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SHORT COMMUNICATION

The generation and characterization of a rat neural cell line overexpressing the α2,6(N) sialyltransferase

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In order to examine the effects of altered protein sialylation on neural cell function, B104 rat neuroblastoma cells were stably transfected with the cDNA coding for α2,6(N) sialyltransferase (ST(6)N). Lectin blot analysis of the clones demonstrated an increase in staining of the *Sambucus nigra* lectin, which detects α2,6 linked sialic acid, in parallel with enzyme activity. There was a concomitant decrease in staining by the *Maackia amurensis* lectin which labels α2,3-linked sialic acid, indicating that the individual sialyltransferase enzymes may compete for penultimate galactose acceptor sites. While there was an initial increase in protein-bound sialic acid in parallel with enzyme activity, the sialylation of the cells was demonstrated to be saturable. There was an inverse relationship between cell adhesion to a fibronectin substrate and ST(6)N activity suggesting that the negatively charged sugar acts to modulate cell-substrate interaction. These cells will provide an ideal model system with which to further investigate the effect of altered sialic acid on neural cell function.

***Keywords*: sialyltransferase, sialoglycoproteins, transfection, adhesion, neuron**

Introduction

Sialic acid is a negatively charged sugar which can be attached to penultimate galactose (Gal), *N*-acetylglucosamine (GlcNAc) or *N*-acetylgalactosamine (GalNAc) groups of both glycoproteins and glycolipids. In addition, it can form polysialic acid chains of up to 50 residues in length via the 8-hydroxyl group [1]. The transfer of the activated form of sialic acid, CMP-NeuNAc, onto the acceptor sugar is catalysed by the sialyltransferase (ST) group of enzymes. These are membrane-bound proteins located almost exclusively in the *trans*-Golgi and trans Golgi network, with the catalytic domain facing into the lumen. Because there is a specific enzyme for the mediation of each transfer reaction, the existence of up to 19 individual ST enzymes has been proposed [2]. Despite the similarity in the reaction catalysed, all of the ST enzymes cloned to date exhibit very little homology except for a short consensus sequence, called the sialyl motif, to which the activated sugar donor is thought to bind [3].

Because of its negative charge, sialic acid, particularly in the form of α2,8-linked polysialic acid (PSA), plays a particularly important role in the modulation of cell–cell interaction in the nervous system. During the early stages of development coincident with neurite outgrowth, PSA is expressed on the neural cell adhesion molecule (NCAM) at high levels to provide a permissive substrate along which the neurites can travel. Coincident with synaptogenesis, however, the levels of PSA decrease dramatically thus allowing the generation of synaptic contacts [4].

To date, relatively little is known about the control of ST enzyme expression and activity in the brain and particularly how an alteration in this expression may alter neural function. We have previously demonstrated that neural ST activity is under the constitutive control of corticosteroids *in vivo* [5] and that this activity may be altered in certain disease states [6, 7]. However, it is difficult in these cases to attribute any functional changes solely to alterations in ST activity. Therefore, the aim of this study was to generate a number of stable neural cell lines transfected with the Galβ1-4GlcNAc α2,6 ST (ST6(N)) enzyme which could then be used to examine in detail the effect of altered enzyme activity on neural cell function.

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Materials and methods

The B104 rat neuroblastoma cell line was maintained as previously described [8] in Dulbecco's modification of Eagle's medium (DMEM, Gibco) containing 10% (by vol) fetal calf serum, 1% (by vol) penicillin and 1% (by vol) streptomycin.

The rat ST6(N) cDNA under the control of a CMV promoter (STMYC plasmid) was a kind gift of Dr Sean Munro, MRC, Cambridge [9]. Because this construct did not contain any antibiotic resistance markers, the cells were co-transfected with a trace amount of the pcNeo plasmid which codes for the neomycin resistance gene (Stratagene). The cells were transfected using calcium chloride precipitation of DNA [10]. Geneticin (Sigma) was subsequently added to the cells at 500 $\mu g\,ml^{-1}$ in order to select for the cells that had taken up the pcNeo plasmid and after 7 days, individual colonies were removed by trypsinization and sub-cloned. For biochemical analysis, the clones were cultured to confluency and harvested by scraping.

The total sialyltransferase activity of the cell pellets was determined using cytidine-5-monophosphate-4,5,6,7,8,9-^{14}C-*N*-acetylneuraminic acid (CMP-^{14}C-NeuNAc, Radiochemical Centre, Amersham; specific activity 293 $mCi\,mmol^{-1}$) as the sialic acid donor and asialofetuin (Sigma) as an exogenous acceptor [11]. The expression of cellular sialoglycoproteins was determined by lectin blot analysis as previously described [12] using the *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA) lectins which detect sialic acid in α2,3 and α2,6 linkages respectively (DIG glycan differentiation kit, Boehringer Mannheim). The total cellular sialic acid content was determined using a modification of the thiobarbituric acid assay [13].

The strength of cell adhesion to a fibronectin substratum (20 $\mu g\,ml^{-1}$) was determined using a centrifugal shear assay [14]. The cells were seeded at a density of 2×10^5 cells per cover slip in a six well tissue culture plate. The cells were permitted to adhere to the substrate for 24 h and were then metabolically labelled for 24 h in methionine-free medium (Sigma) containing 2 μCi ^{3}H methionine (Amersham) per well. The cover slips were then removed (at least six for each assay point) and placed in a 30 ml universal container containing 10 ml of phosphate-buffered saline and were centrifuged at $4500 \times \mathbf{g}$ for 10 min. The radioactivity remaining on the cover-slips was expressed as a percentage of the radioactivity on coverslips which were not spun (total cell count).

Results

A significant number of clones with varying ST activities were obtained in the study with activities ranging up to 10 times control activity. A subset of clones of varying enzyme activity were chosen for detailed study. An increase in total ST activity was accompanied by an increase in SNA lectin staining of α2,6 sialoglycoproteins (Figure 1a,c). This increase in ST activity was accompanied by a decrease in MAA lectin staining of α2,3-linked sialoglycoproteins (Figure 1b,c). While there was an initial increase in the total cellular protein-bound sialic acid pool with enzyme activity, this was seen to be saturable and further increases in enzyme activity did not alter the total protein sialic acid content (Figure 1d).

The functional effects of altered ST activity were investigated by determining the adhesion of the cells to a fibronectin substrate. There was a significant negative correlation between the total ST activity and the adhesion of the cells to the substrate (Figure 2) indicating that an increase in the negatively-charged cell surface sialic acid results in a decrease in cell adhesion.

Discussion

While sialic acid has been proposed to play a major role in nervous system function, few studies have attempted to further investigate this role by altering its cellular expression levels. While certain drugs can alter ST activity, these also have other cellular effects so it is difficult to dissociate these from the ST-related effects [5, 15]. Therefore, we have generated a neural cell line which overexpresses the ST6(N) enzyme to examine the effect of altered ST action and sialic acid on cellular function.

Overexpression of the enzyme resulted in an increase in the SNA lectin labelled α2,6 sialoglycoproteins. This demonstrates that the transfected enzyme is functional and is expressed, at least in part, in the correct subcellular compartment so that it can transfer sialic acid onto the penultimate galactose acceptor residues. The increase in SNA lectin staining corresponded well with the total cellular ST activity. However, the increase in SNA lectin staining was associated with a parallel decrease in MAA lectin staining, representing a down-regulation of the terminal α2,3-linked sialic acid residues (Figure 1c). This suggests that the ST3(N) and ST6(N) enzymes may compete for a limited pool of penultimate galactose acceptor sites and thus, as the ST6(N) enzyme activity increases, it has the ability to sialylate more galactose residues, leaving fewer available for the transfer of the α2,3-linked sialic acid. This is in good agreement with previous results both *in vitro* [16, 17] and *in vivo* [12] which proposed a functional interaction between the two controlling ST enzymes. This would further explain why, despite a significant increase in cellular ST6(N) activity, there was only a modest increase in total cell-surface sialic acid which appeared saturable (Figure 1d). A small increase in ST activity over control levels resulted in an increase in cellular sialic acid of approximately 50%. Subsequent increases in ST activity did not further increase sialic acid levels indicating that the cells may now have reached saturation level, again suggesting that the majority of the N-linked sugars

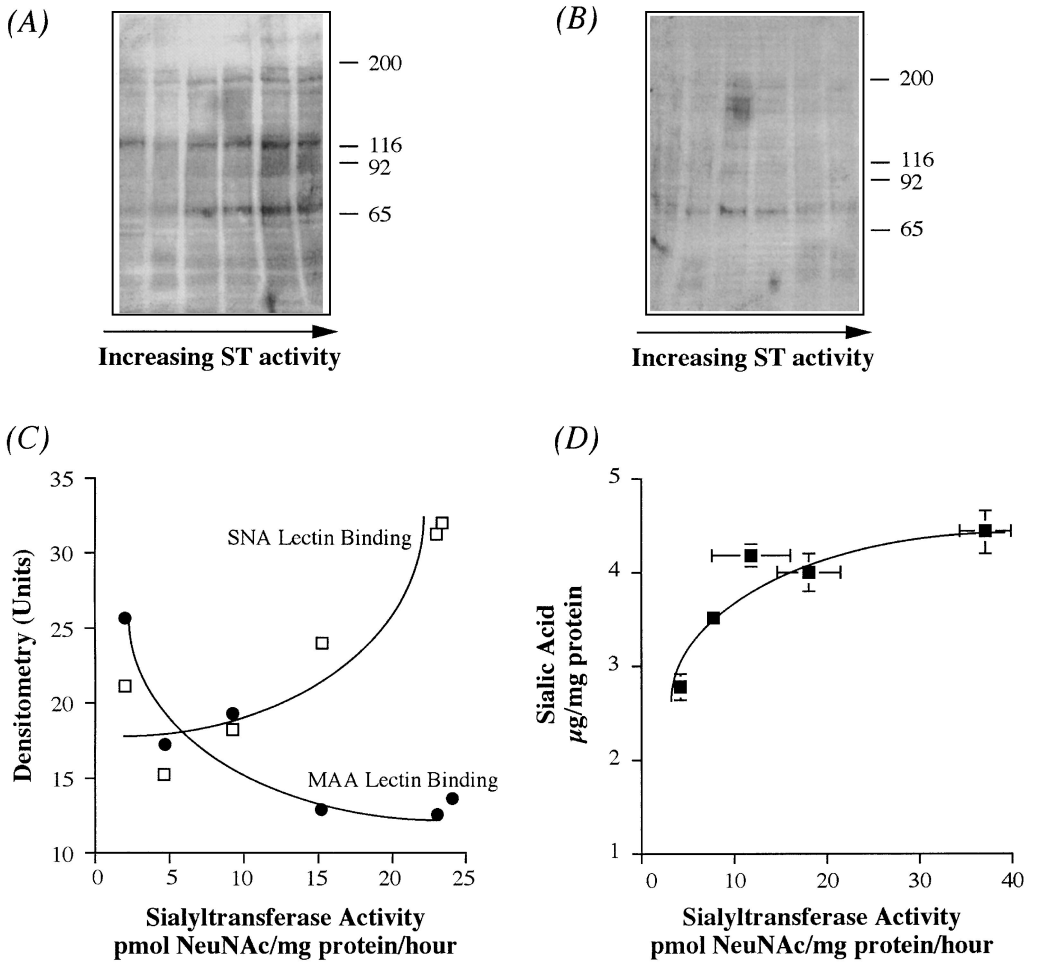

Figure 1. (a) SNA lectin and (b) MAA lectin staining of cellular sialoglycoproteins (50 μg per lane) in transfected B104 mouse neural cells with increasing ST activities; (c) correlation between densitometric analysis of the lectin-labelled sialic acid and ST activity; (d) relationship between protein-bound sialic acid and cellular ST activity.

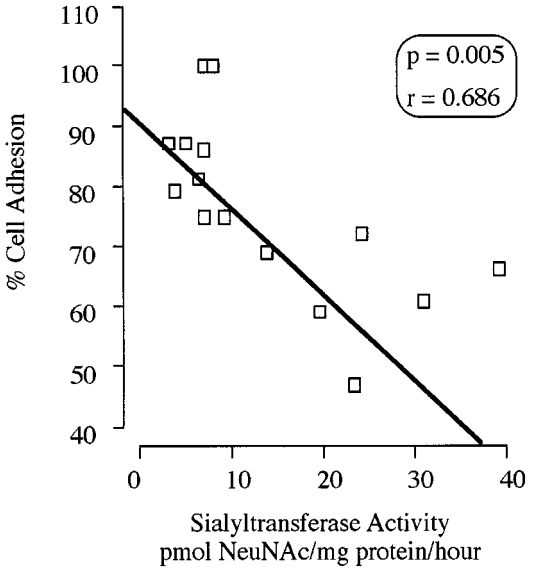

Figure 2. The relationship between cellular ST activity and adhesivity to a fibronectin substrate.

may already contain a terminal sialic acid residue in the control (untransfected) cells. Therefore, rather than observing a major change in total sialic acid levels in the transfected cells, there is rather a subtle change in the linkages of the sialic acid residues present.

Because of its negative charge and its presence on cell surface glycoproteins, sialic acid has been demonstrated to modulate the binding properties of adhesion ligands. The integrin family of cell surface glycoconjugates mediate fibronectin adhesion and these have been demonstrated to contain sialic acid residues which may modulate their adhesive potential [18, 19]. Therefore, an increase in ST activity may increase the sialylation of the cell surface integrins and thus alter their ability to bind to fibronectin. It is also possible that the change from α2,3- to α2,6-linked sialic acid may alter the functionality of the cell surface proteins that play a role in the modulation of cell adhesion.

The increase in cellular sialic acid levels in the clonal cell lines generated in this study has been demonstrated to

modulate the adhesive characteristics of the cells. This demonstrates that the transfected ST6(N) is functionally active and is capable of modulating the cell sialylation state. Further studies will be required in order to investigate other functional consequences of the increase in ST6(N) activity.

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