Separating Complex Carbohydrates

Founded in 1985, the Complex Carbohydrate Research Center (CCRC) at the University of Georgia, Athens, is dedicated to advancing knowledge of the structure and functions of oligo- and polysaccharides. The Center employs about 100 scientists and staff and actively pursues research using a variety of techniques in an area regarded by many as the last frontier of molecular biochemistry. More information regarding the CCRC can be obtained by visiting the world wide web site at **http://www.ccrc.uga.edu**.

Developing separation methodology is an integral part of the CCRC's research effort. Chromatographic separations of closely related oligosaccharides help elucidate structural details to obtain essential metabolic information. The structure and chromatograms in Figures 1 and 2 are examples of such separations.

In this case the objective was the structural characterization of xyloglucan, a major hemicellulosic component of the primary cell wall of higher plants. Xyloglucan polymer obtained from the medium of a bean cell culture was digested with a fungal *endo*-β-1,4-glucanase, yielding a variety of xyloglucan oligosaccharides. The maximum structure of the oligosaccharides is depicted in Figure 1. The backbone of the molecule is a string of four glucopyranose residues. To the first three of these are linked successively xylose, galactose, and fucose residues in a stairlike arrangement. The galactose residues are often acetylated at the 3, 4, or 6 positions. In solution, acetyl groups migrate slowly between the three positions, reaching in about half a day an equilibrium that favors the 6 position (80%) with the remainder equally divided between 3 and 4. Smaller oligosaccharides obtained from the culture medium are indicated by dashed lines. They can be minus one or more residues including the fucosyl, galactosyl, the leftmost xylosyl, and the leftmost glucosyl. To structurally characterize the different oligosaccharides (particularly the location of the O-acetate) via spectroscopic methods (mass spectrometry, NMR, etc.) it was imperative to purify the native, underivatized oligosaccharides.

As shown by the chromatograms of Figure 2, reversed-phase chromatography on Vydac 218TP or 238TP columns can separate most of these molecules. The presence of the fucose residue and acetylation of galactose at the 6 position markedly increase retention, causing the molecules to emerge within the indicated time groups. Within these groups, presence or absence of additional sugar residues and acetylation of galactose at the 3 or 4 positions have less dramatic effects, but are nonetheless sufficient to produce resolution in most instances. The samples were chromato-

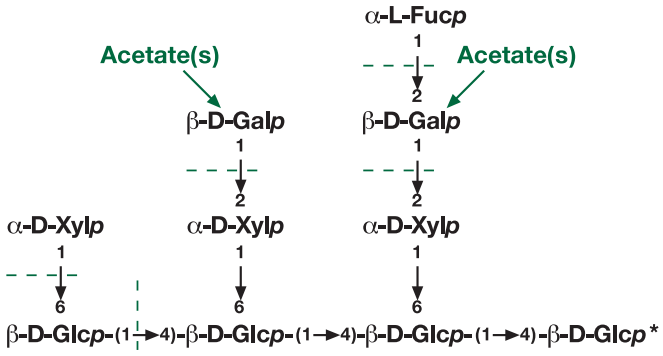

Figure 1. Maximum structure of a cell-wall-component xyloglucan oligosaccharide isolated from bean cell culture. An asterisk designates the reducing end. Underivatized variants of this structure differing in acetate position on the galactose residues or arising by cleavage separate in distinct retention-time groups on two Vydac reversed-phase columns.

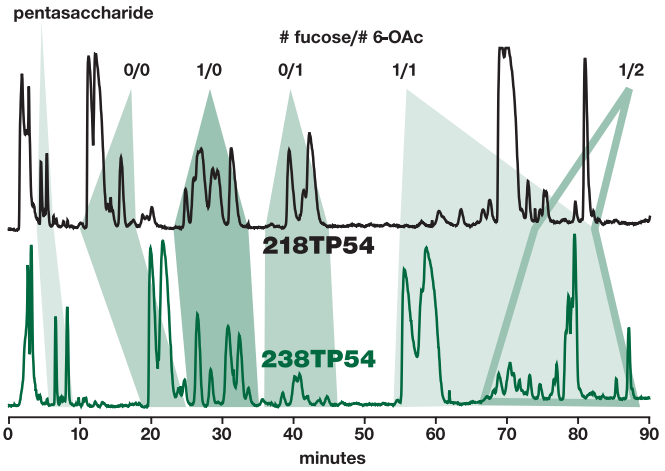

Figure 2. Separation of oligosaccharides represented by the decasaccharide structure of Figure 1 on two Vydac reversed-phase columns. Presence of fucose and/or at least one acetate at the 6 position on galactose produce major retention effects. Columns: 218TP54 "polymeric" reversed-phase and 238TP54 "monomeric" reversed-phase, both with 5µm spherical packing in 4.6mm ID x 250mm L columns. Flow rate: 1 mL/min. Mobile phase: methanol in water. Gradient: linear, 7% to 10% MeOH in 60 minutes, then to 25% at 90 minutes. Detection: ELSD. The logarithm of the detector signal has been plotted to accentuate smaller peaks.

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graphed without prior derivatization, and peaks were detected using a SEDEX 55 evaporative light scattering detector (ELSD) purchased from Richard Scientific, Novato, CA. Peaks were collected and were further analyzed by mass spectrometry and NMR after evaporation of the volatile solvents.

The Vydac 218TP and 238TP are both C18 reversed-phase columns, but produced using "polymeric" and "monomeric" bonding chemistries, respectively. These two columns provide subtly different selectivities, and one or the other may produce better resolution of specific analyte pairs.

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For a novel way to separate smaller oligosaccharides and monomeric sugars, contact Vydac Technical Support at 800.347.6378.

ORDERING INFORMATION FOR COLUMNS USED IN THIS WORK:

Cat. No.	Description
238TP54	Column, Octadecyl (C18), Monomeric, 5µm, 300Å, 4.6mm ID x 250mm L
218TP54	Column, Octadecyl (C18), Polymeric, 5µm, 300Å, 4.6mm ID x 250mm L

Other analytical and preparative column dimensions available upon request.

To place an order, call The Nest Group, 800.347.6378 your local Vydac distributor.