Molecular basis for polysialylation: A novel polybasic polysialyltransferase domain (PSTD) of 32 amino acids unique to the α2,8-polysialyltransferases is essential for polysialylation

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Abstract To determine the molecular basis of eukaryotic polysialylation, the function of a structurally unique polybasic motif of 32 amino acids (pI~12) in the polysialyltransferases (polySTs), ST8Sia II (STX) and ST8Sia IV (PST) was investigated. This motif, designated the “polysialyltransferase domain” (PSTD), is immediately upstream of the sialylmotif S (SM-S). PolyST activity was lost in COS-1 mutants in which the entire PSTD in ST8Sia IV was deleted, or in mutants in which 10 and 15 amino acids in either the N- or C- terminus of PSTD were deleted. Site-directed mutagenesis showed that Ile275, Lys276 and Arg277 in the C-terminus of PSTD in ST8Sia IV, which is contiguous with the N-terminus of sialylmotif-S, were essential for polysialylation. Arg252 in the N-terminus segment of the PSTD was also required, as was the overall positive charge. Thus, multiple domains in the polySTs can influence their activity. Immunofluorescent microscopy showed that the mutated proteins were folded correctly, based on their Golgi localization. The structural distinctness of the conserved PSTD in the polySTs, and its absence in the mono-oligoSTs, suggests that it is a “polymerization domain” that distinguishes a polyST from a monosialyltransferases. We postulate that the electrostatic interaction between the polybasic PSTD and the polyanionic polySia chains may function to tether nascent polySia chains to the enzyme, thus facilitating the processive addition of new Sia residues to the non-reducing end of the growing chain. In accord with this hypothesis, the polyanion heparin was shown to inhibit recombinant human ST8Sia II and ST8Sia IV at 10 μM.

Keywords Polysialic acid (polySia) · Polysialyltransferase domain (PSTD) · ST8Sia II (STX) · ST8Sia IV (PST) · Neurobiology (neural cell adhesion molecules; N-CAM) · Cancer metastasis

Abbreviations

Sia sialic acid
polySia polysialic acids
N-CAM neural cell adhesion molecules
DP degree of polymerization
ST8Sia II (STX) ST8 α-N-acetylneuraminide
α-2,8-sialyltransferase II
ST8Sia IV (PST) ST8 α-N-acetylneuraminide
α-2,8-sialyltransferase IV
PolySTs polysialyltransferases
Endo-N Endo-N-acetylneuraminidase

Introduction

In mammalian cells, the α2-8-linked polysialic acid (polySia) glycotope is an oncodevelopmental, tumor-associated antigen that plays a key role in modulating cell-cell interactions, principally during embryonic development, neural plasticity and tumor metastasis [reviewed in 1,2]. The major carrier protein of polySia is the neural cell adhesion molecule (N-CAM), in which polySia extends tri- and tetraantenary N-linked glycans [3–5]. Polysialyltransferases (polySTs) are members of the gene family of sialyltransferases that catalyze synthesis of polySia chains by transferring multiple Sia residues from the donor substrate, CMP-Neu5Ac, to N- and O-linked oligosaccharides on acceptor glycoprotein [6–10]. During polysialylation, the growing polySia chains appear to remain bound to the polyST, a feature in common with processive enzymes,
e.g. DNA polymerases. The processive mechanism of polysialylation is in contrast to the distributive mechanism of monosialylation wherein the monosialyltransferases (monoSTs) catalyze the transfer of single Sia residue to their acceptor substrate before release [10]. A comparison between the enzymatic properties of the membrane-bound α2,8-polySTs and the monoST activities in 14-day old embryonic chick brain revealed a number of distinct differences [10].

Prior to molecular cloning of the eukaryotic polySTs in 1995 [11–14], extensive biochemical studies in the preceding decade led to identification in fetal rat brain of the first mammalian α2,8-polyST [6]. This activity was responsible for polysialylation of N-CAM. These studies were made possible by the earlier development in our laboratory of prokaryotic-derived probes that specifically recognized α2,8-linked polySia chains, and which allowed the temporal expression of polySia in developing neural tissue to be determined [15]. Subsequent studies using these probes led to the discovery that extended polySia chains (DP > 55 Sia residues) were expressed on N-CAM in human neuroblastoma cells [16]. Following the development of a new strategy to determine the DP of polySia chains on N-CAM that avoided acid hydrolysis prior to chromatographic profiling, sub-populations of chains extending up to DP~400 have now been shown to decorate N-CAM [17].

The two key mammalian polySTs that control polySia synthesis are designated ST8Sia II (STX) and ST8Sia IV (PST). Both enzymes have been cloned, sequenced and shown to be responsible for the polysialylation of N-CAM, the major carrier protein of polySia in vertebrates [11–14]. Both enzymes also share ~59% identity at the amino acid level, and their catalytic domains are located in the lumen of the Golgi complex [18]. The amino acid sequence of the human ST8Sia IV is 97% homologous with the hamster and mouse and codes for a protein with a predicted molecular mass of 41.2 kD [12]. Conserved disulfide bonds in the monooand polySTs have been described [reviewed in 19]. Chemical modification studies using the thiol-directed alkylating reagents, N-ethylmaleimide and iodoacetamide showed that at least one cysteinyln residue in the embryonic chick brain polyST was critical for polysialylation, but was of lesser importance for monosialylation catalyzed by the α2,3-, α2,6-, and α2,8- monoSTs [10]. This finding led to the hypothesis that a sulfhydryl residue may be involved as a “reactive thiol” in the initiation of polySia chain synthesis [20].

Both mono- and polySTs share at least four conserved amino acid motifs, sialylmotif L (long; SM-L), sialylmotif S (short; SM-S), sialylmotif VS (very short; SM-VS) and motif III consisting of 48, 23, 6 and 4 amino acids, respectively [21–25]. Studies on the monoST, ST6Gal I, led to the suggestion that SM-S was involved in the binding of CMP-Neu5Ac, the common donor substrate for all the sialyltransferases, whereas SM-L was postulated to participate in binding both CMP-Neu5Ac and acceptor glycoproteins [22,23]. While substitution of His residues (His348 in ST8Sia II and His331 in ST8Sia IV) in SM-VS eliminated polyST activity [9], the function of SM-VS in catalysis remains unknown. More recently, a possible fourth sialylmotif unique to the ST8Sia gene family was identified by computational analysis [26]. This 46 amino acid sequence, KTxxxTxNPSx(33)PAF, was postulated to be a linkage-specific sequence motif. But, since no direct biochemical or mutational analyses were carried out, it remained to be determined experimentally the possible role of this sequence in substrate recognition or N-CAM polysialylation.

To further identify protein domains within the polyST that are required for N-CAM polysialylation, Angata et al. constructed chimeric enzymes using the “catalytic domain” of ST8Sia IV with the corresponding segments of ST8Sia II and ST8Sia III [27]. While ST8Sia III has some modest homology with ST8Sia II and ST8Sia IV, it lacks the PSTD and cannot polysialylated N-CAM, although it may oligosialylate itself. The catalytic domain of ST8Sia IV was defined as comprizing amino acid residues 62 to 356 in the protein, and was identified initially on the basis of deletion analysis. These studies revealed that multiple protein domains within the polySTs appeared to be required for N-CAM polysialylation, and that these domains were distinct from those required for N-CAM recognition. This latter finding confirms the earlier conclusions reached by Colley et al. [28,29].

In contrast to the monoSTs, we discovered that both ST8Sia II and ST8Sia IV contain a structurally unique poly-basic motif (calculated pI ~12) of 32 amino acids immediately upstream and contiguous with SM-S [10]. We have now designated this domain as the “polysialyltransferase domain” (PSTD), since it is a structural motif unique to ST8Sia II and ST8Sia IV, and which distinguishes the polySTs from the mono-oligoSTs. Organization of the PSTD within ST8Sia IV is shown in the top of Fig. 1.

Discovery of PSTD in ST8Sia II and ST8Sia IV led us to hypothesize that the polybasic motif may be important for the processive synthesis of polySia to take place by tethering nascent polySia chains to the polySTs during the addition of new sialyl residues to the non-reducing termini of the growing chains [10]. To test experimentally the potential functional importance of the PSTD in polysialylation, we have determined the mono- and polyST activities in a series of mutants obtained by deletion and site-directed substitutions within the PSTD in ST8Sia IV. The results reported herein show that the PSTD is an essential motif required for polysialylation. Furthermore, the C-terminal Ile275, Lyg276 and Arg277 residues located at the juncture between the PSTD and SM-S are key residues of critical importance for polymer synthesis, as is the overall positive charge of the domain. Neither the monoSTs or the putative oligoST, ST8Sia III, contain