Glucuronyl C5-epimerases catalyze the conversion of D-glucuronic acid (GlcUA) to L-iduronic acid (IdceA) units during the biosynthesis of glycosaminoglycans. An epimerase implicated in the generation of heparin/heparan sulfates was previously purified to homogeneity from bovine liver (Campbell, P., Hannesson, H. H., Sandbäck, D., Rodén, L., Lindahl, U., and Li, J.-p. (1994) J. Biol. Chem. 269, 26953–26958). The present report describes the molecular cloning and functional expression of the lung enzyme. The cloned enzyme contains 444 amino acid residues and has a molecular mass of 49,905 Da.

The coding cDNA insert was cloned into a baculovirus expression vector and expressed in SF9 insect cells. Cells infected with recombinant epimerase showed a 20–30-fold increase in enzyme activity, measured as release of 3H2O from a polysaccharide substrate containing C5-3H-labeled hexuronic acid units. Furthermore, incubation of the expressed protein with the appropriate (GlcUA-GlcNNSO3)substituted substrate resulted in conversion of ~20% of the GlcUA units into IdceA residues. Northern analysis implicated two epitope transcripts in both bovine lung and liver tissues, a dominant ~9-kilobase (kb) mRNA and a minor ~5-kb species. Mouse mastocytoma cells showed only the ~5-kb transcript. A comparison of the cloned epimerase with the enzymes catalyzing an analogous reaction in alginate biosynthesis revealed no apparent amino acid sequence similarity.

Heparin and heparan sulfate are complex sulfated glycosaminoglycans composed of alternating glucosamine and hexuronic acid residues. The two polysaccharides are structurally related but differ in composition, such that heparin is more heavily sulfated and shows a higher ratio of L-iduronic acid (IdceA)1 to D-glucuronic acid (GlcUA) units (2, 3). Heparin is produced by connective tissue-type mast cells, whereas heparan sulfate has a ubiquitous distribution and appears to be expressed by most cell types. The biological roles of heparin and heparan sulfate are presumably largely due to interactions of the polysaccharides with proteins, such as enzymes, enzyme inhibitors, extracellular matrix proteins, growth factors/cytokines, and others (2–5). The interactions tend to be more or less selective especific with regard to carbohydrate structure and thus depend on the amounts and distribution of the various sulfate groups and hexuronic acid units. Notably, IdceA units are believed to generally promote binding of heparin and heparan sulfate chains to proteins, due to the marked conformational flexibility of these residues (6).

Heparin and heparan sulfate are synthesized as proteoglycans (for reviews, see Refs. 3–5 and 7). The process is initiated by glycosylation reactions that generate saccharide sequences composed of alternating GlcUA and GlcNAc units (8) covalently bound to peptide core structures. The resulting (GlcUA1,4-GlcNAc1,4)n disaccharide repeats are modified, probably along with chain elongation, by a series of enzymatic reactions that is initiated by N-deacetylation and N-sulfation of GlcNAc units, continues through C5-epimerization of GlcUA to IdceA residues, and is concluded by the incorporation of O-sulfate groups at various positions. The N-deacetylation/N-sulfation step has a key role in determining the overall extent of modification of the polymer chain since the GlcUA C5-epimerase as well as the various O-sulfotransferases depend all on the presence of N-sulfate groups for substrate recognition. While the GlcNAc N-deacetylation and N-sulfation reactions are both catalyzed by the same protein (9), the isolation and molecular cloning of N-deacetylase/N-sulfotransferases from mouse mastocytoma and rat liver implicated two distinct forms of the enzyme (10–12). The two enzyme types differ with regard to kinetic properties, with the mastocytoma enzyme being more efficient in introducing N-sulfate groups in the growing polymer (13).

A GlcUA C5-epimerase was purified to apparent homogeneity (~1 million-fold) from bovine liver (1). This report describes the molecular cloning, using a bovine lung cDNA library, as well as the functional expression of this enzyme.

EXPERIMENTAL PROCEDURES

Peptide Purification and Sequencing—The 52-kDa epimerase protein (~1 µg), purified from a detergent extract of bovine liver by chromatography on O-desulfated heparin-Sepharose, red-Sepharose, phenyl-Sepharose, and concanavalin A-Sepharose (1), was subjected to direct N-terminal sequencing using an Applied Biosystems Model 470A protein sequenator equipped with an on-line Model 120 phenylthiohydanto-
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tain analyzer (14). Another sample (~1 μg) was applied to preparative (12%) SDS-polyacrylamide gel and was then transferred to a polyvinylidenefluoride membrane. After staining the membrane with Coomassie Brilliant Blue, the enzyme band was excised. Half of the material was subjected to direct N-terminal sequence analysis, whereas the remaining material was cleaved with Lys-C and sequenced using the automated Edman degradation method with a gas-phase analyzer (16). A rabbit was immunized with 240 μg (see “Results”) was chemically synthesized by Åke Engström (Department of Medical and Physiological Chemistry, Uppsala University, Uppsala, Sweden) and was then conjugated to ovalbumin using glutaraldehyde (16). A rabbit was immunized with 240 μg of the peptide conjugates emulsified in complete Freund’s adjuvant. After six booster injections (each with 240 μg of conjugated peptide), blood was collected, and the antisera was recovered. IgG was isolated from the antiserum by affinity chromatography on a protein A-Sepharose column (Pharmacia Biotech Inc.), and used for immunoblotting.

Samples of GlcUA C5-epimerase were separated under denaturing conditions by 12% SDS-polyacrylamide gel electrophoresis and were then transferred to a nitrocellulose membrane (Hybond™ ECL). ECL immunoblotting was performed according to the protocol of the manufacturer (Amersham Corp.). Briefly, the membrane was blocked with 5% (w/v) nonfat dry milk, washed 3 times with TBS-T, and then incubated with anti-peptide antibodies (1:5000). The membrane was then incubated with alkaline phosphatase-conjugated anti-rabbit antibody (1:10 000). The membrane was washed 3 times with TBS-T, and then incubated with NBT/BCIP solution for 1 h. The membrane was then rinsed 3 times with TBS-T and gel-stained with Coomassie Brilliant Blue. The bands were visualized using an ECL luminometer, and the intensity was quantified using a Fuji LAS-1000 diode array detector. Selected peptides were then subjected to sequence analysis as described above.

Probes for Screening—Total RNA was isolated from bovine liver by the LiCl/urea/SDS method (15). Single-stranded cDNA was synthesized by incubating ~5 μg of bovine liver total RNA (denatured at 65 °C for 3 min) in 10 mM Tris-HCl, pH 9.0, 0.5 mM dNTPs, 100 μg/ml of yeast tRNA, and 200 U/ml of M-MLV reverse transcriptase (Perkin-Elmer), 1 μl each dNTP, 5 μg random nucleotide hexamer, and 1.25 units of murine leukemia virus reverse transcriptase (Perkin-Elmer). The mixture was kept at 42 °C for 45 min and then at 95 °C for 5 min. Degenerated oligonucleotide primers were designed based on the amino acid sequence determined for one of the internal peptides derived from the purified epimerase (see Table I). Single-stranded bovine liver cDNA was subjected to PCR together with 1 pmol of primers 1 (sense) and 3 (antisense) in a total volume of 100 μl of 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl₂, 100 μM dNTPs, 1 unit of Taq polymerase (Pharmacia Biotech Inc.). The reaction products were separated on a 12% polyacrylamide gel. An ~100-bp fragment, was isolated, inserted into a pUC119 plasmid, and sequenced. The DNA fragment cleaved from the plasmid was labeled with [32P]dCTP (NEN Life Science Products) using a random-primer DNA labeling kit (Boehringer Mannheim).

Screening of cDNA Library—A bovine lung cDNA library constructed in a Agt10 vector (CLONTECH) was screened with the 108-bp PCR fragment as hybridizing probe. The nitrocellulose filters were prehybridized in 6 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 5 × Denhardt’s solution containing 0.1% SDS and 0.1 mg/ml denatured salmon DNA for 2 h at 65 °C. Hybridization was carried out at 42 °C in the same solution containing [32P]-labeled probe for 18–18 h. The filters were washed two times with 2 × SSC and 0.5% SDS and two times with 0.5 × SSC and 0.1% SDS at the same temperature. Finally, the cDNA phage library was subjected to PCR amplification using an epimerase cDNA-specific primer (5′-GCTGATTCTTT-CATAGCTATTCCAAAG-3′, sense) together with Agt10 forward or reverse primers (CLONTECH).

Subcloning and Sequencing of cDNA Inserts—cDNA inserts, isolated by preparative agarose gel electrophoresis (15) after EcoRI restriction cleavage of recombinant bacteriophage DNA, were subcloned into a pUC119 plasmid. The complete nucleotide sequence was determined for one of the internal peptides derived from the purified epimerase (see Table I). Single-stranded cDNA was subjected to PCR together with 100 pmol of primers 1 (sense) and 3 (antisense) together at 27 °C for 5 days. The cells from one dish were used for total RNA extraction followed by Northern blot analysis, performed as described above. Cells from the other dish were lysed in a buffer of 100 mM KCl, 15 mM EDTA, 1% Triton X-100, and 50 mM HEPES, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml of proteinase inhibitors (19). After exposure, the applied protein molecular standards (LMW molecular calibration kit, Pharmacia Biotech Inc.) were visualized by staining the gel with Coomassie Brilliant Blue.

Expression of GlcUA C5-Epimerase—GlcUA C5-epimerase was expressed using a BacPAK8™ baculovirus expression system (CLONTECH) according to the instructions of the manufacturer. Two oligonucleotides, one at the 5′-end of the cDNA clone (5′-GCCACCGTCTCAAGCTGATCTTCTCATAGCTATTCCAAAG-3′, sense) and the other at the 3′-end of the coding sequence (5′-CTAGTTGTGCTTGTGGCC-3′, antisense), were used to PCR amplify the C5-epimerase cDNA clone.

The resulting fragment was cloned into the BacPAK8 vector. Sf9 insect cells, maintained in Grace’s insect medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum and penicillin/streptomycin, were then coinfected with the C5-epimerase construct along with viral DNA. Control transfections were performed with a β-glucuronidase cDNA construct included in the expression kit and a mouse cDNA coding for the GlcNAc N-deacetylase/N-sulfotransferase implicated in the biosynthesis of glucurono-D-mannose (10, 11). Single plaques of each cotransfected recombinant were picked and propagated. Two 60-mm Petri dishes of Sf9 insect cells were infected with each recombinant virus stock and incubated at 27 °C for 5 days. The cells from one dish were used for total RNA extraction followed by Northern blot analysis, performed as described above. Cells from the other dish were lysed in a buffer of 100 mM KCl, 15 mM EDTA, 1% Triton X-100, and 50 mM HEPES, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml of proteinase inhibitors (19). After exposure, the applied protein molecular standards (LMW molecular calibration kit, Pharmacia Biotech Inc.) were visualized by staining the gel with Coomassie Brilliant Blue.

Demonstration of GlcUA C5-Epimerase Activity—Epimerase activity was assayed by detecting [3H]release by using a biaphasic liquid scintillation procedure essentially as described by Campbell et al. (1). The reaction mixtures (total volume, 55 μl) contained 25 μl of cell lysate or medium, 25 μl of 2 × epimerase assay buffer (20 mM HEPES, 50 mM EDTA, 0.02% Triton X-100, and 200 mM KCl, pH 7.4), and 5 μl of substrate (10 000 cpm [3H]). Alternatively, the reaction mixtures were applied to a PD-10 column (Pharmacia Biotech Inc.), and the released [3H]in the H₂O form was separated from the polysaccharide substrate. The fractions containing [3H]O were evaporated by lyophilization. The substrate was a chemically N-deacetylated and N-sulfated polysaccharide, obtained from Escherichia coli K5 according to the procedure of Campbell et al. (1), except that 3-[3H]glucuronic acid was substituted for 4-[3H]glucose.

Enzymatic conversion of D-glucuronic to l-iduronic acid was demonstrated using the metabolically 1-[3H]-labeled substrate (N-deacetylated and N-sulfated capsular polysaccharide from E. coli K5) and the analytical procedure described by Campbell et al. (1). A sample (~20 μg, 200 000 cpm) of the material was incubated with 250 μl of cell lysate in a total volume of 300 μl of epimerase assay buffer at 37 °C for 6 h. The incubation was terminated by heating at 100 °C for 5 min. The sample was mixed with 50 μl of carrier heparin and reacted with nitrous acid at pH 1.5 (21), followed by reduction of the products with NaBH₄. The resultant hexuronallyhydromannitol disaccharides were recovered by gel chromatography on a column (1 × 200 cm) of Sephadex
TABLE I
Peptide and primer sequences

| Peptide sequences of isolated C5-epimerase | N-terminal sequences of isolated C5-epimerase |
|------------------------------------------|-------------------------------------------|
| 1. PFDXWVPGKCFMA (purified protein)      | 5′-ccgaattcAARGCNATGTYNCCNTYTT-3′        |
| 2. PXDWTVPFGXAF (band excised from PVDF membrane) | 5′-ccgaattcGAYTNMGCAYTTATG-3′       |

| Peptide sequences | Primer (sense) | Degeneracy |
|------------------|---------------|------------|
| 1. PNDATVPK      | 5′-ccgaattcAARGCNATGTYNCCNTYTT-3′    | 512        |
| 2. XXIAPETSEGXLQL | 5′-ccgaattcGAYTNMGCAYTTATG-3′       | 512        |
| 3. GGNPIMWTRK    | 5′-ccgaattcGAYTNMGCAYTTATG-3′       | 32         |

| Primer (sense) | Degeneracy |
|----------------|------------|
| Primer 2 (sense) | Primer 3 (antisense) |
| Primer 1 (sense) | Primer 2 (sense) | Primer 3 (antisense) |

| Primer 1 (sense) | Primer 2 (sense) | Primer 3 (antisense) |
|------------------|------------------|----------------------|
| 5′-ccgaattcAARGCNATGTYNCCNTYTT-3′ | 5′-ccgaattcGAYTNMGCAYTTATG-3′ | 5′-ccgaattcGAYTNMGCAYTTATG-3′ |

a PVDQ, polyvinylidene difluoride.
b R, A, or G; Y, T, or C; M, C, or A; N, A, or C or G or T.

Characterization of cDNA and Predicted Protein Structure—The cDNA structure indicates the occurrence of three potential N-glycosylation sites (Fig. 1). A potential transmembrane region is underlined in Fig. 1. The predicted protein contains two cysteine residues, only one of which occurs in the isolated (truncated) protein.

Functional Expression of GlcUA C5-Epimerase—The cDNA corresponding to nucleotides 1–1407, with an added ATG codon at the 5′-end, was cotransfected with baculovirus into Sf9 insect cells. The expressed protein thus is larger, by a 25-amino acid sequence, than the predicted 444-amino acid-long protein (Fig. 1). In two separate experiments, the lysates from cells infected with the same recombinant epimerase virus stock showed >10-fold higher enzyme activities, on a mg of protein basis, than the corresponding fractions from cells infected with control recombinant virus stock (Table II). The conditioned media of cells infected with recombinant epimerase showed 20–30-fold higher enzyme activities than the corresponding fractions from cells infected with control plasmid virus stock. Transfections with cDNA encoding other enzymes, such as a β-glucuronidase or the mouse mastocytoma GlcNAc N-deacetylase/N-sulfotransferase involved in hepargin biosynthesis (12), did not significantly increase the epimerase activity beyond control levels. Notably, the higher 3H2O release recorded for control samples as compared with heat-inactivated expressed enzyme (Table II) suggests that the insect cells constitutively produce endogenous C5-epimerase, although the amount of enzyme activity is less than that in mammalian cells (data not shown). Furthermore, isolation of the 3H-containing fraction from the C5-labeled polysaccharide substrate after incubation with the recombinant epimerase followed by evaporation of the isolated fraction confirmed that 3H2O was indeed formed during incubation (data not shown).

The polysaccharide substrate used for routine assays of epimerase activity was obtained by chemical N-deacetylation and N-sulfating the capsular polysaccharide (GlcUA1,4-GlcNAc1,4)] of E. coli K5 that had been grown in the presence of [5-3H]glucose. The data in Table II thus reflect the release of 3H2O from 5-3H-labeled GlcUA units in the modified polysaccharide, due to enzyme action (22, 23). More direct evidence for the actual conversion of GlcUA to IdceA residues was obtained by incubating the expressed enzyme with an analogous substrate, obtained following incubation of E. coli K5 with [1-3H]glucose. This substrate will retain the label through the epimerization reaction and can therefore be used to demonstrate the formation of IdceA-containing disaccharide units. Following incubation with the recombinant epimerase, 21% of the glucuronic acid residues were converted to IdceA, as dem-
Cloning of d-Glucuronyl C5-Epimerase

**Fig. 2. In vitro transcription-translation.** The epimerase cDNA was inserted into a pcDNA3 expression vector and linearized with XhoI at the 3'-end. It was then subjected to in vitro transcription-translation in a rabbit reticulocyte lysate system in the presence of [35S]methionine as described under “Experimental Procedures.” The translation product of epimerase cDNA (Epi) has a molecular mass of ~50 kDa by comparison with the low-molecular mass protein standard. A control sample of β-galactosidase (C; 118 kDa), expressed in the same system, is shown for comparison.

**Table II. Expression of hexuronyl C5-epimerase in Sf9 cells**

Sf9 cells (1 x 10^6 in 4 ml of medium) were seeded in 60-mm Petri dishes and allowed to attach for 3 h at 27 °C. The medium was then removed, and 200 µl of recombinant virus stock was added to infect the cells. After incubation at room temperature for 1 h, the virus suspension was aspirated off, and 4 ml of medium was added to each dish. The cells were incubated at 27 °C for 5 days. The medium was then transferred into a sterile tube and centrifuged. The supernatant was saved for analysis (“Medium”), whereas the pellet was combined with the cells collected from the dish. After washing twice with phosphate-buffered saline, the cells were lysed with 300 ml of homogenization buffer as described under “Experimental Procedures.” Aliquots (25 µl) of cell lysate and medium were assayed for epimerase activity. Enzyme activity is expressed as release of 3H from the K5 polysaccharide substrate per h of incubation and per mg of protein or ml of medium. The data are the means ± S.D. of three independent assays.

| Construct                        | Cell lysate | Medium  |
|----------------------------------|-------------|---------|
| | cpmp/mg/h | | |
| HexUA C5-epimerase (Exp. 1) | 102,670 ± 5540 | 45,200 ± 1770 |
| HexUA C5-epimerase (Exp. 2) | 123,270 ± 4660 | 52,610 ± 810 |
| HexUA C5-epimerase (Exp. 1, heat-inactivated) | 240 | 610 |
| N-Deacetylase/N-sulfotransferase | 9520 ± 620 | 1350 ± 280 |
| β-Glucuronidase | 8460 ± 1270 | 1610 ± 440 |
| Empty plasmid | 5150 ± 880 | 2820 ± 690 |
| No plasmid | 7250 ± 370 | 550 ± 120 |

*HexUA, hexuronyl.*

**Fig. 1. Nucleotide sequence and predicted amino acid sequence of C5-epimerase.** The predicted amino acid sequence is shown below the nucleotide sequence. The numbers on the right indicate the nucleotide residues and the amino acid residues (*boldface italic*) in the respective sequence. The five sequenced peptides are shown in *boldface* type. The N-terminal sequence of the purified protein is shown in *boldface italic* type. The potential N-glycosylation sites are shown (*). The potential transmembrane region is *underlined.*

**Northern Analysis.** Total RNAs from bovine liver and lung...
bolically 3H-labeled K5 polysaccharide was N-deacetylated and N-sulfated with HNO₂/NaBH₄, and the resultant hexuronylanhydromannitol di-saccharides were recovered and separated by paper chromatography. The structural diversity of heparin and heparan sulfate is generated through selective modification of the GlcUA (b) and GlcNAc (a) blocks in addition to mannuronic acid blocks and mixed blocks in the intact polymer, is the only reaction that cannot as yet be conducted without the aid of a biological catalyst, i.e. the C5-epimerase. The molecular cloning and functional expression of this enzyme are important steps toward elucidating the mechanism of an intriguing reaction that has so far been elusive.

As discussed above, the native molecular size of 49,905 Da of the epimerase remains somewhat uncertain due to the lack of an in-frame stop codon upstream of the assigned initiating ATG codon (Fig. 1). Expression of this protein in the baculovirus system with 25 amino acids added at the N terminus, as well as in Chinese hamster ovary cells, COS-1 cells, all reproduced the lung/liver pattern (data not shown). Whether also the C5-epimerase occurs in genetically and/or catalytically distinct enzyme forms with different catalytic properties and that are present in most cells of two N-deacetylaselSulfotransferase and alginates composed of guluronic acid blocks and mannuronic acid/guluronic acid blocks, whereas an epimerase from Pseudomonas aeruginosa yields a product with mannuronic acid blocks and guluronic acid/guluronic acid blocks, is still larger than the calculated ~42 kDa. This discrepancy may be due to the presence of additional bound carbohydrate, such as O-linked oligosaccharides. Besides, it is also recalled that proteins differ in their ability to bind SDS, resulting in differences in mobility in SDS-polyacrylamide gel electrophoresis (26).

The structural difference between heparin and heparan sulfate is best explained in terms of the various biosynthetic polymer modification reactions, which are consistently more extensive in the case of heparin (3, 5, 7). The N-acetylgalcosaminyl N-deacetylaselSulfotransferase is a key regulatory enzyme, which initiates polymer modification and commits the subsequent series of reactions toward formation of either heparin or heparan sulfate. Recent studies have revealed the occurrence in most cells of two N-deacetylaselSulfotransferase transcripts, ~4 and ~8 kb in size (11, 12), that encode distinct enzyme forms with different catalytic properties and that are derived from different genes.2 Transcription of GlcUA C5-epimerases involves the same biological system, as Northern blotting of bovine lung and liver (using C5-epimerase cDNA as a probe) resulted in a predominant ~9-kb hybridizing band and a faint ~5-kb band, whereas murine mastocytoma RNA contained the ~5-kb transcript only (Fig. 4). RNAs from a number of other cell types, such as human embryonic kidney cells, Chinese hamster ovary cells, and COS-1 cells, all reproduced the lung/liver pattern (data not shown). Whether also the C5-epimerase occurs in genetically and/or catalytically distinct forms remains to be elucidated. Interestingly, the C-terminal portion of a hypothetical Caenorhabditis elegans protein (27) shows amino acid sequence similarities to the cloned GlcUA C5-epimerase (data not shown).

Enzymes catalyzing C5-inversion of hexuronic acid residues in polysaccharides occur also in other biosynthetic systems. Thus, C5-epimerases converting 6-mannuronic acid to l-guluronic acid residues in alginate have been described in brown algae and in bacteria (28). A C5-epimerase isolated from Azotoxobacter vinelandii generates alginates composed of guluronic acid blocks in addition to mannuronic acid blocks and mixed mannuronic acid/guluronic acid blocks, whereas an epimerase from Pseudomonas aeruginosa yields a product with mannuronic acid blocks and mannuronic acid/guluronic acid blocks.

FIG. 3. Effect of recombinant C5-epimerase on N-deacetylated and N-sulfated capsular polysaccharide from E. coli K5. Metabolically 3H-labeled K5 polysaccharide was N-deacetylated and N-sulfated and then incubated with lysate from SF9 cells infected with recombinant C5-epimerase (A) or with lysate from SF9 cells infected with recombinant β-glucuronidase (B). The incubation products were treated with HNO₂/NaBH₄, and the resultant hexuronylanhydromannitol di-saccharides were recovered and separated by paper chromatography. The arrows indicate the migration positions of glucuronylanhydromannitol (GM) and iduronylanhydromannitol (IM) di-saccharide standards.

FIG. 4. Northern blot analysis of C5-epimerase mRNA. Total RNAs from bovine lung (BL) and mouse mastocytoma (MCT) cells were separated by agarose gel electrophoresis and hybridized with a 32P-labeled 2460-bp fragment of the epimerase cDNA clone as described under “Experimental Procedures.” The bars indicate the positions of RNA markers.

DISCUSSION

The structural diversity of heparin and heparan sulfate is generated through selective modification of the GlcUA (1,4-GlcNAc1,4), polymer formed during the initial stage of the biosynthetic process (3, 5, 7). Most of these reactions, i.e. N-deacetylation, N-sulfation, and the various O-sulfation reactions, can be reproduced, albeit in a poorly controlled fashion, through chemical modification (24, 25). In fact, the conversion of GlcUA to IdceA units, in the intact polymer, is the only reaction that cannot as yet be conducted without the aid of a biological catalyst, i.e. the C5-epimerase. The molecular cloning and functional expression of this enzyme are important steps toward elucidating the mechanism of an intriguing reaction that has so far been elusive.

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but no guluronic acid blocks (29, 30). The two types of enzymes showed no significant amino acid sequence homology (31). Contrary to the GlcUA C5-epimerase(s) involved in the biosynthesis of heparin/heparan sulfate, which show no requirement for divalent cations, the algal and bacterial mannuronate C5-epimerases all depend on Ca\textsuperscript{2+} for catalytic activity. Finally, an additional GlcUA C5-epimerase has been implicated in the biosynthesis of dermatan sulfate and thus also acts on a glycosaminoglycan substrate, i.e. chondroitin (GlcUAβ1,3-GalNAcβ1,4), (32, 33). Notably, this latter enzyme again requires divalent cations for activity. The two GlcUA C5-epimerases committed to glycosaminoglycan biosynthesis show no cross-reactivity with regard to polysaccharide substrates (chondroitin versus N-sulfoheparosan) and thus are believed to be distinct entities. Further information regarding the relationship between these two enzymes will emerge when also the dermatan epimerase has been subjected to molecular cloning.

Acknowledgments—We thank Dr. Wing-fai Cheung for valuable discussions. The technical assistance of Lena Nylund is gratefully acknowledged.

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