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Activities and Specificities of *N*-Acetylneuraminyltransferases in Leukemic Cells *

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Recently, interest in glycosyltransferases has increased due to the finding that differentiation and neoplastic transformation of cells may be accompanied by alterations in the composition and metabolism of cellular glycoconjugates. Previous studies using lactose as exogenous acceptor have shown apparent differences in the expression of the activities of fucosyl- and *N*-acetylneuraminyltransferases in various leukemic cells arrested at different stages of cell maturation [1].

The aim of this study was to determine the activities and linkage specifities of different *N*-acetylneuraminyltransferases in various leukemic and normal cell populations. Normal and leukemic cells were isolated from human blood by differential and/or gradient centrifugation and characterized by surface membrane marker analysis as described recently [1].

N-acetylneuraminyl(NeuAc)-transferase activites were assayed in cell lysates by measuring the transfer of [^{14}C]NeuAc from CMP-[^{14}C]NeuAc to different exogenous glycoprotein acceptors. The reaction mixture for the standard *N*-acetylneuraminyltransferase assay contained 50 µl lysate (10^8 sonicated cells or platelets/ml of 0.05 *M* HEPES-buffer, pH 6.8, containing 0.1% Triton X-100), 100 µl acceptor glycoprotein solution (2 mg asialo-α_1-acid glycoprotein/acceptor sequence: Gal-β1,4-Glc NAc-oligosaccharide glycoprotein, 1 mg asialo-ovine submaxillary mucin/acceptor sequence: GalNAc-Ser/Thr or 0.7 mg asialo-afuco-porcine submaxillary mucin/acceptor sequence: Gal-β1,3-GalNAc-Ser/Thr in 100 µl of 0.05 *M* HEPES-buffer, pH 6.8), and 2148 pmol/25 µl CMP-[^{14}C]NeuAc (CFB 165:50 µCi/ml, spec. activity 291 mCi/mmol, Amersham Buchler) in a total reaction volume of 220 µl. The reaction mixture was made 10 m*M* with $MgCl_2$ and the final concentration of Triton X-100 was 0.023%. After incubation from 0 to 3 h at 37 °C in a shaking water bath, aliquots of 25 µl of the reaction mixture were immediately subjected to high-voltage paper electrophoresis for 70 min at 2000 V in 0.05 *M* sodium tetraborate buffer, pH 9.0, according to Roseman [2], to separate the neuraminylated exogenous glycoprotein acceptors. The paper was cut and the radioactivity due to [^{14}C] NeuAc was determined by scintillation counting. *N*-acetylneuraminyltransferase activities toward exogenous glycoprotein acceptors were expressed as transfer of pmol NeuAc/(h×mg lysate protein). *N*-acetylneuraminyltransferase activity toward endogenous acceptors of the lysates was found to be negligible.

The linkage specificity of the CMP-NeuAc: β-galactosyl [1, 4] *N*-acetylglucosaminide *N*-acetylneuraminyltransferase was establishd after transfer of NeuAc from CMP-NeuAc to the terminal [^{3}H] galactose residues of asialo-α_1-acid [^{3}H] Gal glycoprotein using a micromethodology based on methylation and hydrolysis of the neuraminylated product, followed by analysis of the emerging trimethyl-[^{3}H] galactosides, as described recently [3].

* Dedicated to Prof. Dr. K. D. Bock on the occasion of his 60th birthday

Cell type	Activity (pmol/h × mg lysate protein)	Linkage specificity
Normal T-lymphocytes (> 94% E-R positive)		
252	52	α (2,6)
257	94	α (2,6)
473	110	α (2,6)
Normal B-lymphocytes (> 91% EAC-R positive)		
253	78	α (2,6)
258	68	α (2,6)
478	110	α (2,6)
Normal platelets		
467	1425	α (2,6) and α (2,3)
526	2373	α (2,6) and α (2,3)
Normal leukocytes		
466	< 30	Not detectable
Cell lines		
D.G.-75	840	α (2,6)
MOLT-4	198	α (2,6)
K-562	62	α (2,6)
Leukemic cells		
281 (AML)	59	α (2,6)
400 (AML)	37	α (2,6)
408 (non-T/non-B ALL)	< 30	Not done
433 (non-T/non-B ALL)	< 30	Not done
249 (T-ALL)	280	α (2,6)
183 (CLL)	709	α (2,6)
274 (IC)	194	α (2,6)
379 (CLL)	320	α (2,6)
431 (CLL)	268	α (2,6)
432 (CC)	115	α (2,6)

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia (B-cell type); IC, leukemic immunocytic non-Hodgkin lymphoma (LP immunocytoma); CC, leukemic centrocytic non-Hodgkin lymphoma

Table 1. Activity and specificity of β-galactosyl(1,4)N-acetylglucosaminide-N-acetylneuraminyltransferase in human normal T- and B-lymphocytes, platelets, and various leukemic cells (exogenous acceptor: asialo α_1-acid glycoprotein)

A. Serum Type of Glycoprotein *N*-Acetylneuraminyltransferase

As shown in Table 1 *N*-acetylneuraminyltransferase activity toward asialo-α_1-acid glycoprotein was low in both isolated normal T- and B-lymphocytes, whereas the transferase activity of various leukemic and culture cell line cells varied from almost not detectable to high.

Leukemic myeloid (No. 281 and 400) and non-T/non-B lymphoid cells (No. 408 and 433) as well as K-562 cells were apparently arrested at an early stage of differentiation and displayed low levels of activity, whereas T-lymphoblasts (No. 249) and the MOLT-4 cells (Thy-ALL) were further developed along the T-cell lineage and transferred 280 and 198 pmol NeuAc/(h × mg lysate protein), respectively. Neoplastic

B-lymphocytes and the D.G.-75 cells (B-lymphoblasts) were further developed along the B-cell lineage, showing varying levels of activity of *N*-acetylneuraminyltransferase of the serum glycoprotein type.

When analyzed for the linkage specificity of this *N*-acetylneuraminyltransferase it appeared that the leukemic and culture cell line cells as well as normal T- and B-lymphocytes exclusively transferred NeuAc to position C-6 of the terminal [^{3}H] galactose residues of asialo-α_1-acid[^{3}H]Gal glycoprotein, indicating that these cells only expressed α2,6-*N*-acetylneuraminyltransferase activity.

However, normal platelets displayed in addition to high α2,6- also high α2,3-*N*-acetylneuraminyltransferase activity.

B. Mucin Type of Glycoprotein *N*-Acetylneuraminyltransferase

When tested with asialo-ovine submaxillary mucin (GalNAc-Ser/Thr) and asialo-afuco-porcine submaxillary mucin (Gal-β1,3-GalNAc-Ser/Thr) as exogenous glycoprotein acceptors, neither the various leukemic cells nor normal T- and B-lymphocytes revealed significant activities [$<$30 pmol NeuAc/(h $\times$ mg lysate protein)] of *N*-acetylneuraminyltransferases of the mucin glycoprotein type.

C. Discussion

Transfer of NeuAc to the labeled terminal [^{3}H] galactosyl units of the acceptor molecule asialo-α_1 acid [^{3}H] Gal glycoprotein can involve the positions C-2, C-3, C-4, and C-6, depending on the specificity of the CMP-NeuAc: β-galactosyl (1,4)*N*-acetylglucosaminide (α2,2-, α2,3-, α2,4- or α2,6-) *N*-acetylneuraminyltransferase involved. Our data establish α2,6-*N*-acetylneuraminyltransferase as the only *N*-acetylneuraminyltransferase of that type in various leukemic cells and normal lymphocytes.

Leukemic non-T/non-B lymphoid cells were almost devoid of this enzyme activity whereas leukemic T and B cells apparently further developed along the T- or B-cell lineages varied from low activity, as found in normal T- and B-lymphocytes, to high activity, indicating that the expression of α2,6-*N*-acetylneuraminyltransferase activity occurs concomitantly with the differentiation of lymphoid cells. However, no evidence was found for an additional α2,3-*N*-acetylneuraminyltransferase which had been detected in fetal calf liver and in human placenta [4], giving rise to speculations on the possible existence in leukemic cells of an oncofetal α2,3-*N*-acetylneuraminyltransferase. Only platelets displayed α2,3-*N*-acetylneuraminyltransferase activity in addition to high α2,6-*N*-acetylneuraminyltransferase activity. These data establish CMP-NeuAc: β-galactosyl (1,4) *N*-acetylglucosaminide α2,3-*N*-acetylneuraminyltransferase as a marker enzyme for human platelets.

Using lectins such as soybean agglutinin (specificity: GalNAc), peanut agglutinin (specificity: Gal-β1,3-GalNAc), and lobster agglutinin (specificity: NeuAc), changes in the carbohydrate moieties of surface glycoconjugates concomitant with differentiation of lymphocytes have been demonstrated, reflecting alterations in the expression of cellular glycosyltransferases. During the process of cell maturation the surface lectin-binding sites exposing terminal GalNAc or Gal-β1,3-GalNAc units are masked by NeuAc residues. However, our experiments did not reveal any activities of *N*-acetylneuraminyltransferases of the mucin glycoprotein type (acceptor specificity: GalNAc or Gal-β1,3-GalNAc) in lysates of normal lymphocytes and of various leukemic cells arrested at different stages of maturation.

Since the expression of α2,6-*N*-acetylneuraminyltransferase activity of the serum glycoprotein type occurs concomitantly with the differentiation of lymphocytes, masking of the described lectin-binding sites on the surface of lymphocyte seems not to be due to direct chemical linkage of NeuAc to either terminal GalNAc or Gal-β1,3-GalNAc units, but rather to a charge or steric effect of NeuAc residues linked to Gal-β1,4-GlcNAc units by α2,6-*N*-acetylneuraminyltransferase of the serum glycoprotein type.

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