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Note

Phenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside, a substrate for sialyltransferase

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Serum or plasma sialyltransferase may be increased in rats and humans having malignant tumors[1–4], but little is known about the nature or function of these enzymes. Although sialyltransferases have been isolated from other sources[5,6], none of the serum sialyltransferases of patients with metastasising tumours or normal individuals have been purified, and in most studies desialylated fetuin was employed as the acceptor molecule for the sialyltransferase reaction. Several sialyltransferases may be involved in the transfer of sialic acid to the asialofetuin molecule[7,8].

p-Nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside was described recently[9]. The parent disaccharide unit [β-Gal-(1→3)-GalNAc], which is found in the "antifreeze" glycoproteins, as well as in several other glycoproteins including asialofetuin[8,10,11], is an efficient acceptor for certain sialyltransferase enzymes found in rat-liver and rat-mammary adenocarcinoma[12] and in porcine submaxillary glands[13].

Since *p*-nitrophenyl glycopyranosides can easily be linked to agarose for affinity chromatography[14,15], the β-Gal-(1→4)-GalNAc derivative may be useful for the isolation and characterisation of the enzymes from pathological sera.

We now report on the sialyltransferase acceptor activity of the phenyl glycoside of β-Gal-(1→3)-GalNAc. This derivative was used in place of the *p*-nitrophenyl analogue due to its greater aqueous solubility.

The disaccharide portion β-Gal-(1→3)-GalNAc of the antifreeze glycoprotein, as well as the free disaccharide, can serve as an efficient acceptor for solubilised sialyltransferases from rat-liver and rat-mammary adenocarcinomas[11]. The ability of the phenyl glycoside of this disaccharide to act as an acceptor for sialyltransferase from normal human serum was tested (Fig. 1a). When sialyltransferase was assayed in the presence of the glycoside, a radioactivity scan of the acid-soluble products of the reaction consistently showed a third peak which was not observed in the controls. Incorporation into the third peak was linear during incubation for 2 h. The following data suggest that this third peak represents a trisaccharide composed of [^{14}C]sialic

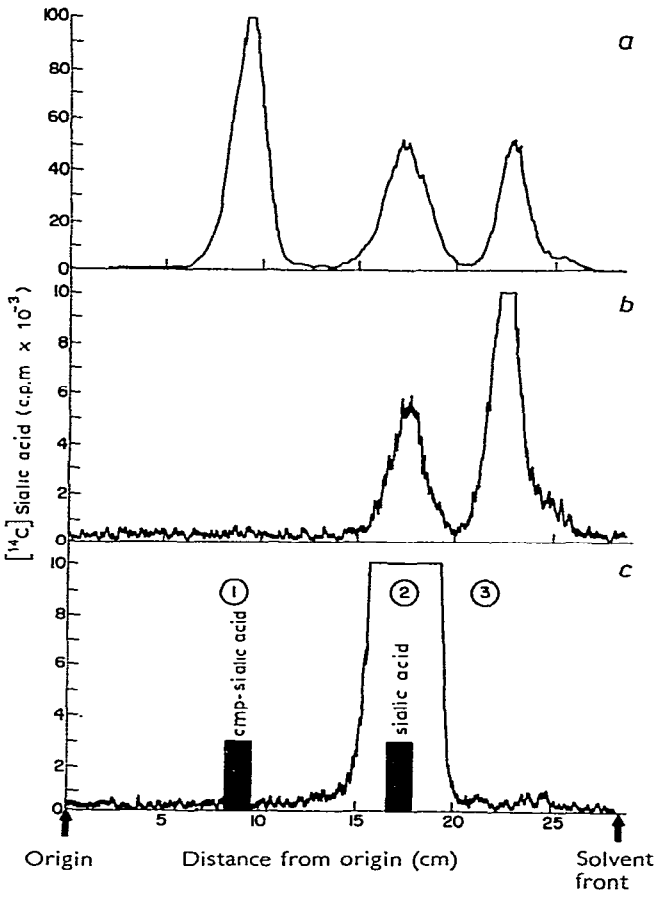

Fig. 1. Transfer of [^{14}C]sialic acid to the phenyl glycoside of β-Gal-(1→3)-GalNAc: radioactivity scans of chromatograms (*a*) after incubation with sialyltransferase and CMP-*N*-acetyl-[^{14}C]neuraminic acid, 2 h at 37°, (*b*) after breakdown of the sialo-containing third peak by neuraminidase, (*c*) after mild, acid hydrolysis of the sialo-containing third peak.

acid attached to the phenyl glycoside. Incubation of the trisaccharide with *Vibrio cholerae* neuraminidase (1 h, 37°; 0.1M cacodylate buffer, pH 5.5) effected partial degradation and released sialic acid (Fig. 1b). No degradation occurred in controls from which neuraminidase was omitted. Mild, acid hydrolysis[16] of the trisaccharide under conditions (12.5mM H_2SO_4, 1 h, 80°) used for preparation of sialic acid-free fetuin released all of the sialic acid (Fig. 1c).

The specific serum sialyltransferase which transfers sialic acid from CMP-sialic acid to the phenyl glycoside showed no requirement for divalent cation. Enzyme activity was observed over a broad range of pH, with optimal activity occurring at 6.0 (Fig. 2). The addition of 5mM sialic acid to the assay medium resulted in no detectable alteration in enzyme activity, whereas the addition of mM CMP completely abolished enzyme activity. A K_m value of 20.4 μM was observed for the phenyl glycoside, using a Lineweaver–Burke double-reciprocal plot (Fig. 3).

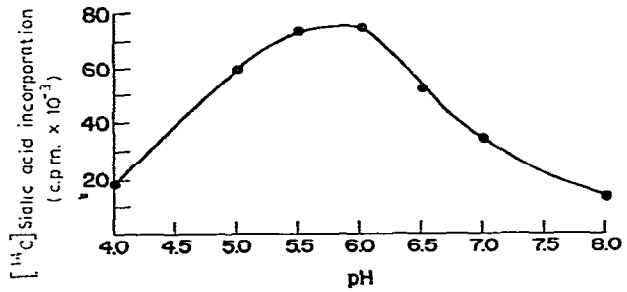

Fig. 2. The effect of pH on sialyltransferase activity with the phenyl glycoside of β-Gal-(1→3)-GalNAc as acceptor.

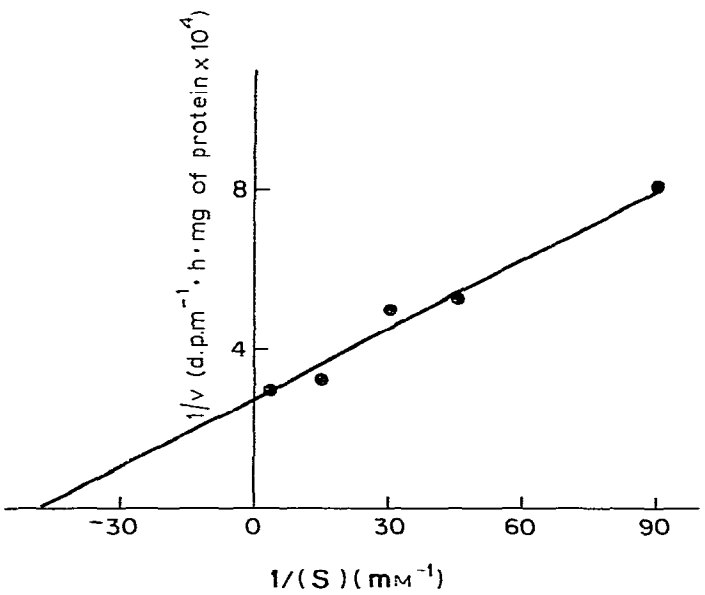

Fig. 3. Lineweaver–Burk double-reciprocal plot of CMP-sialic acid transfer to the phenyl glycoside of β-Gal-(1→3)-GalNAc.

When sialyltransferase activity to asialofetuin, asialo-ovine submaxillary mucin, or asialo-α_1-acid glycoprotein (all at 3 mg/ml) was measured in the presence of the phenyl glycoside (also at a final concentration of 3 mg/ml), the activity was diminished by 28, 35, and 0%, respectively.

The location of the sialic acid moiety was not determined and it could be attached both to galactosyl and 2-acetamido-2-deoxygalactosyl residues as in the oligosaccharide chains of fetuin[8]. The inhibition of sialic acid incorporation into asialofetuin, but not into asialo-α_1-acid glycoprotein, by the phenyl glycosides is consistent with their structures, since the carbohydrate chains of asialofetuin terminate in either β-Gal-(1→3)-GalNAc or β-Gal-(1→4)-GlcNAc, whereas α_1-acid glycoprotein contains only the latter disaccharide unit. Furthermore, since the phenyl

glycoside inhibits incorporation of sialic acid into asialo-ovine submaxillary mucin, a glycoprotein composed preponderantly of GalNAc-α-Thr-/Ser groups[5] suggests that at least some of the sialic acid attached to the phenyl glycoside is (2→6)-linked to GalNAc. The structure of the trisaccharide is being studied further.

The level of sialyltransferase activity in the bloodstream may be important in such disease states as muscular dystrophy[17], α_1-antitrypsin deficiency[18], cystic fibrosis[18], various liver diseases[19], and cancer[1-4]. In the last disease, serum sialyltransferase levels are significantly elevated in animals and patients bearing malignant tumors[1-4]. However, in these studies, asialofetuin was the only acceptor employed in the sialyltransferase assays, and no attempt was made to determine which of the sialyltransferases present in the sera were elevated. We have begun a study aimed at the purification and characterisation of the sialyltransferases which contribute to the increased enzyme levels observed in sera from patients having metastatic tumors. Attachment of such a synthetic substrate as the phenyl glycoside described above to a gel matrix should be useful for the purification of specific sialyltransferases.

EXPERIMENTAL

Human blood from normal individuals was allowed to clot. Serum was obtained by centrifugation and stored at −20°. Serum sialyltransferase assays were performed by using a modification of the procedure described by Bernacki[20]. The assay media consisted of 0.1M cacodylate buffer (pH variously 4–8), 3.0 mg/ml of phenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside[9], 1.0–3.0 mg of serum protein, and 9.65μM CMP-*N*-acetyl-[^{14}C]neuraminic acid (259 mCi/mmole). Incubations were performed at 37° in a shaker bath usually for 30 min, but occasionally up to 2 h. The results (average of 3 expts.) were as follows:

Acceptor	±*Phenyl glycoside*	*Specific activity*	*Inhibition* (%)
Asialofetuin	—	17,754	28
	+	12,808	
Asialo-α_1-acid glycoprotein	—	14,490	0
	+	14,420	
Asialo-ovine submaxillary mucin	—	2,360	35
	+	1,529	

When asialofetuin was used as the acceptor, the reaction was terminated by the addition of 1% phosphotungstic acid in 0.5M HCl. Samples were washed twice in 10% aqueous trichloroacetic acid and once in 95% ethanol–ether (2:1), and counted in a Packard Tri-Carb β-γ liquid scintillation counter. Protein was deter-

mined by the procedure of Lowry *et al.*[21]. Enzyme activity was calculated as the difference between the exogenous and endogenous activity and expressed as d.p.m. of *N*-acetyl-[^{14}C]neuraminic acid incorporated per h per mg of protein.

When the phenyl glycoside of β-Gal-(1→3)-GalNAc was used as the acceptor, reactions were terminated by the addition of 95% ethanol. The precipitate was removed by centrifugation, and the supernatant material was chromatographed on S + S Orange Ribbon Paper (Schleicher and Schuell) with 95% (ethanol)–M ammonium acetate (7:3). Radioactivity on the chromatogram was detected by using an Actagraph strip scanner (Searle).

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