Communication

Tissue-specific Expression of Sialyltransferases*

(Received for publication, February 9, 1989)

James C. Paulson, Jasminde Weinstein, and Ashley Schauer
From the Department of Biological Chemistry, and Molecular Biology Institute, UCLA School of Medicine, Los Angeles, California 90024

Three sialyltransferases which attach terminal sialic acids to glycoprotein sugar chains are shown to exhibit striking differential expression in seven tissues of the rat. Using cDNA probes for the Galβ1,4GlcNAc α-2,6-sialyltransferase which forms a NeuAcα2-6Galβ1-4GlcNAc sequence on N-linked sugar chains, three different sized mRNAs are detected, two of which (4.7 and 4.3 kilobases (kb)) have high homology along the full length, and a third (3.6 kb, in kidney) which is missing the 5′ region corresponding to 45% of the NIH-α-terminal coding sequence. The 4.7- and 4.9-kb mRNAs exhibit differential expression of over 50-fold with the highest levels in liver and lowest in brain and heart.

Assays for enzyme activity in tissue homogenates show high correspondence to the levels of mRNA. Evidence of tissue-specific expression was also obtained for two other sialyltransferases which form the NeuAcα2-3Galβ1-4GlcNAc and NeuAcα2-3Galβ1-3GalNAc sequences on N-linked and O-linked sugar chains, respectively. Comparison of the ratios of the three enzymes in several tissues suggests that they are expressed independently. The results are discussed for their relevance to cellular control of terminal glycosylation sequences on glycoproteins and glycolipids.

Tissue and cell type-specific expression of terminal glycosylation sequences of glycoproteins and glycolipids is well documented (1–3). Indeed, antibodies directed to specific carbohydrate structures detect cell surface antigens on some normal tissues but not others and on tumor tissues where the same carbohydrate antigens are not present on surrounding normal tissue (1, 2). Some of these carbohydrate structures are described as onco/fetal antigens because they are most abundant in early fetal development, and their expression is developmentally regulated (1-3). Similarly, expression of ganglioside G_{33}^3 in the “undifferentiated” mesenchyme of the developing kidney appears to be required to signal the invasion and branching of the ureter bud into the mesenchyme (6).

The basis for the developmentally regulated and cell type-specific expression of the terminal carbohydrate sequences of glycoproteins and glycolipids is at present poorly understood. Because the carbohydrate structures of these glycoconjugates are largely determined by the specificities of the glycosyltransferases that synthesize them (15), it is presumed that differential expression of these enzymes is most likely the way in which this is regulated (1, 16–18). However, few reports have systematically investigated the degree to which specific glycosyltransferases are differentially expressed in normal tissues (19) or how such expression might be regulated in tissue-specific and developmentally regulated fashion.

Cloned cDNAs for several terminal glycosyltransferases have recently become available (20–23) permitting molecular approaches to the analysis of glycosyltransferase expression.

In this report, differential expression of a Galβ1,4GlcNAc α-2,6-sialyltransferase (23) in various rat tissues by Northern analysis and direct enzyme assays. Enzyme assays of two other glycoprotein sialyltransferases also reveal differential expression in rat tissues and a lack of coordinate expression with the Galβ1,4GlcNAc α-2,6-sialyltransferase.

The results support the concept that expression of glycosyltransferases is a predominant factor in regulating cell type-specific sugar structures.

**EXPERIMENTAL PROCEDURES**

Materials—Lacto-N-tetraose, free of lacto-N-neo-tetraose, was purified from human milk (24, 25), and antifreeze glycoprotein 8 was purified as described earlier (26) from the serum of Dissostichus mawsoni, a gift of Dr. A. L. DeVries (University of Illinois at Urbana). α: Acid glycoprotein was purchased from Sigma and was desialylated by mild acid treatment as previously described (27). CMP-[14C]NeuAc was obtained from Du Pont-New England Nuclear. The Galβ1,4GlcNAc α-2,6-sialyltransferase, the Galβ1,3(4)GlcNAc α-2,3-sialyltransferase, and the Galβ1,3GlcNAc α-2,3-sialyltransferase were purified as reported previously (28, 29). Affinity-purified polyclonal antibodies to the Galβ1,3(4)GlcNAc α-2,6-sialyltransferase were prepared as described previously (28, 30).

Northern Analysis—Total mRNA was prepared from freshly dissected rat tissues as previously described (31). Gel electrophoresis of total RNA (15 μg/lane) was done in 1% agarose gels containing formaldehyde, and Northern hybridizations were performed as reported earlier (29). Radiolabeled cDNAs (632 × 10^6 cpm/μg) were used as probes, prepared from agarose gel-purified fragments of the sialyltransferase cDNA (32) subcloned into Bluescript (Stratagene). Blots were exposed to x-ray film for 2–5 days at approximately 80 °C. Sizes of mRNAs were determined by co-electrophoresing total RNA samples mixed with commercial RNA standards (Bethesda Research Laboratories) and comparing the migration of standards with mRNAs detected with a CDNA probe.

Preparation of Tissue Homogenates—White Sprague-Dawley rats were anesthetized and killed by decapitation. Tissues were quickly dissected, rinsed in cold 0.9% saline solution, blotted, weighed, minced with scissors, and homogenized in a glass Teflon homogenizer with 5 ml of 0.1 M potassium phosphate buffer, pH 7.0.

1 The abbreviations used are: G_{33}, NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ-ceramide; kb, kilobase(s); CHO, Chinese hamster ovary; Gal α-2,6-ST, Galβ1,4GlcNAc α-2,6-sialyltransferase (EC 2.4.9.1); Gal α-2,3-ST, Galβ1,3(4)GlcNAc α-2,3-sialyltransferase (EC 2.4.99.6); Gal α-2,3(4)-ST, Galβ1,3(4)GlcNAc α-2,3-sialyltransferase (EC 2.4.99.4).
Characterization of Tissue-specific mRNAs of the Galα1,4GlcNAc α-2,6-Sialyltransferase—Preliminary Northern analysis surveying mRNAs from different rat tissues showed that a cDNA probe corresponding to the coding sequence of the Galα1,4GlcNAc α-2,6-sialyltransferase (Gal α-2,6-ST) hybridized to mRNAs of different size (4.7, 4.3, and 3.6 kb) in a tissue-specific manner. An abundant mRNA of 4.3 kb was detected only in liver,3 one of 4.7-4.8 kb was seen in spleen, lung, ovary, heart, kidney, and brain, and a predominant mRNA of 3.6 kb was found only in kidney (see Fig. 1). All three species are polyadenylated mRNAs evidenced by their purification on oligo-dT columns (not shown).

To examine the relationship of the different sized mRNAs to the cDNA of the enzyme originally cloned from a rat liver library (23), five identical Northern blots containing liver, spleen, and kidney mRNAs were hybridized to probes corresponding to different regions along the length of the sialyltransferase cDNA. The results, shown in Fig. 1, indicate that the 4.3- and 4.7-kb mRNAs are highly homologous along their entire length and likely represent alternative processing of the sialyltransferase mRNA in the various tissues. In contrast, the 3.6-kb mRNA showed no homology to two probes stemming from the 5′ end of the liver sialyltransferase cDNA. These probes covered regions corresponding to the 5′-untranslated sequence and approximately 45% of the coding sequence of the enzyme (Fig. 1). The results indicate that the predominant kidney mRNA (3.6 kb) cannot code for the Gal α-2,6-ST. Preliminary evidence suggests that the 3.6-kb kidney mRNA appears to be derived from the same sialyltransferase gene as the other two messages.4 However, whether or not it codes for a functional protein remains to be determined.

Tissue-specific Expression of the Galα1,4GlcNAc α-2,6-Sialyltransferase—From the results in Fig. 1, it is immediately apparent that liver and spleen contain very different levels of sialyltransferase mRNA. Comparison of the level of mRNAs

FIG. 1. Homology of mRNAs in liver (L), kidney (K), and spleen (S) to cDNA of the Gal α-2,6-ST. Five different Northern blots with total RNA (20 μg) from liver, spleen, and kidney were probed with cDNAs representing different regions of the liver sialyltransferase mRNA. At the top is a diagram representing the full-length cDNA of the Gal α2,6-ST (4.3 kb) cloned from rat liver cDNA libraries in λgt11 (20). The coding region of the enzyme is indicated in the cross-hatched box. The thick black lines beneath numbers 1-5 show the origin of the cDNA fragments used to make radiolabeled probes for the corresponding five Northern blots at the bottom. cDNA probes were derived from subcloned fragments of the λgt11 clones (23) as follows: 1, 5′-EcoRI fragment of ST3; 2, 5′-PstI fragment of ST1; 3, 3′-PstI fragment of ST1; 4, ST2; and 5, ST5.

3 The size of the sialyltransferase mRNA in liver was previously estimated to be approximately 4.7 kb (23).

4 E Svensson, J. Weinstein, X. Wen, and J. Paulson, unpublished data.
Fig. 2. Differential expression of the Gal α-2,6-ST in various rat tissues. Northern blot of total RNA from liver, brain, ovary, kidney, spleen, and lung probed with a radiolabeled cDNA probe corresponding to the 5' end of the sialyltransferase mRNA in liver (same as probe 1 used in Fig. 1).

SIALYLTRANSFERASE  SEQUENCE FORMED

Gal α2,6 ST  NeuAca2,6Galβ1,4GlcNAc-R
(E.C. 2.4.99.1)
Gal α2,3 ST  NeuAca2,3Galβ1,3/GlcNAc-R
(E.C. 2.4.99.6)
Gal α2,3(O) ST  NeuAca2,3Galβ1,3GlcNAc-O-Thr/Ser
(E.C. 2.4.99.4)

Fig. 3. Terminal sequences formed by the three glycoprotein sialyltransferases examined in this report.

from several additional tissues is shown in Fig. 2 using a probe from the 5' end of the sialyltransferase cDNA which detects only the 4.3- and 4.7-kb mRNAs containing the complete coding sequence. The blot shown in overexposed to detect minor amounts of message (compare Figs. 1 and 2). Again, it is apparent that these tissues differ dramatically in the amount of sialyltransferase mRNA detected with the lowest levels seen in brain, intermediate levels in ovary, kidney, spleen, and lung, and the highest levels in liver. In a separate experiment, sialyltransferase mRNA was found to be barely detectable in total RNA from heart (not shown). The relative levels of mRNA in liver, spleen, and brain are approximately 50:2.5:1, as determined from different exposure times of blots from several different experiments.

Comparison of the Levels of Activity of Three Sialyltransferases in Rat Tissues—To directly examine the relationship between the levels of mRNA and enzyme activity for the tissues examined here, tissue homogenates were assayed for the Gal α-2,6-ST. At the same time, two other sialyltransferases were also examined, the Galβ1,3(4)GlcNAc α-2,3-sialyltransferase (Gal α-2,3,-ST) and the Galβ1,3GalNAc α-2,3-sialyltransferase (Gal α-2,3(O)-ST). The sequences elaborated by these enzymes are given in Fig. 3.

The levels of sialyltransferase activities observed in homogenates of seven rat tissues are summarized in Table I. Specific activities for the Gal α,2,6-ST varied by over 100-fold, with the highest activity seen in liver and the lowest (undetectable) in brain and heart. Qualitative comparison of the results in Table I with the Northern analysis in Fig. 2 shows a good correlation between the levels of Gal α,2,6-ST activity and the levels of the sialyltransferase mRNA in each tissue. This suggests that the primary reason for variation in levels of sialyltransferase activity is the regulation of the steady state levels of sialyltransferase mRNA.

Another striking finding from the results in Table I is that the three sialyltransferases examined are not coordinately expressed. This is demonstrated by comparison of the ratios of the activities of two enzymes. Indeed, the Gal α-2,3-ST and

| Tissue     | Gal α-2,6-ST | Gal α-2,3-ST | Gal α-2,3(O)-ST |
|------------|--------------|--------------|----------------|
| Liver      | 6600         | 1900         | 2500           |
| Brain      | 0            | 390          | 140            |
| Ovary      | 120          | 2900         | 2950           |
| Kidney     | 600          | 2100         | 440            |
| Spleen     | 590          | 740          | 1600           |
| Lung       | 260          | 270          | 3700           |
| Heart      | 0            | 65           | 100            |

*Details of homogenate preparation and sialyltransferase assays are given under "Experimental Procedures." Results shown are the average of duplicate assays of one or more experiments. Duplicate assays were typically within 5% of the average, and activities in separate homogenates varied within 25% except at the limits of detection where the variation in background values becomes significant. Thus, while the lowest activities are not as precise, they accurately reflect the differential distribution of the sialyltransferases in different tissues. A value of 0 indicates no activity detected at a level of 25 pmol/h/mg of protein.

| Intestinal homogenate | Sialyltransferase activity* |
|-----------------------|----------------------------|
|                       | Gal α-2,6-ST | Gal α-2,3-ST | Gal α-2,3(O)-ST |
| Duodenum mucosa       |                |              |                |
| Jejunum mucosa        |                |              |                |
| Ileum mucosa          |                |              |                |
| Ileum wall            |                |              |                |
| Intestinal homogenate |                |              |                |
| Gal α-2,6-ST          |                |              |                |
| Gal α-2,3-ST          |                |              |                |
| Gal α-2,3(O)-ST       |                |              |                |

*Details of assays are given under "Experimental Procedures." In this experiment duplicate assays contained 20 μl of homogenate and were incubated for 2 h at 37 °C. A value of 0 indicates no activity observed with a limit of detection of 20 pmol/h/g of tissue.

Gal α-2,6-ST activities in liver yield a ratio of 0.3, while in ovary the same ratio is 33, representing a differential expression of over 100-fold in these two tissues. Similar comparisons suggest that the three enzymes are expressed independently of each other.

Sialyltransferase Activities in the Mucosa and Wall of the Small Intestine—In an earlier report we noted lack of expression of the Gal α-2,6-ST and Gal α-2,3-ST in the small intestine (37) while others have described the presence of sialyltransferase activities in this tissue (38, 39). To examine the possibility that differential expression of sialyltransferase activities might account for these discrepancies, sialyltransferase activities in homogenates of the mucosal surfaces of the duodenum, jejunum, and ileum separated from the intestinal wall by gentle scraping. In addition, a homogenate of the wall of the ileum was also examined. The Gal α-2,3-ST was either not detected or present at very low levels in all homogenates (37) not shown. Activities observed for the other two sialyltransferases are summarized in Table II.

While the Gal α-2,6-ST was undetectable in all of the mucosal homogenates, the Gal α-2,3(0)-ST showed a graded distribution, with highest levels in the duodenum, intermediate levels in the jejunum, and not detectable in the ileum. Perhaps the most striking finding is that neither the Gal α-2,6-ST nor the Gal α-2,3(O)-ST sialyltransferases were detectable in ileum mucosa but were found in relatively high levels in the remaining ileum wall. In this case the two enzymes are differentially expressed within two regions of the same tissue, each having separate functions and unique cell types. Together the results provide another example of tissue-specific and differential expression of sialyltransferases.

TABLE I
Sialyltransferase activities in selected tissues of the rat

| Tissue     | Gal α-2,6-ST | Gal α-2,3-ST | Gal α-2,3(O)-ST |
|------------|--------------|--------------|----------------|
| Liver      | 6600         | 1900         | 2500           |
| Brain      | 0            | 390          | 140            |
| Ovary      | 120          | 2900         | 2950           |
| Kidney     | 600          | 2100         | 440            |
| Spleen     | 590          | 740          | 1600           |
| Lung       | 260          | 270          | 3700           |
| Heart      | 0            | 65           | 100            |

*Details of homogenate preparation and sialyltransferase assays are given under "Experimental Procedures." Results shown are the average of duplicate assays of one or more experiments. Duplicate assays were typically within 5% of the average, and activities in separate homogenates varied within 25% except at the limits of detection where the variation in background values becomes significant. Thus, while the lowest activities are not as precise, they accurately reflect the differential distribution of the sialyltransferases in different tissues. A value of 0 indicates no activity detected at a level of 25 pmol/h/mg of protein.

TABLE II
Sialyltransferase activities in regions of the small intestine

| Intestinal homogenate | Sialyltransferase activity* |
|-----------------------|----------------------------|
|                       | Gal α-2,6-ST | Gal α-2,3-ST | Gal α-2,3(O)-ST |
| Duodenum mucosa       |                |              |                |
| Jejunum mucosa        |                |              |                |
| Ileum mucosa          |                |              |                |
| Ileum wall            |                |              |                |

*Details of assays are given under "Experimental Procedures." In this experiment duplicate assays contained 20 μl of homogenate and were incubated for 2 h at 37 °C. A value of 0 indicates no activity observed with a limit of detection of 20 pmol/h/g of tissue.
What is the consequence of the differential expression of terminal glycosyltransferases? Most terminal glycosyltransferases compete for common acceptor substrates (15). Thus, the type of terminal glycosyltransferases expressed by a cell should influence the types of terminal sequences found on the carbohydrate groups it produces.

To take a relevant case for the enzymes studied here, the Gal α-2,6-ST and the Gal α-2,3-ST both compete for the Galβ1-4GlcNAc-R sequence on the terminal branches of N-linked oligosaccharides of glycoproteins to form the NeuAca2-6Galβ1-4GlcNAc-R and NeuAca2-3Galβ1-4GlcNAc-R sequences, respectively. In vitro, either enzyme can completely resialylate asialo-glycoproteins which contain di-, tri-, and tetra-branched oligosaccharides (35), although the Gal α-2,6-ST has been shown to have considerable branch specificity, sialylating some branches easier than others (41). Structure analysis shows that the N-linked oligosaccharides of some glycoproteins have exclusively the NeuAca2-6Gal linkage providing the enzyme is present, as 40% by a cell. Thus, it appears that CHO cells have only the NeuAca2-6Gal linkage (43, 10934). This principle is supported by recent results examining the preparation of several of the mRNAs and Eric Svensson and Xiao-hong Wen for insightful discussions and critical reading of the manuscript.

ACKNOWLEDGMENTS—We would like to thank Xiao-hong Wen for preparation of several of the mRNAs and Eric Svensson and Xiao-hong Wen for insightful discussions and critical reading of the manuscript.

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* E. U. Lee and J. C. Paulson, unpublished results.