Analytical Biochemistry 381 (2008) 224–232

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Dual-gradient high-performance liquid chromatography for identification of cytosolic high-mannose-type free glycans

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ARTICLE INFO

Article history:
Received 8 April 2008
Available online 9 July 2008

Keywords:
Reversed-phase HPLC
Free oligosaccharide
N-Glycan
Peptide:*N*-glycanase

ABSTRACT

It has been shown that free oligosaccharides derived from N-linked glycans accumulate in the cytosol of animal cells. Most of the glycans have only a single GlcNAc at their reducing termini (Gn1 glycans), whereas the original *N*-glycans retain *N,N′*-diacetylchitobiose at their reducing termini (Gn2 glycans). Under the conditions of high-performance liquid chromatography (HPLC) mapping established for pyridylamine (PA)-labeled Gn2 *N*-glycans, Gn1 glycans are not well retained on reversed-phase HPLC, making simultaneous analysis of Gn1 and Gn2 glycans problematic. We introduced a dual gradient (i.e., pH and butanol gradient) for the separation of Gn1 and Gn2 glycans in a single reversed-phase HPLC. Determination of elution time for various standard Gn2 high-mannose-type glycans, as well as Gn1 glycans found in the cytosol of animal cells, showed that elution of Gn1 and Gn2 glycans could be separated. Sufficient separation for most of the structural isomers could be achieved for Gn1 and Gn2 glycans. This HPLC, therefore, is a powerful method for identification of the structures of PA-labeled glycans, especially Gn1-type glycans, isolated from the cytosol of animal cells.

Pyridylamination of the reducing end of oligosaccharides is widely used for highly sensitive labeling of oligosaccharides [1,2]. Two- and three-dimensional mapping is a powerful characterization method to identify oligosaccharide structure [2–5]. Using this method, various structures can be deduced based on the elution position of given *N*-glycans by two- or three-dimensional high-performance liquid chromatography (HPLC)[3] methods (anion exchange, reversed phase, and size fractionation). This method has proven to be sufficiently powerful to analyze the structure of Gn2 *N*-glycans retaining *N,N′*-diacetylchitobiose at their reducing termini. The same strategy cannot be applied for Gn1-type glycans bearing only a single GlcNAc at their reducing termini because the glycans are not retained well in the octadecylsilica (ODS) column under the conditions usually employed. The separation method for Gn1-type glycans has been reported [6], but there is no established method to obtain sufficient separation for structural isomers of Gn1 and Gn2 glycans using a single analytical method.

Besides butanol concentration, pH is a key factor for the partition of pyridylamine (PA)-labeled oligosaccharides with the ODS column [7]. Using this principle, we established an HPLC condition in which a gradient of butanol concentration and pH was generated. Under this condition, we analyzed various Gn1- and Gn2-type, high-mannose-type oligosaccharides, which are the major free oligosaccharides found in animal cells [6,8–14]. Our results clearly showed that Gn1 and Gn2 glycans give reproducible retention times, expressed as glucose units (GUs), with sufficient separation of structural isomers. Although the empirical additive rule with regard to the reversed-phase HPLC had been used successfully [15–18], ratio comparison was found to be more consistent than the additive scale when compared with Gn1 and Gn2 glycans with the same isomeric oligosaccharide structures. Through ratio analysis of Gn1/Gn2 glycans, the value for G3M9A was found to be considerably higher than that for the other glycans examined. The

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[3] *Abbreviations used:* HPLC, high-performance liquid chromatography; Gn2 glycan, *N*-glycan-derived free glycan with *N,N′*-diacetylchitobiose at its reducing terminus; ODS, octadecylsilica; Gn1 glycan, *N*-glycan-derived free glycan with a single GlcNAc at its reducing terminus; PA, pyridylamine; GU, glucose unit; MALDI–TOF, matrix-assisted laser desorption/ionization time-of-flight; RNase B, ribonuclease B; PNGase, peptide:*N*-glycanase; MS, mass spectrometry; ENGase, *endo*-β-*N*-acetylglucosaminidase; ESI–MS, electrospray ionization mass spectrometry.

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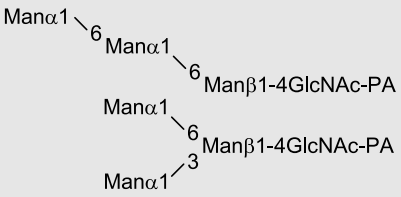
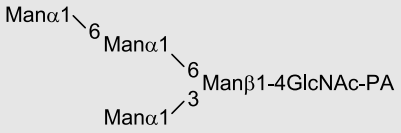
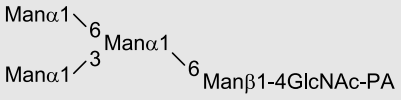
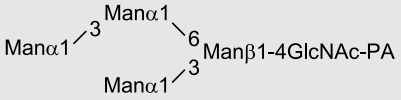
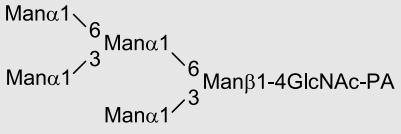
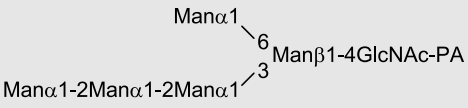
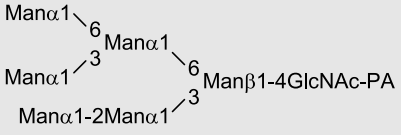
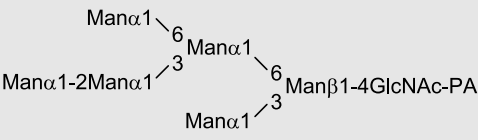
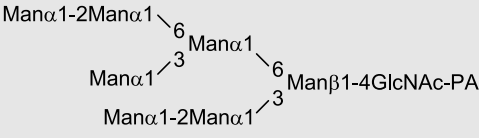
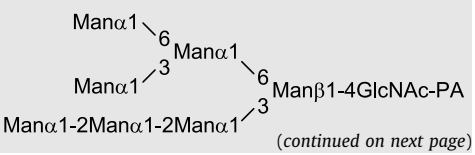
doi:10.1016/j.ab.2008.07.002

Table 1
Structures of Gn1 PA-oligosaccharides used and their abbreviations

Abbreviation	Structure
GN	GlcNAc-PA
M3A′ M3B′	
M4A′	
M4B′	
M4C′	
M5A′	
M5B′	
M6B′	
M6C′	
M7A′	
M7B′	

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