tn*916* elements in the *P. multocida* acapsular mutants H and L were not integrated directly in the HAS gene but rather were located in the *KfaA* homolog gene. The resulting disruption probably caused a polar mutation resulting in no expression of the downstream genes encoding the HAS or the UDP-Glc dehydrogenase. Potential ribosome-binding sites preceded these latter two ORFs, but there were no obvious promoter elements. The *S. pyogenes* HAS gene, *hasA*, is followed by an UDP-Glc dehydrogenase, *hasB*, and an UDP-Glc pyrophosphorylase, *hasC*; in this case, however, the promoter element is at the 5′ end of *hasA* (28).

At the amino acid level, PmHAS is not as similar to the other cloned HASs as one would initially expect. Two potential short motifs, DGS(S/T) at residues 477–480 and DSD at residues 527–529 of PmHAS, are present in HasA. Another similar DGS-containing motif is found repeated at residues 196–198 of

PmHAS. The DG of the first motif and the DSD are conserved in all HASs. On the other hand, several absolutely conserved motifs ((S/G)GPL*X*X*Y*, GDDR*X*LTN, and L*X*QQ*X*RW*X*KS(Y/F/W)(F/C)RE) found in *all* previously cloned HASs are *absent* from PmHAS (7, 12). Instead, a variety of bacterial glycosyltransferases align more closely with the sequence in the central portion of the *P. multocida* HAS protein. These enzymes, which have been either shown or predicted to transfer GlcNAc, galactose, or GalNAc groups, are roughly one-third the size of the *P. multocida* enzyme; it is interesting to note that their amino termini sequences align together with the middle of the PmHAS polypeptide (residues 430–540). Sections of the first 420 residues of PmHAS show some similarity to portions of the mammalian UDP-GalNAc:polypeptide GalNAc-transferase. These observations may be a reflection of a possible domain structure within PmHAS. The last ~340 residues of this new HAS are not very similar to other entries in the sequence data bases. Therefore, the *P. multocida* HAS may be unique or may be the prototype of a new class of HAS.

PmHAS is roughly twice the size of the streptococcal, viral, or vertebrate HASs (972 *versus* 417–588 residues, respectively; Ref. 7). Furthermore, the hydropathy plots of PmHAS and the other known HASs are dissimilar. Using the TMPRED program (29), PmHAS is predicted to have only two candidate transmembrane helices (centered on residues 170 and 510), and both termini of the protein may be located in the cytoplasm. Topologically, this speculation implies that perhaps one-third of the *P. multocida* polypeptide (~340 residues) is located outside of the cytoplasm. On the other hand, a different topology is predicted for the other class of HASs (7, 9). Reporter enzyme fusion analysis of streptococcal HasA concurs with these predictions; the topological arrangement appears to consist of (i) two transmembrane helices near the amino terminus, (ii) a putative cytoplasmic domain, followed by (iii) three membrane-associated regions at the carboxyl half of the protein (30). The connecting loops between membrane-associated regions are rather short (4–10 residues); therefore, the vast majority of the polypeptide chain is probably *not* extracellularly exposed. In the future, it will be interesting to elucidate if these two distinct structural classes of HASs utilize the same or different mechanisms to polymerize a product with the identical composition and glycosidic linkages.

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