Journal of Pharmaceutical Analysis 7 (2017) 87–94

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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

Original Research Article

N-glycans released from glycoproteins using a commercial kit and comprehensively analyzed with a hypothetical database

Xue Sun[a], Lei Tao[a], Lin Yi[a], Yilan Ouyang[a], Naiyu Xu[a], Duxin Li[a,*], Robert J. Linhardt[b], Zhenqing Zhang[a,*]

[a] *Jiangsu Key Laboratory of Translational Research and Therapy for Neuro-Psycho-Diseases and College of Pharmaceutical Sciences, Soochow University, Suzhou, Jiangsu 215021, China*

[b] *Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA*

ARTICLE INFO

Keywords:
N-glycan
Hypothetical database
Glycoproteins
Gastric carcinoma cells
Glycomics

ABSTRACT

The glycosylation of proteins is responsible for their structural and functional roles in many cellular activities. This work describes a strategy that combines an efficient release, labeling and liquid chromatography–mass spectral analysis with the use of a comprehensive database to analyze *N*-glycans. The analytical method described relies on a recently commercialized kit in which quick deglycosylation is followed by rapid labeling and cleanup of labeled glycans. This greatly improves the separation, mass spectrometry (MS) analysis and fluorescence detection of *N*-glycans. A hypothetical database, constructed using GlycResoft, provides all compositional possibilities of *N*-glycans based on the common sugar residues found in *N*-glycans. In the initial version this database contains > 8,700 *N*-glycans, and is compatible with MS instrument software and expandable. *N*-glycans from four different well-studied glycoproteins were analyzed by this strategy. The results provided much more accurate and comprehensive data than that had been previously reported. This strategy was then used to analyze the *N*-glycans present on the membrane glycoproteins of gastric carcinoma cells with different degrees of differentiation. Accurate and comprehensive *N*-glycan data from those cells was obtained efficiently and their differences were compared corresponding to their differentiation states. Thus, the novel strategy developed greatly improves accuracy, efficiency and comprehensiveness of *N*-glycan analysis.

1. Introduction

Glycosylation is one of the most common forms of posttranslational modification (PTM) in eukaryotic proteins. This PTM involves the enzymatic attachment of glycans to asparagine (*N*-linked glycans), serine or threonine (*O*-linked glycan) residues of a protein [1]. Protein glycosylation is involved in a number of important structural and functional roles such as protein folding, cell-cell recognition, cancer metastasis, and immune system activation [2]. Potential sites for *N*-glycosylation can be readily identified from the consensus sequences, AsnXSer or AsnXThr, where X can be any amino acid except proline [3]. *N*-glycans generally contain a common pentasaccharide core structure and can be classified into three types: high mannose, complex and hybrid types of glycans. *O*-glycans are oligosaccharides connected to peptide chains through an *N*-acetyl galactosamine (GalNAc) residue. Their structures are variable and *O*-glycosylation sites could be either Ser or Thr residues on the peptide chains which cannot be easily predicted [3].

The analysis of *N*-glycosylation in proteins can be achieved at several levels of detail. The simplest and most direct strategy involves the release of glycans from protein followed by analysis using mass spectrometry (MS) [4,5]. Typically, *N*-glycans are released using an enzyme, peptide-*N*-glycosidase F (PNGase F), which liberates non-fucosylated and core 6-fucosylated *N*-glycans [6]. In some studies, these released *N*-glycans are analyzed directly without modification [7]. More commonly, the released *N*-glycans are derivatized to enhance their analysis by liquid chromatography–mass spectrometry (LC–MS) [8–10]. The most common way to label *N*-glycans is through reductive amination using a fluorescent tag [11–13] such as 2-aminobenzamide (2-AB) [11]. A kit for labeling glycans based on 2-AB is commercially available, but while the resulting fluorescently labeled glycans are often readily detected by fluorescence, they can be difficult to be detected by MS due to their poor ionization efficiency [14].

In glycomic analysis, it is useful to rely on a comprehensive database containing compositional information and accurate molecular weights (MWs) of all possible glycans for mass searching. However, an

Peer review under responsibility of Xi'an Jiaotong University.

* Corresponding authors.
E-mail addresses: duxin.li@suda.edu.cn (D. Li), z_zhang@suda.edu.cn (Z. Zhang).

http://dx.doi.org/10.1016/j.jpha.2017.01.004
Received 8 November 2016; Received in revised form 9 January 2017; Accepted 10 January 2017
Available online 11 January 2017

actual spectral database can only be established based on a large number of experiments on a variety of samples, making this approach both time and labor intensive.

In the current study, a strategy for *N*-glycan analysis was developed that took advantage of the recently available commercial glycan-labeling kit and a hypothetical *N*-glycan database prepared using GlycReSoft. GlycoWorks RapiFluor-MS *N*-Glycan kit was used for the fast enzymatic release and rapid labeling of *N*-glycans [15]. This innovative sample preparation kit uses optimized de-glycosylation conditions and reagents for fast release. In addition, this kit contains a novel rapid labeling reagent called RapiFluor-MS, which is designed to provide both benefits of sensitive fluorescence and sensitive MS detection [15]. GlycReSoft, established by Maxwell and co-workers in 2012 [16], allows a convenient method to establish a hypothetical *N*-glycan database. Furthermore, using GlycReSoft it is possible to rapidly extract the glycan composition and abundance from MS data after deconvolution and the conversion of spectral data to numerical data [16]. The hypothetical database constructed using GlycReSoft can be easily opened and read by MS software, such as Masshunter from Agilent. Mammalian *N*-glycans are composed primarily of four different monosaccharide residues, hexoses (Hex) (including mannose (Man) and galactose (Gal)), *N*-acetyl hexosamine (HexNAc), deoxyhexose (dHex, i.e., fucose (Fuc)), and *N*-acetylneuraminic acid (NeuAc) [17]. The hypothetical database constructed from these common monosaccharide residues contained most possibilities for *N*-glycans composition in mammalian-derived glycoproteins and their corresponding accurate masses.

Four well-studied glycoprotein standards having well-established *N*-glycan structures [1,7,18,19] were analyzed using our new strategy. A flowchart of the approach developed is shown in Fig. 1. Then, our new strategy was applied on the *N*-glycans analysis of three gastric carcinoma (GC) cell lines (AGS, SGC-7901, NCI-N87) having different differentiation-states.

2. Materials and methods

2.1. Materials and reagents

Glycoworks RapiFluor-MS *N*-Glycan kit was purchased from Waters Corporation (MA, USA). Minute™ plasma membrane protein isolation kit was purchased from Invent Biotechnologies (MN, USA).

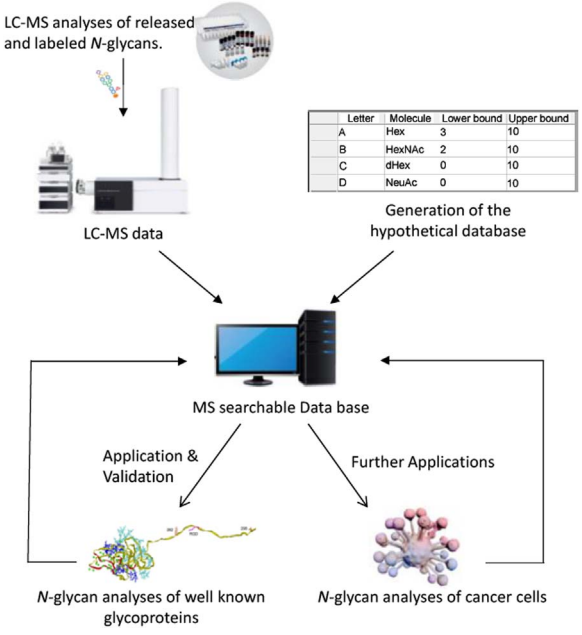

Fig. 1. Work flow chart of development of *N*-glycans analysis strategy.

IgG from porcine serum, fetuin from fetal bovine serum, lactoferrin from bovine milk and ribonuclease B from bovine pancreas were all purchased from Sigma-Aldich (St. Louis, MO USA). Acetonitrile of LC–MS grade was purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared by ELGA LabWater (resistivity ≥18.2 MΩ×cm, 25 °C).

2.2. Establishing of the hypothetical N-glycan database

GlycResoft software was used to establish a hypothetical *N*-glycan database. Four sugar residues, Hex (Mannose), HexNAc (GlcNAc), dHex (Fucose) and NeuAc, were listed as the components of *N*-glycans. As *N*-glycan contains a core pentasaccharide, Man3GlcNAc2, the lower bound for Hex and HexNAc content was set as 3 and 2, respectively. The upper limit of number of the four sugar residues was set at 10 to include many composition possibilities. The fluorescent tag, RapiFluor-MS, was set as a required component attached to the reducing end of each hypothetical *N*-glycan in the GlycResoft glycan database.

2.3. Isolation of the cancer cell membrane proteins

The AGS cell line was obtained from a sterile segment of a freshly resected adenocarcinoma of the stomach in a patient who had received no prior cancer therapy and was poorly differentiated [20]. The SGC-7901 cell line was obtained from a lymphoglandula metastasis of a gastric carcinoma and was moderately differentiated [21]. NCI-N87 cells were obtained from liver metastasis of gastric carcinoma arising in an American patient and were highly differentiated [22]. These cells were kindly provided by Professor Shiliang Wu, (Soochow University, China).

Minute™ plasma membrane protein isolation kit is designed to rapidly isolate native total membrane proteins (organelle membrane proteins) and native plasma membrane proteins from cultured mammalian cells or tissues. This kit sequentially separates cellular components into four fractions: nuclei, cytosol, organelles and plasma membrane [23]. About 10^6 cells of each cancer cell line were collected with low speed centrifugation (500–600 *g* for 5 min). Following the standard procedure coming with this kit, the cell membrane proteins were isolated from those three cancer cells, respectively.

2.4. Fast enzymatic release and rapid labeling of N-glycans

A standard three-step protocol of the GlycoWorks RapiFluor-MS *N*-Glycan kit, including quick deglycosylation, rapid labeling and SPE clean-up of labeled glycans, was applied to standard proteins and isolated membrane proteins. The standard glycoproteins used were fetuin, IgG, lactoferrin, ribonuclease B (~1 μg for each). The entire process to prepare each sample could be accomplished in 30 min [15]. The labeled glycans from each glycoprotein were stored at 4 °C until LC–MS analysis was performed.

2.5. UHPLC–MS analysis of the labeled N-glycans

The analysis was performed on an Agilent system equipped an ultra-high performance liquid chromatography (UHPLC, 1290 dual pumps) and an electrospray ionization–quadrapole time-of-flight–mass spectrometry (ESI–Q/TOF–MS) (6540, Agilent Technologies, USA). Labeled *N*-glycans were loaded onto an HILIC column (ACQUITY UPLC Glycan BEH Amide 130 Å, Waters, 2.1 mm×150 mm, 1.7 μm, Waters Corp.), running at 0.4 mL/min. The mobile phase A and B were 50 mM ammonium formate aqueous solution (pH 4.4) and acetonitrile, respectively. A gradient of mobile phase B from 75% to 54% was applied over 35 min. The column temperature was set at 60 °C. MS analysis conditions were: gas temperature 300 °C, drying gas 8 L/min, nebulizer 35 psig, sheath gas temp 400 °C, sheath gas flow 12 L/min, capillary voltage 4000 V, nozzle voltage (Expt) 500 V,

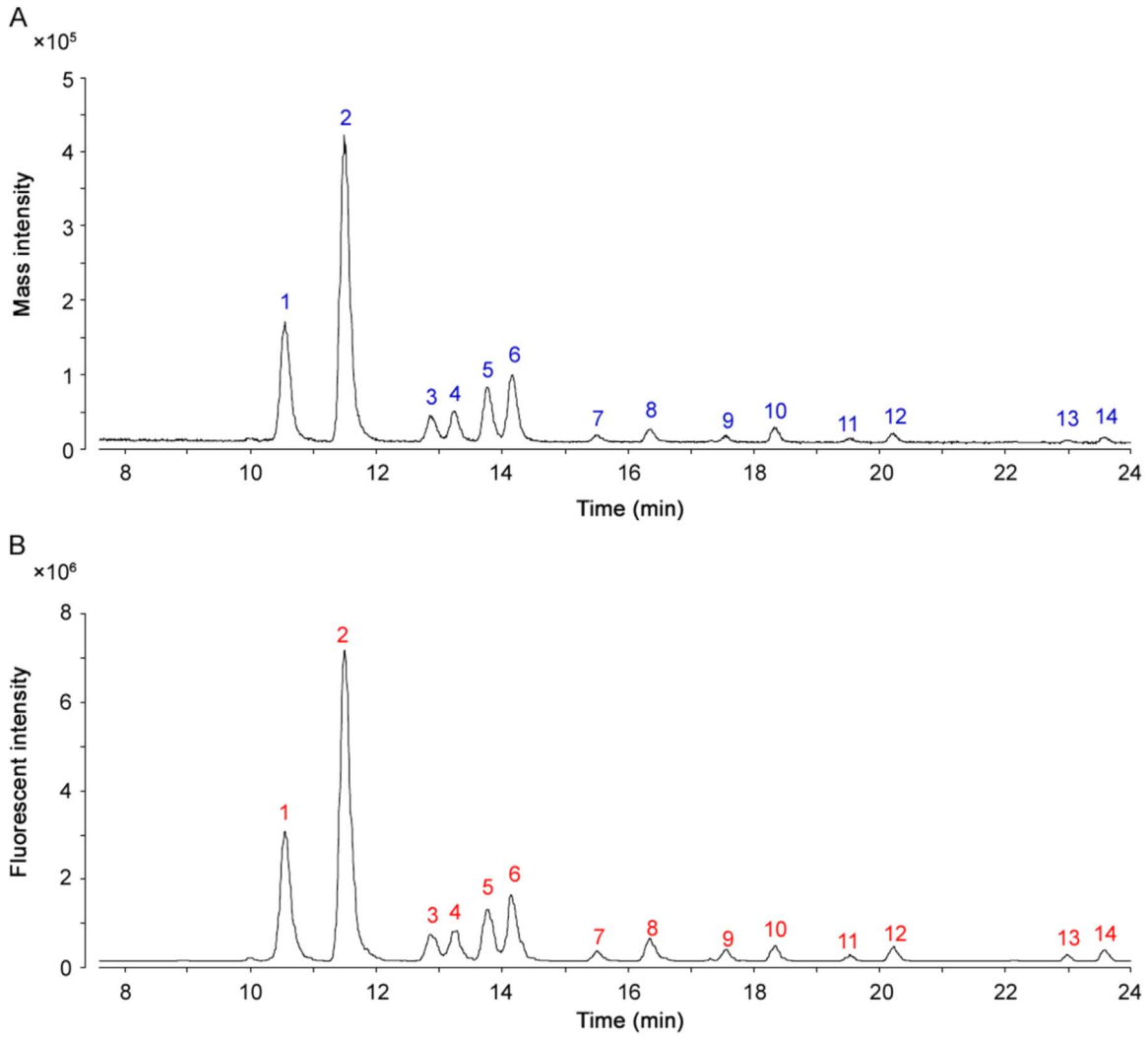

Fig. 2. (A) Total ion chromatogram (TIC) and (B) fluorescent chromatogram of labeled *N*-glycans released from IgG. The assignments of each peak are shown in Fig. 3B.

fragmentor 80 V, skimmer 65 V and mass range 200–2000 m/z. The collision-induced dissociation (CID) energy used in MS/MS to dissociate oligosaccharides was set as 30 V.

3. Results and discussion

3.1. Establishing a hypothetical N-glycan database

The database was based on a typical composition, the number of four types of monosaccharide residues (Hex, HexNAc, dHex and NeuAc) and the fluorescent tag. GlycResoft software calculated every compositional possibility and generated an initial database containing 8712 hypothetical RapiFluor-MS derived *N*-glycans (Mass shift from glycans with free reducing end is 311.1746 Da). In this version of the generated database, the composition and accurate molecular weight of each hypothetical *N*-glycan were included. The composition of each oligosaccharide in the database was described using five numbers in square brackets corresponding to the number of Hex, HexNAc, dHex, NeuAc and water, fixing the value of water as 1. The database table generated in GlycResoft was exported as "csv" file, containing three columns for compound name (Cpd), accurate molecular weight (mass), and molecular formula.

The Agilent MassHunter Qualitative Analysis Software provides a function to search an external database. Using this function, the mass data extracted from the MS profile can be searched in the given database. The mass match tolerance was set at 5 ppm. The matching result lists the composition, charge statement (species), accurate mass, and score. The mass accuracy, isotope abundance and isotope spacing contribute to the score by 50%, 30% and 20%, respectively. Only the chromatographic peaks presented both in total ion chromatography (TIC) and fluorescent chromatography were selected and the corresponding MS data was searched in the hypothetical database to decrease the false-positive results. For example, Fig. 2 contains the fluorescent chromatogram and TIC of the *N*-glycans released from IgG. The 14 chromatographic peaks, labeled in Fig. 2, were selected and their MS data were searched.

3.2. N-glycan analysis of four standard proteins

The *N*-glycans of four well-studied glycoproteins, fetuin, IgG, lactoferrin and ribonuclease B, were analyzed using this strategy. Their TICs are shown in Fig. 3. The label reagent, RapiFluor-MS, significantly improved the separation of the *N*-glycans on LC column and allowed for sensitive MS and fluorescence detection. The labeled *N*-glycans were well separated and shown in the TIC (Fig. 3). The chromatographic separation before MS analysis minimized ion suppression and the formation of artifacts. The effective cleanup step was also very helpful in this regard. Furthermore, the increase of MS sensitivity facilitates the detection of minor *N*-glycans. The current method requires only 35 min, from deglycosylation until injection for

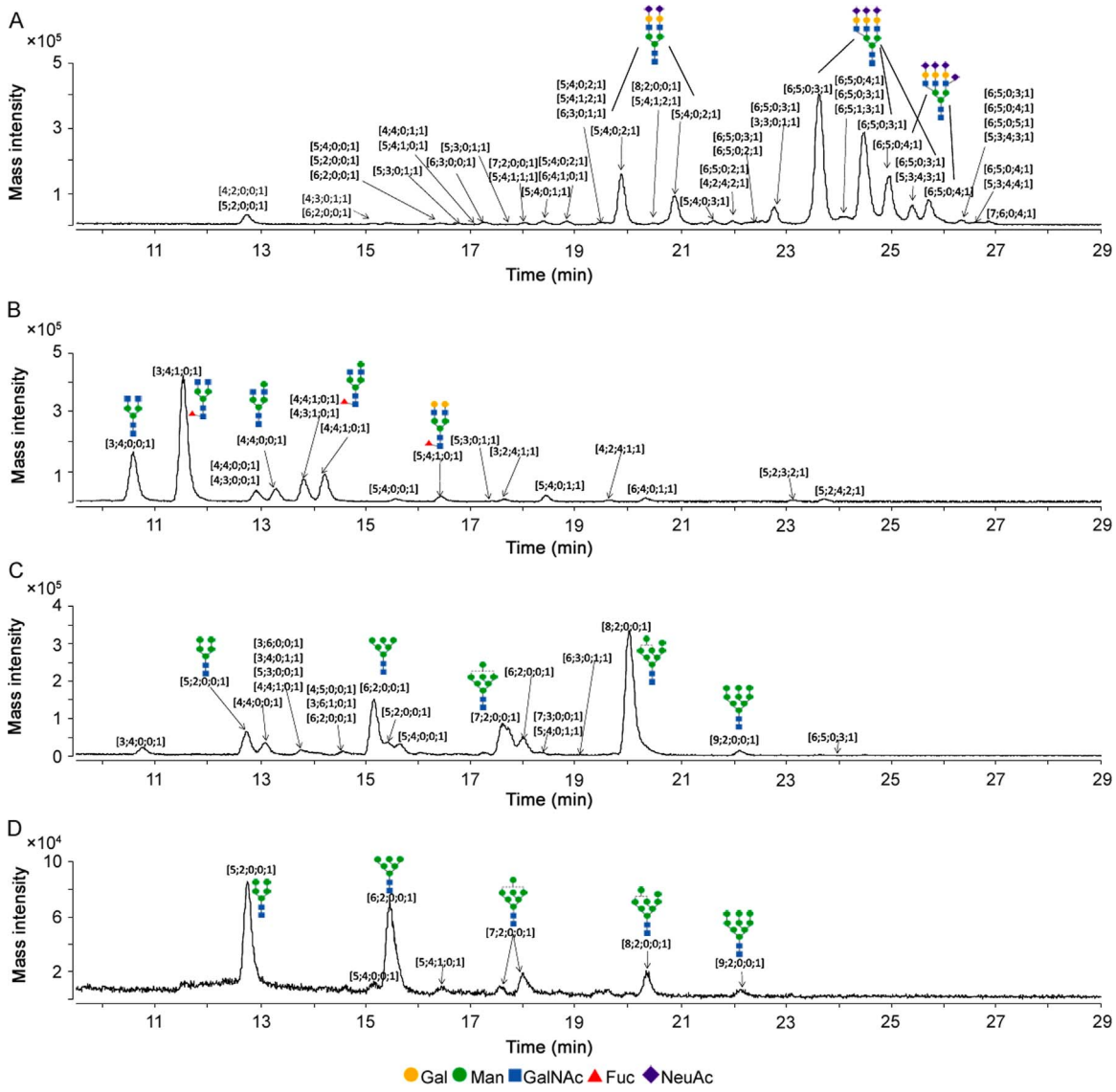

Fig. 3. Total ion chromatogram (TIC) of the labeled *N*-glycans released from four standard proteins. (A) TIC of *N*-glycans from fetuin; (B) TIC of *N*-glycans from IgG; (C) TIC of *N*-glycans from lactoferrin; and (D) TIC of *N*-glycans from ribonuclease B.

LC–MS. This fast operation results primarily from rapid deglycosylation, which takes only 5 min at 50 °C and does not require the use of dithiothreitol and iodoacetamide [24,25]. Furthermore, the mild, aqueous and efficient tagging reaction can be accomplished in a few minutes [15]. Desialylation often occurs using the harsher traditional reductive amination of the aldehyde group at the reducing end residue that requires released glycans to undergo multiple chemical conversions and lengthy high temperature incubation steps [11]. Such harsh conditions are not required in the current method [15].

The mass spectra of each chromatographic peak in TICs of the *N*-glycans recovered from the standard glycoproteins were searched against the initial hypothetical *N*-glycan database containing 8,712 structures using MassHunter Qualitative Analysis. The results of this search are shown in Fig. 3 and Table 1. The data were confirmed based on both mass and fluorescence detection, and the mass spectra were also manually examined. The assignments of *N*-glycans were extremely accurate and no mass signal corresponding to an *N*-glycan was missed using automated searching of the hypothetical glycan database. The *N*-glycans having 37, 15, 18 and 7 compositions were observed from fetuin, IgG, lactoferrin and ribonuclease B, respectively (Table 1). Previous papers [1,7,18,19,26–31], using other methods had only reported 3–6, 3–5, 7–10 and 5 *N*-glycans, respectively, for these same four glycoproteins (Table 1). Only the major *N*-glycans had been observed in previous reports and these are assigned with symbolic structures in Fig. 3. The minor *N*-glycans, found at lower abundance corresponding to rare compositions, such as those having high fucose content, were observed only using the current strategy. Some chromatographic peaks, corresponding to the same composition observed in the *N*-glycan profiles in the current strategy, were unassigned, which implied that additional structures, corresponding to sequence isomers, were also present. Thus, the excellent separation, high-sensitivity detection, clean background and comprehensive database provided a more in depth analysis of the *N*-glycans present in these glycoproteins.

3.3. *N*-glycan analysis of cancer cells

The abnormal expression of glycosyl transferases has been reported to respond to the invasion and metastasis of cancer cells, and some glycosyltransferases are used as biomarkers to detect the extent of cancer differentiation [21]. Profiling *N*-glycans might represent an alternative approach for revealing the extent of cancer cell differentia-

Table 1
N-glycans found in standard proteins.

Protein	*N*-glycans ([Hex; HexNAc; dHex; NeuAc; Water])			
	Observed in this work	References	References	References
IgG from porcine serum	[3;4;0;0;1][3;4;1;0;1][3;2;4;1;1][4;4;0;0;1][4;3;0;0;1][4;4;1;0;1] [4;3;1;0;1][4;2;4;1;1][5;4;0;0;1][5;4;1;0;1][5;3;0;1;1][5;4;0;1;1] [5;2;3;0;2][5;2;4;2;1][6;4;0;1;1]	[3;4;1;0;1][4;4;1;0;1][4;5;1;0;1][5;4;1;0;1] [5;5;1;0;1] [7]	[3;4;1;0;1] [4;4;1;0;1] [5;4;1;0;1] [26]	[3;3;0;0;1] [4;3;0;0;1] [4;3;1;0;1] [4;4;1;0;1] [5;4;1;0;1] [27]
Fetuin from fetal bovine serum	[3;3;0;1;1][4;2;0;0;1][4;3;0;1;1][4;4;0;1;1][4;2;4;2;1][5;2;0;0;1] [5;4;0;0;1][5;3;0;1;1][5;4;1;0;1][5;3;4;3;1][5;4;0;1;1][5;4;1;1;1] [5;4;0;2;1][5;4;1;2;1][5;4;0;3;1][6;2;0;0;1][6;3;0;0;1][6;5;0;4;1] [6;4;0;0;1][6;3;0;1;1][6;5;4;0;1][6;4;1;0;1][6;4;2;0;1][6;5;1;2;1] [6;4;1;1;1][6;4;0;1;1][6;5;0;3;1][6;5;0;2;1][6;4;0;2;1][6;5;1;3;1] [6;5;1;4;1][6;5;0;5;1][7;2;0;0;1][7;4;1;0;1][7;6;0;4;1][8;2;0;0;1] [9;2;0;0;1]	[5;4;0;1;1][5;4;0;2;1][6;5;0;1;1][6;5;0;2;1] [6;5;0;3;1][6;5;0;4;1] [19]	[5;4;0;2;1] [6;5;0;3;1] [6;5;0;4;1] [28]	[5;4;0;2;1] [6;5;0;3;1] [6;5;0;4;1] [29]
Lactoferrin from bovine milk	[3;4;0;0;1][3;6;0;0;1][3;4;0;1;1][3;6;1;0;1][4;4;0;0;1][4;4;1;0;1] [4;5;0;0;1][5;2;0;0;1][5;3;0;0;1][5;4;0;0;1][5;4;0;1;1][6;2;0;0;1] [6;3;0;1;1][6;5;0;3;1][7;2;0;0;1][7;3;0;0;1][8;2;0;0;1][9;2;0;0;1]	[3;4;0;0;1][4;4;0;0;1] [4;3;0;0;1][5;2;0;0;1] [5;3;0;0;1][6;2;0;0;1][6;3;0;0;1][7;2;0;0;1] [8;2;0;0;1][9;2;0;0;1] [1]	[5;2;0;0;1] [6;2;0;0;1] [7;2;0;0;1] [8;2;0;0;1] [9;2;0;0;1] [4;4;0;0;1] [4;5;0;0;1] [5;4;0;0;1] [30]	[6;2;0;0;1] [7;2;0;0;1] [8;2;0;0;1] [5;4;0;1;1] [5;4;1;1;1] [5;4;1;2;1] [5;4;0;2;1] [31]
Ribonuclease B from bovine pancreas	[5;2;0;0;1][5;4;0;0;1][5;4;1;0;1][6;2;0;0;1][7;2;0;0;1][8;2;0;0;1] [9;2;0;0;1]	[5;2;0;0;1][6;2;0;0;1] [7;2;0;0;1][8;2;0;0;1] [9;2;0;0;1] [18]	[5;2;0;0;1] [6;2;0;0;1] [7;2;0;0;1] [8;2;0;0;1] [9;2;0;0;1] [26]	[5;2;0;0;1] [6;2;0;0;1] [7;2;0;0;1] [8;2;0;0;1] [9;2;0;0;1] [29]

Note: The value of water was fixed as 1.

tion. The *N*-glycans from three GC cells, displaying different degrees of cellular differentiation, were analyzed. These were AGS, SGC-7901 and NCI-N87 with a low, medium and high degree of differentiation, respectively.

The membrane proteins were recovered from three subtypes of GC cells (10^6) and their *N*-glycans were released and analyzed using our newly developed strategy. The TICs obtained in these analyses are shown in Fig. 4. With the aid of our hypothetical *N*-glycan database, approximately 200 *N*-glycans were identified in these three cell lines as shown in Tables S1-S3. The 19 major *N*-glycans are labeled in Fig. 4. Seven novel glycans were observed in manual interpretation and were assigned as [2;2;1;0;1], [2;2;0;0;1], [1;0;0;2;1], [1;1;1;1;1], [0;0;0;1;1], [0;1;0;0;1], and [0;1;1;0;1]. Their mass spectra are shown in Fig. 5, and their molecular ions were observed at m/z 603.7578 (doubly charged), 530.7294 (doubly charged), 537.7196 (doubly charged), 566.7439 (doubly charged), 621.2838 (singly charged), 533.2702 (singly charged), and 679.3292 (singly charged), respectively. These are new compositions for *N*-glycans. None of these have the typical core pentasaccharide structure. Compositional information from these novel glycans was manually input into our hypothetical database. The *N*-glycan analysis results of these three cancer cells were again searched with this expanded database (8719 compositions of *N*-glycans). All the manually added glycans afforded high scores (Tables S1-S3), further confirming their structural assignment.

The profiles of *N*-glycans from these three GC cells were different, although all contained high-mannose *N*-glycan types. These were assigned as [3;2;0;0;1], [4;2;0;0;1], [5;2;0;0;1], [6;2;0;0;1], [7;2;0;0;1], [8;2;0;0;1], [9;2;0;0;1] and [10;2;0;0;1], in which the peaks corresponded to [7;2;0;0;1] and [8;2;0;0;1] and were used to normalize the TICs of these three GC cells. The chromatographic peaks corresponding to fucose-enriched and non-sialylated *N*-glycans at TIC retention times of 16.5, 17.2 and 22.6 min were exclusively observed in the SGC-7901 cells (Fig. 4A and C). These were assigned as [5;4;1;0;1], [5;4;2;0;1] and [7;6;1;0;1]. The chromatographic peaks corresponding to sialic acid enriched *N*-glycans were observed at relatively high intensities in the TIC at 18.6–19.7 min in the NCI-N87 cells (Fig. 4A and D). These were assigned as [5;4;0;2;1], [5;4;1;1;1] and [5;4;1;2;1], respectively. In addition, the chromatographic peaks corresponding to sialic acid enriched *N*-glycans were observed in the TIC at 26.0–26.5 min exclusively in the NCI-N87 cells (Fig. 4A and D). These were assigned as [7;6;0;4;1] and [7;6;1;4;1]. According to these assignments, some of these sialic acid enriched *N*-glycans from the NCI-N87 cells are also fucosylated. Most of *N*-glycans observed in AGS cells were of the high-mannose type with the exception of a few fucosylated *N*-glycans with low intensities, eluting at 5.5 and 8.2 min and assigned as [2;2;1;0;1] and [3;2;1;0;1] (Fig. 4A and B). Thus, the *N*-glycans are rarely fucosylated or sialylated in the GC cells showing a low degree of differentiation, some of *N*-glycans are fucosylated in moderately differentiated GC cells, and some of *N*-glycans are sialylated in the highly differentiated GC cells.

4. Conclusion

In this work, a strategy that combines an efficient method of analysis and the use of a comprehensive database was applied for *N*-glycan analysis. This analytical method depends on a kit for the rapid release of *N*-glycan from a glycoprotein, tagging and recovery. The total analysis time, from sample preparation to collection of LC–MS data using this kit and proper UHPLC–MS conditions, was only 1 h. Moreover, the separation efficiency and sensitivity have been significantly improved compared to previous reports. An *N*-glycan database was initially constructed from a hypothetical library and then expanded

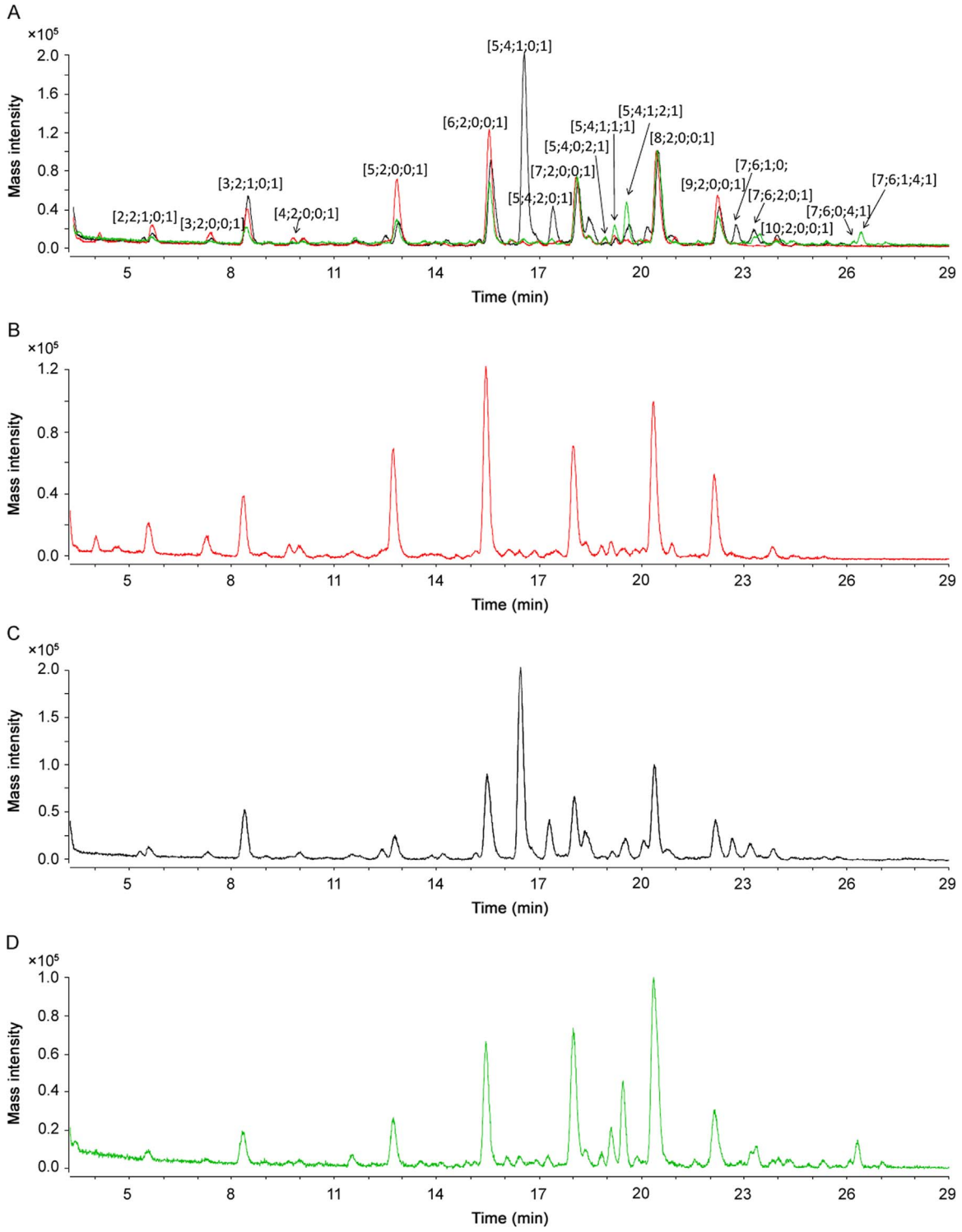

Fig. 4. Total ion chromatogram (TIC) of labeled *N*-glycans released from the membrane proteins of three different subtypes of gastric cancer cells. (A) Overlaid chromatograms; (B) TIC of labeled N-glycans from AGS cells; (C) TIC of labeled *N*-glycans from SGC-7901; and (D) TIC of labeled *N*-glycans from NCI-N87.

with data obtained from subsequent experiments. The final database had 8719 *N*-glycans and contained their compositions, molecular formulas, accurate molecular weights and isotope information. This comprehensive, accurate and expandable database was applied to *N*-glycans analysis of a number of standard glycoproteins. The *N*-glycans on fetuin, IgG, lactoferrin and ribonuclease B were characterized and the numbers of *N*-glycans observed in these glycoproteins was much greater than that had been previously reported. Finally, the *N*-glycans from three types of GC cells were profiled using our strategy. Several unusual glycans having lengths shorter than the typical *N*-glycan core pentasaccharide were identified in these GC cells and subsequently input into the database. The *N*-glycans from those three cell lines also differed based on their degree of differentiation. Thus, the novel strategy for the accurate, efficient and comprehensive analysis of *N*-glycans from glycoproteins and cells has been developed that greatly simplifies the process of *N*-glycan analysis.

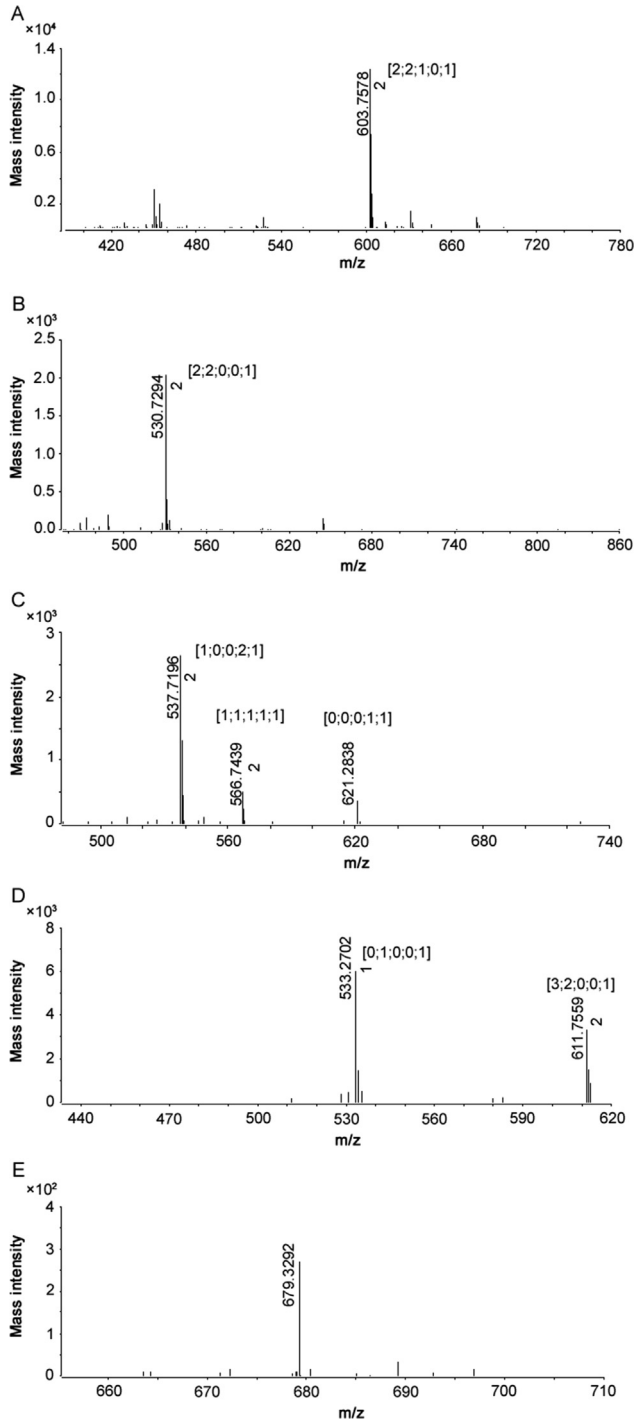

Fig. 5. MS spectra of special glycans observed in GC cells.

Acknowledgments

The authors appreciate Prof. Shiliang Wu (Soochow University) providing GC cell lines. The authors are grateful to the National Natural Science Foundation of China (81473179 and 81673388), Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, YX13200111), and the funding for Jiangsu Key Laboratory of Translational Research and Therapy for Neuro-Psycho-Diseases (BM2013003).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2017.01.004.

References

[1] C.C. Nwosu, R.R. Seipert, J.S. Strum, et al., Simultaneous and extensive site-specific N- and O-glycosylation analysis in protein mixtures, J. Prote. Res. 10 (2011) 2612–2624.
[2] K. Ohtsubo, J.D. Marth, Glycosylation in cellular mechanisms of health and disease, Cell 126 (2006) 855–867.
[3] R.G. Spiro, Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds, Glycobiology 12 (2002) 43R–56R.
[4] G. Yu, Y. Zhang, Z. Zhang, et al., Effect and limitation of excess ammonium on the release of O-glycans in reducing forms from glycoproteins under mild alkaline conditions for glycomic and functional analysis, Anal. Chem. 82 (2010) 9534–9542.
[5] J. Yuan, C. Wang, Y. Sun, et al., Nonreductive chemical release of intact N-glycans for subsequent labeling and analysis by mass spectrometry, Anal. Biochem. 462 (2014) 1–9.
[6] V. Tretter, F. Altmann, L. März, Peptide-N4-(N-acetyl-beta-glucosaminyl) asparagine amidase-F cannot release glycans with fucose attached alpha-1-3 to the asparagine-linked N-acetylglucosamine residue, Eur. J. Biochem. 199 (1991) 647–652.
[7] D.J. Harvey, L. Royle, C.M. Radcliffe, et al., Structural and quantitative analysis of N-linked glycans by matrix-assisted laser desorption ionization and negative ion nanospray mass spectrometry, Anal. Biochem. 376 (2008) 44–60.
[8] I. Ciucanu, F.A. Kerek, Simple and rapid method for the permethylation of carbohydrates, Carbohydr. Res. 131 (1984) 209–217.
[9] A.S. Weiskopf, P. Vouros, D.J. Harvey, Characterization of oligosaccharide composition and structure by quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 11 (1997) 1493–1504.
[10] I. Ciucanu, C.E. Costello, Elimination of oxidative degradation during the per-O-methylation of carbohydrates, J. Am. Chem. Soc. 125 (2003) 16213–16219.
[11] J.C. Bigge, T.P. Patel, J.A. Bruce, et al., Nonselective and efficient fluorescent labeling of glycans using 2-amino benzamide and anthranilic acid, Anal. Biochem. 230 (1995) 229–238.
[12] P.M. Rudd, G.R. Guile, B. Küster, et al., Oligosaccharide sequencing technology, Nature 388 (1997) 205–207.
[13] Y. Mechref, Y. Hu, J.L. Desantos-Garcia, et al., Quantitative glycomics strategies, Mol. Cellu. Proteo. 12 (2013) 874–884.
[14] R.P. Kozak, C.B. Tortosa, D.L. Fernandes, et al., Comparison of procainamide and 2-aminobenzamide labeling for profiling and identification of glycans by liquid chromatography with fluorescence detection coupled to electrospray ionization-mass spectrometry, Anal. Biochem. 486 (2015) 38–40.
[15] M.A. Lauber, Y.Q. Yu, D.W. Brousmiche, et al., Rapid preparation of released N-glycans for HILIC analysis using a labeling reagent that facilitates sensitive fluorescence and ESI-MS detection, Anal. Chem. 87 (2015) 5401–5409.
[16] E. Maxwell, Y. Tan, Y. Tan, et al., GlycReSoft: a software package for automated recognition of glycans from LC/MS data, PLoS One 7 (2012) e45474.
[17] M. Bern, Y.J. Kil, C. Becker, Byonic: advanced peptide and protein identification software, Curr. Protoc. Bioinforma. Chapter 13, Unit 20 (2012).
[18] Y.Q. Yu, J. Fournier, M. Gilar, et al., Identification of N-linked glycosylation sites using glycoprotein digestion with pronase prior to MALDI tandem time-of-flight mass spectrometry, Anal. Chem. 79 (2007) 1731–1738.
[19] Y. Huang, Y. Mechref, M.V. Novotny, Microscale nonreductive release of O-linked glycans for subsequent analysis through MALDI mass spectrometry and capillary electrophoresis, Anal. Chem. 73 (2001) 6063–6069.
[20] S.C. Barranco, C.M. Townsend, C. Casartelli, et al., Establishment and characterization of an invitro model system for human adenocarcinoma of the stomach, Cancer Res. 43 (1983) 1703–1709.
[21] Y.P. Zhao, X.Y. Xu, M. Fang, et al., Decreased core-fucosylation contributes to malignancy in gastric cancer, PLoS One 9 (2014) e94536.
[22] J.G. Park, H. Frucht, R.V. Larocca, et al., Characteristics of cell-lines established from human gastric-carcinoma, Cancer Res. 50 (1990) 2773–2780.
[23] R. Terrasse, P. Tacnet-Delorme, C. Moriscot, et al., human and Pneumococcal cell surface Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) proteins Are both ligands of human C1q protein, J. Biol. Chem. 287 (2012) 42620–42633.
[24] W. Morelle, J.C. Michalski, Analysis of protein glycosylation by mass spectrometry, Nat. Protoc. 2 (2007) 1585–1602.
[25] J. Zaia, R. Boynton, D. Heinegård, et al., Posttranslational modifications to human bone sialoprotein determined by mass spectrometry, Biochemistry 40 (2001) 12983–12991.
[26] L. Royle, M.P. Campbell, C.M. Radcliffe, et al., HPLC-based analysis of serum N-glycans on a 96-well plate platform with dedicated database software, Anal. Biochem. 376 (2008) 1–12.
[27] M. Thaysen-Andersen, S. Mysling, P. Højrup, Site-specific glycoprofiling of N-linked glycopeptides using MALDI-TOF MS: strong correlation between signal strength and glycoform quantities, Anal. Chem. 81 (2009) 3933–3943.
[28] M. Melmer, T. Stangler, A. Premstaller, et al., Comparison of hydrophilic-interaction, reversed-phase and porous graphitic carbon chromatography for glycan

analysis, J. Chromatogr. A. 1218 (2011) 118–123.
[29] W. Ding, H. Nothaft, C.M. Szymanski, et al., Identification and quantification of glycoproteins using ion-pairing ormal-phase liquid chromatography and mass spectrometry, Mol. Cellu. Proteo. 8 (2009) 2170–2185.
[30] D. Garrido, C. Nwosu, S. Ruiz-Moyano, et al., Endo-beta-N-acetylglucosaminidases from infant gut-associated Bifidobacteria release complex N-glycans from human milk glycoproteins, Mol. Cellu. Proteo. 11 (2012) 775–785.
[31] M. Lopez, B. Coddeville, J. Langridge, et al., Microheterogeneity of the oligosaccharides carried by the recombinant bovine lactoferrin expressed in Mamestra brassicae cells, Glycobiology 7 (1997) 635–651.