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Nano-scale liquid chromatography-mass spectrometry of 2-aminobenzamide-labeled oligosaccharides at low femtomole sensitivity

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Abstract

Conventional normal-phase high performance liquid chromatography of fluorescently labeled oligosaccharides with or without on-line mass spectrometry is an established tool for the structure characterization of protein and lipid derived glycans. Here we describe the miniaturization of such a system to the nano-scale using a 75 μm internal diameter normal-phase amide column on-line with electrospray ionization ion-trap mass spectrometry. 2-Aminobenzamide-labeled oligosaccharides have a predictable retention on this normal-phase column that can be expressed as glucose units by comparison to the retention of a standard 2-aminobenzamide glucose polymer mixture. Isobaric compounds are separated on the basis of their structural differences, and by on-line electrospray ionization ion-trap mass spectrometry, the sequence of the monosaccharides can be deduced. The major improvement of the on-line nano-LC-MS system in comparison to conventional systems is the gain in sensitivity with detection of low femtomole amounts of glycans. This implies that LC-MS of 2-aminobenzamide-labeled oligosaccharides can now be performed at higher sensitivity than their analysis with fluorescence detection.
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Keywords: 2-Aminobenzamide; Glucose units; Ion trap; Keyhole limpet hemocyanin; N-Glycans

1. Introduction

High performance liquid chromatography (HPLC) of oligosaccharides is a widely used technique in the analysis of protein glycosylation [1–3]. Fluorescent labeling of the oligosaccharides is often beneficial to the analysis because it improves sensitivity and selectivity of the detection as well as the chromatographic behavior of the glycans. Fluorescent reagents commonly applied for labeling of oligosaccharides include 2-aminobenzamide (AB) [4–6], 2-aminopyridine [7–9], and anthranilic acid [3]. Normal-phase (NP) HPLC techniques have been applied to fluorescently labeled N-glycans [4,10], O-glycans [5,11,12] as well as oligosaccharide moieties of glycosphingolipids [6,13]. In NP-HPLC the elution positions of the oligosaccharides can be expressed in glucose units (GU) by comparison to a dextran ladder standard [4]. This system allows the prediction of glycan structures from experimentally deduced GU increments or decrements upon elongation or cleavage of the oligosaccharides chains. In particular in combination with enzymatic cleavage [4,14] or partial hydrolysis [15,16] of glycans or glycan pools, standardized NP-HPLC with fluorescence detection can provide a wealth of structural information. Important additional techniques for separation, mapping and structural assignment of labeled oligosaccharides are reversed-phase (RP) HPLC for which elution positions can similarly be standardized using glucose polymers [17–20], and anion-exchange chromatography [8].

In addition to chromatographic characterization combined with enzymatic or chemical cleavage, mass spectrometry (MS) is often applied for the structural analysis of oligosaccharides. Although MALDI-TOF-MS [21] and off-line electrospray ionization (ESI) MS [22–27] are also frequently used for high-sensitivity carbohydrate analysis, (microbore)

Abbreviations: AB, 2-aminobenzamide; ESI, electrospray ionization; F, fucose; GU, glucose units; H, hexose; HPLC, high performance liquid chromatography; IT, ion trap; KLH, keyhole limpet hemocyanin; LC, liquid chromatography; N, *N*-acetylhexosamine; NP, normal-phase; RP, reversed phase

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HPLC methods with on-line ESI-MS are most valuable for the extensive structural analysis of complex oligosaccharide mixtures [5,28–30].

NP-HPLC of fluorescently labeled oligosaccharides allows the glycosylation analysis of proteins separated by sodium dodecyl sulfate polyacrylamide electrophoresis and thus provides a valuable tool for the characterization of post-translational modifications in proteomics [31–34]. While NP-HPLC of AB-labeled glycans using fluorescence detection displayed a detection limit of 10–20 fmol for a labeled diluted standard [5], mass spectrometric detection appeared to be 10–20 times less sensitive [33]. In addition to its comparatively low sensitivity, MALDI-TOF-MS analysis of manually collected peaks often required additional steps like glycan desalting by small-scale solid-phase extraction [35]. In order to overcome these limitations, we developed nano-scale NP-HPLC analysis of AB-labeled oligosaccharides with on-line ESI-MS. This system is characterized by a low femtomole detection limit, high tolerance of salt and various contaminants, and the possibility of obtaining additional structural information by mass spectrometric analysis of fragment ions.

2. Experimental

2.1. Preparation of glycan samples

N-Glycans from keyhole limpet hemocyanin (KLH), asialofetuin and ribonuclease B (Sigma) were released by PNGase F treatment after reduction with β-mercaptoethanol and denaturation by addition of sodium-dodecylsulfate and CHAPS, following the manufacturers instruction (Roche Diagnostics, Mannheim, Germany). Released N-glycans were purified by gel filtration. A dextran ladder was prepared by incubating dextran (25 mg of Dextran 10, Amersham Biosciences, Freiburg, Germany) in 500 μl 0.1 M trifluoroacetic acid for 60 min at 100 °C followed by lyophilization. Type 2-chain diantennary N-glycan was a gift from Prof. Dirk van den Eijnden, Free University of Amsterdam. Glycans were labeled with AB [36]. The sample was then applied to a reversed-phase cartridge (500 mg Bakerbond Octadecyl; Baker, Phillipsburg, NJ). After a 5 ml wash with water, AB-labeled glycans were eluted with 2 ml of 50% methanol and dried under N_2.

2.2. Nano-liquid chromatography ESI-ion trap (IT)-MS

AB-labeled N-glycans were separated on a nano-scale Amide-80 column (5 μm, 80 Å; 75 μm × 100 mm; Tosohaas, Montgomeryville, PA) packed by Grom Analytik (Rottenburg, Germany) using an Ultimate nano-LC system with a Famos autosampler (LC Packings, Amsterdam, The Netherlands). Solvent A was 50 mM formic acid adjusted to pH 4.4 with ammonia solution. Solvent B was 20% solvent A in acetonitrile. The following gradient conditions were used: $t = 0$ min, 100% solvent B; $t = 152$ min, 52% solvent B; $t = 153$ min, 100% solvent B; $t = 200$ min, 100% solvent B. The flow was approximately 300 nl/min. Samples were injected in 80% acetonitrile [28]. The system was directly coupled with an Esquire 3000 ESI-IT-MS (Bruker Daltonik, Bremen, Germany) equipped with an on-line nanospray source operating in the positive-ion mode. For electrospray (900–1200 V), capillaries (360 μm o.d., 20 μm i.d. with 10 μm opening) from New Objective (Cambridge, MA) were used. The solvent was evaporated at 100 °C with a nitrogen stream of 6 l/min. Ions from *m/z* 50 to 3000 were

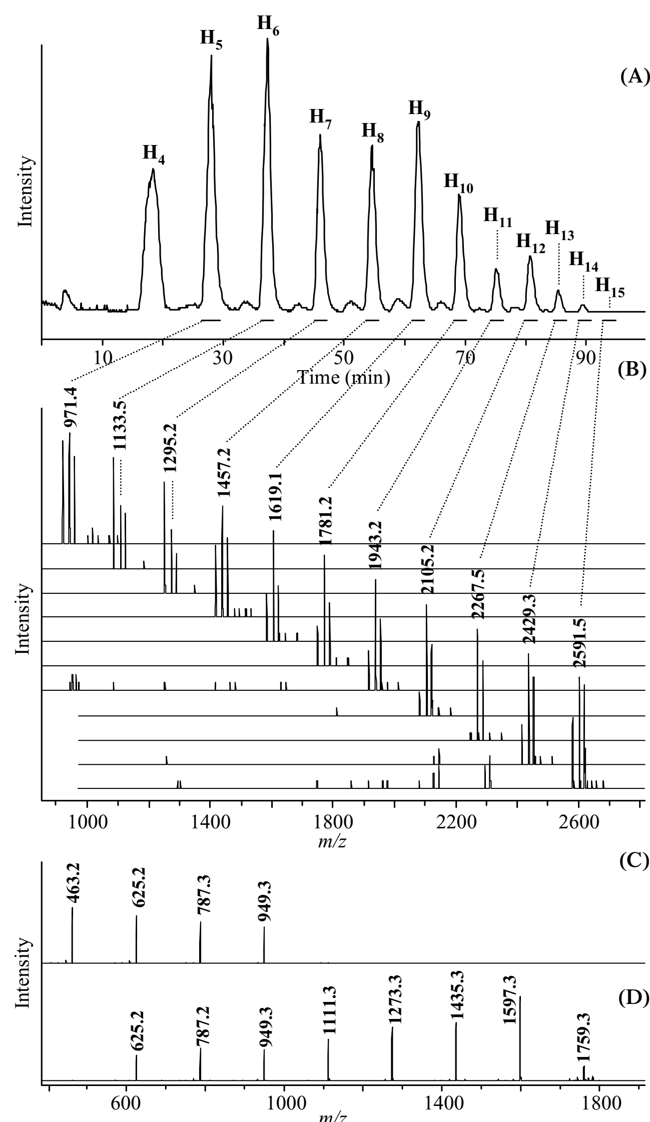

Fig. 1. Normal-phase nano-LC-ESI-MS of a dextran ladder labeled with 2-aminobenzamide. Analyses were performed on an amide column (75 μm × 100 mm). (A) Base-peak chromatogram (*m/z* 700–3000). Peaks are marked with glucose numbers (H_4 contains four glucosyl residues, etc.). (B) Mass spectra of the AB-labeled glucose oligomers obtained as indicated by horizontal bars in (A). Proton, sodium and potassium adducts where observed, resulting in peak triplets. Sodium adducts were labeled with the measured mass. (C, D) Fragment spectra obtained for the proton adducts of H_5 and H_{10} at *m/z* 949 and 1759, respectively.

registered. For the analysis of the AB-labeled hexamannosidic N-glycan ($Man_6GlcNAc_2$) the following fast gradient was applied instead of the above-described slow gradient: $t = 0$ min, 100% solvent B; $t = 20$ min, 52% solvent B; $t = 23$ min, 52% solvent B; $t = 24$ min, 100% solvent B; $t = 40$ min, 100% solvent B.

2.3. HPLC with fluorescence detection of AB-labeled glycans

AB-labeled N-glycans were separated on an conventional-scale Amide-80 column (5 μm, 80 Å; 4 mm × 250 mm; Tosohaas, Montgomeryville, PA) using a ÄktaPurifier

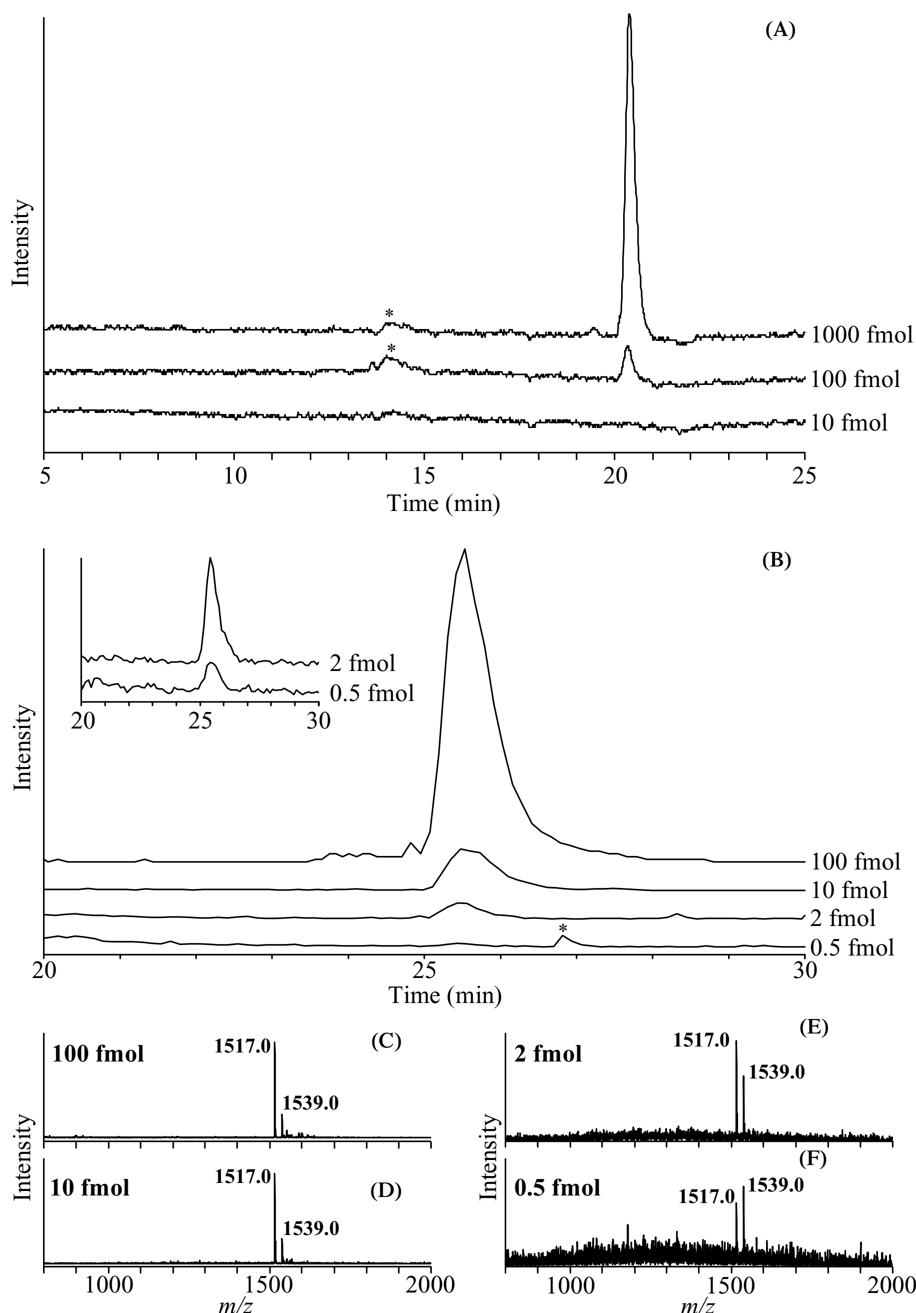

Fig. 2. Sub-femtomole sensitivity of nano-LC-ESI-MS in the analysis of an AB-labeled hexamannosidic N-glycan ($Man_6GlcNAc_2$). A AB-labeled hexamannosidic N-glycan was analyzed by normal-phase HPLC either on an conventional-scale column (4 mm × 250 mm) using fluorescence detection (A), or on a nano-scale column (75 μm × 100 mm) using mass spectrometric detection (B–F). Base-peak chromatograms of a dilution series allowed the detection of as little as 2 fmol of AB-tagged $Man_6GlcNAc_2$ (B). Sum spectra of the elution range of $Man_6GlcNAc_2$ (25.2–26 min; C–F) revealed the detection of proton adducts (*m/z* 1517) and sodium adducts (*m/z* 1539) from down to 500 amol (0.5 fmol) of injected standard (F). Corresponding selected ion chromatograms for *m/z* 1517 and *m/z* 1539 likewise allowed the detection of the standard injecting a 2 fmol as well as a 500 amol aliquot (inset in B). *, contaminant.

pump system (Amersham Biosciences) equipped with a Jasco FP-1520 fluorescence detector (Jasco, Maarssen, The Netherlands), according to a modification of the method established by Guile et al. [4]. Solvent A was 50 mM formic acid adjusted to pH 4.4 with ammonia solution. Solvent B was 20% solvent A in acetonitrile. The following gradient conditions were used: $t = 0$ min, 100% solvent B; $t = 20$ min, 52% solvent B; $t = 23$ min, 52% solvent B; $t = 24$ min, 100% solvent B; $t = 40$ min, 100% solvent B. The flow was 1 ml/min. Samples were injected in 80% acetonitrile. Fluorescence (360/425 nm) was registered at maximal sensitivity (gain 1000) of the detector.

3. Results and discussion

Nano-scale NP-HPLC of AB-labeled oligosaccharides on-line coupled with electrospray mass spectrometry was first evaluated using a dextran ladder. The system was characterized by high resolution (Fig. 1A) and allowed

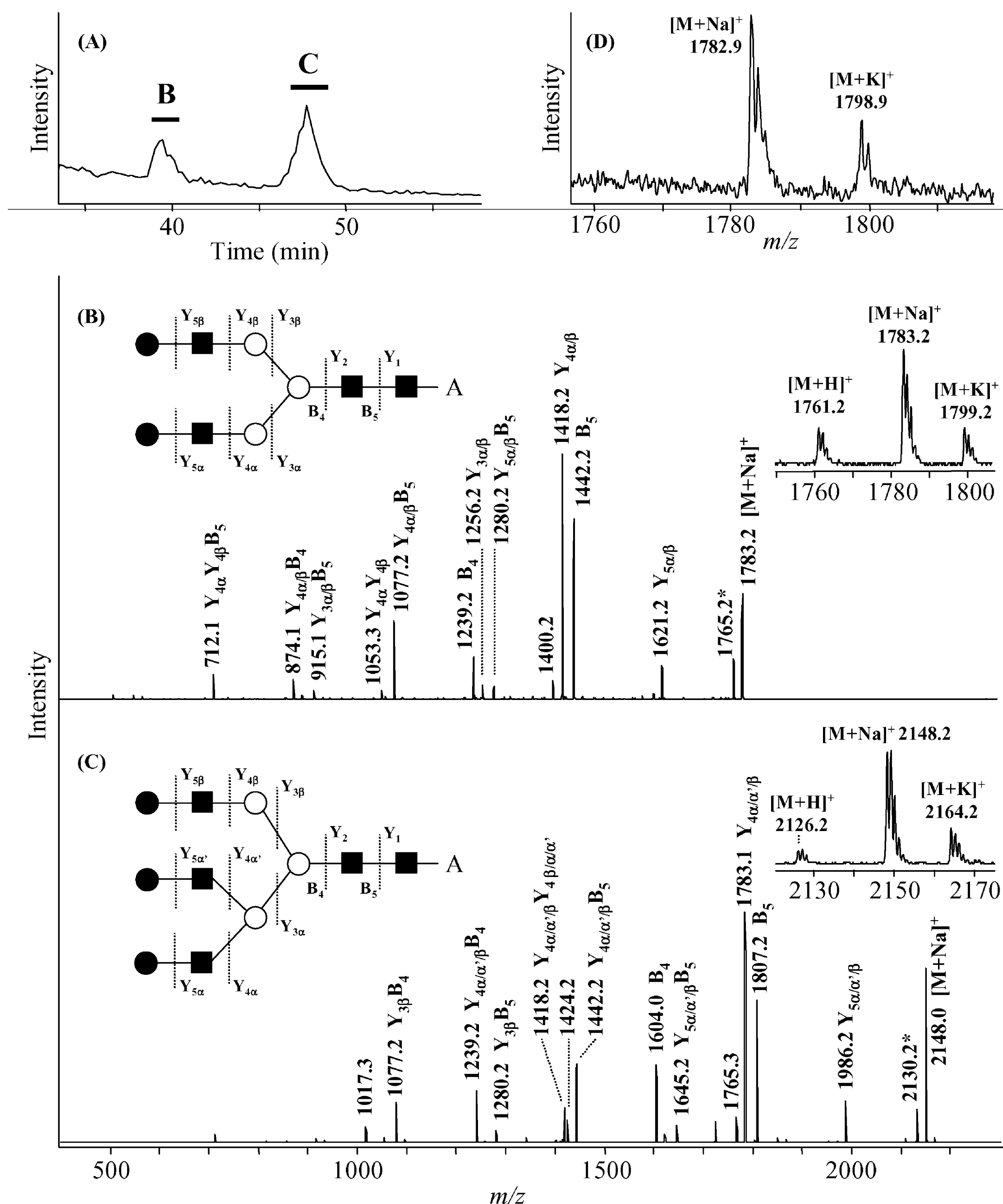

Fig. 3. Normal-phase nano-LC-ESI-MS of asioalofetuin-derived N-glycans labeled with 2-aminobenzamide. (A) Base-peak chromatogram (BPC; mass range m/z 350–3000) obtained with 250 fmol AB-N-glycans from asialofetuin. (B, C) Mass spectra of asialofetuin diantennary (B) and triantennary (C) AB-N-glycans obtained for the time windows indicated by horizontal bars in (A). (D) Mass spectrum obtained after injection of a 10 fmol aliquot of 2-aminobenzamide-labeled type 2-chain diantennary structure. A, 2-aminobenzamide; open circle, mannose; solid circle, galactose; solid square, *N*-acetylglucosamine.

the registration of intact oligosaccharides (Fig. 1B) and characteristic fragments thereof (Fig. 1C and D).

In order to compare the sensitivity of conventional-scale fluorescence detection with the nano-scale HPLC-ESI-MS system, we analyzed a 2-aminobenzamide-labeled hexamannosidic N-glycan isolated from ribonuclease B ($Man_6GlcNAc_2$) on both systems (Fig. 2). While 100 fmol of this standard compound could still be detected by fluorescence, a 10 fmol aliquot clearly was below the detection limit (Fig. 2A). Thus, sensitivity of fluorescence detection using our conventional-scale system seemed to be close to the 16 fmol detection limit for an 2-aminobenzamide-labeled oligosaccharide reported by Royle et al. [5]. Sensitivity of the nano-LC-MS system for the 2-aminobenzamide-tagged $Man_6GlcNAc_2$, was determined using 500 amol, 2 fmol, 10 fmol and 100 fmol aliquots. Down to a sample amount of 2 fmol, the AB-labeled $Man_6GlcNAc_2$ standard could clearly be seen in the basepeak chromatogram (Fig. 2B). Sum mass spectra of the chromatographic range of 25.2–26.0 min allowed the detection of the proton adduct (*m/z* 1517) as well as sodium adduct (*m/z* 1539) of as little as 0.5 fmol (500 amol) of the glycan standard (Fig. 2C–F). Combined extracted-ion chromatograms for the proton and sodium adducts likewise visualize the detection of sub-femtomole amounts of the AB-labeled hexamannosidic N-glycan (inset in Fig. 2B). Taken together, analysis of the labeled oligosaccharide standard with the here presented nano-LC system with on-line mass spectrometric registration is one to two orders of magnitude more sensitive than its analysis by conventional fluorescence detection.

The method was applied to N-glycans released from asialofetuin (Fig. 3), which allowed the mass spectrometric characterization of the two major N-glycan species, which showed a composition of H_5N_4 (H: hexose, N: *N*-acetylhexosamine) and H_6N_5, from a 250 fmol aliquot of a glycan pool (Fig. 3B and C, respectively). Mass spectrometric data are in accordance with the diantennary (H_5N_4) and triantennary (H_6N_5) complex-type structures described for asialofetuin in literature [37]. Injection of a 10 fmol aliquot of an isolated type 2-chain diantennary N-glycan standard still allowed its detection in base-peak chromatogram (not shown) and mass spectrum (Fig. 3D).

Furthermore, AB-labeled N-glycans derived from KLH were analyzed by NP-HPLC (Fig. 4). In a first run, KLH AB-labeled glycans could be separated over a range of more than 30 min (Fig. 4A). Individual glycans were detected as triplets of proton adducts, sodium adducts and potassium adducts (Fig. 4C–J), which allowed the assignment of glycan compositions in terms of hexose, *N*-acetylhexosamine, and deoxyhexose. After a second injection, KLH glycans were further characterized by fragment ion analysis, as exemplified for the $H_4N_2F_1$ species (Fig. 5). Indications for isobaric structures of this composition, which differed in retention, were given by the ion tracks of *m/z* 1361 (sodium adducts) and *m/z* 1339 (proton adducts; Figs. 4A and 5A). Fragment ion analysis of the sodium adducts (Fig. 5B and C) gave evidence for the presence of two major $H_4N_2F_1$ N-glycans derived from KLH. Using HPLC fractionation, mass spectrometry, linkage analysis and exoglycosidase treatment, these species were recently

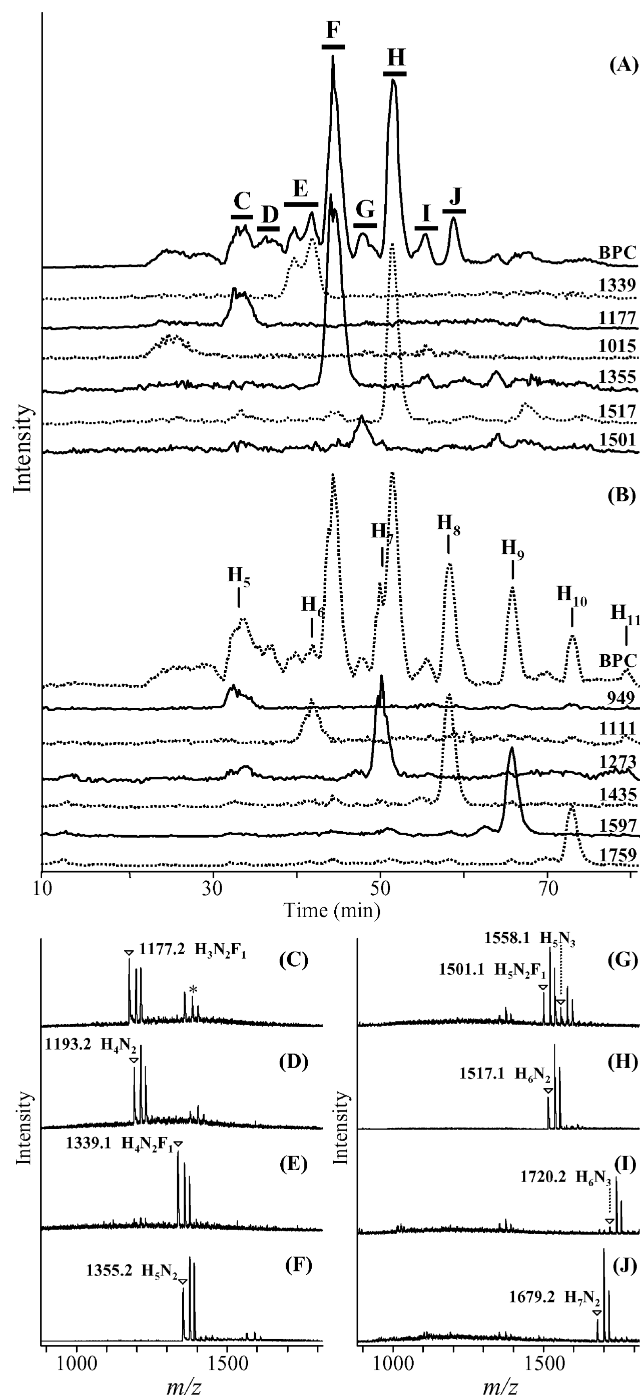

Fig. 4. Normal-phase nano-LC-ESI-MS of KLH N-glycans labeled with 2-aminobenzamide. (A) Base-peak chromatogram (BPC; mass range *m/z* 800–3000) as well as extracted-ion chromatograms labeled with the selected mass for KLH AB-N-glycans without (A) and with (B) the addition of a dextran ladder. (C–J) Mass spectra of KLH AB-N-glycans obtained for the time windows indicated by horizontal bars in (B). H, hexose; F, fucose; N, *N*-acetylglucosamine; ∇, proton adduct; *, contaminant.

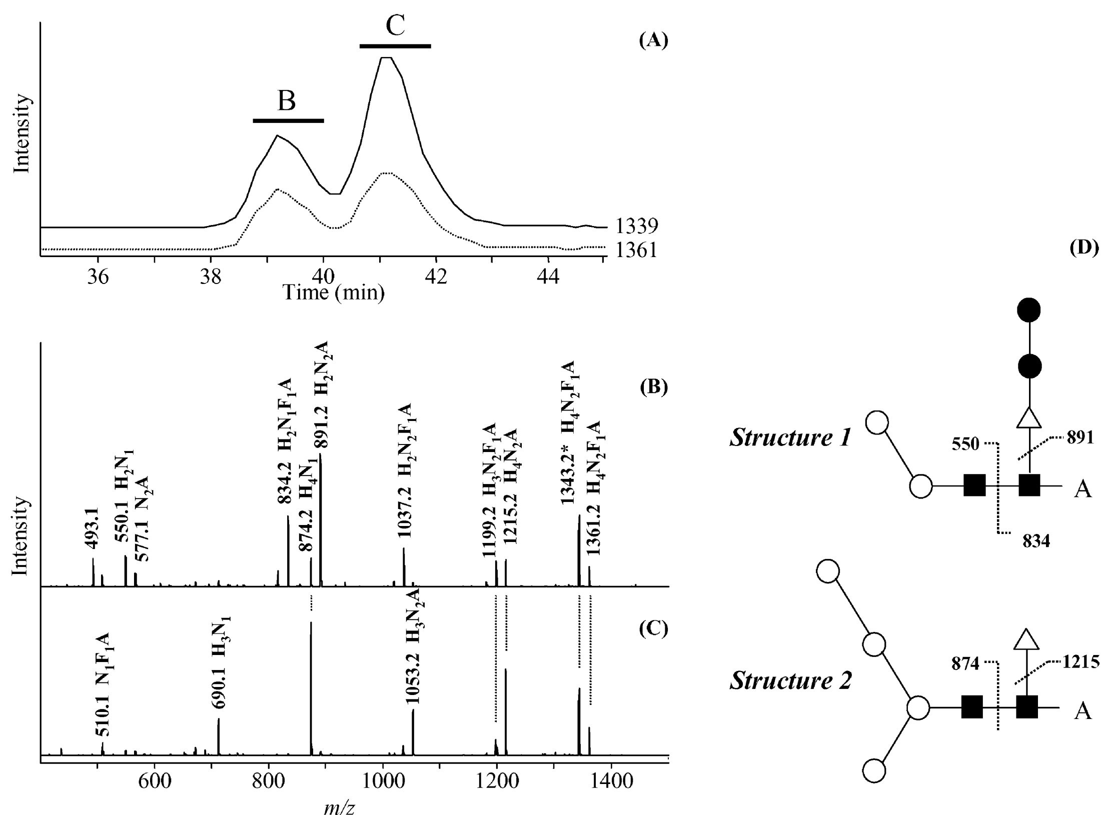

Fig. 5. ESI-IT mass spectrometric fragment ion analysis of the KLH AB-labeled N-glycans of composition $H_4N_2F_1$. (A) Extracted-ion chromatograms of *m/z* 1339 (proton adducts of $H_4N_2F_1$) and *m/z* 1361 (sodium adducts of $H_4N_2F_1$). (B, C) Mass spectra obtained on collision-induced dissociation of precursors of *m/z* 1361 in two different time windows as indicated in (A). (D) Schematic representations of Structure 1 and Structure 2, which are the main precursors in (B) and (C), respectively. A, 2-aminobenzamide; H, hexose; N, *N*-acetylhexosamine; F, fucose. Open circle, mannose; closed circle, galactose; square, *N*-acetylglucosamine; triangle, fucose.

identified as Man(α1–6)Man(β 1–4)GlcNAc(β1–4)[Gal(β 1–4)Gal(β1–4) Fuc(α1–6)]GlcNAc-AB (Structure 1) and Man(α1–6)Man(α1–6)[Man(α1–3)]Man(β1–4)GlcNAc(β 1–4)[Fuc(α1–6)]GlcNAc-AB (Structure 2) [38]. Both structures are schematically shown in Fig. 5D, the former being characterized by an unusual core Gal(β1–4)Gal(β1–4)Fuc (α1–6)-modification. The mass spectra of the two peaks (Fig. 5B and C) revealed major ions due to the loss of the α1–6 attached fucose and cleavage of the chitobiose core, as shown in Fig. 5D. This indicated Structure 1 to be the major precursor in Fig. 5B. The fragment ion spectrum in Fig. 5C, in contrast, is dominated by Structure 2. A further isobaric KLH N-glycan characterized as Gal(β1–6)Man(α1–6) [Man(α1–3)]Man(β1–4)GlcNAc(β 1–4)[Fuc(α1–6)]GlcNAc [39] may likewise contribute to this spectrum. The intense signals at *m/z* 690 and 1053 in Fig. 5C can be correlated with another $H_4N_2F_1$ KLH N-glycan structure Man(α1–6)[Man(α1–3)] Man(β1–4)Glc NAc(β1–4)[Gal(β1–4)Fuc(α1–6)]GlcNAc which likewise was structurally characterized recently [38], and seems to elute in the second peak of $H_4N_2F_1$ species (region C in Fig. 5A).

Conventional-scale NP-HPLC of AB-oligosaccharides using calibration with an AB-labeled dextran ladder allows the determination of retention in glucose units [4]. A comparison of glucose units determined for KLH AB-labeled glycans by nano-scale HPLC with mass spectrometric detection (Fig. 5B) and conventional-scale HPLC with fluorescence detection is presented in Table 1. Comparison of retention times was done for the two structural isomers of composition $H_4N_2F_1$ (structures given in Fig. 5D), for pentamannosidic, hexamannosidic and heptamannosidic N-glycans ($H_{5-7}N_2$) [38,39], for a H_6N_3 species, which was formerly elucidated as a hybrid-type structure with a type 1-chain lower an-

Table 1
Comparison of glucose units for KLH N-glycans determined by nano-scale vs. conventional-scale NP-HPLC

Species	Glucose units (GU)	
	Nano	Conventional
$H_4N_2F_1$ Structure 1	5.75	5.72
$H_4N_2F_1$ Structure 2	6.02	5.97
H_5N_2	6.32	6.28
$H_5N_2F_1$	6.72	6.69
H_6N_2	7.12	7.13
H_6N_3	7.64	7.57
H_7N_2	8.08	8.07

tenna [39], and for a peak containing species of composition $H_5N_2F_1$, which seems to be a mixture of isobaric structures with or without galactosylation of the core fucose [38]. In order to overcome variations in retention times which are routinely observed in nano-scale HPLC, we chose for co-injection of an AB-labeled dextran ladder with the KLH N-glycans (Fig. 4B). The good agreement of GU obtained in the two different systems indicated that GU determined by nano-scale HPLC can be directly compared to those obtained by conventional-scale HPLC and thus can be used as characteristic parameters for structural assignment.

4. Conclusions

Miniaturization of NP-HPLC of AB-labeled glycans to the nano-scale allowed mass spectrometric detection of AB-labeled oligosaccharides with low femtomole sensitivity, thus closing the sensitivity gap between fluorescence detection and mass-spectrometric analysis which has been observed so far [33]. Normal-phase nano-LC-ESI-MS was applied to a complex mixture of AB-labeled N-glycans from KLH yielding a high-resolution separation. The method allowed the determination of characteristic glucose units for individual species as well as the differentiation between isobaric structures based on retention time and fragment ion spectrum. Taken together, normal-phase nano-LC-ESI-MS provided a powerful, highly sensitive alternative to conventional chromatographic and mass-spectrometric techniques for analysis of AB-labeled glycans.

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References

[1] V.K. Dua, V.E. Dube, C.A. Bush, Biochim. Biophys. Acta 802 (1984) 29.
[2] J.U. Baenziger, Methods Enzymol. 230 (1994) 237.
[3] K.R. Anumula, S.T. Dhume, Glycobiology 8 (1998) 685.
[4] G.R. Guile, P.M. Rudd, D.R. Wing, S.B. Prime, R.A. Dwek, Anal. Biochem. 240 (1996) 210.
[5] L. Royle, T.S. Mattu, E. Hart, J.I. Langridge, A.H. Merry, N. Murphy, D.J. Harvey, R.A. Dwek, P.M. Rudd, Anal. Biochem. 304 (2002) 70.
[6] D.R. Wing, B. Garner, V. Hunnam, G. Reinkensmeier, U. Andersson, D.J. Harvey, R.A. Dwek, F.M. Platt, T.D. Butters, Anal. Biochem. 298 (2001) 207.
[7] S. Hase, Methods Mol. Biol. 14 (1993) 69.
[8] S. Hase, Methods Enzymol. 230 (1994) 225.
[9] S. Natsuka, S. Hase, Analysis of *N*- and *O*-glycans by pyridylamination, in: E.F. Hounsell (Ed.), Glycoanalysis Protocols, Humana Press, Totowa, NJ, 1998.
[10] S. Oka, K. Terayama, K. Imiya, S. Yamamoto, A. Kondo, I. Kato, T. Kawasaki, Glycoconj. J. 17 (2000) 877.
[11] N. Kuraya, S. Hase, Anal. Biochem. 233 (1996) 205.
[12] H. Iwase, I. Ishii, Karakasa, E. Fujii, K. Hotta, Y. Hiki, Y. Kobayashi, Anal. Biochem. 206 (1992) 202.
[13] K. Ohara, M. Sano, A. Kondo, I. Kato, J. Chromatogr. A 586 (1991) 35.
[14] P.M. Rudd, G.R. Guile, B. Küster, D.J. Harvey, G. Opdenakker, R.A. Dwek, Nature 388 (1997) 205.
[15] Y. Makino, N. Kuraya, K. Omichi, S. Hase, Anal. Biochem. 238 (1996) 54.
[16] Y. Makino, K. Omichi, S. Hase, Anal. Biochem. 264 (1998) 172.
[17] K. Yanagida, H. Ogawa, K. Omichi, S. Hase, J. Chromatogr. A 800 (1998) 187.
[18] Z. El Rassi, D. Tedford, J. An, A. Mort, Carbohydr. Res. 215 (1991) 25.
[19] A. Kurosaka, A. Yano, N. Itoh, Y. Kuroda, T. Nakagawa, T. Kawasaki, J. Biol. Chem. 266 (1991) 4168.
[20] G.R. Guile, D.J. Harvey, N. O'Donnell, A.K. Powell, A.P. Hunter, S. Zamze, D.L. Fernandes, R.A. Dwek, D.R. Wing, Eur. J. Biochem. 258 (1998) 623.
[21] D.J. Harvey, Mass Spectrom. Rev. 18 (1999) 349.
[22] M. Okamoto, K. Takahashi, T. Doi, Rapid Commun. Mass Spectrom. 9 (1995) 641.
[23] K. Yoshino, T. Takao, H. Murata, Y. Shimonishi, Anal. Chem. 67 (1995) 4028.
[24] U. Bahr, A. Pfenninger, M. Karas, B. Stahl, Anal. Chem. 69 (1997) 4530.
[25] W. Mo, H. Sakamoto, A. Nishikawa, N. Kagi, J.I. Langridge, Y. Shimonishi, T. Takao, Anal. Chem. 71 (1999) 4100.
[26] D.J. Harvey, Analyst 125 (2000) 609.
[27] D.J. Harvey, J. Am. Soc. Mass Spectrom. 11 (2000) 900.
[28] B. Garner, A.H. Merry, L. Royle, D.J. Harvey, P.M. Rudd, J. Thillet, J. Biol. Chem. 276 (2001) 22200.
[29] L.A. Gennaro, D.J. Harvey, P. Vouros, Rapid Commun. Mass Spectrom. 17 (2003) 1528.
[30] T.S. Mattu, L. Royle, J. Langridge, M.R. Wormald, P.E. Van den Steen, J. Van Damme, G. Opdenakker, D.J. Harvey, R.A. Dwek, P.M. Rudd, Biochemistry 39 (2000) 15695.
[31] K.R. Anumula, Anal. Biochem. 283 (2000) 17.
[32] M. Oh-eda, E. Tominaga, Y. Nabuchi, T. Matsuura, N. Ochi, M. Tamura, S. Hase, Anal. Biochem. 236 (1996) 369.
[33] P.M. Rudd, C. Colominas, L. Royle, N. Murphy, E. Hart, A.H. Merry, H.F. Hebestreit, R.A. Dwek, Proteomics 1 (2001) 285.
[34] K.R. Anumula, P. Du, Anal. Biochem. 275 (1999) 236.
[35] S. Kim, S.K. Hwang, R.A. Dwek, P.M. Rudd, Y.H. Ahn, E.H. Kim, C. Cheong, S.I. Kim, N.S. Park, S.M. Lee, Glycobiology 13 (2003) 147.
[36] J.C. Bigge, T.P. Patel, J.A. Bruce, P.N. Goulding, S.M. Charles, R.B. Parekh, Anal. Biochem. 230 (1995) 229.
[37] E.D. Green, G. Adelt, J.U. Baenziger, S. Wilson, H. van Halbeek, J. Biol. Chem. 263 (1988) 18253.
[38] M. Wuhrer, M.L.M. Robijn, C.A.M. Koeleman, C.I.A. Balog, R. Geyer, A.M. Deelder, C.H. Hokke, Biochem. J. (2003), in press.
[39] T. Kurokawa, M. Wuhrer, G. Lochnit, H. Geyer, J. Markl, R. Geyer, Eur. J. Biochem. (2002) 5459.