

Developmental control of N-CAM sialylation state by Golgi sialyltransferase isoforms

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Summary

A rat brain Golgi sialyltransferase activity capable of the differentiation-dependent control of N-CAM sialylation state is described. The specific activity of Golgi sialyltransferase was found to be developmentally regulated with respect to both endogenous and exogenous protein acceptors, with a particular elevation on postnatal days 10–12 when the heavily sialylated or 'embryonic' form of N-CAM is re-expressed. The subsequent developmental decrease in activity was associated with a significant decrease in apparent K_m for the CMP-NeuNAc substrate, but not for the asialofetuin exogenous acceptor, which could not be attributed to the temporal expression of an endogenous competitive inhibitor. The apparent V_{max} remained constant for CMP-NeuNAc but was significantly reduced for asialofetuin. Sialyltransferase activity, which was optimal at pH 7.0–7.5, was also modulated by various cations. Zinc abolished enzyme function, in contrast to ferric ions which stimulated

activity fourfold–sevenfold. The marked activation of the adult form of the enzyme by potassium and magnesium ions, together with the alterations in kinetic constants, suggested this activity to be distinct from that derived from postnatal day-12 tissue. The kinetics of [14 C]sialic acid incorporation into immunoprecipitated N-CAM demonstrated the individual polypeptides to be sialylated, possibly by addition of polysialosyl units, in a developmental sequence. The presence of four distinct sialyltransferase activities was demonstrated by non-denaturing gel electrophoresis followed by solid-phase enzyme assay. These isoforms were temporally expressed during development, two being correlated with the postnatal re-expression of the 'embryonic' form of N-CAM.

Key words: N-CAM, sialylation, Golgi sialyltransferase, isoforms, postnatal development, rat brain.

Introduction

The neural cell adhesion molecule (N-CAM) is believed to be a morphoregulator of developmental and functional neuroplasticity (Edelman, 1986; Daniloff *et al.* 1986). In the central nervous system it comprises three polypeptides whose extracellular domains are identical but which differ in those regions associated with membrane attachment (Cunningham *et al.* 1987). The two largest polypeptides (N-CAM₁₈₀ and N-CAM₁₄₀) are integral membrane proteins with substantial cytoplasmic domains. The smallest polypeptide (N-CAM₁₂₀) lacks the membrane-spanning segment and is attached to the cell surface by a phosphatidylinositol-containing anchor. These polypeptides are believed to mediate their cell-adhesive actions by a homophilic binding

mechanism the strength of which is inversely proportional to the sialylation state of apposing molecules (Hoffman & Edelman, 1983). During early development, embryonic N-CAM (E-form) contains up to 30% (wt/wt) of α -2,8-linked polysialic acid attached to a tri- or tetra-antennary carbohydrate core typical of those normally encountered in N-linked glycoproteins (Finne 1982; Hoffman *et al.* 1982; Finne *et al.* 1983; Finne & Makela, 1985). This sialylation decreases with age, thereby increasing the adhesivity necessary for final morphogenesis. This embryonic to adult conversion (E to A) has been demonstrated to be regulated by a Golgi-associated poly α -2,8-sialosyl-sialyltransferase activity (McCoy *et al.* 1985).

Postnatal histogenesis is also associated with alterations in N-CAM sialylation state (Meier *et al.* 1984;

Chuong & Edelman, 1984). This is controlled by a developmentally regulated Golgi sialyltransferase activity and no cell surface modulation of N-CAM sialylation has been demonstrated (Breen & Regan, 1986; Breen *et al.* 1987). During postnatal development, a unique differentiation-dependent sialylation of the individual N-CAM polypeptides occurs (Breen & Regan, 1988a). N-CAM₁₂₀ is sialylated at all postnatal periods, N-CAM₁₄₀ at times of cell migration and N-CAM₁₈₀ with differentiation and final synaptogenesis. Further the embryonic form (HMr) was found to be re-expressed at times of extensive fibre outgrowth. This sialylation pattern is consonant with suggestions that N-CAM polypeptides are functionally distinct, as N-CAM₁₄₀ is highly mobile in the plane of the membrane (Pollerberg *et al.* 1986) whereas N-CAM₁₈₀ is associated with differentiated neurones (Pollerberg *et al.* 1985; Chuong *et al.* 1987) and points of cell-cell contact (Pollerberg *et al.* 1985).

To date, the mechanism(s) which would mediate a differentiation-dependent sialylation of N-CAM polypeptides remain obscure. In approaching this problem, we have now demonstrated that the developmental regulation of Golgi sialyltransferase is not mediated by an endogenous inhibitor as previously suggested (Duffard & Caputto, 1972) but rather by the sequential appearance of four isozymes, two of which are represented only at times when 'embryonic' (HMr) N-CAM is re-expressed postnatally.

Materials and methods

Golgi-enriched fractions were obtained from Wistar rat brain of increasing postnatal age as previously described (Breen *et al.* 1987). The sialyltransferase activity associated with these fractions was measured using cytidine 5'-monophospho-*N*-acetyl-4,5,6,7,8,9-[¹⁴C]neuraminic acid (CMP-¹⁴C-NeuNAc; sp. act. 290 mCi mmol⁻¹, Radiochemical Centre, Amersham) as sugar donor with an asialofetuin (ASF; Sigma Chemical) acceptor for exogenous transfer and Golgi membrane acceptors for endogenous transfer. The exogenous transfer was carried out in 50 mM-Tris-HCl, pH 7.0, containing 10 mM-MgCl₂, 3 mg ml⁻¹ Triton X-100, 1 mg ml⁻¹ ASF and 2 mg ml⁻¹ of the Golgi preparation and was initiated by the addition of 50 nCi CMP-¹⁴C-NeuNAc. Endogenous transfer was carried out in 50 mM-Tris-HCl, pH 7.0, containing 10 mM-MgCl₂, 2 mg ml⁻¹ of the Golgi preparation and 0.5 mM of the sialidase inhibitor 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (dehydro-NeuNAc; Sigma Chemical) and was initiated by the addition of 1 μCi CMP-¹⁴C-NeuNAc. Exogenous transfer was carried out for 45 min at 37°C in a final volume of 420 μl and endogenous transfer for 6 h at 37°C in a final volume of 200 μl. Each reaction was linear with respect to time and protein concentration. Both reactions were terminated by making the solution 6% with trichloroacetic acid and 0.5% with phosphotungstic acid. The precipitated protein was gathered by centrifugation (12 000, 3 min), washed and

solubilized overnight in Soluene-350 (United Technologies, Packard), and counted by liquid scintillation spectrometry. The controls contained a heat-inactivated enzyme preparation. The effect of various ions on Golgi sialyltransferase was ascertained by replacing the MgCl₂ in the reaction buffer with the relevant chloride salt at a final concentration of 10 mM.

Endogenous transfer of [¹⁴C]sialic acid to Golgi-associated N-CAM was carried out using 10- and 12-day fractions as previously described (Breen *et al.* 1987; Breen & Regan, 1988a). Briefly ¹⁴C-sialylated Golgi membranes were solubilized with 2% Nonidet-P40 (NP-40) in 100 mM-Tris-HCl, pH 7.0, containing 5 mM dehydro-NeuNAc. The solubilized N-CAM was immunoprecipitated with a N-CAM-specific polyclonal antibody (final dilution 1:150) (Sheehan *et al.* 1986) and the antigen-antibody complexes isolated using *Staphylococcus aureus* (Cowan strain)-immobilized protein-A. After washing, the immunocomplexes were solubilized by boiling in electrophoresis sample buffer and separated on 10% acrylamide gels essentially according to the procedure of Liu & Greengard (1976). After electrophoresis, the gel lanes were horizontally sliced into 2 mm sections, solubilized with NCS tissue solubilizer (Amersham, UK) and counted by liquid scintillation spectrometry. The gels were calibrated using ¹⁴C-methylated protein molecular weight markers (average sp.act. 36 μCi μg⁻¹ protein; Amersham International).

The presence of an endogenous competitive inhibitor was ascertained by including up to 1 mg ml⁻¹ of cytosolic protein in the exogenous transfer assay. The cytosolic fraction was obtained during the preparation of the Golgi fractions as previously described (Breen & Regan, 1987). Briefly the S2 supernatant obtained in the preparation of the P2 pellet was layered onto a cushion of 1.6 M-sucrose and centrifuged (100 000 g, 2 h) and the resulting supernatant used as a cytosolic fraction. The cytosol was dialysed for 18 h against 2 l of distilled water at 4°C to remove the sucrose and then concentrated 10-fold with a Minicon Macrosolute Concentrator (Amicon Corp.) which gave a protein recovery of approximately 85%. Aliquots of concentrated cytosol were stored at -20°C until required.

Individual Golgi sialyltransferase activities were demonstrated by a combination of non-denaturing gel electrophoresis and solid-phase enzyme assay. Golgi fractions of increasing postnatal age were solubilized in 50 mM-Tris-HCl, pH 7.0, containing 2% NP-40. The samples were then separated on 10% acrylamide gels as described above. Both the gel and running buffer contained 0.1% sodium dodecyl sulphate (SDS) which enhanced separation but had no effect on enzyme activity. After separation the proteins were electrophoretically transferred (Towbin *et al.* 1979) to nitrocellulose which had been previously been coated for 1 h with 1 mg ml⁻¹ asialofetuin in 50 mM-Tris-HCl, pH 7.0. This was incubated at 37°C for 1 h in buffer containing 1 nCi ml⁻¹ CMP-¹⁴C-NeuNAc. The paper was then washed extensively in Tris-HCl, pH 7.0, containing 1% NP-40 and 0.9% NaCl, dried and the gel lanes cut into 2 mm slices. The radioactivity associated with each slice was determined by liquid scintillation counting.

All protein concentrations were estimated according to the procedures of Lowry *et al.* (1951).

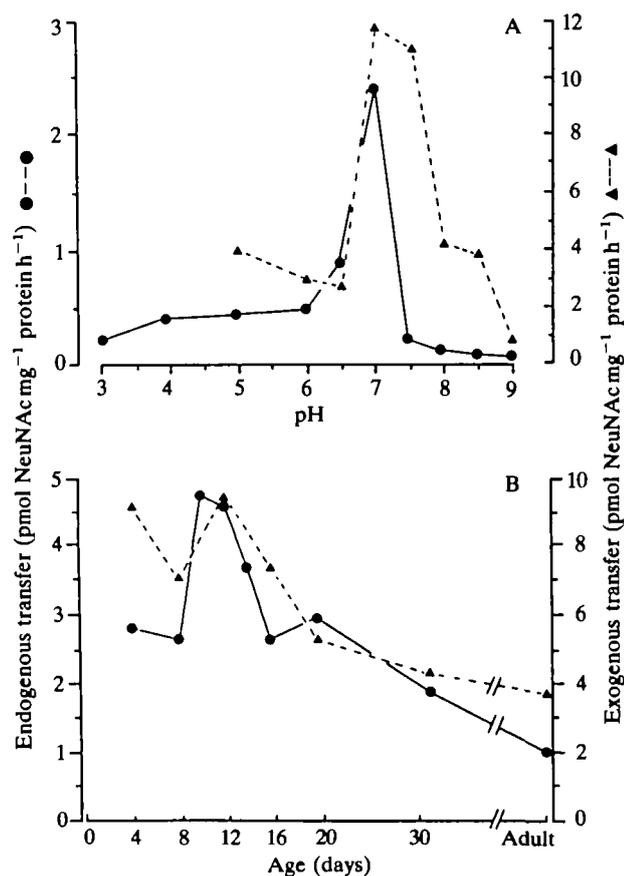


Fig. 1. The pH dependence (A) and developmental regulation (B) of exogenous and endogenous Golgi sialyltransferase activity. The pH dependence was estimated using 12-day Golgi fractions. Values are expressed as pmol NeuNAc transferred to either ASF (exogenous) or Golgi acceptors (endogenous) mg^{-1} enzyme protein h^{-1} and are the mean of three independent observations. In no case did the standard errors exceed 10% of the mean.

Results

Golgi sialyltransferase activity was found to be strictly pH dependent and developmentally regulated with respect to both exogenous and endogenous protein acceptors (Fig. 1A,B). In general exogenous transfer was found to be 5–6 times greater than that seen for endogenous transfer but both showed a fivefold increase in activity between pH 7.0–7.5 (Fig. 1A). Exogenous and endogenous activities were also developmentally regulated (Fig. 1B). A sharp and transient rise in activity was apparent from postnatal days 8–12 and this was particularly marked in the case of endogenous transfer. Following this the activity decreased to an adult level which was approximately 20% and 30% of maximal activity at day 12 for endogenous and exogenous transfer, respectively. The most intense phase of this decrease occurred between postnatal days 12 and 16.

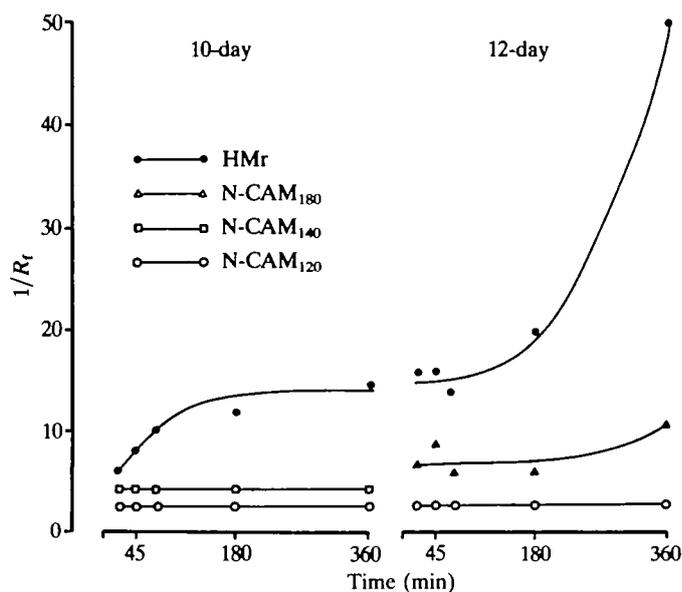


Fig. 2. The effect of time-dependent transfer of [¹⁴C]sialic acid on N-CAM polypeptide mobility. Golgi N-CAM was [¹⁴C]sialylated by endogenous Golgi sialyltransferase activity, immunoprecipitated and separated on 10% acrylamide gels. Polypeptide position was located by counting solubilized gel slices (2 mm).

Sialyltransferase-mediated sialylation of N-CAM polypeptides was demonstrated in Golgi fractions from postnatal days 10 and 12. As previously described (Breen & Regan, 1988a) only N-CAM₁₄₀ and N-CAM₁₂₀ were sialylated at postnatal day 10 and N-CAM₁₈₀ and N-CAM₁₂₀ on postnatal day 12 (Fig. 2). The HMr or 'embryonic' form was also produced at both time points and thus was derived from N-CAM₁₄₀ and/or N-CAM₁₂₀ on day 10 and N-CAM₁₈₀ and/or N-CAM₁₂₀ on day 12. The kinetics of [¹⁴C]sialic acid incorporation also varied between postnatal days 10 and 12. Production of HMr from N-CAM₁₄₀ and/or N-CAM₁₂₀ showed an initial sharp increase in mobility followed by a plateau which was suggestive of saturation. In contrast, the HMr produced from N-CAM₁₈₀ and/or N-CAM₁₂₀ showed little change in mobility up to 3 h but this was followed by a sharp increase which did not appear to be saturable over the time period examined. The molecular weight differences in the initial appearance of the HMr form at days 10 and 12 are assumed to reflect the size differences of the contributing polypeptides. Further at no time were intermediate molecular weights noted between the HMr form and its contributing polypeptides.

In three independent observations, this developmental decrease in Golgi sialyltransferase activity was associated with a significant decrease in affinity (apparent K_m) for CMP-NeuNAc but not asialofetuin (Table 1). In contrast the apparent V_{max} remained constant with respect to the CMP-NeuNAc substrate

Table 1. Kinetic parameters for Golgi sialyltransferase activity in 12-day and adult animals

Substrate	12-day		Adult	
	K_m	V_{max}	K_m	V_{max}
CMP-NeuNAc	0.80 ± 0.24	1.25 ± 0.16	2.54 ± 0.95*	1.13 ± 0.28
Asialofetuin	0.47 ± 0.28	0.77 ± 0.09	0.28 ± 0.04	0.41 ± 0.05*

The apparent K_m is as mM-CMP-NeuNAc or mg ml⁻¹ ASF and the apparent V_{max} as nmoles NeuNAc mg⁻¹ protein h⁻¹. All values are the mean ± s.e.m. ($n = 3$) and those significantly ($P < 0.05$) different from the adult value are indicated by an asterisk.

Table 2. Inhibition of 12-day Golgi sialyltransferase activity by a soluble endogenous inhibitor

Addition	% Control activity
12-day cytosol	64 ± 6
Adult cytosol	56 ± 6
Adult cytosol + 0.5 mM dehydro NeuNAc	53 ± 5
Boiled adult cytosol	91 ± 8

In all cases, the reaction tubes contained 1 mg of total soluble protein and the values (mean ± s.e.m., $n = 3$) are expressed as a percentage of the control activity to which an equal volume of 0.9% NaCl had been added.

Table 3. Ion dependence of Golgi sialyltransferase obtained from 12-day and adult animals

Ion	12-day	Adult
K ⁺	62 ± 10	167 ± 15*
Na ⁺	100 ± 17	133 ± 25
Ca ²⁺	91 ± 7	76 ± 15
Mg ²⁺	116 ± 1	173 ± 9*
Mn ²⁺	83 ± 6	77 ± 44
Zn ²⁺	6 ± 5	2 ± 2
Fe ³⁺	417 ± 25	694 ± 49*

The chloride salt (10 mM) was used in all cases and the values (mean ± s.e.m., $n = 3$) are expressed as a percentage of the control activity in which no ion was present. Activities differing significantly ($P < 0.05$) between 12-day and adult samples are indicated by an asterisk.

but was significantly decreased ($P < 0.05$) for the asialofetuin acceptor protein (Table 1). As these findings suggested the presence of a competitive endogenous inhibitor, the effect of a cytosol fraction on enzyme activity was ascertained. Concentrated cytosol from 12-day and adult animals contained an inhibitor which decreased sialyltransferase activity but was not found to be developmentally regulated (Table 2). This inhibition could not be attributed to the presence of a cytosolic sialidase as an inhibitor of this enzyme had no effect (Table 2). This inhibitor appeared to be a protein as boiling destroyed its activity.

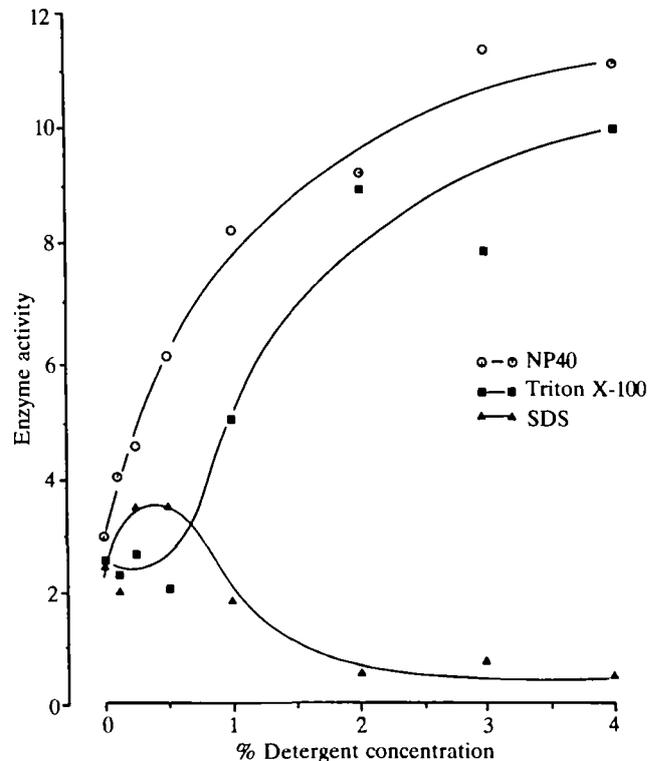


Fig. 3. The effect of varying detergent concentration on 12-day Golgi sialyltransferase activity. Values are expressed as pmol NeuNAc mg⁻¹ ASF h⁻¹ and are the mean ± s.e.m. ($n = 3$).

In addition to altered enzyme kinetics, the developmental regulation of Golgi sialyltransferase was also associated with differential ion sensitivities (Table 3). Optimal sialyltransferase activity showed a marked ion dependency. In both 12-day and adult preparations, Zn²⁺ totally abolished enzyme activity. In contrast, the trivalent ferric ion had a fourfold–sevenfold stimulatory effect which was most pronounced in the adult preparation. However both K⁺ and Mg²⁺ were found to stimulate differentially the sialyltransferase activity derived from adult animals. This difference was most marked with K⁺ which also had an inhibitory effect on 12-day preparations. These observations support the kinetic parameters in that they suggest Golgi sialyltransferase to be developmentally regulated by a temporal expression of alternating isozyme patterns.

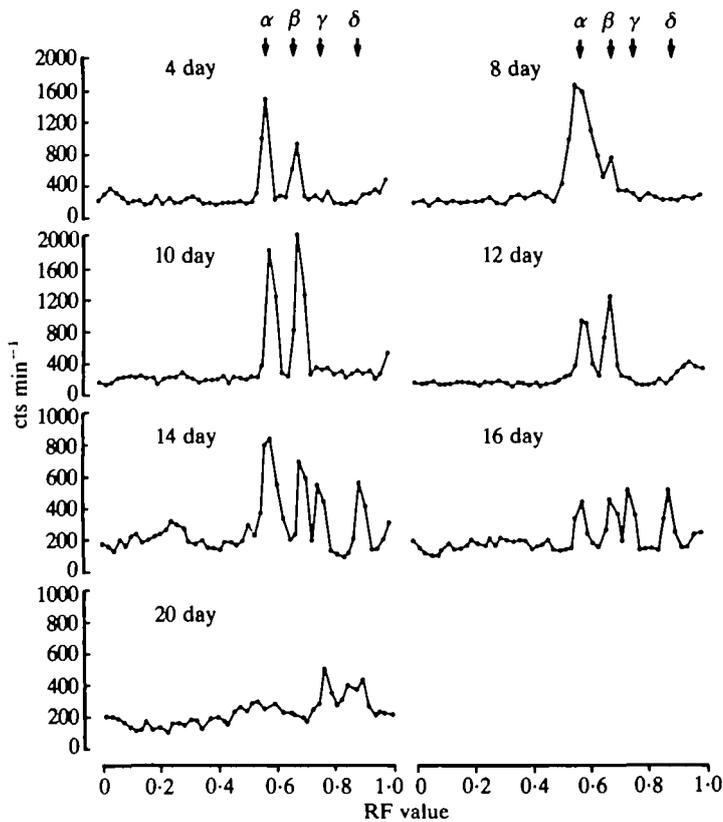


Fig. 4. Electrophoretic analysis of individual sialyltransferase activities in Golgi fractions of increasing developmental age. The protein samples (50 μ g) were fractionated on 10% acrylamide gels, transferred to an ASF-coated nitrocellulose sheet and enzyme activity estimated by incubating the paper with 1 μ Ci CMP- 14 C-NeuNAc ml $^{-1}$ 50 mM Tris-HCl, pH 7.0. Enzyme activity is as cts min $^{-1}$ transferred to immobilized ASF by the individual sialyltransferase isoforms.

The presence of multiple sialyltransferase forms in Golgi fractions was unequivocally demonstrated using non-denaturing gel electrophoresis combined with a solid-phase enzyme assay. The samples were solubilized in 2% NP-40 which resulted in a two-fold–threefold increase in enzyme activity and this was similar to the increase obtained with Triton X-100 which was used in all exogenous transfer reactions (Fig. 3). The 10% acrylamide gels contained 0.1% SDS and, at this concentration, no loss of enzyme activity was apparent (Fig. 3). Electrophoretic transfer from the gel to the ASF-coated nitrocellulose sheet appeared to be complete as judged by staining of the gel after transfer. The position of enzyme forms was then identified by transfer of [14 C]sialic acid from CMP- 14 C-NeuNAc to immobilized ASF. With this procedure, four activities with different mobilities were apparent and these were designated α , β , γ and δ (Fig. 4). The individual enzyme activities were also developmentally regulated. Up to and including post-

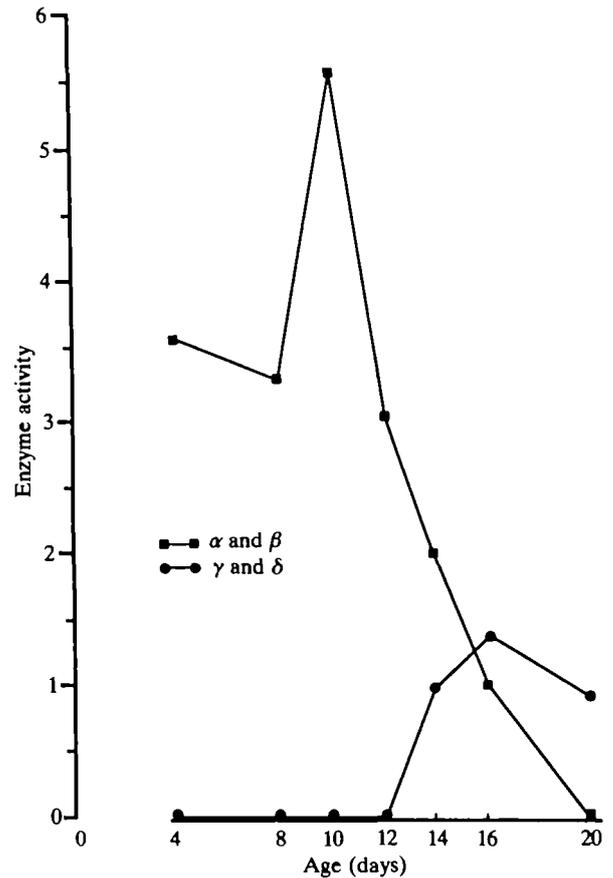


Fig. 5. Summed activities of α and β and γ and δ forms of Golgi sialyltransferase during postnatal development. The experimental conditions are as described in Fig. 4 and enzyme activity is as pmol NeuNAc transferred to immobilized ASF by the individual sialyltransferase isoforms.

natal day 12 only the α and β forms were expressed. Between postnatal days 14 and 16 all isozymes were present but thereafter only the γ and δ forms were apparent. It is unlikely that these are proteolysed fragments of a single enzyme activity as they are developmentally regulated and protease inhibitors (aprotinin, 25 K.i.u./ml $^{-1}$; phenylmethylsulphonyl-fluoride, 100 μ M) were included during the preparation and solubilization of the Golgi fractions. Further all enzyme activities were completely inhibited by 10 mM-ZnCl $_2$ at each developmental age examined which is in accord with the effects of this cation on Golgi-associated sialyltransferase activity. The summed activities of the sialyltransferase isoforms closely paralleled the regulation of the endogenous activity at each developmental time point (compare Fig. 1 and Fig. 5). Furthermore, the combined α and β activities accounted for total Golgi sialyltransferase activities at the times when the 'embryonic' form (HM r) of N-CAM is re-expressed during postnatal development (compare Fig. 2 and Fig. 5) (Breen & Regan, 1988a).

Discussion

The findings reported here extend our previous observations on a Golgi-associated sialyltransferase activity which is known to regulate N-CAM sialylation state (Breen *et al.* 1987; Breen & Regan, 1988a). This activity is strictly pH-dependent (pH 7.0) and its optimum differs from that used (pH 6.0) to characterize the poly- α -2,8-sialosyl-sialyltransferase of chick neural tissue (McCoy *et al.* 1985). Endogenous and exogenous transfer to Golgi acceptors and asialofetuin, respectively, decreased developmentally in parallel with postnatal N-CAM desialylation (Jorgensen, 1981; Meier *et al.* 1984) and prevalence (Choung & Edelman, 1984; Linneman *et al.* 1985). In all cases, exogenous transfer was 5–6 times greater than that seen for endogenous transfer. This presumably reflects increased access of the enzyme to its substrate in the presence of Triton X-100 as the developmental regulation and pH optimum mirrored that for endogenous transfer. Both exogenous and endogenous activity showed a marked stimulation at postnatal days 10–12, a time coincident with the re-expression of the HMr or 'embryonic' form of N-CAM (Breen & Regan, 1988a). Consistent with our previous observations (Breen & Regan, 1988a), N-CAM sialylation is mediated in a differentiation-dependent manner. N-CAM₁₄₀ is only sialylated up to and including postnatal day 10, N-CAM₁₈₀ from postnatal day 12 onwards and N-CAM₁₂₀ at all postnatal time points. Both N-CAM₁₄₀ and/or N-CAM₁₂₀ and N-CAM₁₈₀ and/or N-CAM₁₂₀ were capable of forming the HMr form of the molecule. The mobility changes overtime of the HMr form generated suggest that the sugar may be incorporated as polysialosyl units. The absence of intermediate molecular weight forms at later time points further suggests that the system is committed to transfer of additional units, the number of which may be restricted in N-CAM₁₄₀, to those polypeptides which have initially accepted one unit. This suggestion is not unprecedented. The capsular K1 antigen of certain *E. coli* strains is comprised of poly- α -2-8-sialosyl units which the sialyltransferase complex initially forms on a monophosphoryl-undecaprenol lipid intermediate before transferring the 'oligomerized' units to the cell envelope (Troy *et al.* 1975; Rohr & Troy, 1980). Further, the addition of preformed oligosaccharides *via* lipid-linked donors has been demonstrated for polymannose attachment to nascent polypeptides in the rough endoplasmic reticulum in mammalian cells (Farquhar, 1985). As only a single polysialosyl unit is believed to be attached to the tri- or tetraantennary glycan core of N-CAM (Finne & Makela, 1985), these results suggest that the sialic acid chains may be differentially linked to one or more attachment points (Crossin *et*

al. 1984; Hemperley *et al.* 1986; Barthels *et al.* 1987) depending on the individual N-CAM polypeptide which is being sialylated. The results also demonstrate that N-CAM may be initially sialylated before the attachment of the preformed units as the constitutive polypeptides are sialylated without a concomitant change in electrophoretic mobility.

The apparent K_m and rate changes observed for sialyltransferase during postnatal development suggests that this enzyme activity may be regulated by the temporal expression of an endogenous competitive inhibitor. Such inhibitor(s) have previously been reported to developmentally regulate the expression of the CMP-NeuNAc lactosyl ceramide sialyltransferase which is associated with ganglioside biosynthesis (Duffard & Caputto, 1972). Although we have also demonstrated the presence of an endogenous inhibitor in concentrated cytosol, we could not demonstrate the necessary change in its developmental expression. The inhibitor appeared to be a heat-labile protein and was not a soluble sialidase which could influence the transfer of sialic acid to protein acceptors (Breen & Regan, 1988b). This inhibitor may be a glycoprotein as previous studies have indicated that sialic-acid-containing conjugates are capable of inhibiting sialyltransferase action (Kijima-Suda *et al.* 1986), presumably by interfering at the CMP-NeuNAc binding site. To date, the physiological significance of such inhibitors remains obscure.

In contrast to other glycosyltransferases, the sialyltransferases are consistently reported to have no absolute requirement for divalent cations (Schachter & Roseman, 1980). Our observations support this conclusion. However they also demonstrate sialyltransferase activity to be markedly modulated in the presence of different cations. The respective stimulatory and inhibitory action of the ferric and zinc cations was most profound. The marked stimulation of the adult form of the enzyme by potassium and magnesium suggested that this activity was distinct from that seen in 12-day fractions. It was possible to rationalize this observation once the separate sialyltransferase isoforms were resolved by gel electrophoresis. The sequential expression of these isoforms during development is of particular interest as the α and β forms are only apparent during the postnatal period when N-CAM sialylation is dramatically modulated. Furthermore, they are particularly active when the HMr or 'embryonic' form of N-CAM is re-expressed. These isoforms are considered to be separate entities. The observed molecular weights in the gel system employed indicated the α , β , γ and δ forms to be 73, 60, 52 and 40×10^3 , respectively. Given the nondenaturing gel system employed, these values cannot be considered absolute but they are in good accord with previous observations which have

demonstrated the molecular weights of purified sialyltransferases to range from $37\text{--}76 \times 10^3$ (Paulson *et al.* 1982; Miyagi & Tsuiki, 1982; Hesford *et al.* 1984 and Ivanov *et al.* 1984).

The presence of $\alpha 2\text{--}3$, $\alpha 2\text{--}4$, $\alpha 2\text{--}6$ and $\alpha 2\text{--}8$ sialic acid linkages in N-glycosidically attached carbohydrates demands, at least, four sialyltransferase activities (Corfield & Schauer, 1982). Given that N-CAM is presumed to contain $\alpha 2\text{--}3$ and $\alpha 2\text{--}8$ sialic acid linkages (Finne, 1982; Finne *et al.* 1983; Finne & Makela, 1985) it is likely that the α and β forms will regulate its sialylation state. It is unlikely that these isoforms are N-CAM polypeptide-specific and that the observed differentiation-dependent sialylation of these reflects prevalence modulation of individual polypeptides (Breen & Regan, 1988a; Prentice *et al.* 1987; Choung *et al.* 1987; Pollerberg *et al.* 1987).

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