A Small-Molecule Modulator of Poly-α2,8-Sialic Acid Expression on Cultured Neurons and Tumor Cells


Poly-α2,8-sialic acid (PSA) has been implicated in numerous normal and pathological processes, including development, neuronal plasticity, and tumor metastasis. We report that cell surface PSA expression can be reversibly inhibited by a small molecule, N-butanoylmannosamine (ManBut). Inhibition occurs through a metabolic mechanism in which ManBut is converted to unnatural sialic acid derivatives that effectively act as chain terminators during cellular PSA biosynthesis.

The biological functions of cell surface oligosaccharides have been difficult to elucidate owing to the complexity of achieving genetic control over a molecule that is the product of multiple enzymes and thus of multiple genes. In a few well-studied cases, the function of a specific oligosaccharide epitope has been determined, enhancing our understanding of cell-cell recognition (I). Still, few such structures have been assigned a specific purpose. Small molecules that disrupt or activate a target process in a cellular context have provided insights in systems that are difficult to manipulate with traditional genetic methods (2). The ability to block the expression of a specific oligosaccharide epitope by use of a small molecule would facilitate the study of oligosaccharide function.

PSA (Fig. 1A), a linear homopolymer of α2,8-linked sialic acid residues, is found mainly on the neural cell adhesion molecule (NCAM) (3, 4). Its biosynthesis is mediated by polylactosyltransferases, the best-characterized human homologs of which are ST8Sialid (STX) and ST8SialIV (PST) (5–7). Both enzymes catalyze the iterative formation of α2,8-sialic acid linkages using cytidine 5′-monophosphate (CMP)–sialic acid as a substrate. PSA is abundant in the central nervous system during fetal development but is restricted to those regions of the adult brain associated with synaptic plasticity (8–10).

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In addition, PSA is a marker of several tumors including neuroblastomas, small cell lung carcinomas, and Wilms tumor (11, 12). It has been implicated in tumor metastasis and the complex neural processes involved in learning and memory (3, 13). We report here a small-molecule modulator of PSA expression.

The cellular machinery for conversion of N-acetylmannosamine (ManNac, Fig. 1B) to CMP–sialic acid tolerates conservatively altered N-acyl substituents (14). Thus, administration of N-propanoylmannosamine (ManProp, Fig. 1B) or N-butanoylmannosamine (ManBut) to cultured cells and laboratory animals results in biosynthesis of the corresponding CMP–sialic acid analogs and the appearance of unnatural sialic acid residues on cell surface glycoproteins. In most glycoconjugates, sialic acid residues occupy terminal α2,3- or α2,6-linkages to galactose; replacement of some fraction of these residues with an unnatural variant has no discernible effect on their abundance (15–17).

By contrast, in PSA sialic acid occupies both α2,3- and α2,6- linkages to galactose; replacement of some fraction of these residues with an unnatural variant has no discernible effect on their abundance (15–17).

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lated NCAM normally migrates on SDS–polyacrylamide gel electrophoresis as a diffuse band with an apparent molecular size greater than 200 kD; without PSA chains, NCAM appears as up to three isoforms with apparent molecular sizes of 120, 140, and 180 kD (22). ManProp had no effect on the apparent molecular size of polysialylated NCAM (PSA-NCAM) (Fig. 2A), suggesting that the polymer’s length, and therefore biosynthesis, is unaffected. NT2 neurons cultured with 10 mM ManProp lost PSA immunoreactivity, but OB11 staining confirmed the presence of the polymer at its normal size. The loss of PSA immunoreactivity is likely due to incorporation of N-propanoyl sialic acid into the polymer and concomitant disruption of antibody recognition (23).

Unlike ManProp, ManBut did inhibit PSA biosynthesis on NCAM in NT2 neurons. PSA mAb 735 staining was reduced in the presence of 1 mM ManBut (Fig. 2B), and NCAM mAb OB11 staining confirmed a corresponding reduction in the molecular size of the glycoprotein. Inhibition was essentially complete in the presence of 3 mM ManBut, as demonstrated by the appearance of the 140- and 180-kD isoforms of unmodified NCAM.

To determine the effects of ManBut treatment on other cell types, we incubated SH-SY5Y cells (human neuroblastoma) (12), H345 cells (human small cell lung carcinoma) (24), and HeLa cells (human cervical carcinoma) stably transfected with the 140-kD isoform of NCAM and the human polysialyltransferase STX (HeLa-NCAM-STX) (25) with ManProp or ManBut. In all cases, ManProp had no effect on PSA biosynthesis, whereas ManBut inhibited the process (19). We confirmed that ManBut is not a general inhibitor of sialylation by analyzing its effects on total cellular sialosides using the periodate-resorcinol assay (19, 26).

Inhibition of PSA biosynthesis by ManBut was reversible. PSA expression was completely inhibited on HeLa-NCAM-STX cells treated with 5 mM ManBut for 24 hours, but returned 24 hours after ManBut was removed from the medium (Fig. 3, A and B). Thus, ManBut disrupts polysialylation in a time-dependent and reversible manner.

Given that ManBut is readily converted to the corresponding unnatural sialoside in other linkage forms (i.e., α2,3 and α2,6), we reasoned that inhibition of PSA biosynthesis is exerted at the level of polysialyltransferase activity. The polysialyltransferases may use unnatural variants of their donor (CMP–sialic acid) and acceptor (NCAM-bound sialic acid) substrates with reduced efficiency according to the size of the N-acyl groups. To examine this possibility,
we investigated unnatural PSA biosynthesis in vitro using CMP–N-propanoyl sialic acid (CMP-SiaProp) or CMP–N-butanoyl sialic acid (CMP-SiaBut) (27), recombinant protein A fusions of the STX and PST catalytic domains (28), and the extracellular domain of NCAM expressed as a Fc fusion (NCAM-Fc) (29). The apparent molecular size of NCAM-Fc after enzymatic modification provided a measure of PSA biosynthesis.

CMP-SiaBut was used by the polysialyltransferase STX less efficiently than either STX or PST. NCAM-Fc primed with unnatural sialic acids by treatment with STX and either CMP-SiaProp (lanes 3 and 4 and lanes 9 and 10, Prop) or CMP-SiaBut (lanes 5 and 6 and lanes 11 and 12, But). The primed NCAM-Fc was then isolated and reacted with either STX or PST and 10 nmol CMP-Sia (+) or buffer (–) for 4 hours at 37°C, and compared with unprimed NCAM-Fc controls (lanes 1 and 2 and lanes 7 and 8, U). Samples were analyzed by Western blot as before.

References and Notes
7. Supplementary Web material is available on Science Online at www.sciencemag.org/cgi/content/full/294/5541/380/DC1.
9. Simple N-acyl mannosamine derivatives exhibit no toxicity with cultured cells, even at high millimolar concentrations (17). The peracetylated forms of these sugars can be used at much lower concentrations (10 to 20 μM) to achieve similar effects.
13. The CMP–sialic acid analogs were synthesized as described (30). Experimental details are provided in (19).
14. Either enzyme is sufficient for PSA biosynthesis. NT2 neurons and SH-SY5Y cells express both enzymes (12). The expression of STX and PST in H345 cells has yet to be determined.
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