An Improved Method for the Determination of Glycosyltransferases Using \textit{para}-Nitrophenyl-Glycosides as Substrates

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Due to their easy purification nitrophenyl-glycosides have become of increasing interest as acceptor substrates for the assay of glycosyltransferase activity. In this communication reversed-phase chromatography is shown to provide a convenient means for the isolation of the radioactively labelled nitrophenylglycoside(s) formed in this reaction, superior to the methods hitherto used with regard to speed and specificity.

\textit{(Keywords: Glycosyltransferase assay; Reversed-phase chromatography)}

Eine verbesserte Methode zur Bestimmung von Glykosyltransferase-Aktivitäten unter Verwendung von Nitrophenyl-Glykosiden als Substrate


Introduction

Glycosyltransferase activity is determined usually by testing the amount of monosaccharide transferred from the respective sugar nucleotide onto suitable precursor substances. Oligosaccharides and glycoproteins are commonly used as sugar acceptors (see e.g.\textsuperscript{1–5}), only in a few cases monosaccharides are applicable. Recently it was found\textsuperscript{6} that also phenyl- and nitrophenylglycosides can act as acceptor substrates in glycosyltransferase assays. As they are easier available and well defined in their composition and structure, these substrates
proved to be far more comfortable glycosyl acceptors, and so they have replaced to a high degree most of the other substances mentioned above (e.g. 6–11).

In the investigations presented in this communication, reversed-phase chromatography was tested for its usefulness in glycosyltransferase assays. In contrast to the usual silica gel chromatography, the hydrophilic components of an aqueous solution are not adsorbed in "reversed-phase chromatography", whereas the lipophilic substances are retained on the column material. They can be eluted with dilute methanol.

This method promised to offer a simple means for isolating nitrophenyl-glycosides out of the incubation mixtures.

Materials and Methods

The preparation of the following materials has been described previously: β-galactosyltransferase from human milk12, hog gastric mucosal microsomes3, N-acetyl-galactosaminyl-1-phosphate13, and GDP-fucose14.

p-Nitrophenyl β-D-galactosyl-(2-N-acetyl)-β-D-glucosaminide was synthesized by enzymatic transfer of 14C-galactose from UDP-galactose onto p-nitrophenyl N-acetyl-β-D-glucosaminide (see below) using paper chromatography for purification of the reaction product. UDP-galactose, UDP-N-acetylgalcosamine as well as p-nitrophenyl β-D-galactoside, p-nitrophenyl N-acetyl-β-D-glucosaminide, D-galactose, L-fucose, and N-acetyl-D-glucosamine were purchased from Sigma Chemical Company, St. Louis, MO, USA; UDP-1-(14C)galactose was supplied by the Radiochemical Centre, Amersham.

SEP-PAK-C18 cartridges (columns containing about 0.8 ml of conventional silica gel particles coated with C18-hydrocarbon compounds) were obtained from Waters Associates Inc., Milford, MA, USA.

Concentrations of galactose and fucose were determined by the cysteine-sulphuric acid method15, acetylaminohexoses according to Reissig et al.16, N-acetylgalactosaminyl-1-phosphate being previously hydrolysed in 0.01M hydrochloric acid (15 minutes at 100 °C). Sugar nucleotides and p-nitrophenyl-glycosides were assayed by measuring their light absorption at 280 and 300 nm, respectively.

Galactosyltransferase action was measured by incubating the respective enzyme preparation (about 10–8 I.U.) with UDP-(14C)galactose (30 nmol, 10 mCi/mmole), MnCl2 (1 μmol), and p-nitrophenyl N-acetyl-β-D-glucosaminide (200 nmol) in an imidazole—HCl buffer (0.02 mol/I, pH 7.0) at 37 °C; the final volume was 85 μl. The incubation was terminated by the addition of an equal volume of 96% ethanol; protein thereby denatured was removed by centrifugation and the supernatant tested for the presence of p-nitrophenyl-disaccharide

a) by descending paper chromatography6 on Whatman 3MM using ethylacetate/pyridin/water (10:4:3) as the solvent. Sugar nucleotides and sugar phosphates remained near the origin, free sugars showed Rf values between 0.25 and 0.40, and the p-nitrophenyl-disaccharides migrated with Rf values between 0.5 and 0.7. Radioactivity peaks were localized with the aid of a