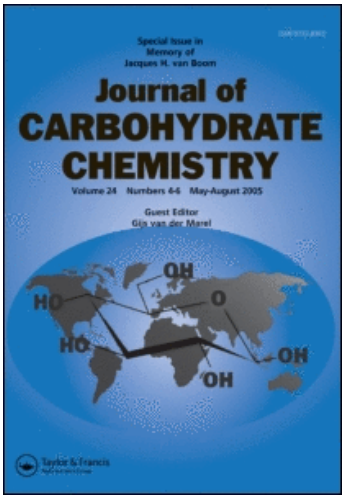

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NEW POLYMERIC INHIBITOR OF GALACTOSYL TRANSFERASE[1]

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ABSTRACT

2',3'-Di-*O*-acetyluridine 5'-*p*-styrenesulfonate was synthesized by the reaction of 2',3'-di-*O*-acetyluridine with *p*-styrenesulfonyl chloride and polymerized. After removal of acetyl groups, the polymeric product was shown by NMR spectroscopy and gel permeation chromatography to be poly(uridine 5'-*p*-styrenesulfonate). This uridine-containing polymer was tested against the galactosyl transferase that synthesizes lactose in the presence of α-lactalbumin. The polymeric compound did inhibit the enzyme with 75% inhibition requiring 120 μM which is only one percent of the concentration of glycosyl donor substrate (UDP-galactose, 12 mM).

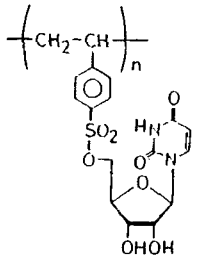
INTRODUCTION

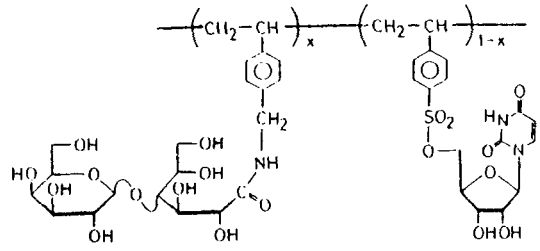
Many natural oligosaccharides in glycoproteins and glycolipids participate in a variety of biochemical functions *in vivo*. It has been noted that the chemical structure of the oligosaccharide is important for the biochemical functions. Therefore, control of activities of enzymes responsible for biosynthesis of oligosaccharides must be important for a better understanding of the functions of oligosaccharides. For this purpose, inhibition of biosynthetic pathway is quite effective and thus it is important to synthesize new enzyme inhibitors.

Many inhibitors of the processing enzymes (glycosidases) for N-linked oligosaccharide are known. For example, swainsonine inhibits mannosidase II,[2] castanospermine inhibits glucosidase I[3] and glucosidase II,[4] and so on. On the other hand, not many glycosyl transferase inhibitors such as tunicamycin, that inhibits the first step in the lipid-linked saccharide pathway,[5] have been found. In this investigation, we attempted to prepare a new inhibitor of a glycosyl transfer reaction by chemically attaching a *p*-styrenesulfonyl group to the 5'-OH of uridine followed by polymerization and deprotection.

RESULTS AND DISCUSSION

In the previous study, 2',3'-*O*-isopropylideneuridine 5'-*p*-styrenesulfonate was synthesized and polymerized.[6] However, the deprotection of the polymer gave an irregular structure due to a side reaction. In this investigation, the acetyl derivative was used as a protecting group because acetyl groups of poly(2',3'-di-*O*-acetyluridine 5'-*p*-styrenesulfonate) could be removed under mild condition without side reactions. The polymeric product was shown by NMR spectroscopy and gel permeation chromatography to be poly(uridine 5'-*p*-styrenesulfonate).





Three kinds of synthetic polymers, namely poly(uridine 5'-*p*-styrenesulfonate), poly{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}, and copoly[(uridine 5'-*p*-styrenesulfonate)-{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}], were incubated with galactosyl transferase to test their inhibitory effectiveness against this enzyme. The standard incubation mixtures for assay of galactosyl transferase contained 0.1 U of galactosyl transferase (EC 2.4.1.22, SIGMA Chemical Co.), 0.12 or 1.2 μmol of UDP-galactose, 0.12 μmol of *p*-nitrophenyl α-D-glucopyranoside, 0.2 ng of α-lactalbumin, 0.5 μmol of $MnCl_2$, and various amounts of inhibitors in 0.1 M cacodylate buffer (pH 7.5), all in a total volume of 100 μL. After incubation at 37 °C for 24 h (Time course studies are shown in FIG. 1.), the reaction was terminated by heating on a boiling

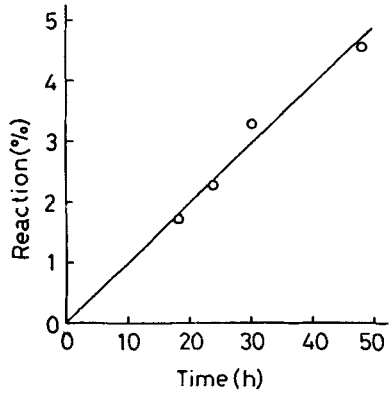


FIG. 1. Time course of the galactosyl transferase reaction.

water bath for 3 min. The reaction mixture was applied to a gel filtration column (Sephadex G-50, Pharmacia). A low-molecular-weight fraction was separated from a high-molecular-weight fraction which contained enzyme and the synthetic polymer. The obtained low-molecular-weight fraction was analyzed using reverse phase HPLC (column: CAPCELL PAK C18 SG120, Shiseido Research Center, Yokohama, Japan). The yield of the galactosyl transfer reaction was calculated from the peak intensities of *p*-nitrophenyl α-D-glucopyranoside and *p*-nitrophenyl 4-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside.

The results are shown in FIG. 2. The data showed that uridine-containing polymer and copolymer inhibited the galactosyl transferase, while galactose-containing polystyrene did not inhibit this enzyme. It can be speculated that the galactosyl transferase interacts with uridine residue much more strongly than with galactose residue, while the substrate specificity of this enzyme should be high for both uridine and galactose residues. Poly(uridine 5'-*p*-styrenesulfonate) proved to be a reasonable inhibitor of the galactosyl transferase with almost complete inhibition occurring at the concentration of less than 1.2 mM, which is the same concentration as glycosyl donor substrate (UDP-galactose). Furthermore, poly(uridine 5'-*p*-styrenesulfonate) inhibited the galactosyl transferase with 75% inhibition requiring the concentration of 0.12 mM which is only one percent of glycosyl donor substrate (UDP-galactose, 12 mM) as shown in FIG. 3.

Known competitive inhibitors such as UDP, UMP, and uridine did inhibit the galactosyl transferase. However, their inhibitory effects were not as strong as synthetic polymeric inhibitors. It can be presumed that the slower molecular motion of polymer chain than that of low-molecular-weight compound may increase the apparent affinity of

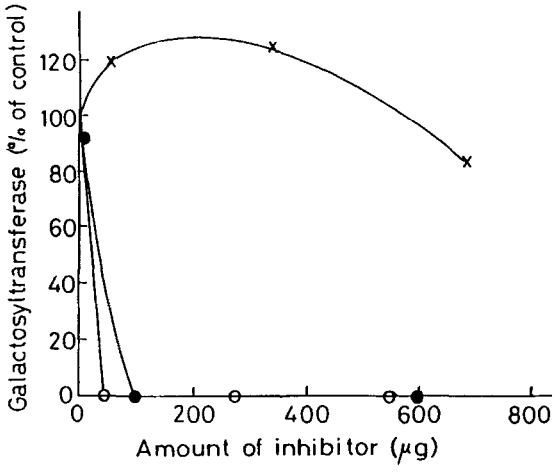


FIG. 2. Effect of substituent on the inhibition of galactosyl transferase by polystyrene derivatives. O: poly(uridine 5'-*p*-styrenesulfonate); ●: copoly[(uridine 5'-*p*-styrenesulfonate)-{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}]; X: poly{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}

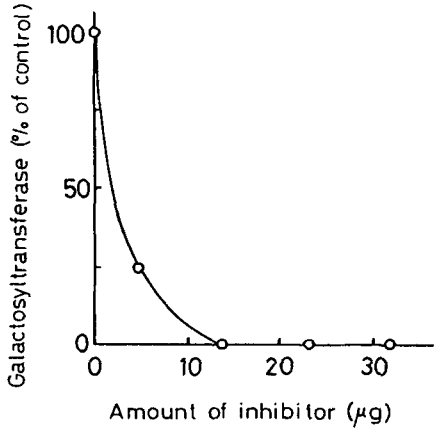


FIG. 3. Inhibition of galactosyl transferase by poly(uridine 5'-*p*-styrenesulfonate).

the enzyme for polymeric compounds. Therefore, poly(uridine 5'-*p*-styrenesulfonate) and copoly[(uridine 5'-*p*-styrenesulfonate)-{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}] showed much stronger inhibitory effect than low-molecular-weight compounds such as UDP. The investigation of the mechanism of inhibition is now in progress.

EXPERIMENTAL

To the solution of 2',3'-di-*O*-acetyluridine[7] (1.89 g, 5.76 mmol) and 4-dimethylaminopyridine (1.35 g, 11.05 mmol) in pyridine (30 mL), which had been refluxed over potassium hydroxide for several hours and then distilled, was added dropwise *p*-styrenesulfonyl chloride (1.73 g, 8.5 mmol), which had been synthesized by the reaction of sodium *p*-styrenesulfonate and thionyl chloride, with stirring at 0 °C. The reaction mixture was maintained at 0 °C for 2 days and neutralized with a cold saturated solution of sodium hydrogen carbonate. After extraction with chloroform, the organic layer was washed with water several times and dried over sodium sulfate. After solvent evaporation, the polymerizable product was chromatographed on silica gel, with chloroform-acetone (5:1 v/v) as eluent, and purified by precipitation using the acetone-hexane system to afford powdery 2',3'-di-*O*-acetyluridine 5'-*p*-styrenesulfonate.

Polymerization of 2',3'-di-*O*-acetyluridine 5'-*p*-styrenesulfonate was carried out in dimethyl sulfoxide with 2,2'-azobisisobutyronitrile as initiator at 70 °C under high vacuum. The polymerization was terminated by the addition of methanol. The obtained reaction mixture was used for the next step without purification. The polymer was deacetylated with methanol saturated with ammonia. After dialysis (3 days), the poly(uridine 5'-*p*-styrenesulfonate) was obtained by freeze drying the water.

Poly{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide} was prepared according to Kobayashi's method.[8] Copoly[(uridine 5'-*p*-styrenesulfonate)-{*N*-*p*-vinylbenzyl-4-*O*-(β-D-galactopyranosyl)-D-gluconamide}] was synthesized by copolymerization of uridine-containing monomer and galactose-containing monomer (1:1) followed by deprotection.

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