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The Sialyltransferase "Sialylmotif" Participates in Binding the Donor Substrate CMP-NeuAc*

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All members of the sialyltransferase gene family cloned to date contain a conserved region, the "sialylmotif," consisting of 48-49 amino acids in the center of the coding sequence. To investigate the function of this motif, mutant constructs of the Galβ1,4GlcNAc α2,6-sialyltransferase were designed by site-directed mutagenesis, replacing 11 individual conserved amino acids with alanine. Each of the mutants was expressed in COS-1 cells, and eight of these retained sialyltransferase activity, allowing comparison of their enzymatic properties with that of the wild type enzyme. Kinetic analysis showed that six of eight mutants had a 3-12-fold higher K_m for the donor substrate CMP-NeuAc relative to the wild type enzyme, while the K_m values for the acceptor substrate were within 0.5-1.2-fold of the wild type for all eight mutants evaluated. The K_i of the donor substrate analog CDP was also evaluated for the recombinant sialyltransferase with the Val to Ala mutation at residue 220, which produced a 6-fold increase in K_m of CMP-NeuAc. A corresponding increase in K_i of 3.4-fold was observed for CDP, indicating a decreased affinity for the cytidine nucleotide. Taken together, these results suggest that the conserved sialylmotif in the sialyltransferase gene family participates in the binding of the common donor substrate, CMP-NeuAc.

The family of sialyltransferases that transfers sialic acid from CMP-sialic acid to carbohydrate groups of glycolipids and glycoproteins has been predicted to consist of a group of 10-12 enzymes (1, 2). So far cDNA clones have been obtained for eight distinct members of this family (3-19). Analysis of their protein sequences has revealed that all of the sialyltransferases have structural features common to all glycosyltransferases, which are relevant to their function as enzymes involved in post-translational glycosylation of glycoproteins through their transit through the Golgi apparatus. They each have a short NH_2 cytoplasmic domain, a hydrophobic signal-anchor sequence that serves as the membrane-spanning domain, a luminal "stem" region not required for catalytic activity, and a large luminal catalytic domain. However, apart from their common topological features, there is little sequence homology among the sialyltransferases except for 10-12% of the sequence in the middle of the catalytic domain and a smaller sequence of 23 residues at the carboxyl end of the sequence (17, 20). The largest conserved region, termed the "sialylmotif" (12), consists of 48 or 49 amino acids in the center of each molecule, which exhibits 40-60% identity between any two enzymes and contains 8 invariant residues among all the cDNAs cloned to date.

The sialylmotif is presumed to contribute to a structural feature related to the common function of the sialyltransferases. Each enzyme transfers sialic acid from the common donor substrate CMP-NeuAc to an oligosaccharide acceptor substrate. Thus, the sialylmotif could form part of the binding sites for either the donor or acceptor substrates, or both. Alternatively, it could be a structural nucleus that maintains the conformation and spatial relationship of variable substrate binding pockets in the sialyltransferase family.

To explore the function of the sialylmotif, we prepared single point mutants of the Galβ1,4GlcNAc α2,6-sialyltransferase (EC 2.4.99.1) as a model in this study. This enzyme forms the NeuAcα2,6Galβ1,4GlcNAc- sequence common to many Asn-linked oligosaccharides (21, 22). The cDNA encoding this enzyme was cloned from rat liver (3), and its kinetic properties have been established (21-23). In this study, we constructed 11 recombinant enzymes by site-directed mutagenesis, changing selected conserved amino acids in the sialylmotif to alanine, and evaluated the expressed enzymes for changes in their enzymatic properties. Most of the mutants retained sialyltransferase activity and had increases in the K_m of CMP-NeuAc without corresponding changes in the K_m of the acceptor substrate. Thus, the results suggest that the sialylmotif of the Galβ1,4GlcNAc α2,6-sialyltransferase participates in binding the donor substrate, CMP-NeuAc.

EXPERIMENTAL PROCEDURES

Construction of Mutants—Single alanine mutations were introduced in spST-2, a construct of expression vector pSVL (Pharmacia Biotech Inc.) containing cDNA for rat Galβ1,4GlcNAc α2,6-sialyltransferase (24). The mutations in the cDNA for the corresponding nucleotide(s) were introduced by a two-step polymerase chain reaction following the procedure of Sarkar and Sommer (25) with modifications as follows: the cDNA for the Galβ1,4GlcNAc α2,6-sialyltransferase (GenBank accession no. M18769) previously subcloned in pBluescript KS+ plasmid (26) was used as a template. The mutagenic antisense oligonucleotides (the substituted nucleotides are underlined) used for construction of the corresponding cDNAs were (5'-3'): for C181A, AGACGACGGCAGC-CCTTTGCCAAGG (nt[1] 553-529); for V184A, GCAGAAGAGGCGACG-GCACACCTTT (nt 560-536); for L190A, GGAGTTTTCGCAGATCCT-GCAGAAG (nt 579-554); R207A, CCCATTAAACGCCAGAACTG-CATCA (630-606); for D219A, TTTTGAGCCCACAGCCTGTTG-GAAGTTGT (nt 669-641); for V220A, GTTTTTGAGCCCGCATCCT-GTTGG (nt 671-648); for G221A, GGTAGTTTTTGAGGCCACATCCT-GTT (nt 675-651); for S222A, GGTAGTTTTTGCGCCCACATCCT-GTTG (nt 675-649); for K223A, CGAATGGTAGTTGCTGA-GCCCACATC (nt 680-655); for T224A, CGAATGGTAGCTTTTGAGC-CCACAT (nt 680-656); for T225A, TTAGGCGAATGGCAGTTTTT-GAGCCC (nt 685-660). In the first step of PCR, the 5'-end oligonucleotide primer, CCCAGGTTGAATTCTCAAACAGC (5'-3'; nt 170-192), and the mutant antisense oligonucleotide for the desired mutation were first used to generate a megamer using Pfu DNA polymerase (Stratagene); the conditions used were: 94 °C, 30 s, 56 °C, 1 min, 73 °C, 2 min, 25 cycles. The gel analysis showed the generation of a single band for about 450 bp depending on the position of the oligomer. This megamer

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[1] The abbreviations used are: nt, nucleotide(s); bp, base pair(s); PCR, polymerase chain reaction.

was purified from the PCR mixture using Geneclean II (Bio101). It was then used in the second step of PCR as follows. The tube containing the megamer, the above template, and all the other reagents including Pfu polymerase except the 3'-primer was placed in the thermocycler (Perkin-Elmer 9600), and the reaction was performed for five cycles at 94 °C, 1 min, 73 °C, 3 min. While the tubes were at 73 °C, the tubes were taken out and briefly centrifuged; the 3'-end antisense oligonucleotide primer, CCAGGAGAGGATCCATAAAATGAC (5'–3'; nt 1270–1247) was added, and the mixture was briefly vortexed and centrifuged and then placed in the thermocycler to continue the polymerase chain reaction at 94 °C, 1 min, 68 °C, 1 min, 73 °C, 3 min for 20 cycles. This modification of the conditions was found to be optimum for a good yield of the final products. The products were analyzed by agarose gel electrophoresis that showed the generation of a major single band of 1.1 kilobase. This band was purified by agarose gel electrophoresis followed by Geneclean II (Bio101) to separate from the megamer. For the cDNAs of D219A, V220A, G221A, S222A, K223A, T224A, and T225A, the gel-purified product was digested with two unique restriction enzymes, *Apa*I (at nt 629) and *Bst*BI (at nt 824), and the smaller 195-bp fragment was purified by the MERmaid kit (Bio101). This fragment was then subcloned into a similarly digested and finally purified larger fragment of spST-2 (prior to this treatment this plasmid DNA was propagated through the *dam* negative strain GM2163 of *Escherichia coli* to inhibit the inhibitory methylation at the *Apa*I site) following standard techniques (27). Similarly, for C181A, V184A, L190A, and R207A, the gel-purified product was digested with *Dra*III (at nt 477) and *Bst*BI, and the 350-bp fragment was purified and subcloned into a similarly digested and purified larger fragment of spST-2. The mutation was confirmed by dideoxy double-stranded sequencing (28) of the entire fragment that has been subcloned, including the restriction sites used. Using Pfu polymerase, we found that all the clones obtained have only the desired mutation.

Expression of the Wild Type and Mutant Sialyltransferases—COS-1 cells obtained from ATCC were routinely maintained as described (29). COS-1 cells (1–2 × 10^6 cells/100-mm dish) were transfected using 2.0 μg of plasmid DNA using LipofectAmine™ reagent according to the supplier (Life Technologies, Inc.). Expression of transfected proteins was typically allowed to continue for 36–48 h post-transfection before harvesting the cells. After 36–48 h of post-transfection, the cell culture media were collected and concentrated 10-fold by ultrafiltration using microconcentrators (MWCO 10; Amicon Inc., Beverly, MA). The concentrated media containing the soluble expressed sialyltransferase were used directly for analysis of the enzymatic activity as described below. It was observed that the wild type as well as the mutant recombinant sialyltransferases are quite stable at 4 °C for at least 2 weeks. In this study, the enzyme assays were typically performed within 1 week after collecting the media. Following collection of the media, the transfected cells were subsequently used for radiolabeling of the expressed proteins when desired. Transfections of each mutant were done at least three times with plasmid DNAs from different preparations to determine the degree to which variation in expression was transfection-dependent.

Sialyltransferase Assay—Enzyme assays were performed in duplicate as described previously (21). Unless otherwise stated, 10 μl of concentrated COS-1 cell medium containing expressed recombinant sialyltransferases was assayed in a 60-μl reaction mixture containing 9 nmol of CMP-[^{14}C]NeuAc (DuPont NEN; 6600 cpm/nmol) as donor, 50 μg of asialo α_1-acid glycoprotein (α_1-acid glycoprotein from Sigma was desialylated by mild acid hydrolysis following the procedure of Schmid (30)), 50 μg of bovine serum albumin, 50 mM sodium cacodylate (pH 6.0) with 0.5% Triton CF-54. After incubation at 37 °C for 1 h, the radiolabeled product was isolated and quantitated as described previously (22, 31) employing chromatography on Sephadex G-50 (Sigma). The assays were repeated in duplicate at least three times from different transfection experiments.

Pulse-Chase Labeling of Transfected COS-1 Cells and Analysis of the Transiently Expressed Proteins—Metabolic labeling of cells using Trans^{35}S-Express protein label (DuPont NEN; 100 μCi/ml) and immunoprecipitation of expressed proteins with rabbit anti-rat α2,6-sialyltransferase were performed using media from cells of 36–48 h post-transfection essentially as described earlier (32).

Western Blot—The protein samples from the concentrated media were boiled for 5 min in 1× Laemmli gel sample buffer containing 10% β-mercaptoethanol. These were run using 10% SDS-polyacrylamide gel electrophoresis in Tris/glycine-SDS buffer. The electrophoresed proteins were transferred to nitrocellulose membrane following the usual procedure (33). The blot was developed by adsorption of the rabbit anti-rat Galβ1,4GlcNAc α2,6-sialyltransferase antibody (1:500) followed by horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:1000; Amersham Corp.). The protein bands were finally visualized by following the chemiluminescence (ECL) technique as suggested by the supplier of the reagents (Amersham Corp.).

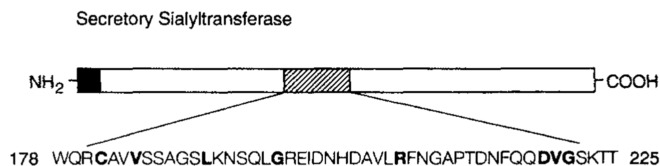

FIG. 1. **spST-2 construct and its mutants in relation to the domain structure of sialyltransferase.** The cleavable signal anchor domain from dog pancreatic insulin is fused with the catalytic domain of Galβ1,4GlcNAc α2,6-sialyltransferase converting this membrane-bound enzyme into the soluble form (32). The "sialylmotif" in this enzyme spans from amino acid 178 to 225 and contains eight invariant amino acids, which are indicated by *boldface letters*. The *underlined* amino acid residues were changed to alanine by single point mutagenesis. ■, cleavable signal sequence; ▨, sialyl motif; □, catalytic domain.

Protein Determination—The concentration of proteins in the media was determined using the bicinchoninic acid protein assay reagent kit (Pierce) with bovine serum albumin as standard.

Enzyme Assay for Kinetic Analysis—For kinetic measurements, initial rate data using asialo α_1-acid glycoprotein as acceptor were obtained. All sialyltransferase assays were done in duplicate. To determine K_m of the donor substrate, a fixed concentration of asialo α_1-acid glycoprotein (50 μg) and five concentrations of unlabeled CMP-NeuAc (Sigma) were added to assay mixtures containing a fixed amount of CMP-[^{14}C]NeuAc (DuPont NEN; 315 μCi/μmol; 58,500 cpm/assay) to give a final concentration of 20–200 μM (unless otherwise specified). Similarly, to determine K_m of asialo α_1-acid glycoprotein, assays containing six concentrations were examined ranging from 40 to 400 μM based on galactose content (1 μmol of asialo α_1-acid glycoprotein or 40 μg is equivalent to 16.6 μmol of Gal acceptor unit), using a fixed concentration of CMP-[^{14}C]NeuAC (6433 cpm/mmol). Kinetic constants were obtained from double-reciprocal plots (for details see Ref. 34) by linear regression analysis of Lineweaver-Burk plots (35). In all cases consumption of substrates was kept below 20% to ensure accurate initial rates by adjusting the amount of enzyme added if necessary.

RESULTS

Expression of the Sialylmotif Mutants and Comparison of Their Enzyme Activity—The plasmid cDNAs used to examine the sialyltransferase activity of selected mutations in the sialylmotif were constructed as shown in Fig. 1. The mutants were derived from an expression vector of the wild type enzyme in which the N-terminal cytoplasmic tail, signal anchor domain, and the stem regions (first 71 amino acids) were replaced with a cleavable signal peptide of dog pancreas insulin (32). Thus, this vector results in the expression of the Galβ1,4GlcNAc α2,6-sialyltransferase protein in the media as a soluble form. The nomenclature for the mutant sialyltransferases reflects the residue number for which alanine was substituted (*e.g.* for D219A, aspartic acid 219 is replaced by alanine, etc.).

Expression vectors containing mutant sialyltransferase constructs were transfected into COS-1 cells and evaluated for expression of the recombinant proteins and for expressed sialyltransferase activity. Immunoprecipitation of radiolabeled sialyltransferases using the anti-sialyltransferase antibody, followed by analysis by SDS-polyacrylamide gel electrophoresis, indicated that all mutant sialyltransferases were expressed and exhibited similar molecular weight to the wild type sialyltransferase (not shown). To determine the enzymatic properties of the mutant sialyltransferases, concentrated media from the transfected cells were used as a source of crude enzyme. Media from COS-1 cells "mock" transfected using the expression vector (pSVL) only contained low levels of endogenous sialyltransferase, which were typically less than 2% that of media containing the expressed soluble sialyltransferases. All the mutants showed Galβ1,4GlcNAc α2,6-sialyltransferase activities, which varied from 25 to 85% that of the wild type

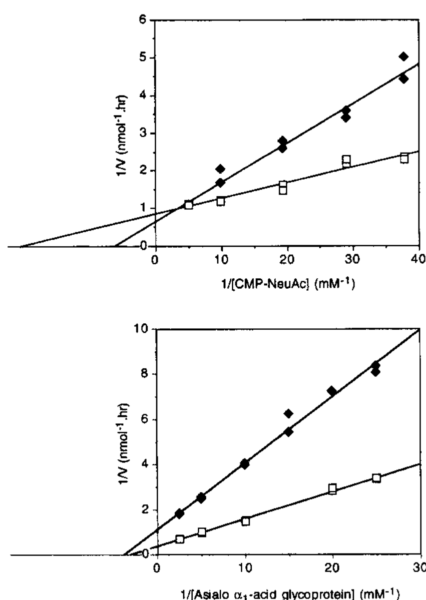

FIG. 2. **Double-reciprocal plots of initial rate data with CMP-NeuAc (*top*) or asialo α_1-acid glycoprotein (*bottom*) as the varied substrate.** *Top*, rate data with CMP-NeuAc as the donor (26.5–201 μM) were determined at a fixed concentration of the acceptor asialo α_1-acid glycoprotein, 50 μg. *Bottom*, the concentration of the acceptor asialo α_1-acid glycoprotein was varied (0.04–0.4 mM) at a fixed concentration of the donor, 0.15 mM. The plot was shown only for T225A (♦) in *top* and V220A (♦) in *bottom*, as a representative of the mutants and compared with that of the wild type (spST-2) of sialyltransferase (□). The K_m (apparent) values were determined from the x intercept ($-1/K_m$ (apparent)) (35).

enzyme except for the mutants, C181A, R207A, and S222A, which showed very low activity (5% or less).

By comparing sialyltransferase expression by Western blot to the enzymatic activity observed it was noted that the expression level was apparently similar to the wild type enzyme for V184A, L190A, D219A, V220A, K223A, and T225A, while the enzyme activities obtained were 45, 28, 56, 30, 32, and 27%, respectively. While the mutation of Cys[181], Arg[207], and Ser[222] to Ala reduced the enzyme activity to 5% or less, the protein expression appeared to be reduced only 2–3-fold.

Kinetic Analysis of the Mutant Enzymes—Analysis of the kinetics of wild type and each mutant sialyltransferase expressed in COS-1 cells was performed as described under "Experimental Procedures," except for C181A, R207A, and S222A, which had low activity and were not evaluated. The K_m values for each sialyltransferase were determined for the donor substrate, CMP-NeuAc, and best glycoprotein acceptor substrate of the wild type enzyme, asialo α_1-acid glycoprotein. This glycoprotein acceptor contains five *N*-linked oligosaccharides, which are predominately tri- and tetra-antennary structures, each branch of which is terminated with the acceptor sequence Galβ1,4GlcNAc. Data obtained for each of the enzymes produced Lineweaver-Burk plots with correlation coefficients of 0.95 or greater (*e.g.* Fig. 2).

As summarized in Table I, the K_m values obtained for asialo α_1-acid glycoprotein were within 0.5–1.2-fold that of the wild type enzyme for each of the mutant enzymes examined. In contrast, the K_m values for the CMP-NeuAc were significantly altered, particularly for V184A, L190A, D219A, V220A, K223A, and T225A, with increases in K_m (apparent) of 3–12-fold observed.

Increase in K_i of CDP for V220A—Since decreased binding affinity of CMP-NeuAc would be reflected as an increase in the K_m for this substrate, it seemed reasonable to expect that the amino acids altered in the mutants that exhibited this change

TABLE I

Kinetic constants for the wild type and mutants of Galβ1,4GlcNAc α2,6-sialyltransferase

The kinetic analyses were performed using 10-fold concentrated media of COS-1 cells transfected with cDNAs of wild type secretory sialyltransferase (SpST-2) and mutants (for the methods, see "Experimental Procedures" and also described in the text).

Sialyltransferase constructs	Apparent K_m values[a]			
	CMP-NeuAc	-fold	Acceptor (ASGP)[b]	-fold
	μM		μM	
Wild type[c]	50	1.0	331	1.0
Mutants				
V184A	343	6.8	372	1.1
L190A	597	11.9	188	0.6
D219A	540	10.8	160	0.5
V220A	300	6.0	260	0.8
G221A	79	1.6	280	0.8
K223A	330	6.6	400	1.2
T224A	64	1.3	320	1.0
T225A	160	3.2	200	0.6

[a] The apparent K_m listed was the average of three experiments. In each case the range of values observed was found to be within ±0.5-fold of the indicated value.

[b] Asialo α_1-acid glycoprotein.

[c] The literature values of K_m were 50 μM for CMP-NeuAc (23) and 0.18 mM for the asialo α_1-acid glycoprotein (22). For the K_m determination, the conditions were identical for the wild type as well as the mutants as shown in Fig. 2, except for the mutant V220A (see Fig. 3).

participated in the binding of the cytidine or NeuAc moiety of CMP-NeuAc. Previous reports have indicated that the cytidine analog CDP is often a potent inhibitor of sialyltransferases, exhibiting equilibrium dissociation constants similar to that of CMP-NeuAc (34). To determine if the increase in K_m for CMP-NeuAc would also be reflected in a decrease in affinity for CDP, CDP was tested as a competitive inhibitor of CMP-NeuAc for mutant V220A (Fig. 3). The K_i for V220A was determined to be 16.1 μM, representing a 3.4-fold increase over the wild type enzyme (4.7 μM). Thus, the increase in K_i for CDP observed for V220A is comparable in magnitude with the increase in K_m for CMP-NeuAc, suggesting that Val[220] participates in binding the common cytidine moiety in these two ligands.

DISCUSSION

The sialylmotif of the sialyltransferase gene family has been identified as a conserved stretch of 48–49 amino acids found roughly in the center of the coding sequence of these enzymes. Based on the premise that this motif was restricted to the sialyltransferase gene family, five sialyltransferase genes have been successfully cloned using a PCR homology approach, bringing the total number of distinct members of sialyltransferases cloned to date to eight (3–19). Further evidence that the sialylmotif is unique to the sialyltransferase gene family is that no other gene in GenBank, including representative cloned cDNAs from the fucosyltransferase, galactosyltransferase, *N*-acetylglucosaminyltransferase, or *N*-acetylgalactosaminyltransferase families, has been found to have a homologous sequence. These facts alone argue that the sialyltransferase genes described to date originate from a common gene and that the sialylmotif participates in a structural feature or function common to all of these enzymes.

The aim of this present study was to evaluate the functional role of the conserved sialylmotif of the sialyltransferase gene family. As described under "Results," the Galβ1,4GlcNAc α2,6-sialyltransferase was used as a representative enzyme in these studies, substituting alanine for conserved amino acid residues in the sialylmotif by site-directed mutagenesis to see if the alteration had an effect on sialyltransferase activity. The 11 mutants reported in this study include 7 of the 8 amino acid residues found to be invariant in all the sialyltransferase genes

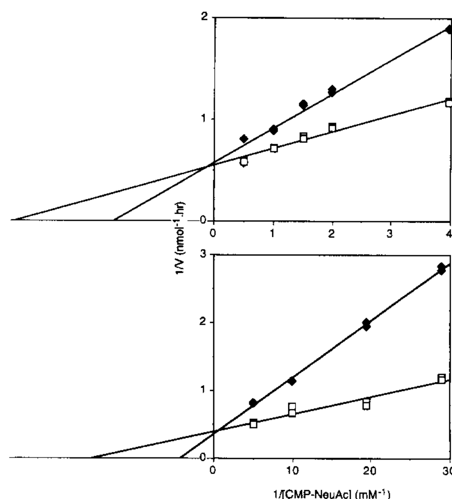

FIG. 3. **Inhibition kinetics of sialyltransferase by CDP.** Initial rate data for the wild type enzyme *(top panel)* were determined in the presence of varied concentrations of the donor substrate CMP-NeuAc (0.025–0.2 mM) and in the absence (□) and presence (◆) of a fixed concentration of 12.5 μM CDP. Similarly, kinetics for the mutant V220A *(bottom panel)* were determined in the presence of the varied concentrations of CMP-NeuAc (0.25–2.0 mM) and in the absence (□) or presence (◆) of a fixed concentration of 16.7 μM CDP. The concentration of the acceptor asialo α_1-acid glycoprotein was kept constant at 50 μg throughout for both enzymes. The K_i values were extracted from the x intercept in the presence of inhibitor (35), where the x intercept $= -(1/K_m)(1 + [I]/K_i)$ and where K_m is obtained in the absence of the inhibitor CDP and [I] is the concentration of CDP.

cloned to date (see Fig. 1). In addition, several other highly conserved amino acids were examined.

The results suggest that the sialylmotif participates in the binding of the donor substrate, CMP-NeuAc, and has little contribution to the binding of the oligosaccharide acceptor substrate. Indeed, of the eight mutant sialyltransferses that exhibited sialyltransferase activity, six exhibited increased K_m values of 3–12-fold over that of the wild type for the donor substrate, CMP-NeuAc (Table I). In contrast, all of the mutant enzymes had K_m values for the acceptor substrate (asialo-α_1 acid glycoprotein) that were within 0.5–1.2-fold that of the wild type sialyltransferase. The mutations spanned the length of the sialylmotif (see Fig. 1), and the magnitude of the change in K_m for CMP-NeuAc did not appear to be dependent on the location of the amino acid in this conserved domain. Further examination of one of the mutants, V220A, revealed that it exhibited a decreased affinity (increased K_i) for the competitive substrate CDP relative to the wild type enzyme.

The conclusions drawn from these studies are in keeping with observations made previously by others. Wen *et al.* (10) suggested that the sialylmotif may form a conserved surface feature of the sialyltransferases, consistent with a substrate binding pocket, due to the large number of conserved charged residues found in the sialylmotif. Kurosawa *et al.* (16) further suggested that the conserved region was less likely to be an acceptor oligosaccharide binding site considering that the cloned enzymes transferred sialic acid to galactose, *N*-acetylgalactosamine, and sialic acid found in a variety of glycoprotein and glycolipid carbohydrate groups. The fact that the sialylmotif appears to participate in binding the common donor substrate, CMP-NeuAc, suggests that the PCR homology approach to cloning sialyltransferase cDNAs may continue to have utility in defining this diverse gene family.

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REFERENCES

1. Corfield, A. P., and Schauer, R. (1982) in *Sialic Acids: Chemistry, Metabolism and Function* (Schauer, R., ed) *Cell Biology Monographs*, Vol. 10, pp. 195–261, Springer-Verlag, New York
2. Paulson, J. C., and Colley, K. J. (1989) *J. Biol. Chem.* **264,** 17615–17618
3. Weinstein, J., Lee, E. U., McEntee, K., Lai, P., and Paulson, J. C. (1987) *J. Biol. Chem.* **262,** 17735–17743
4. Grundmann, U., Nerlich, C., Rein, T., and Zettlmeissl, G. (1990) *Nucleic Acids Res.* **18,** 667
5. Bast, B. J. E. G., Zhou, L.-J., Freeman, G. J., Colley, K. J., Ernst, T. J., Munro, J. M., and Tedder, T. F. (1992) *J. Cell Biol.* **116,** 423–435
6. Hamamoto, T., Kawasaki, M., Kurosawa, N., Nakaoka, T., Lee, Y-C., and Tsuji, S. (1993) *Bioorg. & Med. Chem. Lett.* **1,** 141–145
7. Kurosawa, N., Kawasaki, M., Hamamoto, T., Nakaoka, T., Lee, Y.-C., Arita, M., and Tsuji, S. (1994) *Eur. J. Biochem.* **219,** 375–381
8. Gillespie, W., Kelm, S., and Paulson, J. C. (1992) *J. Biol. Chem.* **267,** 21004–21010
9. Lee, Y.-C., Kurosawa, N., Hamamoto, T., Nakaoka, T., and Tsuji, S. (1993) *Eur. J. Biochem.* **216,** 377–385
10. Wen, D. X., Livingston, B. D., Medzihradszky, K. F., Kelm, S., Burlingame, A. L., and Paulson, J. C. (1992) *J. Biol. Chem.* **267,** 21011–21019
11. Kitagawa, H., and Paulson, J. C. (1993) *Biochim. Biophys. Acta* **194,** 375–382
12. Livingston, B. D., and Paulson, J. C. (1993) *J. Biol. Chem.* **268,** 11504–11507
13. Lee, Y.-C., Kojima, N., Wada, E., Kurosawa, N., Nakaoka, T., Hamamoto, T., and Tsuji, S. (1994) *J. Biol. Chem.* **269,** 10028–10033
14. Kitagawa, H., and Paulson, J. C. (1994) *J. Biol. Chem.* **269,** 1394–1401
15. Sasaki, K., Watanabe, E., Kawashima, K., Sekine, S., Dohi, T., Oshima, M., Hanai, N., Nishi, T., and Hasegawa, M. (1993) *J. Biol. Chem.* **268,** 22782–22787
16. Kurosawa, N., Hamamoto, T., Lee, Y.-C., Nakaoka, T., Kojima, N., and Tsuji, S. (1994) *J. Biol. Chem.* **269,** 1402–1409
17. Sasaki, K., Kurata, K., Kojima, N., Kurosawa, N., Ohta, S., Hanai, N., Tsuji, S., and Nishi, T. (1994) *J. Biol. Chem.* **269,** 15950–15956
18. Nara, K., Watanabe, Y., Maruyama, K., Kasahara, K., Nagai, Y., and Sanai, Y. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7952–7956
19. Haraguchi, M., Yamashiro, S., Yamamoto, A., Furukawa, K., Takamiya, K., Lloyd, K. O., Shiku, H., and Furukawa, K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 10455–10459
20. Drickamer, K. (1993) *Glycobiology* **3,** 2–3
21. Weinstein, J., Silva, U., and Paulson, J. C. (1982) *J. Biol. Chem.* **257,** 13835–13844
22. Weinstein, J., Silva, U., and Paulson, J. C. (1982) *J. Biol. Chem.* **257,** 13845–13853
23. Gross, H. J., Rose, U., Krause, J. M., Paulson, J. C., Schmid, K., Feeney, R. E., and Brossmer, R. (1989) *Biochemistry* **28,** 7386–7392
24. Colley, K. J., Lee, E. U., and Paulson, J. C. (1992) *J. Biol. Chem.* **267,** 7784–7793
25. Sarkar, G., and Sommer, S. S. (1990) *BioTechniques* **8,** 404–407
26. Lee, E. U., Roth, J., and Paulson, J. C. (1989) *J. Biol. Chem.* **264,** 13848–13855
27. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5463–5467
29. Datta, A. K., and Lehrman, M. A. (1993) *J. Biol. Chem.* **268,** 12663–12668
30. Schmid, K. (1975) in *The Plasma Proteins* (Putnam, F. W., ed) 2nd Ed., Vol. I, pp. 183–228, Academic Press, New York
31. Paulson, J. C., Beranek, W. E., and Hill, R. L. (1977) *J. Biol. Chem.* **252,** 2356–2362
32. Colley, K. J., Lee, E. U., Adler, B., Browne, J. K., and Paulson, J. C. (1989) *J. Biol. Chem.* **264,** 17619–17622
33. Harlow, E., and Lane, D. (1988) *Antibodies*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
34. Paulson, J. C., Rearick, J. I., and Hill, R. L. (1977) *J. Biol. Chem.* **252,** 2363–2371
35. York, J. L. (1986) in *Text Book of Biochemistry* (Devlin, T. M., ed) 2nd Ed., pp. 117–175, John Wiley & Sons, Inc., New York