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Identification and Molecular Cloning of a Heparosan Synthase from *Pasteurella multocida* Type D*

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Pasteurella multocida **Type D, a causative agent of atrophic rhinitis in swine and pasteurellosis in other domestic animals, produces an extracellular polysaccharide capsule that is a putative virulence factor. It was reported previously that the capsule was removed by treating microbes with heparin lyase III. We molecularly cloned a 617-residue enzyme, pmHS, which is a heparosan (nonsulfated, unepimerized heparin) synthase. Recombinant *Escherichia coli*-derived pmHS catalyzes the polymerization of the monosaccharides from UDP-GlcNAc and UDP-GlcUA. Other structurally related sugar nucleotides did not substitute. Synthase activity was stimulated about 7–25-fold by the addition of an exogenous polymer acceptor. Molecules composed of ~500–3,000 sugar residues were produced *in vitro*. The polysaccharide was sensitive to the action of heparin lyase III but resistant to hyaluronan lyase. The sequence of the pmHS enzyme is not very similar to the vertebrate heparin/heparan sulfate glycosyltransferases, EXT1 and 2, or to other *Pasteurella* glycosaminoglycan synthases that produce hyaluronan or chondroitin. The pmHS enzyme is the first microbial dual-action glycosyltransferase to be described that forms a polysaccharide composed of β4GlcUA-α4GlcNAc disaccharide repeats. In contrast, heparosan biosynthesis in *E. coli* K5 requires at least two separate polypeptides, KfiA and KfiC, to catalyze the same polymerization reaction.**

Glycosaminoglycans (GAGs)[1] are long linear polysaccharides consisting of disaccharide repeats that contain an amino sugar (1, 2). Heparin/heparan (β4GlcUA-α4GlcNAc)$_n$, chondroitin (β4GlcUA-β3GalNAc)$_n$, and hyaluronan (β4GlcUA-β3GlcNAc)$_n$ are three prevalent GAGs. In the former two polymers, usually $n = 20–100$, although in the case of HA, typically $n = 10^{3–4}$. In vertebrates, one or more modifications including *O*-sulfation of certain hydroxyls, deacetylation and subsequent *N*-sulfation, or epimerization of glucuronic acid to iduronic acid are found in most GAGs except HA (3). A few clever microbes also produce GAG chains, however sulfation or epimerization has not been described. The GAGs in pathogenic bacteria are found as extracellular polysaccharide coatings, called capsules, which serve as virulence factors (4). The capsule is thought to assist in the evasion of host defenses such as phagocytosis and complement. As the microbial polysaccharide is identical or very similar to the host GAG, the antibody response is either very limited or non-existent.

The invasiveness and pathogenicity of certain *Escherichia coli* strains has been attributed to their polysaccharide capsule (4). Two *E. coli* capsular types, K5 and K4, make polymers composed of GAG-like polymers. The K5 capsular material is a polysaccharide called heparosan, *N*-acetylheparosan, or desulfoheparin, which is identical to mammalian heparin/heparan sulfate except that the bacterial polymer is nonsulfated, and there is no epimerization of GlcUA to iduronic acid (5). The *E. coli* K4 polymer is a nonsulfated chondroitin backbone decorated with fructose side branches on the C3 position of the GlcUA residues (6).

The *E. coli* K5 capsule biosynthesis locus contains the open reading frames *KfiA–D* (also called *Kfa* in some reports; GenBank™ accession number X77617). At first, KfiC was stated to be a dual-action glycosyltransferase responsible for the alternating addition of both GlcUA and GlcNAc to the heparosan chain (7). However, a later report by the same group reported that another protein, KfiA, was actually the αGlcNAc-transferase required for heparosan polymerization (8). Therefore, at least these two enzymes, KfiA and KfiC, the βGlcUA-transferases, work in concert to form the disaccharide repeat. Another deduced protein in the operon, KfiB, has been reported to stabilize the enzymatic complex during elongation *in vivo* but perhaps not participate directly in catalysis (8). The identity and the sequence of the *E. coli* K4 capsular glycosyltransferase(s) has not yet been reported.

Many *Pasteurella multocida* isolates produce GAG or GAG-like molecules as assessed by enzymatic degradation and removal of the capsule of living bacterial cells (9, 10). Carter Type A *P. multocida*, the major causative agent of fowl cholera and pasteurellosis, makes an HA capsule (11). A single polypeptide, the HA synthase pmHAS, polymerizes the HA chain by transferring both GlcUA and GlcNAc (12). Type F *P. multocida*, the minor fowl cholera pathogen, produces a capsule composed of a nonsulfated chondroitin sensitive to *Flavobacterium* chondroitin AC lyase (9, 13, 14). Again, a dual-action chondroitin synthase, pmCS, polymerizes the chondroitin chain (14). The capsule of another distinct *P. multocida*, Type D, was reported to be sensitive to heparin lyase III (9). In this report, we describe the identification and characterization of pmHS, a dual-action heparosan synthase.

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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) AF425591 and AF439804.

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[1] The abbreviations used are: GAG, glycosaminoglycan; HA, hyaluronan, hyaluronate, or hyaluronic acid; pmCS, *P. multocida* chondroitin synthase; pmHS, *P. multocida* heparosan synthase; pmHAS, *P. multocida* HA synthase; ORF, open reading frame.

EXPERIMENTAL PROCEDURES

Materials and Pasteurella Strains—Unless otherwise noted, all chemicals were from Sigma or Fisher, and all molecular biology reagents were from Promega. The wild-type encapsulated Type D *P. multocida* isolates P-934 (swine), P-3881 (bovine), P-4058 (rabbit), and P-5695 (swine) were obtained from the United States Department of Agriculture collection (Ames, IA). The strains were grown in brain heart infusion (Difco) at 37 °C.

Analysis of Genomic DNA and Isolation of Capsule Biosynthesis Locus DNA—Preliminary data from a Southern blot analysis using pmHAS-based hybridization probes (12) suggested that the Type A synthase and the putative Type D synthase were not very similar at the DNA level. However, a PCR suggested that the UDP-glucose dehydrogenase genes, which encode an enzyme that produces the UDP-GlcUA precursor required for both HA and heparin biosynthesis, were very homologous. In most encapsulated bacteria, the precursor-forming enzymes and the transferases are located in the same operon. To make a hybridization probe predicted to detect the capsule locus, Type D chromosomal DNA served as a template in PCR reactions utilizing degenerate oligonucleotide primers (sense: GARTTYBTIMRIGARGGIAARGCIYTITAYGAY; antisense: RCARTAICCICCRTAICCRAAISWXGGRTTRTTRTARTG, where I is Ino; R is Ado or Guo; S is Cyd or Guo; W is Ado or Thd; and Y is Cyd or Thd) corresponding to a conserved central region in many known UDP-glucose dehydrogenase genes. The ~0.3-kb amplicon was generated using *Taq* DNA polymerase (Fisher), gel-purified, and labeled with digoxigenin (High Prime system, Roche Molecular Biochemicals).

A lambda library of *Sau*3A partially digested P-3881 DNA (~4–9-kb average length insert) was made using the *Bam*HI-cleaved λZap Express™ vector system (Stratagene). The plaque lifts were screened by hybridization (5× SSC, 50 °C; 16 h) with the digoxigenin-labeled probe using the manufacturer's guidelines for colorimetric development. *E. coli* XLI-Blue MRF′ was co-infected with the purified, individual positive lambda clones and ExAssist helper phage to yield phagemids. The resulting phagemids were transfected into *E. coli* XLOLR cells to recover the plasmids. Sequence analysis of the plasmids using a variety of custom primers as well as the GPS-1 Genome Priming System (New England Biolabs) revealed a novel open reading frame, which we called pmHS (DNA sequence facilities at Oklahoma State University and the University of Oklahoma Health Sciences Center). We amplified and sequenced the ORF from several highly encapsulated isolates (see next section); very similar sequences were obtained.

Expression of Recombinant P. multocida Heparosan Synthase—The pmHS ORF (617 amino acids) was amplified from the various Type D genomic DNA templates by 18 cycles of PCR (16) with *Taq* polymerase. For constructing the full-length enzyme, the sense primer (ATGAGCTTATTTAAACGTGCTACTGAGC) corresponded to the sequence at the deduced amino terminus of the ORF and the antisense primer (TTTACTCGTTATAAAAAGATAAACACGGAATAAG) encoded the carboxyl terminus including the stop codon. In addition, a truncated version of pmHS was produced by PCR with the same sense primer but a different antisense primer (TATATTTACAGCAGTATCATTTTCTAAAGG) to yield a predicted 501-residue protein, DcbF (GenBank™ accession number AAK17905; Ref. 15); this variant corresponds to residues 1–497 of pmHS followed by the residues TFRK.

The amplicons were cloned using the pETBlue-1 acceptor system (Novagen) according to the manufacturer's instructions. The *Taq*-generated single A overhang is used to facilitate the cloning of the open reading frame downstream of the T7 promoter and the ribosome binding site of the vector. The ligated products were transformed into *E. coli* NovaBlue and plated on LB carbenicillin (50 μg/ml) and tetracycline (13 μg/ml) under conditions for blue/white screening. White colonies were analyzed by PCR-based screening and restriction digestion. Plasmids with the desired ORF were transformed into *E. coli* Tuner, the T7 RNA polymerase-containing expression host, and maintained on LB medium with carbenicillin and chloramphenicol (34 μg/ml) at 30 °C. Mid-log phase cultures were induced with β-isopropylthiogalactoside (0.2 mM final) for 5 h. The cells were harvested by centrifugation and frozen, and the membranes were prepared according to a cold lysozyme/sonication method (16) except that 0.1 mM mercaptoethanol was included during the sonication steps. Membrane pellets were suspended in 50 mM Tris, pH 7.2, 0.1 mM EDTA, and protease inhibitors.

Assays for Heparosan Synthase Activity—The incorporation of radiolabeled monosaccharides from UDP-[^{14}C]GlcUA and/or UDP-[^{3}H]GlcNAc precursors (PerkinElmer Life Sciences) was used to monitor heparosan synthase activity. Samples were usually assayed in a buffer containing 50 mM Tris, pH 7.2, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 0–0.6 mM UDP-GlcUA, and 0–0.6 mM UDP-GlcNAc at 30 °C. Depending on the experiment, a Type D acceptor polymer processed by extended ultrasonication of a capsular polysaccharide preparation (isolated by cetylpyridinium chloride precipitation of the spent Type D culture media, Ref. 14) was also added to the reaction mixture. The reaction products were separated from substrates by descending paper (Whatman 3M) chromatography with ethanol, 1 M ammonium acetate, pH 5.5, development solvent (65:35). The origin of the paper strip was cut out, eluted with water, and the incorporation of radioactive sugars into polymer was detected by liquid scintillation counting with BioSafe II mixture (Research Products International). The metal preference of pmHS was assessed by comparing the signal from a no metal control reaction (0.5 mM EDTA) to reactions containing 10–20 mM manganese, magnesium, or cobalt chloride. To test the transfer specificity of pmHS, various UDP-sugars (UDP-GalNAc, UDP-GalUA, or UDP-Glc) were substituted for the authentic heparosan precursors. The data from the recombinant construct containing the *pmHS* gene from the P-4058 strain are presented, but the results are similar to constructs derived from the P-934 or P-5695 strains.

Size Analysis and Enzymatic Degradation of Labeled Polymers—Gel filtration chromatography was used to analyze the size distribution of the labeled polymers. Separations were performed with a Polysep-GFC-P 4000 column (300 × 7.8 mm; Phenomenex) eluted with 0.2 M sodium nitrate at 0.6 ml/min. Radioactivity was monitored with an inline Radioflow LB508 detector (EG & G Berthold; 500-μl flow cell) using a Unisafe I mixture (1.8 ml/min; Zinsser). The column was standardized with fluorescein-labeled dextrans of various sizes.

To further characterize the radiolabeled polymers, depolymerization tests with specific glycosidases was performed. The high molecular weight product was purified by paper chromatography. The origin of the strips was washed with 80% ethanol and air-dried and then extracted with water. The water extract was lyophilized, resuspended in a small volume of water, and split into three aliquots. Two aliquots were treated with glycolytic enzymes for 2 days at 37 °C (*Flavobacterium* heparin lyase III, 6.7 milliunits/μl, 50 mM sodium phosphate, pH 7.6, or *Streptomyces* HA lyase, 333 milliunits/μl, 50 mM sodium acetate, pH 5.8). The last aliquot was mock-treated without enzyme in acetate buffer. The aliquots were quenched with SDS and subjected to paper chromatography, and the radiolabel at the origin was measured by liquid scintillation counting.

RESULTS

Molecular Cloning of the Type D P. multocida Heparosan Synthase—A PCR product that contained a portion of the Type D UDP-glucose dehydrogenase gene was used as a hybridization probe to obtain the rest of the Type D *P. multocida* capsular locus from a lambda library. We found a functional heparosan synthase, which we named pmHS, in several distinct Type D strains from different host organisms isolated around the world. In every case, an open reading frame of 617 residues with very similar amino acid sequence (98–99% identical) was obtained.

In the latter stages of our project, another group deposited a sequence from the capsular locus of a Type D organism in GenBank™ (15). In their annotation, the carboxyl terminus of the pmHS homolog is truncated and mutated to form a 501-residue protein that was called DcbF (GenBank™ accession number AAK17905). No functional role for the protein except glycosyltransferase was described, and no activity experiments were performed. As we describe later, membranes or cell lysates prepared from *E. coli* with the recombinant *dcbF* gene do not possess heparosan synthase activity.

Another deduced gene recently uncovered by the University of Minnesota in their Type A *P. multocida* genome project (17) called *pglA* (GenBank™ accession number AAK02498) and encoding 651 amino acids is also similar to pmHS (73% identical in the major overlapping region). However, the *pglA* gene is not located in the putative capsule locus. This group made no annotation of the function of pglA. We have preliminary evidence that this protein also polymerizes GlcUA and GlcNAc residues to form heparosan (data not shown). We found that a Type D strain also appears to contain a homologous *pglA* gene as shown by PCR and activity analysis (data not shown).

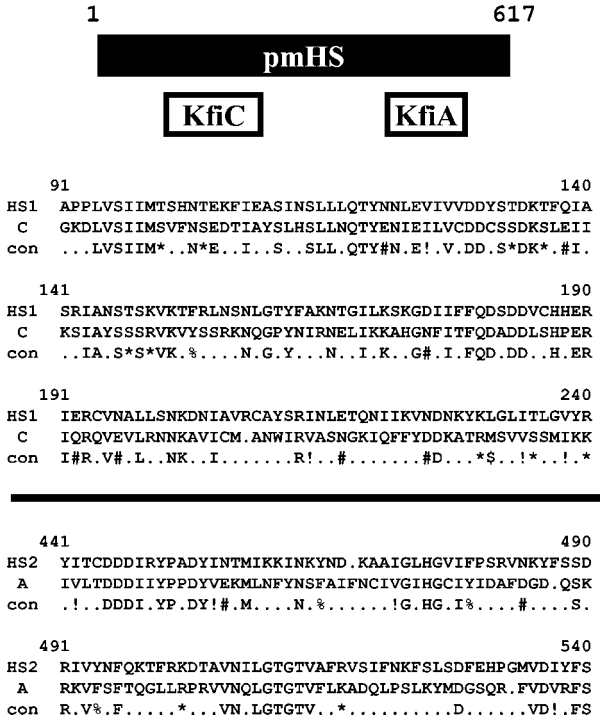

FIG. 1. **Sequence similarity of pmHS with KfiA and KfiC.** Elements of the *Pasteurella* heparosan synthase, *HS1* (containing residues 91–240) and *HS2* (containing residues 491–540), are very similar to portions of two proteins from the *E. coli* K5 capsular locus (*A*, residues 75–172 of KfiA; *C*, residues 262–410 of KfiC) as shown by this modified Multalin alignment (Ref. 21; numbering scheme corresponds to the pmHS sequence). The HS1 and HS2 elements may be important for hexosamine transferase or for glucuronic acid transferase activities, respectively. *con*, consensus symbols; *asterisks*, (Lys or Arg) and (Ser or Thr); %, any one of Phe, Tyr, or Trp; $, any one of Leu or Met; !, any one of Ile or Val; #, any one of Glu, Asp, Gln, or Asn.

The next best heterologous matches for the pmHS enzyme in the data base are KfiA and KfiC proteins from *E. coli* K5; these two proteins work together to make the heparosan polymer (7, 8). There is a good overall alignment of the enzyme sequences if smaller portions of the pmHS ORF are aligned separately with KfiA and KfiC (Fig. 1). Some of the most notable sequence similarities occur in the regions containing variants of the D*X*D amino acid sequence motif.

Heterologous Expression of a Functional P. multocida Heparosan Synthase—Membrane extracts derived from *E. coli* Tuner cells containing the plasmid encoding pmHS but not samples from cells with the vector alone synthesized polymer *in vitro* when supplied with both UDP-GlcUA and UDP-GlcNAc simultaneously (Table I). The identity of the polymer as heparosan was verified by its sensitivity to *Flavobacterium* heparin lyase III (~97% destroyed after treatment) and its resistance to the action of *Streptomyces* HA lyase. No substantial incorporation of radiolabeled [^{14}C]GlcUA into polymer was observed if UDP-GlcNAc was omitted or if UDP-GalNAc was substituted for UDP-GlcNAc. Conversely, in experiments using UDP-[^{3}H]GlcNAc, substantial incorporation of radiolabel into the polymer was only noted when UDP-GlcUA was also present. UDP-GalUA or UDP-Glc did not substitute for UDP-GlcUA. No polymerization or transferase activity was detected if the divalent metal ions were chelated with EDTA. Maximal activity was observed in reactions that contained Mn^{2+}, but Mg^{2+} also supported substantial incorporation (65–85% maximal). Cobalt was a weaker cofactor (~30% maximal).

The addition of the heparosan polymer acceptor increased sugar incorporation catalyzed by pmHS at least 7–25-fold in comparison to parallel reactions without acceptor (Fig. 2), analogous to observations of pmHAS (18) and pmCS (14). The acceptor stimulation of activity is probably due to the lower efficiency or slower rate of initiation of a new polymer chain in comparison to the elongation stage *in vitro*. The exogenous acceptor sugar probably associates with the recombinant enzyme's binding site for the nascent chain and then is elongated rapidly.

Analysis by gel filtration chromatography indicated that recombinant pmHS produced long polymer chains (~1–3 × 10^3 monosaccharides or ~200–600 kDa) *in vitro* without acceptor (Fig. 3). If an acceptor polymer was supplied to parallel reaction mixtures, then higher levels of shorter chains (~0.1–2 × 10^3 monosaccharides or ~20–400 kDa added to acceptor) were more rapidly produced. Radioactivity from both labeled GlcUA and GlcNAc sugars co-migrated as a single peak in all chromatography profiles. Some chains also appear to be initiated *de novo* in reactions with an acceptor as evidenced by the small

TABLE I

Transferase specificity of recombinant pmHS for sugar nucleotides

Crude membranes from cells with plasmid encoding the full-length pmHS enzyme, pmHS-(1–617) (120 μg of total protein), or no insert, vector (210 μg of total protein), were incubated in 50 μl of assay buffer with 0.6 μg (based on uronic acid) of heparosan oligosaccharide acceptor for 20 min either with UDP-[^{3}H]GlcNAc (top section), *or* UDP-[^{14}C]-GlcUA (bottom section). The radiolabeled sugar (300 μM, 0.04 μCi) was used in the presence of the indicated second unlabeled sugar nucleotide (600 μM). The incorporation into polymer was assessed by paper chromatography. A representative experiment is shown. The recombinant pmHS incorporated only the authentic heparin precursors into polysaccharide.

2nd Sugar	[^{3}H]GlcNAc incorporation	
	pmHS-(1–617)	Vector
	dpm	
None	350	40
UDP-GlcUA	4,360	40
UDP-GalUA	330	50
UDP-Glc	390	30
2nd Sugar	**[^{14}C]GlcUA incorporation**	
	pmHS-(1–617)	Vector
	dpm	
None	140	60
UDP-GlcNAc	14,990	70
UDP-GalNAc	150	80
UDP-Glc	70	60

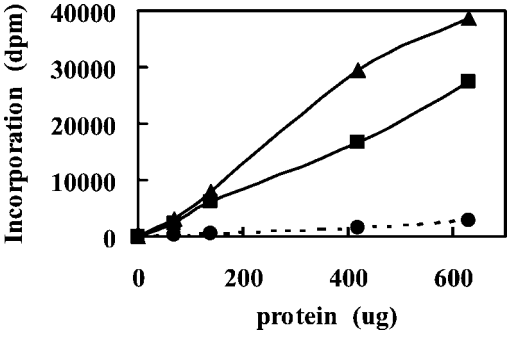

FIG. 2. **pmHS activity dependence on acceptor and enzyme concentration.** Various amounts of crude membranes containing the full-length enzyme, pmHS-(1–617), were incubated in 50 μl of buffer containing 50 mM Tris, pH 7.2, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 500 μM UDP-[^{14}C]GlcUA (0.15 μCi), and 500 μM UDP-GlcNAc. Three parallel sets of reactions were performed with either no acceptor (*circles*) or two concentrations of heparosan polymer acceptor (uronic acid: 0.6 μg, *squares*; 1.7 μg, *triangles*). After 40 min, the reaction was terminated and analyzed by paper chromatography. The background incorporation due to vector membranes alone (630 μg of total protein; not plotted) with the high concentration of the acceptor was 75 dpm [^{14}C]GlcUA. The activity of pmHS is greatly stimulated by exogenous acceptor.

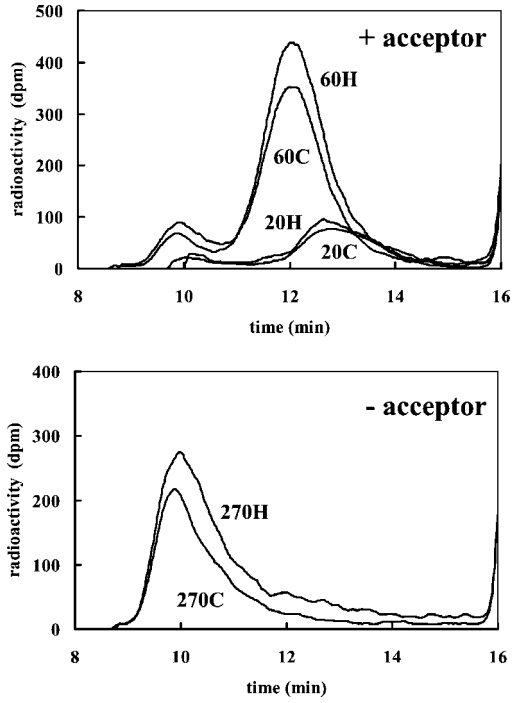

FIG. 3. **Gel filtration analysis of radiolabeled polymer synthesized *in vitro*.** The crude membranes containing pmHS (0.7 mg of total protein) were incubated with UDP-[^{14}C]GlcUA and UDP-[^{3}H]GlcNAc (500 μM, 0.4 μCi each) in a 200-μl reaction volume either in the presence (*top panel*) or absence (*bottom panel*) of acceptor polymer (1 μg of uronic acid). After various reaction times (denoted on curves: *20*, *60*, or *270* min), portions of the samples (75%) were analyzed on the PolySep column (calibration elution times in minutes: void volume, 9.8; 580 kDa dextran, 12.3; 145 kDa dextran, 12.75, totally included volume, 16.7). The starting acceptor polymer eluted at 12.8 min. Large polymers composed of both radiolabeled sugars (*C*, ^{14}C; *H*, ^{3}H) in an equimolar ratio were synthesized by pmHS.

peak of higher molecular weight material near the void volume. Apparently, once pmHS either starts a new chain or binds an existing chain, rapid elongation is performed.

We found in parallel tests that membranes or lysates prepared from recombinant cells containing the predicted *dcbF* gene (a truncated version of *pmHS* in the same expression vector; Ref. 15) do not exhibit heparosan synthase activity. Even with large amounts of total protein repeated polymerization was not observed, and no significant radiolabel incorporation above background levels was noted.

DISCUSSION

In this report, we have molecularly cloned the dual-action glycosyltransferase responsible for polymerizing the heparosan backbone component of the Type D capsular polysaccharide. As discussed earlier, the first 497 residues of the pmHS protein are virtually identical to the hypothetical DcbF sequence. We have sequenced the DNA from the equivalent P-934 isolate obtained from the same United States Department of Agriculture collection as reported in Ref. 15 as well as several other Type D strains, but our results do not agree with the *dcbF* open reading frame. Adler and co-workers (15) appear to have made a sequencing error that resulted in a frameshift mutation; a conceptual premature termination led to the creation of the erroneously truncated *dcbF* annotation.

Recently, we have determined that the *Pasteurella* hyaluronan synthase, pmHAS, contains two active sites in a single polypeptide by generating mutants that transfer only GlcUA or only GlcNAc (19). Interestingly, mixing the two different mutant pmHAS proteins reconstituted the HA synthase activity. We hypothesized that one domain, called A1, is responsible for GlcNAc transfer and that the other domain, called A2, is responsible for GlcUA transfer (19). The chondroitin synthase, pmCS, transfers a different hexosamine, GalNAc, and also appears to contain a similar two-domain structure (14). The amino acid sequence of the heparosan synthase pmHS, however, is very different from other *Pasteurella* GAG synthases, pmHAS and pmCS. The pmHAS and pmHS enzymes both perform the task of polymerizing the identical monosaccharides; HA and heparin only differ with respect to their linkages. The creation of different anomeric linkages probably requires very distinct active sites because of the disparity between a retaining (to form α-linkages) and an inverting (to form β-linkages) transfer mechanism. The putative dual-action vertebrate heparin synthases, EXT1 and 2, also appear to have two transferase domains, but the amino acid sequences are not similar to pmHS (20).

Two distinct regions of pmHS are similar to the *E. coli* K5 KfiA or KfiC proteins, suggesting the limits of the sugar transfer domains (Fig. 1). On the basis of sequence similarity, if the Kfi studies are correct, then GlcUA transfer and GlcNAc transfer occur at the amino and carboxyl portions of pmHS, respectively. The pmHS protein may be the result of the fusion of two ancestral single-action enzymes. The efficiency and convenience of combining the two required enzyme activities into a single polypeptide seem clear, but as a counterexample, the *E. coli* KfiA and KfiC proteins remain separate entities.

Interestingly, *pglA*, a gene with no reported function from a Type A isolate (17), is similar to the *pmHS* gene of a Type D strain. In parallel expression experiments, PglA from Type A or D strains also appears to be a heparosan synthase (data not shown). We have sequencing and enzymology projects in progress to verify the open reading frames and the activities of PglA. It is quite puzzling that the Type A strain would have a heparosan synthase as well as the known HA synthase. The major Type A capsular polymer was shown to be HA, but in retrospect a small amount of heparosan would be difficult or impossible to detect in these characterization studies (11). A possible scenario for the presence of a heparosan synthase in the Type A bacteria is that the *pglA* gene is repressed or silent and not expressed in this host under laboratory conditions. The *pglA* gene could also be a cryptic remnant from an ancestral organism (*i.e.* before Types A and D diverged) that has been maintained, and the gene product is still functional. Another interesting possibility is that in Type A organisms, either the *pmHAS* or the *pglA* gene is utilized at different times depending on conditions or the stage of infection; using different capsular polymers could serve as a phase-shift mechanism. We are generating antibodies to the PglA and pmHS proteins to examine these hypotheses.

Bacteria-derived heparosan may be converted by epimerization and sulfation into a polymer that resembles the mammalian heparin and heparan sulfate because all the modifying enzymes have been identified (3). *P. multocida* Type D (or an improved recombinant version) may be a more economical and useful source of heparosan than *E. coli* K5 for several reasons. The former microbe has a higher intrinsic biosynthetic capacity for capsule production. The *Pasteurella* capsule radius often exceeds the cell diameter when observed by light microscopy of India ink-prepared cells. On the other hand, visualization of the meager *E. coli* K5 capsule often requires electron microscopy. From a safety standpoint, *E. coli* K5 is a human pathogen, whereas Type D *Pasteurella* has only been reported to cause disease in animals. Furthermore, with respect to recombinant gene manipulation to create better production hosts, the benefits of handling only a single gene encoding pmHS, a dual-action synthase, in comparison to utilizing KfiA and C (and probably KfiB) are obvious.

The discovery of pmHS expands the known GAG biosynthesis repertoire of *P. multocida*. Depending on the Carter capsular type, this widespread species produces HA, heparosan, or chondroitin. The use of the three major vertebrate GAGs as capsular polymers certainly strengthens the case for molecular mimicry or camouflage as a useful strategy for bacterial pathogens.

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