Molecular Cloning and Expression of a Glycosaminoglycan N-Acetylglucosaminyl N-Deacetylase/N-Sulfotransferase from a Heparin-producing Cell Line*

(Received for publication, July 22, 1993, and in revised form, September 21, 1993)

Ariel Orellana and Carlos B. Hirschberg†
From the Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655
Zheng Wei, Stuart J. Swiedler, and Masayuki Ishihara
From Glycomed, Inc., Alameda, California 94501

Heparin has a higher content of N-sulfated glucosamine and l-iduronic acid than heparan sulfate. Deacetylation of N-acetylglucosamine followed by N-sulfation may be important steps differentiating the biosynthesis of these glycosaminoglycans. We have cloned, by cross-hybridization with the cDNA from rat liver heparan sulfate N-deacetylase/N-sulfotransferase, a protein from a heparin synthesizing mastocytoma derived cell line called MST. This protein, which has both N-deacetylase/ N-sulfotransferase activities, has predicted amino acid sequence homology of 70% with the above rat liver enzyme and is unique for the following reasons. 1) It was found to be encoded by a 3.8 kilobase mRNA that was unique to heparin-producing cells; an 8.5-kilobase mRNA encoding the rat liver enzymes has been found to occur in all mammalian cells tested on the basis of nucleic acid cross-hybridization; 2) the protein overexpressed in COS cells in its full-length transmembrane form or as a soluble secreted protein A chimera displayed ratios of N-deacetylase to N-sulfotransferase activities that were 4-8-fold higher than that observed for the enzyme found in liver that is involved in the biosynthesis of heparan sulfate. These results suggest that the MST-derived enzyme is probably unique to the production of heparin in mast cells.

Heparin and heparan sulfate (HS) are complex polysaccharides composed of alternating glucosamine and uronic acid residues some of which are sulfated. A variety of biological functions have been proposed for heparin and HS, among them blood clotting (1), cell recognition (2), cell adhesions (3, 4) viral binding and infection (5), endocytosis (6), regulation of bFGF activity (7–11), and developmental regulation of neural tissues (12). One of the reasons that could account for the broad range of functions of these molecules is their high degree of heterogeneity resulting from the different patterns and extent of sulfation.

Heparan biosynthesis occurs only in connective tissue mast cells, whereas that of HS seems to be ubiquitous (13). In addition, heparin contains more N-sulfate groups, a higher total sulfate content and more iduronic acid than HS (14). These characteristics, which may be relevant to the biological functions of heparin and HS, also point to differences in their biosynthesis. Heparin and HS share biosynthetic pathways (14) beginning with polymerization of GlcNAc and GlcA followed by a series of modification reactions: N-deacetylation of N-acetylglucosamine and further N-sulfation. This is a key step for the next set of reactions, epimerization of GlcA to l-iduronic acid, 2-O sulfation of l-iduronic acid and 3-O sulfation of glucosamine. Since the N-deacetylation/N-sulfation of GlcNAc is an obligatory step for the subsequent reactions, changes in these activities could account for significant alterations in the sulfation pattern of heparin and HS.

Recently, we have cloned, sequenced, and expressed a single protein from rat liver which catalyzes both N-sulfation and N-deacetylation during the biosynthesis of HS (15, 16). In mouse mastocytoma, which produces large amounts of heparin, Pettersson et al. (17) have reported that a protein possessing N-sulfotransferase activity acquires N-deacetylation activity upon addition of a crude factor, histones or polybrene (18), suggesting that in a heparin-synthesizing tissue, there may be a different mechanism for controlling the N-deacetylation and N-sulfation of glucosamine.

Using the rat liver ND/NS cDNA as a probe, we searched for homologous sequences in Northern blot analyses of poly(A*) RNA from MST cells, a stable heparin-producing cell line derived from the Futh murine mastocytoma (19). At low stringency, a message of 3.8 kb was detected, which was absent in non-heparin-producing cells. When the same probe was used to screen a nonamplified cDNA library from MST cells, two full-length clones were isolated. Both have identical sequence and differ only in length at both 5' and 3' ends. The MST cDNA contains a single open reading frame, predicting a type II membrane protein (as all other Golgi membrane proteins cloned so far) which shares high identity with the rat liver ND/NS. Upon transient transfection of COS cells with the MST cDNA, overexpression of both activities in vitro was found as well as higher specific incorporation in vivo of radiolabeled sulfate into heparan sulfate. Cell extracts of transfected COS cells and a purified soluble fusion protein containing the putative Golgi luminal domain of the MST enzyme showed a 4–8-fold higher ND/NS ratio compared with experiments with the rat liver enzyme; this suggests that increased N-deacetylation of GlcNAc followed by N-sulfation may be an important step and perhaps differently regulated in the biosynthesis of heparin compared with HS.

MATERIALS AND METHODS

Cell Culture—MST cells (19) were obtained from Dr. Jeffrey Esko, University of Alabama, Birmingham. Cells were grown in DMEM/F-12

* This work was supported by a PEW Latin American Fellowship (to A. O.), National Institutes of Health Grant GM 34396 (to C. B. H.), and by Glycomed, Inc. The costs of publication of this article were defrayed in part by the payment of page charges. The abbreviations used are: HS, heparan sulfate; ND/NS, N-deacetylase/N-sulfotransferase; kb, kilobase(s); DMEM, Dulbecco’s modified Eagle’s medium; MOPS, 4-morpholinepropanesulfonic acid.

† To whom correspondence should be addressed.

‡ The abbreviations used are: HS, heparan sulfate; ND/NS, N-deacetylase/N-sulfotransferase; kb, kilobase(s); DMEM, Dulbecco’s modified Eagle’s medium; MOPS, 4-morpholinepropanesulfonic acid.

Vol. 269, No. 3, Issue of January 21, pp. 2270–2276, 1994
Printed in U.S.A.
culture medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.5 µg/ml amphotericin B

Preparation of Total RNA and Poly(A+) RNA—Total RNA was extracted from MST cells using the method of Chomczynski et al. (20). To prepare Poly(A+) RNA, total RNA was loaded onto an oligo(dT) column (Life Technologies, Inc.) and fractionated following the manufacturer’s instructions.

Radiolabeling—RNA was denatured at 55 °C for 10 min in a solution containing 34% (v/v) formamide, 4% formaldehyde, 13.5 mM MOPS, pH 7.0, 67 µg/ml ethidium bromide and then fractionated in a 1.2% agarose gel containing 6% (v/v) formaldehyde, 20 mM MOPS, pH 7.0, 5 mM sodium acetate, and 1 mM EDTA. Following treatment of the gel with 50 mM NaOH, the gel was blotted to a Hybond N+ nylon membrane (Amersham) and then baked at 80 °C for 2 h. The blot was then hybridized to a radiolabeled probe at 65 °C for 2 h in a hybridization solution consisting of 6 x SSPE, 5 x Denhardt’s solution, 0.5% SDS, and 100 µg/ml of denatured salmon sperm DNA. Single strand probes were prepared as described by Hashimoto et al. (18). Probes were added to the prehybridization solution (4 x 106 cpm/ml) and used to incubate the membrane for 15-18 h at 65 °C. For low stringency washes, the membrane was incubated twice for 15 min at room temperature with a solution containing 2 x SSC, 0.1% SDS and then twice at 45 °C for 15 min. For high stringency washes, the membrane was initially washed twice with 2 x SSC, 0.1% SDS for 15 min at room temperature, and then twice with 0.1 x SSC 0.1% SDS at 65 °C for 15 min. The membranes were then exposed at -70 °C using screens.

Construction of a γgt10 Library—Poly(A+) RNA from MST cells was obtained as described above. The library was constructed with the SuperScript Choice System (Life Technologies, Inc.) using 5 µg of poly(A) and the manufacturer’s protocol (Life Technologies, Inc.). The library was then ligated to λgt10 arms (Life Technologies, Inc.) and packaged in vitro using λDNA packaging kit from Stratagene.

Screening of the γgt10 Library—Phages obtained as described above and without further amplification were used to infect Escherichia coli C600 hi and plated at 2 x 104 plaque-forming units/150-mm plate. 1.5 x 108 plaques were screened as described by Sambrook et al. (21), using the hybridization conditions described previously for low stringency. After the primary screening, 26 positive signals were obtained.

Analysis of Positive Clones—Seven positive clones were treated with SolI, EcoRI, and NotI, followed by Southern analyses. The size of the inserts was 0.8, 1.7, 1.8, 2.0, 2.2, 3.6, and 3.7 kb. The last two were cloned into the pCMV5 vector as described below; the others were amplified by polymerase chain reaction, cut with EcoRI and cloned into the EcoRI site of M13mp18 and partially sequenced.

Construction of pCMST Plasmids—DNA of the λ clones containing the 3.6 (MST-1) and 3.7 (MST-3)-kb inserts were cut with EcoRI and ligated into the EcoRI site of the pCMV5 expression vector (22). DH-5 α cells were transformed with the ligation mixture and plated in LB ampicillin plates. To distinguish the orientation of the inserts, recombinant plasmids were analyzed by restriction mapping. The plasmids were designated as pCMST1.

DNA Sequence Analysis—DNA sequencing was done according to Sanger et al. (23) using deoxyadenosine 5'-32P-triphosphate and Sequenase (United States Biochemical Corp.). Both strands of pCMST1 plasmids were sequenced according to the manufacturer’s protocol using specific primers. The inserts cloned into M13mp18 were sequenced using the universal primer. These were found to be identical to the pCMST plasmids, demonstrating that they are partial clones of the same cDNA.

Detection of mRNAs Using cDNA of MST as Probe—Poly(A) (+) RNA from MST cells and rat liver was processed for Northern blot analysis as described above. To prepare the probe, the insert of MST-5 (Fig. 2) was amplified by polymerase chain reaction, separated on low melting point agarose gels, and purified using Magic polymerase chain reaction preparation from Promega; 50 ng of this DNA were labeled by random hexamer priming using a labeling kit (Pharmacia LKB Biotechnology Inc.), followed by purification with a Nick column (Pharmacia). Hybridization was done as described above and the membrane was washed at low stringency conditions and exposed with a screen at -70 °C for 18 h.

Transient Expression in COS-1 Cells—Transfection was done as described above. Briefly, COS-1 cells, grown on 100-mm plates, were transfected with different plasmids using the DEAE-dextran method (24). Five ml of DMEM containing 10% Nu serum (Collaborative Biomedical Products) were mixed with 0.2 ml of a solution of phosphate-buffered saline containing 10 mg/ml DEAE-dextran plus 2.5 µM chloroquine; DNA was added, and after vortexing, the mixture was incubated for 15 min. The medium was replaced with 5 ml of 10% dimethyl sulfoxide in phosphate-buffered saline, incubated for 2 min at room temperature, and then changed to 5 ml of DMEM. Following 60 h, cells were scraped off the dish and the homogenate used to measure ND/NS activities.

Radiolabeling of COS Cells with Sulfate and Glucosamine—Wild type COS cells (3 x 106 cells/10-cm plate) and mutant CM-15 COS cells (6 x 106 cells/10-cm plate) were transfected using 5 µg of either the MST or rat liver heparan sulfatase dsDNA. Cells were also transfected with the same amount of vector alone. Forty-eight hours after transfection, cells were radiolabeled for 24 h with 50 µCi/ml of H3SO4 or 50 µCi/ml of D3Hglucosamine (DuPont NEN) in DMEM medium lacking sulfate (Life Technologies, Inc. formula 89-0053 AK) supplemented with 10% bovine fetal bovine serum (dialyzed against water) and 1 µCi/ml of glucose. The culture media was then removed and cells were rinsed twice with ice-cold phosphate-buffered saline and the washes combined with the original culture medium. Cells were then trypanized, plates were washed twice with phosphate-buffered saline, and the cell suspension and phosphate-buffered saline washes were combined and centrifuged to pellet the cells. The supernate was then added to the culture medium pool. The glycosaminoglycans from this fraction and the cell pellet were purified by chromatography on DEAE-cellulose columns. Total incorporation of radiolabel was measured as the radioactivity remaining at the origin on a paper chromatogram following chromatography for 15 h in n-butanol, glacial acetic acid, 1 x NH4OH (2:3:2). The content of COS sulfate was determined in the eluate from each column, as described above. No significant differences were observed with rat liver heparan sulfate or chondroitin sulfate, but MST rat liver homogenate was different from total radiolabel remaining at the origin compared with the label remaining following treatment of the samples with either chondroitinase ABC and chondroitinase AC II.
E, arrowheads. The same membrane was washed at high stringency and exposed at -70 °C for 1 week. The figure represents a composite of the same membrane. The arrowheads indicate the position of different mRNAs: 8.5, 7.0, 4.2, and 3.8 kb. The arrows indicate the position of molecular weight standards. B, 1 μg of poly(A)⁺ RNA from rat liver and MST cells were separated in a 1.2% agarose gel and then blotted to a nylon membrane as described under “Materials and Methods.” The membrane was probed with a random hexamer labeled probe derived from a MST cDNA, generated as described under “Materials and Methods.” The membrane was washed at low stringency conditions and exposed at -70 °C for 15 h.

rat liver ND/NS, we decided to further investigate its significance. For this, a cDNA library from MST cells was constructed in agt10 and screened at low stringency conditions. Twenty-six positive clones were identified, and seven of them were analyzed in detail (Fig. 2). Sequencing analysis showed that all of them were different cDNA clones from the same mRNA, five of them being partial lengths and two full length designated as MST-1 and MST-3. To determine which message gave rise to them being partial lengths and two full length designated as MST-1 and MST-3 were identical except for a minor difference in the 3' untranslated region, perhaps an artifact generated during the construction of the library.

From the nucleotide sequence (Fig. 3), a single open reading frame predicts a protein of 883 residues with a molecular mass of 19,1196 Da. The ATG located at position 1 appears to be the correct initiation site, since it is the first methionine in the open reading frame and fits with the consensus sequence for initiation codons (28). Upstream from that ATG, there is a stop codon in the same reading frame at position -36, -96, and -102. Hydrophobicity plots of the protein showed a highly hydrophobic region between residues 19 and 41 which accounts for the only putative transmembrane domain in the predicted protein (consistent with a type II membrane protein). The protein has seven potential N-glycosylation sites (shown as black dots).

Comparison with the Rat Liver N-DeacetylaselN-Sulfotransferase—The predicted protein sequence was compared with that from rat liver (Fig. 4). Both proteins are highly homologous and share 70.3% identity plus 10% amino acid similarity. There is less homology toward the amino terminus of both proteins. If the amino terminus of both proteins are compared (MST-protein from rat amino acids 1–85 with rat liver amino acids 1–84), only a 26% identity is found with an additional 30% similarity. If both proteins are compared removing the amino terminus, the identity increases to 74.8% and similarity to an additional 14.7%.

Although this sequence similarity suggested that the rat liver and MST protein may have similar functions, it may be significant that it is the transmembrane and stem region where they have lesser identity and homology; these regions are thought to be targeting domains of proteins to the Golgi apparatus (29). This raises the possibility that these proteins are not localized in the same (sub)compartment within the cell. This in turn may be relevant to the hypothesis that the MST protein is involved in the biosynthesis of heparin, whereas the one from liver of heparan sulfate (see below).

Expression of MST Clones in COS-1 Cells: the MST Protein Contains Both N-DeacetylaselN-Sulfotransferase Activities—In order to determine whether the MST clones encoded a protein containing ND/NS activities, both MST-1 and MST-3 were cloned in the expression vector pCMV-6 generating clones pCMST-1 and pCMST-3. Both clones and the vector containing the cDNA from rat liver (Fig. 1A) were transfected into COS-1 cells. N-DeacetylaselN-Sulfotransferase activities were measured in cell extracts 65 h after transfection. Both activities were overexpressed (Table 1). Surprisingly, the ratios of N-deacetylaselN-sulfotransferase was 4–8-fold higher in the cell extracts of MST cDNA transfected cells compared with those with rat liver cDNA. The same lower ND/NS ratio was found when COS-1 cells were transfected with a vector containing a soluble form of the rat liver cDNA, suggesting, as a first approximation, that the differences in the activity ratios with rat liver cDNA are the results of the rat liver protein and not factors of the cell extracts. The MST N-sulfotransferase activity was significantly lower than that of rat liver.

To further substantiate the above hypothesis, a fusion protein was constructed using the putative luminal portion of the MST protein fused, at its amino terminus, to protein A, analogous to the above rat liver fusion protein (16). This generated a secreted soluble protein which was purified by its ability to bind IgG beads. SDS-gel electrophoresis of this preparation showed a single protein band with the expected size (not shown), which contained both N-deacetylaselN-sulfotransferase activity (see below).

The above results suggest that the MST-derived enzyme ex-
Biosynthesis of Heparin/Heparan Sulfate

---

Fig. 3. Nucleotide sequence and the predicted amino acid sequence of the MST ND/NS. A, depicted are the nucleotide sequence on the top and the amino acid sequence on the bottom. Nucleotide position 1 corresponds to the first ATG triplet coding for the initiator methionine. The nucleotides at the 5' side of position 1 are indicated by negative numbers. A consensus sequence for polyadenylation toward the end of the nucleotide sequence is underlined. The putative transmembrane domain is shown within a box. The potential N-glycosylation sites are shown with a black dot. B, the hydrophilicity plot from the predicted protein obtained using the algorithm of Kyte and Doolittle (36) (with a window of 18 amino acids) is shown.
presses both N-deacetylase and N-sulfotransferase activity in a ratio that is unique and different from the liver enzyme involved in heparan sulfate biosynthesis. The effect of PAPS on the ND activity of the MST purified fusion protein was also studied. No effect was seen on either the $K_{m}$ or $V_{max}$ using 100 µM PAPS. A similar lack of PAPS effect had been recently described for the rat liver purified fusion protein (16) and in Chinese hamster ovary cells (25). This is different to the previous observations by Barne et al. (25) and Riesenfeld et al. (35) who found activation using microsomes from mastocytoma.

To further evaluate the differences between the rat liver and MST-derived enzymes, a detailed kinetic analysis was undertaken using purified fusion proteins. As a substrate, the polysaccharide capsule of E. coli K5 was utilized (30), since it contains a repeating backbone of glucuronic acid and N-acetylgalcosamine, the hypothetical form of the endogenous PAPS involved in heparan sulfate biosynthesis. The effect of PAPS was compared with rat liver, whereas the $V_{max}$ for N-sulfation was higher for the latter enzyme. The $K_{m,app}$ for N-deacetylase was the same for both enzymes but the $V_{max}$ for that of MST was higher than that of rat liver.

When the ND/NS $V_{max}$ ratios were compared between the rat liver and MST proteins, the latter were 4-fold higher (Table II), suggesting that, based on kinetic behavior, the rat liver and MST enzymes are different.

**Incorporation of Radiolabeled Sulfate into Macromolecules**

To determine whether expression of the MST cDNA leads to changes in sulfation of proteoglycans, wild type and mutant CM-15 COS cells, deficient in ND/NS (27), were transfected with the MST cDNA followed by radiolabeling of cells with $H_{35}SO_{4}$ and [3H]glucosamine. As shown in Table III, the total amount of radiolabeled sulfate incorporation into glycosaminoglycans in CM-15 cells increased by 25% compared with cells transfected with the vector alone; most of this increase was into heparan sulfate with only negligible increase into chondroitin sulfate. The increase in heparan sulfate radiolabeling was observed in the culture medium, extracellular matrix, and intracellular pool, suggesting that no particular pool of heparan sulfate was sulfated preferentially. Incorporation of [3H]glucosamine did not change significantly in cells transfected with the cDNA compared with controls strongly, suggesting no increase in polymer size had occurred and that the above cDNA encodes for a protein with ND/NS activity in vivo.

When sulfate incorporation was measured into wild type COS cells, no significant changes in sulfate incorporation, compared with cells transfected with the vector alone, were detected into either heparan sulfate or chondroitin sulfate, in agreement with previous studies using rat liver cDNA and wild type COS cells (34).

**DISCUSSION**

We have cloned, overexpressed, and characterized a protein from MST cells which has heparin-heparan sulfate N-deacetylase/N-sulfotransferase activities. Several lines of evidence suggest that this protein may be involved primarily in the biosynthesis of heparin and not in that of heparan sulfate. 1) To MST cells, the source of the mRNA encoding this protein, synthesize large quantities of heparin (19) as does mastocytoma tissue, from which the cell line was derived. 2) When Northern analyses were performed with different tissues, a very abundant message of 3.8 kb was detected in MST at low stringencies, using as probe the cDNA from the rat liver heparan sulfate
TABLE II
Comparative kinetic parameters of soluble MST and rat liver N-deacetylase/N-sulfotransferase

| Substrate                  | Rat liver $K_m$ (μM) | MST $K_m$ (μM) | Rat liver $V_{max}$ (μmol/min/mg) | MST $V_{max}$ (μmol/min/mg) |
|----------------------------|----------------------|----------------|----------------------------------|-----------------------------|
| N-Sulfotransferase         | 108.1 ± 26.6         | 40.7 ± 2.1     | 1.82 ± 0.50                      | 1.00 ± 0.25                 |
| PAPS                      | 0.9 ± 0.1            | 22.4 ± 3.8     | 2.26 ± 0.89                      | 0.50 ± 0.10                 |
| N-Deacetylase K5 polysaccharide | 0.61 ± 0.02       | 0.95 ± 0.15     | 0.37 ± 0.06                      | 0.74 ± 0.12                 |
| Ratio $V_{max}$/ND/NS      |                      |                |                                  | 0.21 ± 0.02                  | 0.84 ± 0.02                 |

ND/NS. This message was not found in other tissues which do not synthesize heparin. At low and high stringencies, a message of 8.5 kb was detected in MST as well as other tissues, which was much less abundant than the above message. Because this message was also detected in rat liver, we believe it codes for the heparan sulfate ND/NS. 3) When a library from MST cells was probed with the rat liver heparan sulfate ND/NS cDNA, a high number of positive clones was found (23 out of 150,000), suggesting that they were derived from an abundant message. 4) The predicted protein encoded by the above 3.8-kb message is a type II membrane protein; the sequence has several portions which are identical to that of rat liver ND/NS, but shows significant difference in the amino terminus, the region which has been shown in other Golgi membrane proteins to be necessary for targeting to this organelle (29). We do not know whether this may result in targeting of the above MST-derived enzyme to an organelle other than the Golgi apparatus, perhaps a different subcompartment. 5) The ratio of N-deacetylase to N-sulfotransferase activity is 7-8-fold higher in cell extracts of COS-1 cells transfected with the MST cDNA compared with those transfected with the rat liver cDNA. A similar difference in enzymatic ratio was also found with the purified fusion protein from MST.

Although we hypothesize that the MST enzyme is responsible for the biosynthesis of heparin in vivo, we have been unable to demonstrate this directly. This could be due to several reasons, the principal one being that the COS system is heterologous and may lack the specialized machinery necessary for the biosynthesis of heparin; the synthesis of this molecule may require additional cellular factors not available in COS cells which presumably exist in MST cells. Some evidence for this exists by the fact that overexpression of both the rat liver and this MST ND/NS into wild type cells does not result in increased sulfation, presumably as a result of unknown regulatory factors in these cells (34).

Further evidence that MST and the rat liver protein may have different roles comes from a comparative analysis of the kinetic parameters of the enzymes, in particular, the very large difference in $K_m$ for N-sulfotransferase activity using deacetylated K5 polysaccharide as substrate. This difference must, however, be taken with some caution: it clearly indicates that both enzymes are different and suggests that they may be involved in the biosynthesis of different glycosaminoglycans such as heparin and heparan sulfate. Detailed mechanistic interpretations are difficult to make even though the substrate used for the N-sulfotransferase reaction, N-deacetylated K5 polysaccharide, appears to be more closely related to the in vivo substrate than N-deacetylated heparin or heparan sulfate.

Deacetylated K5 polysaccharide does not have late biosynthetic modifications such as L-iduronic acid and O-sulfated groups (31) which occur in the above two substrates. N-Deacetylated
K5 polysaccharide is a long nonsulfated polymer (average molecular mass, 86,000 Da) with all N-glycosamines already deacetylated while the longest polymer found in mastocytoma microsomes, following incubations with UDP-GlCNAc and UDP-GlcNAc, in the absence of sulfation, has a molecular mass of 10,000–15,000 Da (32). A recent bioinformatic study suggests that the heparin oligosaccharide backbone is N-deacetylated and N-sulfated as the backbone is lengthened (33). The N-deacetylated K5 polysaccharide is unlikely to be the endogenous heparin, since all glucosamine residues are deacetylated at once. In summary, at this time, it is not clear what the specific substrate for the N-sulfotransferase is in vivo and what features of the polymer are specific for recognition of the protein. Another factor to consider is that protein content of the NDNS in the tissue from which MST cells are derived (17, 19) is much higher than that of rat liver (26), suggesting that the lower binding efficiency for the substrate may be compensated by the high enzyme (protein) concentration within the cell.

Petersson and co-workers (17, 18) have recently reported the purification of a 110-kDa protein from a mastocytoma tumor. The following observations suggest that the MST cDNA clone isolated by us might correspond to the protein purified by Petersson et al. (1). The MST cells used to isolated the mRNA and construct the cDNA library from which the cDNA was obtained are derived from the same mastocytoma tumor (2). The predicted protein from MST has a molecular weight which is in agreement with the one estimated by SDS-gel electrophoresis (3). The 110-kDa protein is a glycoprotein; the protein cloned by us has seven potential N-glycosylation sites (4). However, the isolated protein from MST had, by itself, only N-sulfotransferase but not N-deacetylase activity; the fact that the activity can be recovered by adding histones, polybrene, or an unidentified cellular protein factor, all of which by themselves do not have N-deacetylase activity suggests that these proteins are reactivating a catalytic site in the NDNS protein. It is possible that some unknown modification of the enzyme takes place in COS cells, which is not observed in mastocytoma.

Finally, our Northern analysis suggests that a heparin-synthesizing tissue has two different NDNS. Perhaps both are important in the biosynthesis of heparin or heparan sulfate. The cloning of a cDNA from MST cells encoding for the low abundant message, which is also the same size as that from rat liver, should provide a better understanding of the function of the two putative different messages in these cells. In addition, a detailed understanding of the different regions in these proteins with two catalytic activities should lead to a better understanding of possible mechanisms of regulation of the two activities in vivo.

Acknowledgments—We thank Karen Welch and Annette Stratton for excellent secretarial assistance.

**REFERENCES**

1. Lindahl, U., Thunberg, L., Backstrom, G., Riesenfeld, J., Nordling, K., and Bjork, I. (1984) J. Biol. Chem. 259, 12368–12375
2. Lupa, M. T., Gordon, H., and Hall, Z. W. (1990) Dev. Biol. 142, 31–43
3. LeBaron, R. G., Eko, J. D., Woods, A., Johansen, S., and Hook, M. (1988) J. Biol. Chem. 263, 7950–7956
4. LeBaron, R. G., Hook, A., Eko, J. D., Gay, S., and Hook, M. (1989) J. Biol. Chem. 264, 1900–1908
5. Wolf, D., and Spear, P. G. (1989) J. Virol. 63, 53–62
6. Nagashima, M., and Hascall, V. (1984) J. Biol. Chem. 259, 10270–10283
7. Yayon, A., Klagsbrun, M., Eko, J. D., Eder, E., and Ornitz, D. M. (1991) Cell 65, 443–455
8. Rapraeger, A. C., Kruka, A., and Owlin, B. B. (1991) Science 253, 1705–1708
9. Klagsbrun, M., and Bard, A. (1991) Cell 67, 229–231
10. Ishihara, M., Tyrrell, D. J., Staub, G. B., Brown, S., Cosseau, L. B., and Stack, R. J. (1993) J. Biol. Chem. 268, 4765–4774
11. Tyrrell, D. J., Ishihara, M., Bao, N., Horn, A., Kieser, M. G., Staub, G. B., Lam, L. H., and Stack, R. J. (1993) J. Biol. Chem. 268, 4064–4069
12. Narcombe, V., Ford, M., Wildaucht, J., and Bartlett, P. (1993) Science 260, 103–106
13. Neder, H. B., and Dietrich, C. P. (1985) in Heparin (Lane, D. A., and Lindahl, U., eds) pp. 81–96, Arnold, London
14. Lindahl, U., Kutsche, M., Lidholt, K., and Oscarsson, L. (1989) Ann. N. Y. Acad. Sci. 526, 12–50
15. Hashimoto, Y., Orellana, A., Gil, G., and Hirschberg, C. B. (1992) J. Biol. Chem. 267, 15744–15750
16. Wei, Z., Swicker, S., Ishihara, M., Orellana, A., and Hirschberg, C. B. (1992) Proc. Natl. Acad. Sci. U. S. A. 90, 3885–3888
17. Petersson, I., Kutsche, M., Unger, E., Wlad, H., Nylund, L., Lindahl, U., and Kjellin, L. (1991) J. Biol. Chem. 266, 8044–8050
18. Kjellin, L., Petersson, I., Unger, E., and Lindahl, U. (1992) Adv. Exp. Med. Biol. 313, 107–111
19. Montgomery, R., Lindahl, R., Flax, P., Liang, J., Vertel, B., Lindahl, U., and Eko, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11327–11331
20. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Guide, 2nd ed., pp. 109–117, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Anderson, S., Daris, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1988) J. Biol. Chem. 263, 8223–8229
23. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
24. Anfuso, A. (1991) Current Protocols in Molecular Biology, Suppl. 14, Unit 6.13, Greene Publishing Associates and Wiley Interscience, New York
25. Banne, K., Reddy, R. R., and Eko, J. (1991) J. Biol. Chem. 266, 12461–12468
26. Brandon, E., and Hirschberg, C. B. (1988) J. Biol. Chem. 263, 2417–2422
27. Ishihara, M., Kieser, M., Barr, P. O., and Swicker, S. (1992) Anal. Biochem. 200, 405–407
28. Kazak, M. (1991) J. Cell Biol. 109, 228–241
29. Machaner, A. (1991) Trends Cell Biol. 1, 141–144
30. Vann, W., Schmidt, A., Janan, B., and Jann, K. (1981) Eur. J. Biochem. 116, 319–324
31. Lindahl, U., Feingold, D., and Roden, L. (1986) Trends Biochem. Sci., 12, 221–225
32. Lidholt, K., Kjellin, L., and Lindahl, U. (1986) Biochem. J. 241, 999–1007
33. Lidholt, K., and Lindahl, U. (1992) Biochem. J. 287, 21–29
34. Ishihara, M., Guo, Y., Wei, Z., Swicker, S., Orellana, A., and Hirschberg, C. B. (1992) J. Biol. Chem. 268, 20091–20095
35. Riesenfeld, J., Hook, M., and Lindahl, U. (1982) J. Biol. Chem. 257, 421–425
36. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 103–122

---

*2 M. Grotzinger and J. Eko, unpublished results.*