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Mammalian Sialyltransferase Superfamily: Structure and Function

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Abstract

To elucidate the regulatory mechanism for expression of sialyl-glycoconjugates and their biological functions, ninetheen sialyltransferase cDNAs including eleven by our group or co-works have been cloned and characterized so far. The cloned sialyltransferases are classified into four families according to the carbohydrate linkages they synthesize: α2,3-sialyltransferase (ST3Gal I-VI), α2,6-sialyltransferase (ST6Gal I), GalNAc α2,6-sialyltransferase (ST6GalNAc I-VI), and α2,8-sialyltransferase (ST8Sia I-VI). Each of the sialyltransferase genes is differentially expressed in a tissue-, cell type-, and stage-specific manner. These enzymes differ in their substrate specificity and various biochemical parameters. However, enzymatic analysis conducted in vitro with recombinant enzyme revealed that one linkage can be synthesized by multiple enzymes. We present here an overview of structure and function of sialyltransferases performed by our group and co-works. Genomic structures and transcriptional regulation of two kinds of human sialyltransferase gene are also presented.

Key words: sialyltransferase, cloning, expression, specificity

1. Introduction

Sialic acid (NeuAc) residues occur at the terminal positions of the carbohydrate groups of three types of glycoconjugates (*N*-and *O*-glycosidically linked oligosaccharides of glycoproteins, and glycosphingolipids) and play important roles in a variety of biological processes, such as cell-cell communication, cell-matrix interaction, cell differentiation, invasiveness of a number of pathogenic organisms, clearance of asialo glycoproteins from circulation, protein targetting and adhesion of leukocytes to endothelial cells mediated by selectins [1]. The expression of sialyl-glycoconjugates changes during development, differentiation and oncogenic transformation [1]. The transfer of sialic acid from CMP-sialic acid (CMP-NeuAc) to these glycoconjugates is catalyzed by a family of glycosyltransferases called sialyltransferases [2]. These enzymes so far cloned exhibit acceptor substrate specificities for glycoproteins and glycolipids and show remarkable tissue-specific expression, which is correlated with the existence of cell type-specific carbohydrate structure [2,3]. In general their expression appears to be regulated on the transcriptional level. Precise molecular knowledge of sialyltransferases is vital for understanding the regulatory mechanism for the sialylation of glycoconjugates. This review summarizes our current knowledge on the structure, enzymatic activity and regulated expression of the sialyltransferases cloned by our group and co-works.

1.1. Sialyltransferase family

To account for all these sialylated structures described to date, the mammalian sialyltransferase family is supposed to consist of more than 20 sialyltransferases. Ninetheen sialyltransferasae cDNAs have been so far cloned from various mammalian species, 11 of which have been cloned by our group or co-works

(Table 1). The use of the cloned sialyltransferase cDNAs to produce recombinant proteins has shed light on the substrate specificity of each enzyme in vitro.

Table 1. Sialyltransferases (STs) cloned so far.

Enzymes	Abbreviation	Sources
Galβ1,3GalNAcα2,3-ST	ST3Gal I	Mouse, Pig, Chick, Human
Galβ1,3GalNAcα2,3-ST	ST3Gal II	Mouse, Rat, Human
Galβ1,3(4)GlcNAcα2,3-ST	ST3Gal III	Mouse, Rat, Human
Galβ1,4(3)GlcNAcα2,3-ST	ST3Gal IV	Mouse, Human
GM3 synthase	ST3Gal V	Mouse, Human
Galβ1,4GlcNAcα2,3-ST	ST3Gal VI	Human
Galβ1,4GlcNAcα2,6-ST	ST6Gal I	Mouse, Chick, Rat, Human
GalNAcα2,6-ST	ST6GalNAc I	Chick, Mouse, Human
Galβ1,3GalNAcα2,6-ST	ST6GalNAc II	Mouse, Chick, Human
NeuAcα2,3Galβ1,3GalNAcα2,6-ST	ST6GalNAc III	Mouse, Rat
NeuAcα2,3Galβ1,3GalNAcα2,6-ST	ST6GalNAc IV	Mouse, Human
GD1α synthase	ST6GalNAc V	Mouse
GD1α/GT1aα/GQ1bα synthase	ST6GalNAc VI	Mouse
GD3 synthase	ST8Sia I	Mouse, Human
Polysialic acid synthase	ST8Sia II	Mouse, Rat, Human
NeuAcα2,3Galβ1,4GlcNAcα2,8-ST	ST8Sia III	Mouse, Human
Polysialic acid synthase (PST-1)	ST8Sia IV	Mouse, Hamster, Human
α2,8-ST	ST8Sia V	Mouse, Human
α2,8-ST	ST8Sia VI	Mouse

Underlines indicate works performed by our group or co-works.

2. The cloned mammalian sialyltransfereases and their substrate specificity

2.1. β-galactoside α2,3-sialyltransferase (ST3Gal-family)

Six species of β-galactoside α2,3-sialyltransferases have been cloned from various mammalian species (Table 1). Among them, ST3Gal II was firstly found

by our group [4]. Although the amino acid sequence of ST3Gal I and II from mouse brain showed about 46% identity, recombinant ST3Gal I and II produced in COS-cells exhibited the same acceptor substrate specificity and were only able to synthesize the NeuAc α2,3Gal β1,3GalNAc sequence in gangliosides and glycoproteins as well as in oligosaccharides. The existence of two different kinds of sialyltransferase with same acceptor substrate specificity in the same tissue (mouse brain) leads us to the assumption that these enzymes are responsible for the biosynthesis of the NeuAc α2,3Gal β1,3GalNAc sequence in gangliosides and *O*-glycosidically linked oligosaccharides of glycoproteins, respectively. A kinetic parameter and accepotr competition experiment on ST3Gal I and II clearly demonstrated their different substrate preference, *i.e.* Gal β1,3GalNAc and asialofetuin serve as predominant acceptor for ST3Gal I, whereas gangliosides serve as predominant acceptor for ST3Gal II [5] (Table 2). Northern blot analysis revealed that the expression level of ST3Gal I mRNA is abundant in submaxillary gland which express high amount of mucin-type glycoprotein, but not in brain, whereas that of ST3Gal II mRNA is remarkable in brain which is known to contain a particularly high amount of ganglioside, but not in submaxillary gland.

ST3Gal III exhibited a strong acceptor substrate preference for Gal β1, 3GlcNAc over Gal β1,4GlcNAc, whereas the activity of ST3Gal IV showed an opposite pattern compared to ST3Gal III, *i.e.* a strong preference for Gal β1, 4GlcNAc over Gal β1,3GlcNAc.

ST3Gal V was shown to use only lactosylceramide (Gal β1,4Glc β-Cer) as an acceptor subatrate leading to the synthesis of GM3. Despite the ubiquitous distribution of GM3 in mammalian tissues, the ST3Gal V gene was found to be expressed in a tissue-specific manner, with predominant expression in brain which contains a large amount of gangliosides.

Table 2. Summary of Km, Ki and Vmax values for ST3Gal I and II, obtained in competition experiments, with different types of glycoconjugates.

Acceptor	Inhibitor	Km for Acceptor		Ki for Inhibitor		Vmax	
		ST3Gal		ST3Gal		ST3Gal	
		I	II	I	II	I	III
		(mM)		(mM)		(pmol/h)	
AsialoGM1	None	1.25	0.56	-	-	41.3	111.1
	Galβ1,3GalNAc	5.00	0.58	0.15	>3.0	43.5	50.6
	Asialofetuin	2.10	0.62	0.08	NC	40.3	71.4
	GM1	3.30	1.05	NC	0.51	42.0	108.5
Galb1,3GalNAc	None	0.16	0.50	-	-	125.0	90.9
	AsialoGM1	0.71	0.50	NC	0.11	136.1	42.3
	Asialofetuin	0.58	0.96	0.07	0.08	119.8	87.2
Asialofetuin	None	0.10	0.48	-	-	29.1	16.0
	AsialoGM1	0.26	0.56	NC	0.44	29.6	7.2

2.2. Galβ1,4GlcNAc α2,6-sialyltransferase (ST6Gal I)

ST6Gal I catalyzes the transfer of sialic acid residue with an α2,6-linkage to a terminal Gal residue of disaccharide (Galβ1,4GlcNAc) found as a free disaccharide or as a terminal *N*-acetyllactosamine unit of *N*- or *O*-linked oligosaccharide. ST6Gal I showed 4-7 fold higher activity toward CMP-NeuGc than CMP-NeuAc as donor substrate.

2.3. GalNAc α2,6-sialyltransferase (ST6GalNAc-family)

Six species of GalNAc α2,6-sialyltransferases have been cloned from various mammalian species (Table 1). Among them, ST6GalNAc IV was firstly found by our group [6]. Although the amino acid sequence of ST6GalNAc III and IV from mouse brain showed about 43% similarity, like in the case of ST3Gal I and II, recombinant ST6GalNAc III and IV exhibited the same acceptor substrate specificity, only utilizing the NeuAcα2,3Galβ1,3GalNAc trisaccharide sequence found on either *O*-glycan of glycoproteins or ganglioside GM1b. Their enzymatic

activities showed that ST6GalNAc III prefer ganglioside GM1b to *O*-glycan of glycoproteins, whereas ST6GalNAc IV is opposite, *i.e.* a strong preference for GM1b over *O*-glycan.

2.4. α2,8-sialyltransferase (ST8Sia-family)

Six species of α2,8-sialyltransferases have been cloned from various mammalian species (Table 1). Among them, human ST8Sia III and V have been cloned and characterized by our group. ST8Sia III exhibited activity toward NeuAcα2,3Galβ1,4GlcNAc structure on *N*-glycan of glycoproteins as well as those of glycolipids [7]. ST8Sia V exhibited activity toward gangliosides GD1a, GT1b and GD3, and therefore is involved in the biosynthesis of gangliosides such as GD1c, GT1a, GQ1b and GT3.

3. Structure of sialyltransfereases

Analysis of the deduced protein sequence indicated that sialyltransferases are type II transmembrane glycoproteins with a short NH_2-terminal cytoplasmic tail which is not essential for catalytic activity, and a 16-20 amino acid signal anchor domain that participates to the retention signal for these Golgi luminal enzymes. The stem region, highly variable in length (20-200 amino acids) is followed by a large COOH-terminal catalytic domain that resides in the lumen [2,3]. Despite sharing this topological similarity, the amino acid sequences of the cloned sialyltransferases show very little sequence identity with the exception of the two consensus sequences called sialylmotifs L and S [3].

4. References

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