Glycoconjugate Journal (1995) **12**: 755–761

Large-scale expression of recombinant sialyltransferases and comparison of their kinetic properties with native enzymes

MARK A. WILLIAMS[1], HIROSHI KITAGAWA[1]*, ARUN K. DATTA[1], JAMES C. PAULSON[1] and JAMES C. JAMIESON[2]‡

[1]*Cytel Corporation, 3525 John Hopkins Court, San Diego, California 92121, USA*
[2]*Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2*

Received 2 March 1995, revised 22 June 1995

Values of K_m were determined for three purified sialyltransferases and the corresponding recombinant enzymes. The enzymes were Galβ1-4GlcNAc α2-6sialyltransferase and Galβ1-3(4)GlcNAc α2-3sialyltransferase from rat liver; these enzymes are responsible for the attachment of sialic acid to N-linked oligosaccharide chains; and the Galβ1-3GalNAc α2-3sialyltransferase from porcine submaxillary gland that is responsible for the attachment of sialic acid to O-linked glycoproteins and glycolipids. A procedure for the large scale expression of active sialyltransferases from recombinant baculovirus-infected insect cells is described. For the liver enzymes values of K_m were determined using rat and human asialo$_{\alpha 1}$ acid glycoprotein and *N*-acetyllactosamine as variable substrates; lacto-*N*-tetraose was also used with the Galβ1-3(4)GlcNAc α2-3sialyltransferase. Antifreeze glycoprotein was used as the macromolecular acceptor for the porcine enzyme. Values for K_m were also determined using CMP-NeuAc as the variable substrate.

Keywords: sialyltransferase, glycoprotein, baculovirus

Abbreviations: NeuAc, *N*-acetylneuraminic acid; Gal, galactose; GlcNAc, *N*-acetylglucosamine.

Enzymes: Galβ1-4GlcNAc α2-6sialyltransferase, EC 2.4.99.1; Galβ1-3(4)GlcNAc α2-3sialyltransferase, EC 2.4.99.5; Galβ1-3GalNAc α2-3sialyltransferase, EC 2.4.99.4.

Introduction

The sialyltransferases constitute a group of enzymes that are capable of adding sialic acid to terminal positions of oligosaccharide chains of glycoproteins and glycolipids. The nucleotide sugar CMP-NeuAc acts as the donor for all of these enzymes. The sialyltransferases are highly specific recognizing the sequence of the oligosaccharide acceptor substrate and the anomeric linkage of the sugar to which it is attached. Several sialyltransferases have been isolated, characterized, and in some cases purified to homogeneity [1–11], and the cDNA cloning and expression of many of these enzymes have been reported recently [12–29]. Of the sialyltransferases which have been purified to homogeneity, only three have also been expressed from recombinant sources in large quantities. These enzymes are the Galβ1-4GlcNAc α2-6sialyltransferase of rat liver [11, 22, 30] which forms the terminal NeuAcα2-6Galβ1-4GlcNAc structures on N-linked glycoproteins; the Galβ1-3(4)GlcNAc α2-3sialyltransferase of rat liver [29] which attaches sialic acid to either Galβ1-4GlcNAc or Galβ1-3GlcNAc to form terminal NeuAcα2-3Galβ1-3GlcNAc or NeuAcα2-3Galβ1-4GlcNAc structures on N-linked glycoproteins, and the Galβ1-3GalNAc α2-3sialyltransferase from porcine submaxillary glands [14]; this enzyme adds sialic acid in α2-3 linkage to Galβ1-3GalNAc terminal positions on O-linked glycoproteins or glycolipids.

As more glycosyltransferases derived from recombinant sources will become available in the future, it was of interest to make a direct comparison of the kinetic properties of the recombinant sialyltransferases mentioned above to the corresponding native enzymes. We

*Current address: Department of Chemistry, Kobe Pharmaceutical University, Kobe, Japan.

‡To whom all correspondence should be addressed.

report here the results of these studies using macromolecular and oligosaccharide acceptors and CMP-NeuAc as substrates.

Materials and methods

MATERIALS

CMP-[4,5,6,7,8,9-^{14}C] NeuAc (247 mCi mmol^{-1}) was obtained from New England Nuclear Corp., Triton CF 54, lacto-*N*-tetraose and *N*-acetyllactosamine were from Sigma Chemical Co., St Louis, MO. Human α_1 acid glycoprotein was a gift from K. Schmidt, Boston University School of Medicine, rat α_1 acid glycoprotein was prepared as previously described [31]; sialic acid was removed from the human protein by treatment with neuraminidase as before [6] and from the rat protein by hydrolysis with dilute acid as described previously [31]. Native Galβ1-4GlcNAc α2-6sialyltransferase and Galβ1-3(4)GlcNAc α2-3sialyltransferase were prepared from rat liver by Weinstein *et al.* [32], and were stored in 50 mM cacodylate, buffer, pH 6.0, in the presence of 50% glycerol at −20 °C. Native Galβ1-3GalNAc α2-3sialyltransferase from porcine submaxillary glands was prepared by Sadler *et al.* [7], and was stored as above; antifreeze glycoprotein was the substrate for this enzyme; this was anti-freeze glycoprotein 3 [33]. Recombinant enzymes were the secreted forms of the Galβ1-4GlcNAc α2-6sialyltransferase [34], the Galβ1-3(4)GlcNAc α2-3sialyltransferase [14], and the Galβ1-3GalNAc α2-3sialyltransferase [29].

EXPRESSION AND PURIFICATION OF THE Galβ1-3GalNAc α2-3SIALYLTRANSFERASE

Galβ1-3GalNAc α2-3sialyltransferase was expressed in large quantities using a recombinant baculovirus expression system. A DNA fragment containing the untranslated and coding sequences for the insulin signal/catalytic domain chimeric protein was produced from the vector pSVL-spST [14] by PCR employing amplimers containing terminal *Nhe* I sites:

5′ primer:
5′-GGGCTAGCATGGCCCTCTGGATGCG;

3′ primer:
5′-CCGCTAGCTCATCTGCCCTTGAAGATCC.

The resulting DNA was digested with *Nhe* I, and the fragment was cloned at the unique *Nhe* I site in pBlueBac, a baculovirus expression system transfer vector, under the control of the baculovirus polyhedrin promoter (Invitrogen; San Diego, CA). The nucleotide sequence of the insert DNA was determined, and was found to be identical to the original template DNA. All recombinant DNA manipulations were performed under the conditions recommended by the enzyme manufacturers' instructions using standard protocols [34]. Creation of recombinant baculovirus was done using the MaxBac expression system (Invitrogen) following exactly the protocols recommended by the manufacturer, as described previously [35]. Briefly, Sf-9 insect cells were co-transfected with the transfer vector and wild type viral DNA, and the resulting mixture of wild-type and recombinant virus was shed into the culture medium. Recombinant viruses were isolated by repetitive plaque purification at limiting dilution and analysed by sialyltransferase assays. One clonal plaque was chosen for high levels of secreted enzyme, designated rBv2,3OST, and was expanded to 500 ml by infection of fresh Sf-9 cells. Large amounts of sialyltransferase were produced by infecting Sf-9 cells in monolayer culture with the recombinant virus. Medium was removed from confluent cells, the number of cells was estimated, and fresh medium containing five plaque forming units per cell was added. After 1 h, the inoculum was removed, fresh medium was added, and the culture was returned to the incubator. Typically after 72 h, ≈25 U of sialyltransferase had accumulated per litre of medium. The medium was then collected, and the recombinant sialyltransferase was purified.

Purification method I

Two hundred ml of media containing the Galβ1-3GalNAc α2-3sialyltransferase was filtered and diluted to 400 ml with 200 ml of 50 mM cacodylic acid, 50 mM NaCl, pH 6.5. Samples were then applied to a column (1.5 × 12.0 cm) of CDP-hexanolamine-agarose (14 mmol CDP ml^{-1}). After washing with 50 mM cacodylic acid, 0.1 M NaCl, pH 6.5, the column was eluted with 50 mM cacodylic acid, 1.0 M NaCl, pH 6.5. A single peak of the activity was observed. Fractions containing Galβ1-3GalNAc α2-3sialyltransferase activity were pooled and dialysed overnight against 1 litre of 50 mM cacodylic acid, 0.1 M NaCl, 50% glycerol, pH 6.5, and stored at −20 °C.

Purification method II

Two hundred ml of media containing the Galβ1-3GalNAc α2-3sialyltransferase was filtered and diluted to 400 ml with 200 ml of 50 mM cacodylic acid, 50 mM NaCl, pH 6.5. Samples were then applied to a column (1.0 × 10.0 cm) of CDP-hexanolamine-agarose (14 mmol CDP ml^{-1}). After washing the column with 50 mM cacodylic acid, 0.15 M NaCl, pH 6.5, the column was eluted with 50 mM cacodylic acid, 0.1 M NaCl, 1 mM CTP, pH 6.5. Two peaks of the activity were observed. Fractions containing α2,3-sialyltransferase activity from each peak were pooled separately and dialysed overnight against 1 l of 50 mM cacodylic acid, 0.1 M NaCl, 50% glycerol, pH 6.5, and then stored at −20 °C.

SDS-GEL ELECTROPHORESIS

Samples were boiled in a Laemmli sample buffer prior to loading on the 8–16% SDS-polyacrylamide gel (Novex). Protein bands were visualized by Coomassie Blue.

SIALYLTRANSFERASE ENZYME ASSAYS

Sialyltransferase was assayed based on methods described by Paulson *et al.* [6]. The assay mix contained appropriate acceptor substrate, 50 μg bovine serum albumin, 50 mM cacodylate buffer, pH 6.0, 0.5% Triton CF-54, 0.15–0.4 mU of appropriate sialyltransferase [32] and 9 nmol CMP[^{14}C]NeuAc in a total volume of 60 μl. Sialyltransferase activity was defined as the amount of enzyme transferring 1 μmol of sialic acid per min to acceptors defined by Weinstein *et al.* [10] for the N-glycan sialyltransferases (using asialo α_1 acid glycoprotein substrate) and Rearick *et al.* [36] for the Galβ1-3GalNAc α2-3sialyltransferase. Samples were incubated for 20 min at 37 °C. At the end of incubation samples were treated in two ways. When human and rat asialo α_1 acid glycoprotein and antifreeze glycoprotein were used the reaction was stopped with 0.2 ml ice cold 0.15 M NaCl, 5 mM NaN_3 and transferred to ice; the solution was applied to a 0.7 × 13 cm column of Sephadex G-50 (fine grade) equilibrated with NaCl solution and allowed to soak in and then the column was washed with a further 1.2 ml NaCl which was discarded; the labelled protein which eluted in the next 1.5 ml was collected in a scintillation counting vial and the radioactivity determined. When *N*-acetyllactosamine or lacto-*N*-tetraose were used as substrates the incubation conditions were as described above, but the reaction was stopped by addition of 1 ml cold 5 mM sodium phosphate buffer, pH 6.8, and the solutions applied to 2.5 cm columns of Dowex-1 X 8(PO_4^{2-}) [6]. Effluents were collected in scintillation vials, the incubation tube was washed with 1 ml of phosphate buffer which was applied to the column and the effluent collected as above. When CMP-NeuAc was used as the variable substrate a series of eight solutions were prepared each containing the same amount of radioactive CMP-NeuAc (54 500 cpm); the final concentrations varied from 10 to 70 μM. The substrates were saturating concentrations of *N*-acetyllactosamine for Galβ1-4GlcNAc α2-6sialyltransferase and lacto-*N*-tetraose for Galβ1-3(4)GlcNAc α2-6sialyltransferase. For the Galβ1-3GalNAc α2-3sialyltransferase, the concentrations of CMP-NeuAc ranged from 2.5 to 20 μM, and a saturating concentration of anti-freeze glycoprotein (equivalent to 75 nmol of Gal) was used as acceptor. Values for relative K_m were determined from intercepts of double reciprocal plots; in all determinations five different substrate concentrations were used above and below the K_m values for the acceptors with two or three assays for each point; reproducibility was within ±8%. In all assays the consumption of CMP-NeuAc was limited to less than 10% under these conditions. The amount of sialyltransferase varied with acceptor between 0.1–1.0 mU.

Results

Production and purification of recombinant, secreted forms of sialyltransferases

cDNA clones encoding the Galβ1-4GlcNAc α2-6sialyltransferase, the Galβ1-3(4)GlcNAc α2-3sialyltransferase and the Galβ1-3GalNAc α2-3sialyltransferase have been isolated, and the nucleotide sequences of the clones were determined [11, 14, 29]. To produce the enzymes in more readily purified forms, DNA constructions were made to create clones encoding chimeric proteins containing secretion signal sequences fused to the C-terminal catalytic domains of the enzymes downstream of mammalian cell promoters. Cells transfected with these constructions were shown to produce active enzyme that was secreted into the tissue culture medium [14, 29, 30]. Amplified CHO cell lines produce reasonable amounts of enzyme, and the Galβ1-4GlcNAc α2-6sialyltransferase used in this study was purified by affinity chromatography from CHO cell supernatant [30].

To produce larger quantities of secreted Galβ1-3(4)GlcNAc α2-3sialyltransferase and Galβ1-3GalNAc α2-3sialyltransferase, it was necessary to use the chimeric DNAs encoding the enzymes to create recombinant baculoviruses. A recombinant baculovirus encoding a secreted form of the Galβ1-3GalNAc α2-3sialyltransferase was made by inserting the DNA construction encoding the dog pancreas preproinsulin signal sequence fused to the C-terminal catalytic domain of the enzyme [14] into a baculovirus DNA transfer plasmid vector under control of the strong polyhedrin (*polh*) promoter. This vector was mixed with wild-type *Autographa californica* nuclear polyhedrosis virus (AcNPV) DNA, and the DNAs were used to transfect Sf-9 insect cells. The transfer vector contains sequences allowing recombination with wild-type virus, producing recombinant baculovirus encoding secreted sialyltransferase. The recombinant virus was purified by sequential plaque purification at limiting dilutions, then expanded to approximately 500 ml of high-titre virus, and this virus stock was used to infect confluent monolayers of Sf-9 cells. Enzyme accumulation was monitored over time, and a peak of activity was found approximately 72 h post infection (data not shown). Typically, Galβ1-3GalNAc α2-3sialyltransferase activity accumulated to 25 mU ml^{-1} of cell supernatant.

To purify the Galβ1-3GalNAc α2-3sialyltransferase from conditioned media produced by cells infected with rBv2,3OST, the media was applied to a column of CDP-hexanolamine-agarose. Enzyme strongly adsorbed to the column during application and subsequent wash steps in buffers containing 0.1 M NaCl. The enzyme was

eluted by buffer containing 1 M NaCl (Fig. 1), but yields were unacceptably low. CTP has been shown to be a potent inhibitor of the sialyltransferase [7], thus 1 mM CTP was used instead to elute the enzyme from the affinity column. Chromatography in this condition yielded two overlapping peaks (Peak A and B) of activity (data not shown). The fractions corresponding to each peak were pooled into peak A and peak B preparations, and Fig. 1 shows that the molecular weight of the enzyme from Peak A (33 kDa: lane B) is slightly larger than that of the enzyme from Peak B (32 kDa: lane C). The total recovery of activity resulting from this chromatography procedure is high (≈90%), and the specific activity across both peaks is roughly constant at 3.1 U mg^{-1} for both Peak A and Peak B preparations. The reason for the difference in molecular weights is presently unclear but is possibly due to heterogeneity in the structure of the N-linked carbohydrate moiety.

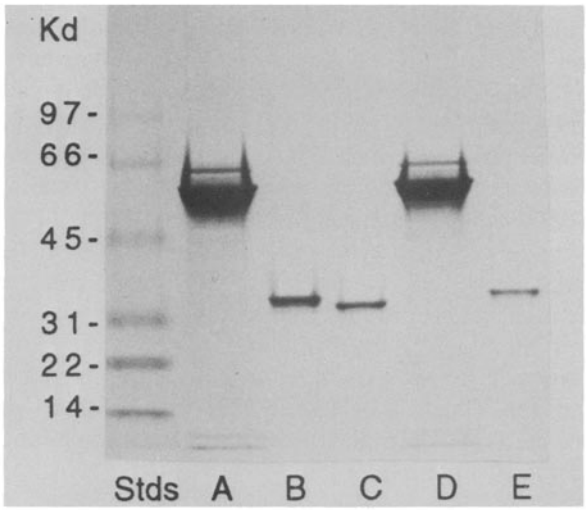

Figure 1. Analysis of the purification of the Galβ1-3GalNAc α2-3sialyltransferase by SDS-gel electrophoresis. The cloned Galβ1-3GalNAc α2-3sialyltransferase DNA was expressed in Sf9 cells using a baculovirus vector. The culture media (lane A and D) and the purified fractions from the CTP elution (lane B from Peak A and lane C from Peak B) and the NaCl elution (lane E) were diluted with Laemmli sample buffer and boiled prior to loading on the gel as described in Methods. Coomassie blue-stained proteins are compared with standards (Stds) indicated to the left.

The production of recombinant baculovirus encoding Galβ1-3(4)GlcNAc α2-3sialyltransferase and the partial purification of enzyme has been described previously [35].

Determination of sialyltransferase enzyme kinetic values

Values have been reported for apparent K_m of native Galβ1-4GlcNAc α2-6sialyltransferase, Galβ1-3(4)-GlcNAc α2-3sialyltransferase [10] and Galβ1-3GalNAc α2-3sialyltransferase [36] with the substrates used in the current work. However, there is no kinetic data on the recombinant enzymes corresponding to these native forms. Since the recombinant forms of these enzymes are now available it is possible to make a direct comparison of the kinetic properties of the native and recombinant enzymes where the assays have been done under identical conditions. Table 1 shows the values found for apparent K_m for the native and recombinant forms of the three enzymes under study using macromolecular and oligosaccharide substrates. Table 2 shows values for K_m with CMP-NeuAc as the variable substrate. Literature

Table 1. Kinetic constants for sialyltransferases, apparent K_m, values in mM[a]

Acceptor	*Galβ1-4GlcNAcα2-6sialyltransferase*		*Galβ1-3(4)GlcNAcα2-3sialyltransferase*		*Galβ1-3GalNAcα2-3sialyltransferase*	
	Native	*Recombinant*	*Native*	*Recombinant*	*Native*	*Recombinant*
Rat asialo α_1 acid glycoprotein (Galβ1-4GlcNAcRandGalβ1-3GlcNAcR)	0.24	0.18	0.18	0.15	–	–
Human asialo α_1 acid glycoprotein (Galβ1-4GlcNAcR)	0.21 (0.18)	0.10	0.26 (0.29)	0.10	–	–
N-acetyllactosamine (Galβ1-4GlcNAc)	1.78 (1.62)	2.38	2.43 (2.66)	2.63	–	–
Lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc)	–	–	0.11 (0.09)	0.22	–	–
Anti-freeze glycoprotein (Galβ1-3GalNAc-O-)	–	–	–	–	0.84 (0.39)	1.18

[a]Values for K_m for the macromolecular acceptors are expressed in mM relative to the terminal Gal residues (see e.g. [32]); on average human asialo α_1 acid glycoprotein contains 18 mol Gal per mol and the rat asialo α_1 acid glycoprotein used in these studies contains 10 residues per mol [47]; it should be noted that the rat protein has been shown to contain both Galβ1-4GlcNAc and Galβ1-3GlcNAc acceptor positions [37]. Values in parenthesis are literature values for the Galβ1-4GlcNAcα2-6sialyltransferase and the Galβ1-3(4)GlcNAcα2-3sialyltransferase from rat liver [10] and for the Galβ1-3GalNAc α2-3sialyltransferase from porcine submaxillary gland [36].

Table 2. Kinetic constants for sialyltransferases with CMP-NeuAc as variable substrate, apparent K_m, values in μM*

Galβ1-4GlcNAcα2-6sialyltransferase	
Native	33.3 (50)
Recombinant	42.7
Galβ1-3(4)GlcNAcα2-3sialyltransferase	
Native	57.3 (70)
Recombinant	74.1
Galβ1-3GalNAcα2-3sialyltransferase	
Native	5.3 (3.0)
Recombinant	5.1

*CMP-NeuAc concentrations were varied as described in the text; values in parenthesis are literature values [48].

values of K_m for the native enzymes as determined by others are given in parenthesis in the Tables.

As can be seen in Tables 1 and 2, K_m values for the native enzymes as determined in the present studies differed little from literature values in most cases. There were two exceptions; the first was with Galβ1-3GalNAc α2-3sialyltransferase where the value for K_m of 0.84 mM using anti-freeze glycoprotein compared with a literature value of 0.39 mM. The reason for this is unclear, but the higher value obtained in the current work is comparable to a value of 1.18 mM found for the corresponding recombinant. The second exception occurred with the K_m values found with CMP-NeuAc as the variable substrate. In the present work the K_m for native Galβ1-4GlcNAc α2-6sialyltransferase was 33.3 μM compared to a literature value of 50 μM and for the native Galβ1-3(4)GlcNAc α2-6sialyltransferase the K_m was 57.3 μM compared to 70 μM reported in the literature, and for the native Galβ1-3GalNAc α2-3sialyltransferase the K_m was 5.3 μm compared with a literature value of 3.0 μm. Again it is unclear why these differences exist, but the variance is not large and may simply reflect differences in experimental technique between the two sets of determinations.

Of greater interest are the values for K_m found for the native enzymes when compared with K_m values for the corresponding recombinants. This is particularly important in order to ensure that the recombinant enzymes are mimicking the catalytic properties of the native enzymes as far as possible. Differences might be expected since the recombinant enzymes are expressed in different cell types compared to their native counterparts. With the oligosaccharide acceptors it was found that the native enzymes generally had lower K_m values than the corresponding recombinants indicating that the native enzymes have a higher affinity for the oligosaccharide acceptors. This was particularly noticable with Galβ1-3(4)GlcNAc α2-3sialyltransferase when lacto-*N*-tetraose was the acceptor; the K_m for the native enzyme was 0.11 mM, half that found for the recombinant. With the macromolecular acceptors there were also differences in values for K_m between the native and corresponding recombinant enzymes. The greatest difference was with the α2-3 and α2-6 sialyltransferases for N-linked structures with human asialo α_1 acid glycoprotein as acceptor. It was found that K_m values for the recombinants were about half those found for the native enzymes using this acceptor (Table 1). With the rat acceptor there was only a small difference in values of K_m between the native and recombinant enzymes, but it should be noted that values of K_m found for the Galβ1-3(4)GlcNAc α2-3 enzyme will be a mixed K_m since the rat acceptor contains both terminal Galβ1-3 and Galβ1-4 positions [37]. There was only a small difference in K_m between native and recombinant forms of Galβ1-3 GalNAc α2-3sialyltransferase acid with anti-freeze glycoprotein as acceptor (Table 1). Similarly, with CMP-NeuAc as variable substrate and sialyltransferases for N-linked structures it was found that both recombinants had a lower affinity for the substrate based on their K_m values while the sialyltransferase for the O-linked structures had little difference in K_m (Table 2).

Discussion

Prior to 1992, more than 320 genes had been expressed through recombinant baculovirus vectors [38], and at least twice that number have now been reported to have been expressed in recombinant baculovirus-infected insect cells. The popularity of this system is likely due to the high yields of protein expression reported in infected cells (0.01–1 $g l^{-1}$ of tissue culture medium) while maintaining eukaryotic post-translational modification. In this work, sialyltransferases purified from tissues were compared to genetically engineered versions of the same proteins produced in recombinant CHO cells and by recombinant baculovirus-infected cells. The dog pancreas preproinsulin signal sequence, cloned in place of the normal N-terminal signal/anchor and cytoplasmic domains of the sialyltransferases so as to direct secretion of the catalytic COOH-terminal domain, was recognized and processed by recombinant baculovirus-infected insect cells in order to produce secreted enzyme. The secreted enzymes contained N-linked carbohydrate chains (data not shown), but the carbohydrate structure may be more heterogeneous than protein secreted from recombinant mammalian cells. Based on K_m values given in Tables 1 and 2 it is clear that some differences exist when the native enzymes are compared with the corresponding recombinants. The recombinants generally express a lower affinity for small molecular weight oligosaccharide acceptors and a higher affinity for macromolecular acceptors except for the α2-3sialyltransferase and anti-freeze glycoprotein; in addition, the recombinants for N-linked carbohydrate substrates have a lower affinity for CMP-

NeuAc. The differences found in K_m values between the native and recombinant enzymes are unlikely to result in major changes in the way these recombinants can be used for glycosylation of glycoproteins. However, the altered affinities found for the recombinants may affect the way they bind to affinity columns; for example, we have found that the recombinant Galβ1-3(4)GlcNAc α2-3sialyltransferase does not bind as well to CDP-hexanolamine affinity columns (unpublished work) which is consistent with the higher K_m value found for this enzyme using CMP-NeuAc as variable substrate. While some recombinant enzymes have identical biological properties and have similar kinetic parameters when compared with their native counterparts (e.g. tyrosine hydroxylase [39, 40]); some behave differently; for example, recombinant human cathepsin L which has a K_m value that is about half of that for the corresponding native protein using a peptide substrate, is less stable and has a higher pH optimum [41].

It is not clear why the recombinant sialyltransferases used in the present study have slightly different kinetic properties compared to the native enzymes. The recombinant sialyltransferases represent secreted forms devoid of the membrane anchor and stem regions [11, 14, 29]; the native enzymes have also lost these regions during preparation [10, 32, 36, 42] so there is little or no difference between the recombinants and native enzymes as far as amino acid sequence is concerned. However, all three enzymes are glycoproteins and it is known that the lack of carbohydrate chains or the presence of carbohydrate chains that differ in structure from those present on the native molecule can influence the biological properties of the proteins in question, presumably because the carbohydrate chains affect protein folding as well as charge in the case of those molecules carrying sialylated chains. The importance of carbohydrate chains for the behaviour of a wide range of biomolecules has recently been reviewed [43], and there is a recent study on native Galβ1-4GlcNAc α2-6sialyltransferase which showed that enzyme activity is reduced on sequential removal of sugar residues from the glycoprotein and that the completely deglycosylated enzyme is inactive [44].

The Galβ1,3GalNAc α2,3sialyltransferase purified from recombinant-baculovirus-infected insect cells described in this work was determined to have a specific activity of 3.1 U mg^{-1}, comparable to the reported value of 10.6 U mg^{-1} for enzyme purified from porcine submaxillary glands [7]. In contrast, Galβ1,4GlcNAc α2,6sialyltransferase expressed in E. coli was found to accumulate in an insoluble, inactive form [45]. Denaturation/refolding experiments recovered enzyme with a specific activity of 0.15 U mg^{-1} [45]. Expression of full length Galβ1,4GlcNAc α2,6sialyltransferase in Saccharomyces cerevisiae has been shown to result in accumulation of sialyltransferase activity [46], suggesting that sufficient carbohydrate addition and maturation occur in yeast to allow folding of the enzyme into an active form. Taken together, these results indicate that eukaryotic host cells capable of glycosylation of the enzyme are required for efficient expression of active sialyltransferases, and show that recombinant baculovirus-infected insect cells are an efficient expression system for production of large amounts of enzyme with specific activity similar to the native enzyme. Although the recombinant sialyltransferases used in this work were expressed in eukaryotic cell lines which possess the biosynthetic machinery for glycosylation there is no definitive information on the extent or structure of the carbohydrate chains attached to the enzymes; clearly, further work is needed to determine whether the activities of the recombinant sialyltransferases may be altered by modification of the oligosaccharide chains' structure.

Acknowledgements

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to J.C.J. and a grant from the National Institutes of Health GM27904 to J.C.P.; J.C.J. also wishes to acknowledge an International Research award from NSERC to allow studies in the United States.

References

1. Hesford FJ, Berger EG, Van Halbeek H (1984) *Glycoconjugate J* **1**: 141–53.
2. Higa HH, Paulson JC (1985) *J Biol Chem* **260**: 8838–49.
3. Josiasse DH, Bergh MLE, ter Hart HGJ, Koppen PL, Hooghwinkel GJM, Van den Eijnden DH (1985) *J Biol Chem* **260**: 4941–51.
4. Melkerson-Watson LJ, Sweeley CC (1991) *J Biol Chem* **266**: 4448–57.
5. Miyagi T, Tsuiki S (1982) *Eur J Biochem* **126**: 253–61.
6. Paulson JC, Beranek WE, Hill RL (1977) *J Biol Chem* **252**: 2356–62.
7. Sadler JE, Rearick JI, Paulson JC, Hill R (1979) *J Biol Chem* **254**: 4434–43.
8. Sadler JE, Rearick JI, Hill R (1979) *J Biol Chem* **254**: 5934–41.
9. Sticher V, Gross HJ, Brossmer R (1988) *Biochem J* **253**: 577–80.
10. Weinstein J, Sousa-e-Silva U de, Paulson JC (1982) *J Biol Chem* **257**: 13845–53.
11. Weinstein J, Lee EU, McEntee K, Lai PH, Paulson JC (1987) *J Biol Chem* **262**: 17735–43.
12. Bast BJEG, Zhou L-J, Freeman GJ, Colley KJ, Ernst TJ, Munro JM, Tedder TF (1992) *J Cell Biol* **116**: 1423–35.
13. Eckhardt M, Muhlenhoff M, Bethe A, Koopman J, Frosch M, Gerardy-Schahn R (1995) *Nature* **373**: 715–18.
14. Gillespie W, Kelm S, Paulson JC (1992) *J Biol Chem* **267**: 21004–10.

15. Hamamoto T, Kawasaki M, Kurosawa N, Nakaoka T, Lee Y-C, Tsugi S (1993) *Bioorg Med Chem Lett* **1**: 141–45.
16. Haraguchi M, Yamashiro S, Yamamoto A, Furukawa K, Takamiya K, Lloyd KO, Shiku H, Furukawa K (1994) *Proc Natl Acad Sci USA* **91**: 10455–59.
17. Kitagawa H, Paulson JC (1993) *Biochem Biophys Res Comm* **194**: 375–82.
18. Kitagawa H, Paulson JC (1994) *J Biol Chem* **269**: 1349–1401.
19. Kurosawa N, Kawasaki N, Hamamoto T, Nakaoka T, Lee Y-C, Arita M, Tsuji S (1994) *Eur J Biochem* **219**: 375–81.
20. Kurosawa N, Hamamoto T, Lee Y-C, Nakaoka T, Kojima N, Tsuji S (1994) *J Biol Chem* **269**: 1402–9.
21. Kurosawa N, Kojima N, Inoue M, Hamamoto T, Tsuji S (1994) *J Biol Chem* **269**: 19048–53.
22. Lee EU, Roth J, Paulson JC (1989) *J Biol Chem* **264**: 13848–55.
23. Lee YC, Kojima N, Wada E, Kurosawa N, Nakaoka T, Hamamoto T, Tsuji S (1994) *J Biol Chem* **269**: 10028–33.
24. Lee YC, Kurosawa N, Hamamoto T, Nakaoka T, Tsuji S (1993) *Eur J Biochem* **216**: 377–85.
25. Livingston BD, Paulson JC (1993) *J Biol Chem* **268**: 11504–7.
26. Nara K, Watanabe Y, Maruyama K, Kasahara K, Nagai Y, Sanai Y (1994) *Proc Natl Acad Sci USA* **91**: 7952–56.
27. Sasaki K, Watanabe E, Kawashima K, Sekine S, Dohi T, Oshima M, Hanai N, Nishi T, Hasegawa M (1993) *J Biol Chem* **268**: 22782–87.
28. Sasaki K, Kurata K, Kojima N, Kurosawa N, Ohta S, Hanai N, Tsuji S (1994) *J Biol Chem* **269**: 15950–56.
29. Wen DX, Livingston BD, Medzihradszky KF, Kelm S, Burlingame AL, Paulson JC (1992) *J Biol Chem* **267**: 21011–19.
30. Colley KJ, Lee EU, Adler B, Browne JK, Paulson JC (1989) *J Biol Chem* **264**: 17619–22.
31. Kaplan HA, Woloski BMRNJ, Hellman M, Jamieson JC (1983) *J Biol Chem* **258**: 11505–9.
32. Weinstein J, Sousa-e-Silva U de, Paulson JC (1982) *J Biol Chem* **257**: 13835–44.
33. DeVries AL, Komatsu SK, Feeney RE (1970) *J Biol Chem* **254**: 2901–8.
34. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* 2nd ed. Plainview, NY: Cold Spring Harbor Laboratory Press.
35. Ichikawa Y, Lin Y-C, Dumas DP, Shen G-J, Garcia-Junceda E, Williams MA, Bayer R, Ketcham C, Walker LE, Paulson JC, Wong CH (1992) *J Am Chem Soc* **114**: 9283–98.
36. Rearick JI, Sadler EJ, Paulson JC, Hill RL (1979) *J Biol Chem* **254**: 4444–51.
37. Yoshima H, Matsumoto A, Mizuochi T, Kawasaki T, Kobata A (1981) *J Biol Chem* **256**: 8476–84.
38. O'Reilly DR, Miller LK, Luckow V (1992) *Baculovirus Expression Vectors: A Laboratory Manual*. New York: W.H. Freeman Co.
39. Ichikawa S, Nasrin S, Nagatsu T (1991) *Biochem Biophys Res Comm* **178**: 664–71.
40. Ribeiro P, Wang Y, Citron BA, Kaufman S (1992) *Proc Natl Acad Sci USA* **89**: 9593–97.
41. Smith SM, Gottesman MM (1989) *J Biol Chem* **264**: 20487–95.
42. Lammers G, Jamieson JC (1988) *Biochem J* **256**: 623–31.
43. Varki A (1993) *Glycobiology* **3**: 97–130.
44. Fast D, Jamieson JC, McCaffrey G (1993) *Biochim Biophys Acta* **1202**: 325–30.
45. Hamamoto T, Lee YC, Kurosawa N, Nakaoka T, Kojima N, Tsuji S (1994) *Bioorg Med Chem* **2**: 79–84.
46. Krezdorn CH, Kleene RB, Watzele M, Ivanov SX, Hokke CH, Kamerling JP, Berger EG (1994) *Euro J Biochem* **220**: 809–17.
47. Jamieson JC, Friesen AD, Ashton FE, Chou B (1972) *Can J Biochem* **50**: 856–70.
48. Gross HJ, Rose U, Krause JM, Paulson JC, Schmid K, Feeney RE, Brossmer R (1989) *Biochemistry* **28**: 7386–92.