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CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase activity in rat brain and in tumour cells that express polysialic acid on neural cell adhesion molecules

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A method for the assay of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase activity was developed. Using a 1-day-old rat brain membrane fraction as an enzyme preparation optimal activity was obtained at pH 6.5, 0.3% Triton X-100, and 5 mM $MnCl_2$. However, no absolute cation requirement was found as EDTA only partially inhibited the activity. Within a concentration range of 0.3–3 mg colominic acid (which consists of a mixture of oligomers of α2 → 8-linked sialic acid) per 50 μl a V of 0.61 nmol per mg protein h^{-1} was estimated while a half-maximal reaction velocity was obtained at a concentration of 1.75 mg per 50 μl. High performance anion-exchange chromatography of the radioactive products formed in the reaction showed that sialic acid oligomers ranging in size from a degree of polymerization (DP) of 2 up to at least DP 9 could serve as acceptor substrates. Comparison of the acceptor properties of DP 3 and DP 6 showed that the larger oligomer was acted upon with a 10-fold higher efficiency. Periodate oxidation of the products followed by reduction and hydrolysis yielded the C_7 analogue of NeuAc as the only radioactive product, indicating that under the conditions of the assay only a single sialic acid residue was introduced into the acceptor molecules. Using the assay it appeared that in rat brain the activity of this sialyltransferase decreased six-fold during postnatal development to the adult stage. The assay method was also applied to lysates of several neuroblastoma and small cell lung tumour cell lines, which differ in the expression of polysialic acid as well as of the neural cell adhesion molecule NCAM, a major carrier of this polymer. Activity of the sialyltransferase appeared to be correlated with the expression of polysialic acid present on NCAM. These results indicate that this sialyltransferase might function in the process of poly-sialylation.

Keywords: polysialic acid, NCAM, sialyltransferase, colominic acid

Abbreviations: DP, degree of polymerization; HPLC, high-performance liquid chromatography; NeuAc, *N*-acetylneuraminic acid; NCAM, neural cell adhesion molecule; PSA, polysialic acid, Sia, sialic acid.

Introduction

In mammals a polymer of α2 → 8-linked NeuAc was described for the first time in developing rat brain as a unique N-linked carbohydrate unit [1]. These units were specifically demonstrated on the neural cell adhesion molecule (NCAM) [2, 3], which functions in embryonic development by mediating cell-cell adhesive interactions in processes like neurite fasciculation, neuromuscular interaction and cell migration [4–6]. The embryonic form of NCAM is particularly rich in polysialic acid (PSA). Upon maturation, however, the degree of sialylation gradually decreases and the adult form of NCAM contains significantly reduced amounts of sialic acid [2, 7].

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This reduction in PSA has been postulated to increase homophilic adhesion between cells [8].

Although polysialylated NCAM is most abundant in nervous tissue, it is not confined to it. For example, temporal expression of the polysialylated form of this molecule has also been reported in rat kidney [9] and muscle [10]. While the amount of polysialylated NCAM is generally highly reduced in adult tissue [2, 7], re-expression of the polysialylated form has been reported in the case of several human tumours such as medulloblastomas [11] and nephroblastomas (Wilms' tumour) [12]. In addition high expression of polysialylated NCAM has been described in human neuroblastoma cells [13, 14] and in small cell lung carcinoma cells [14–16]. High expression of PSA on sublines of the latter cells has recently been demonstrated to be correlated with reduced cell-cell adhesion and a higher incidence of metastasis in nude mice [17]. Thus PSA on NCAM has appeared to be an onco-developmental antigen that, as a result of a decreased homotypic interaction between NCAM molecules when they are polysialylated, may enhance the metastatic potential of malignant cells [17, 18].

In order to study the control of the expression of PSA, methods to assay the activity of the sialyltransferase(s) involved in the synthesis of this polymer have been developed. In one assay endogenously present NCAM or exogenously added NCAM was used as an acceptor [19]. The formation of PSA chains was demonstrated by using a prokaryotic probe (endo-neuraminidase). Applying this assay a developmentally regulated expression of poly-$\alpha 2 \rightarrow$ 8-sialyltransferase activity in rat brain was found [19, 20]. More recently an assay was described that permits the formation of sialic acid oligomers of DP $\geqslant$ 10 catalysed by the rat brain enzyme to be estimated [21]. In addition an assay has been described for the determination of poly-$\alpha 2 \rightarrow$ 8-sialyltransferase activity in unfertilized eggs [22] and ovaries [23] of the rainbow trout by the use of fish egg poly-sialoglycoproteins as exogenous acceptors. Although it has been found that colominic acid (a mixture of oligomers of $\alpha 2 \rightarrow$ 8-linked sialic acid) is an acceptor for the poly-sialyltransferase of *Escherichia coli* K1 *in vitro* [24], it was reported that this oligomeric substrate is not an acceptor for either the fetal rat brain or the trout ovary enzymes [19, 23].

We have re-investigated the possibility of using colominic acid as an exogenous acceptor for sialyltransferase in mammalian tissues and cells because of its ready availability. Using 1-day-old rat brain membranes as an enzyme preparation a convenient assay system was developed with which the enzymatically catalysed transfer of sialic acid from CMP-NeuAc to this acceptor could be demonstrated. Assay conditions were optimized and the product of the reaction was characterized. The method was used to assay CMP-NeuAc:(NeuAc$\alpha 2 \rightarrow 8)_n$ (colominic acid) sialyltransferase activity in rat brain during postnatal development as well as in several neuroblastoma and small cell lung carcinoma cell lines, that vary in the expression of PSA and NCAM.

Experimental procedures

Materials

Colominic acid was purchased from Sigma. CMP-[^{3}H]NeuAc (specific radioactivity 28 Ci mmol^{-1}) was obtained from Du Pont-New England Nuclear. Unlabelled CMP-NeuAc was prepared as described previously [25] and was used to dilute the labelled sugar nucleotide to the desired specific radioactivity. Monoclonal antibody 123C3, directed against NCAM, was obtained as described previously [14]. Monoclonal antibody 735, directed against $\alpha 2 \rightarrow$ 8-polysialic acid [26], was kindly provided by Dr Bitter-Suermann, Hannover, Germany. Rat brains were obtained from litters of white laboratory animals of mixed sex at different ages and from adult females. Brains were homogenized at 0 °C in 10 mM sodium cacodylate, pH 7.0 using a Potter-Elvehjem system with a teflon pestle rotating at 1500 rpm twice for 0.5 min. The homogenates were centrifuged for 10 min at 500 × **g** and the resulting supernatants were centrifuged for 60 min at 120.000 × **g**. The pellets were then re-homogenized in buffer to yield membrane fractions at a protein concentration of 14–38 mg of protein per ml. These preparations were stored frozen at −20 °C until use. The neuroblastoma cell line of CHP-212 was kindly provided by Dr Schlessinger, Philadelphia, PA [27]. The small cell lung cancer cell lines H69 and Alc-3 were generous gifts of Drs Carney (Dublin, Ireland) and Jongsma (Amsterdam, The Netherlands), respectively. The neuroblastoma cell line SK-N-SH, the human ovarian cancer cell line OVCAR-4, and the monkey kidney cell line COS 5/7 cells were obtained from the ATCC (Rockville, MD). All cell lines were maintained in Dubecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. Cells were homogenized as described above for rat brain to give cell lysates containing 15–35 mg of protein per ml.

Standard sialyltransferase assay

Standard assay mixtures contained in a total volume of 50 µl 1 mg colominic acid, 25 nmol CMP-[^{3}H]NeuAc (3.8 Ci mol^{-1}), 5 µmol sodium cacodylate buffer pH 6.5, 0.25 µmol $MnCl_2$, 0.15 µl Triton X-100 and brain membranes (0.2–0.6 mg protein). Incubations were conducted for 4–5 h at 37 °C. Sialyltransferase activities in cell lysates (0.3–0.8 mg protein per incubation) were assayed using the standard incubation system except for the use of 10 mg colominic acid and an incubation time of 16 h. Reactions were stopped by the addition of 200 µl 0.05 M ammonium acetate, pH 5.4, and cooling in ice. Samples

were then centrifuged in an Eppendorf centrifuge for 4 min at maximal speed. Pellets were washed once with the same amount of buffer and centrifuged again. To separate the excess CMP-[^{3}H]NeuAc from the [^{3}H]NeuAc incorporated into colominic acid the combined supernatants were passed over a column (0.7 × 50 cm) of Bio-Gel P-4 (200–400 mesh) equilibrated and eluted with 0.05 M ammonium acetate, pH 5.4, at a flow of 10 ml h^{-1} at room temperature. Fractions of 0.5 ml were collected and assayed for radioactivity by liquid scintillation counting. Incorporation was calculated from the sum of counts in the colominic acid containing fractions (see Fig. 1). The values were corrected for the incorporation into endogenous acceptors by running incubations lacking colominic acid and subtracting the counts found in these samples.

Product characterization

To obtain sufficient product for analysis by HPLC a reaction mixture was prepared containing membranes from 1-day-old rat brains (0.8 mg protein) and 10 mg instead of 1 mg colominic acid. Incubation was conducted for 16 h at 37 °C, whereafter the sample was centrifuged and passed over a column of Bio-Gel P-4 as described above. Fractions containing the products were pooled and aliquots were analysed by HPLC (modified from [28]) on a column (4.7 × 110 mm) of Partisphere 5 Sax (particle size 5 μm; Whatman) using a Kratos Spectroflow 400 solvent delivery system, equipped with a Rheodyne 7105 injection valve. Elution was with 20 mM KH_2PO_4, pH 5.4, for 10 min at a flow of 1 ml min^{-1} after which a discontinuous, convex gradient was applied increasing the buffer concentration to 400 mM at t = 140 min. Detection of oligomers of sialic acid was performed with a Kratos Spectroflow 757 absorbance detector operating at 195 nm connected to a Hitachi D-2000 integrator. Radioactivity was detected by liquid scintillation counting of the eluate which was collected in fractions of 1 ml.

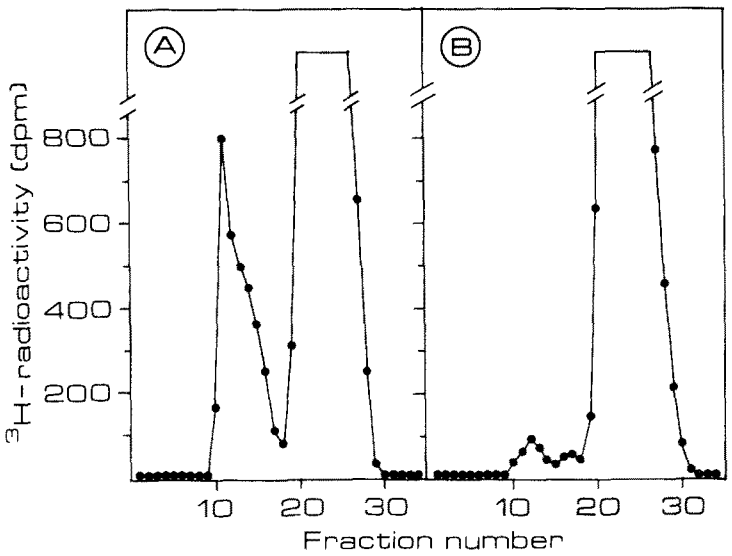

Figure 1. Isolation of the product of the sialyltransferase from 1-day-old rat brain from an incubation mixture (A) with colominic acid as acceptor substrate and (B) without exogenously added acceptor. Mixtures were applied to a column of Bio-Gel P-4 as described under Experimental procedures. Fractions were assayed for radioactivity by liquid scintillation. V_0, fraction 11; products, fractions 10–18; oligomer DP 3, fraction 18; excess precursor label (CMP-[^{3}H]NeuAc), fractions 19–29 (top 23–24); V_t, fraction 33.

Flow cytometry

Cells were dispersed in PBS, pH 7.2, containing 2 mM EDTA, and incubated with excess unlabelled antibody in PBS containing 0.2% BSA at 4 °C. Unbound antibody was washed away with PBS-0.2% BSA and cells were subsequently incubated with a 1:30 dilution of FITC labelled goat anti-mouse antibody (Nordic, Tilburg, The Netherlands). The cells were then washed three times and analysed with a FACS IV cell sorter (Becton and Dickinson, Sunnyvale, CA).

Results

Assay of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase

A membrane preparation of 1-day-old rat brains was used to set up and to optimize the assay of CMP-NeuAc: (NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase. Using the standard incubation conditions, radioactive products were formed which could be separated from the excess CMP-[^{3}H]NeuAc by filtration on Bio-Gel P-4 (Fig. 1). Little product was formed when colominic acid was absent from the incubation. No product was found when the enzyme preparation was omitted, ruling out the possibility of a non-enzymatic transfer. Under sterile conditions as well as in the presence of 0.02% NaN_3 the incorporation of sialic acid appeared unimpaired indicating that the transfer was not due to a contaminating bacterial enzyme. Hence colominic acid appeared to be an acceptor for a sialyltransferase in young rat brain. Although it is known that colominic acid is subject to intramolecular self-cleavage at pH ≤ 5.0 at 37 °C [29], no degradation was observed at the pH and temperature employed during the gel filtration step (pH 5.4, 20 °C). A pH of 5.4 was chosen as buffer solutions with a higher pH resulted in a poorer separation of products and excess CMP-[^{3}H]NeuAc.

Optimal assay conditions

Incorporation of sialic acid into colominic acid catalysed by the rat brain preparation was detected between pH 5.5 and 8.0 with an optimum at pH 6.5 (Fig. 2). Triton

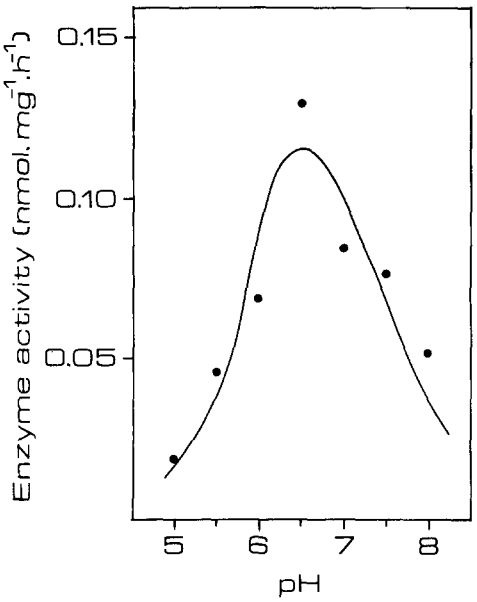

Figure 2. pH dependency of the activity of CMP-NeuAc: (NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase from 1-day-old rat brain.

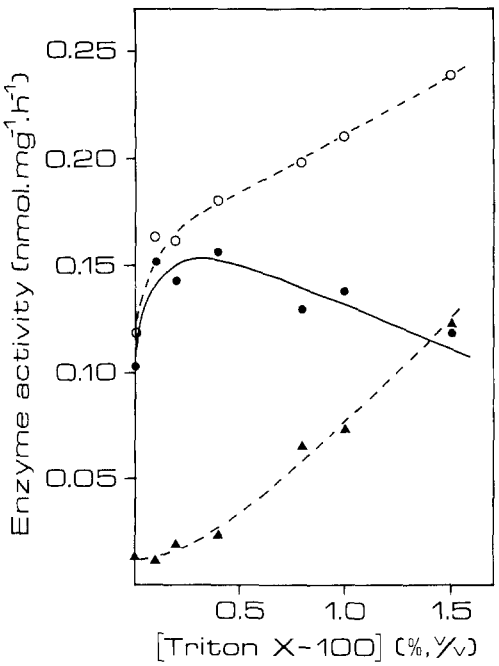

Figure 3. Effect of Triton X-100 concentration on the activity of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase of 1-day-old rat brain membranes. Activity toward endogenous acceptors, (▲); total activity upon addition of exogenous acceptor (colominic acid) (○); incorporation into the exogenous acceptor solely (●) calculated by subtraction.

X-100 up to a concentration of at least 1.5% stimulated the enzyme activity, but also made an endogenous acceptor (presumed to be NCAM) available to the sialyltransferase resulting in an increase in incorporation into this acceptor as the detergent concentration raised (Fig. 3). Subtraction of the values obtained without exogenous acceptor from those obtained with colominic acid added showed that 0.3% Triton X-100 was the optimal concentration to assay the sialyltransferase activity. The sialyltransferase did not appear to have an absolute requirement for divalent cations as EDTA did not abolish the enzyme activity (Table 1). Some cations at a concentration of 5 mM, however, were slightly stimulatory, Mn^{2+} being the most effective, while Zn^{2+} was strongly inhibitory (Table 1). Varying the concentration of $MnCl_2$ indicated that a maximal stimulatory effect was reached at 5 mM and was maintained up to at least 25 mM (Fig. 4).

Table 1. Effect of divalent cations and EDTA on the activity of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase of 1-day-old rat brain. The standard assay conditions were used except for the omission of $MnCl_2$ and the addition of the salts indicated

Addition (5 mM)	*Sialyltransferase activity (%)*
None	100
$MnCl_2$	166
$MgCl_2$	149
$CaCl_2$	130
$ZnCl_2$	7
EDTA	71

Kinetics of the reaction

In order to estimate the lowest level of sialyltransferase activity that could be assayed with reasonable accuracy it was investigated whether increased incubation times and increased amounts of enzyme would still yield reliable kinetics. It was found that the reaction proceeded linear with time up to at least 7 h of incubation (Fig. 5A). Proportionality with enzyme concentration was less but acceptable within the limits of the amounts of enzyme protein added (Fig. 5B). Higher acceptor substrate concentrations also increased the sensitivity of the assay (Table 2). By plotting $1/v$ against 1/[colominic acid] it was found that the reaction did not follow simple Michaelis-Menten kinetics. However, Lineweaver-Burk plots yielded straight lines when the acceptor concentration was varied between 0.3 and 3.0 mg per incubation (not shown). Within these limits a V of 0.61 nmol per mg protein h^{-1} was found while a half-maximal reaction velocity was obtained at a concentration of 1.75 mg acceptor per 50 μl. The anomalous kinetics at concentrations beyond these limits are probably explained by the heterogeneous nature of colominic acid, which is known to consist of a mixture of oligomers of NeuAc differing in size [29] (see also Fig. 6A). Indeed Michaelis-Menten kinetics were followed with defined oligomers; with DP 3 and DP 6 K_m values of 15 and 23 mM and V values of

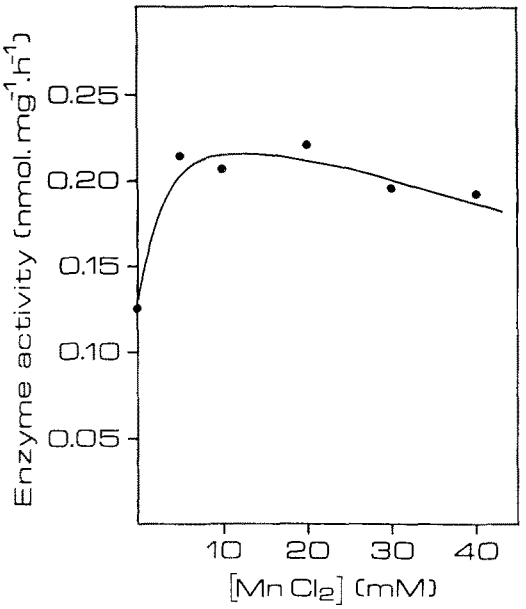

Figure 4. Effect of $MnCl_2$ concentration on the activity of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase from 1-day-old rat brain.

0.19 and 3.45 nmol per mg protein h^{-1}, respectively, were estimated. From these values kinetic efficiencies ($V \cdot K_m^{-1}$) of 0.013 and 0.15 were calculated for DP 3 and DP 6, respectively, showing that the larger oligomers of NeuAc are the better substrates.

Product characterization

Prolonged incubation of 1-day-old rat brain membranes with colominic acid and CMP-[^{3}H]NeuAc yielded a radioactive product that separated into a number of ^{3}H-labelled peaks upon high performance anion-exchange chromatography (Fig. 6B). Each peak coincided with a peak of an oligomer of NeuAc contained in the colominic acid preparation used as detected by absorption at 195 nm (Fig. 6A). Comparison of the two profiles indicated that a fragment as small as DP 2 could serve as an efficient acceptor. This was confirmed with isolated DP 2 which appeared to be an acceptor.

Mild periodate oxidation and subsequent $NaBH_4$ reduction [30] and hydrolysis (0.1 M trifluoroacetic acid, 80 °C, 1 h) of the product yielded a radioactive monosaccharide that could be identified as the C_7 analogue of NeuAc in two HPLC systems [31, 32] (not shown). No intact (C_9) [^{3}H]NeuAc, that would have been found when the sialyltransferase had repeatedly acted on a single acceptor molecule (whether yielding α2 → 8- or α2 → 9-linkages [33]), could be detected.

CMP-NeuAc:(NeuAcα2 → 8)$_n$ sialyltransferase activity in developing rat brain

Brain membrane preparations from rats at different stages of postnatal development were assayed for sialyltransferase activity using the method developed. A six-fold decrease in activity was observed from new born to adult (Fig. 7).

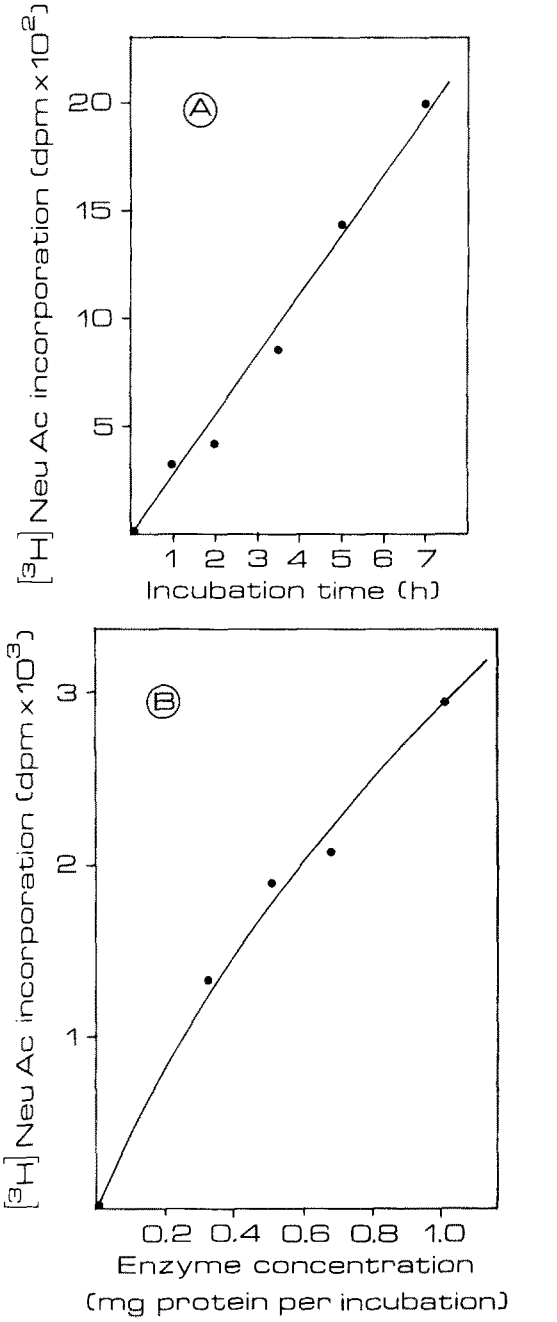

Figure 5. Linearity with time (A) and with enzyme (B) of the reaction catalysed by CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase of 1-day-old rat brain membranes. Assay conditions were as described under Experimental procedures except for the variation in time and amount of enzyme.

Activity of CMP-NeuAc:(NeuAcα2 → 8)$_n$ sialyltransferase and expression of NCAM and polysialic acid (PSA) by tumour cell lines

Several tumour cell lines, differing in the expression of NCAM and PSA, were assayed for sialyltransferase

Table 2. Effect of the amount of colominic acid as acceptor for CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase of 1-day-old rat brain

Colominic acid (mg per 50 µl)	*Enzyme activity (nmol per mg protein h^{-1})*
0.1	0.074
0.3	0.091
1.0	0.23
3.0	0.40
10.0	1.19

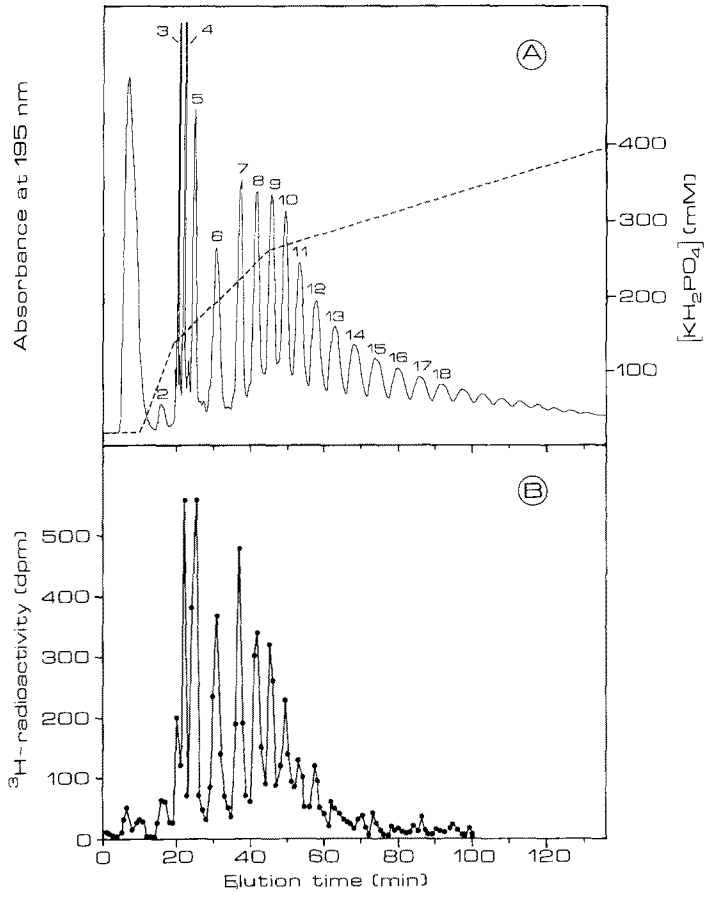

Figure 6. Characterization of the product formed in the reaction with 1-day-old rat brain sialyltransferase using colominic acid as acceptor. The product was isolated (see Fig. 1) and analysed by high performance anion-exchange chromatography on a column of Partisphere Sax as described under Experimental procedures. The eluate was monitored for absorbance at 195 nm (A) and fractions of 1 ml were collected and assayed for radioactivity (B).

activity. High activities were found in the neuroblastoma cell line SK-N-SH and the small cell lung cancer cells H69 and Alc-3, whereas the other cells showed much lower activities (Table 3). Expression of NCAM was high in SK-N-SH, CHP-212 and H69 cells and intermediate in Alc-3 cells, while PSA was abundantly expressed in

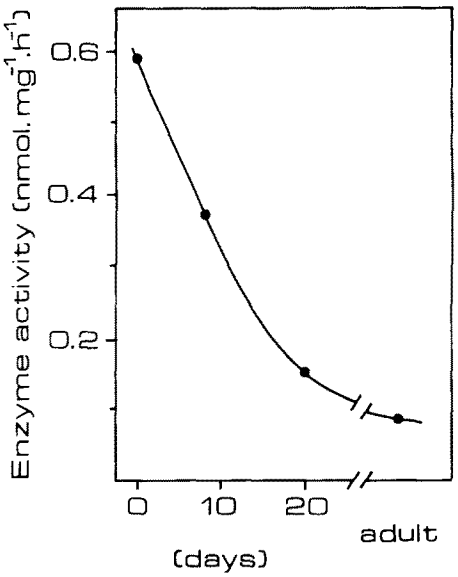

Figure 7. Postnatal developmental changes in the expression of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase in rat brain membranes.

SK-N-SH and H69 cells and intermediate to low in Alc-3 cells (Table 3, Fig. 8). CHP-212 cells along with two unrelated control cell lines (OVCAR and COS cells) were essentially devoid of PSA.

Discussion

We have developed a method to assay the activity of CMP-NeuAc:(NeuAcα2 → 8)$_n$ sialyltransferase by using colominic acid as an acceptor. With this method activity could be readily demonstrated in newborn rat brain and in cells that express PSA on NCAM. Colominic acid is generally considered not to be an acceptor for (poly-) sialyltransferases of eukaryotes such as those present in rat brain [19] and trout ovaries [23]. Also the recombinant form of a developmentally regulated sialyltransferase, cloned from a newborn rat brain cDNA library, was reported to be unable to act on this acceptor [34]. On the other hand colominic acid has been demonstrated to be a substrate for the sialyltransferase of *E. coli* K1 [24]. Although it cannot conclusively be ruled out that we detected a novel sialyltransferase, it is more likely that the assay conditions developed in this study allow the activity of a previously described sialyltransferase(s) to be assayed. An obvious advantage of this method is that colominic acid is a readily available substrate.

It could be estimated that even with the most active enzyme preparation used in this study $\ll 0.1\%$ of the acceptor molecules were acted upon by the sialyltransferase. Therefore the chance that the sialyltransferase would act for a second time on the same sialic acid oligomer yielding a [^{3}H]-NeuAc-[^{3}H]-NeuAc linkage was

Table 3. Expression of CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase, NCAM and polysialic acid (PSA) in some tumour cell lines

Cell line	*Sialyltransferase activity (pmol per mg protein h^{-1})*	*NCAM*	*PSA*
		Mean relative fluorescence intensity (% of cells positive above control)	
SK-N-SH	28	43 (98)	105 (96)
CHP-212	0.7	20 (99)	4 (12)
H69	110	28 (93)	80 (97)
Alc-3	35	12 (56)	9 (29)
OVCAR-4	5.9	6 (5)	5 (6)
COS 5/7	9.2	19 (2)	21 (1)

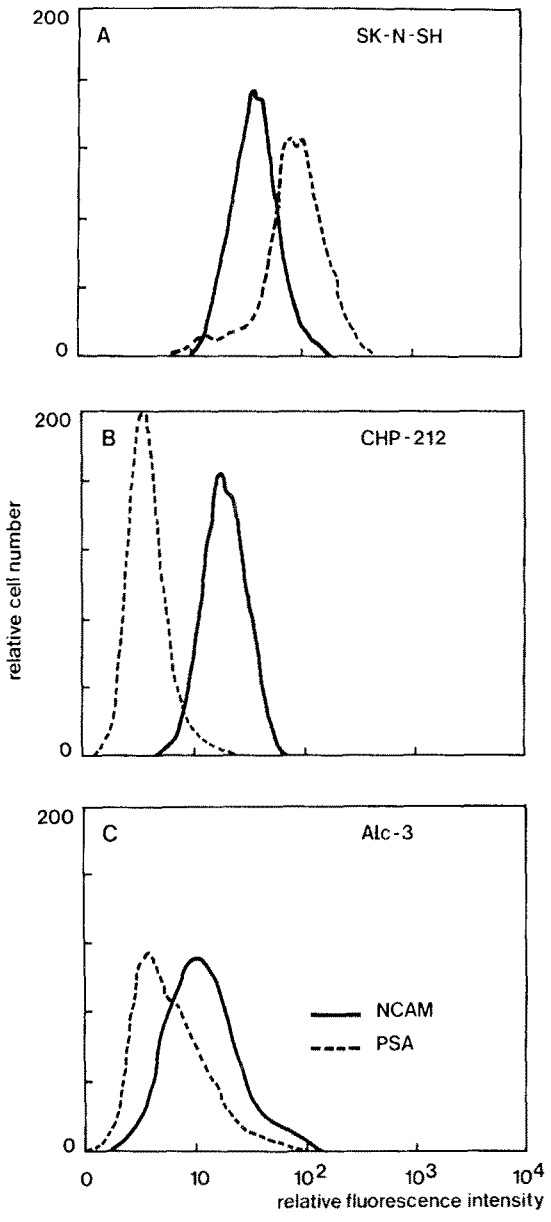

Figure 8. Flow cytometry of cells. Cells were analysed for the expression of NCAM and PSA using the antibodies mentioned under Experimental procedures.

concluded to be extremely small. This explains why only the C_7 analogue of sialic acid was found in the analysis of the products by periodate oxidation. Hence it appears that due to competition by the excess of acceptor molecules only a single sialic acid residue was introduced into the different sialic acid oligomers.

In this respect the rat brain CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid)sialyltransferase seems to differ from the sialyltransferase(s) of *E. coli* K1 that acts on an undecaprenyl carrier to yield a sialic acid polymer of DP 40–45 by the repeated action of the enzyme(s) on a single acceptor molecule [18, 30]. The *E. coli* poly-sialyltransferase can also act on an acceptor present in a membrane fraction of human neuroblastoma CHP-134 cells to yield sialic acid oligomers of DP $\geqslant$ 3 [13]. Whether the *E. coli* enzyme catalyses mono- or poly-sialylation when colominic acid is used as an exogenous acceptor, however, has not been established [24]. Also embryonic rat brain has been demonstrated to contain a sialyltransferase that is capable of synthesizing PSA on endogenous and exogenously added NCAM [19]. Similarly, unfertilized trout oocytes have been reported to contain a poly-sialyltransferase that can catalyse the production of oligomers of sialic acid with a DP $\geqslant$ 7 on fish egg sialoglycoproteins [23]. The mono-sialylation reaction catalysed by rat brain membranes using the method of this study, might have to be explained by postulating that poly-sialylation requires the presence of a lipid or protein aglycon that functions as a scaffold on which the poly-sialic acid chain can be formed. When such a scaffold, with which the poly-sialyltransferase would interact during the elongation reaction, is absent, the enzyme-product complex would dissociate after each transfer step leading to competition between individual acceptor molecules resulting in mono-sialylation only. Our data thus do not permit us to assess unequivocally whether the incorporation of sialic acid into colominic acid is due to the action of a novel (mono)-sialyltransferase or a poly-sialyltransferase.

Evidence is accumulating for the existence of a group of α2 → 8-sialyltransferases that act only once on an

acceptor carrying a terminal sialic acid residue to give a single Siaα2 → 8Sia disaccharide unit. Examples are the recently cloned G_{D3} synthase that adds sialic acid in α2 → 8 linkage to the NeuAcα2 → 3Gal unit in ganglioside G_{M3} [35–37] and a sialyltransferase in trout ovaries that acts on a Siaα2 → 6GalNAc unit to yield a Siaα2 → 8Siaα2 → 6GalNAc sequence [23]. Such 'initiases' have to be distinguished from the actual 'polymerases' that only act on acceptors carrying at least one Siaα2 → 8Sia disaccharide unit [23]. Since we found transfer to sialic acid residues that are in an oligomeric sequence rather than linked to galactose or GalNAc, it seems less likely that the enzyme activity found with colominic acid as an acceptor is due to an 'initiating' sialyltransferase.

The decrease in CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase activity that we observed during postnatal development in rat brain is similar to the decrease in activity of the poly-sialyltransferase assayed with NCAM as an acceptor [20]. This decrease is correlated with the decrease in PSA expression in the nervous system during maturation [2, 7]. In addition, we found a correlation between PSA expression and CMP-NeuAc:(NeuAcα2 → 8)$_n$ sialyltransferase activity in the neuroblastoma SK-N-SH and CHP-212 cells and in the small cell lung carcinoma H69 cell line. The low expression of PSA in the Alc-3 cell line on the other hand appears to be due to a low expression of NCAM rather than a low sialyltransferase activity. These results would suggest that the CMP-NeuAc:(NeuAcα2 → 8)$_n$ (colominic acid) sialyltransferase assayed in rat brain and the tumour cells functions in the process of polysialylation. Therefore, the possibility that the activity found is in fact due to a poly-α2 → 8- or poly-α2 → 9-sialyltransferase is left open. It will, therefore, be of interest to apply the assay method in further studies on the sialyltransferases of fish eggs [22] and oocytes [23] and the recombinant form of the developmentally regulated sialyltransferase from rat brain [34] as well as that of a recently cloned poly-sialyltransferase [37]. This will help to more definitely answer the question of the identity of the sialyltransferase assayed in this study.

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