Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Journal of Chromatography B, 838 (2006) 3–8
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Mass spectrometry for congenital disorders of glycosylation, CDG☆

Yoshinao Wada*

Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan

Received 29 September 2005; accepted 8 February 2006
Available online 6 March 2006

Abstract

Congenital disorders of glycosylation (CDG) constitute a group of diseases affecting N-linked glycosylation pathways. The classical type of CDG, now called CDG-I, results from deficiencies in the early glycosylation pathway for biosynthesis of lipid-linked oligosaccharide and its transfer to proteins in endoplasmic reticulum, while the CDG-II diseases are caused by defects in the subsequent processing steps. Mass spectrometry (MS) produced a milestone in CDG research, by localizing the CDG-I defect to the early glycosylation pathway in 1992. Currently, MS of transferrin, either by electrospray ionization or matrix-assisted laser desorption/ionization, plays the central role in laboratory screening of CDG-I. On the other hand, the glycopeptide analysis recently developed for site-specific glycans of glycoproteins allows detailed glycan analysis in a high throughput manner and will solve problems in CDG-II diagnosis. These techniques will facilitate studying CDG, a field now expanding to O-linked glycosylation and to acquired as well as inherited conditions that can affect protein glycosylation.

Keywords: Mass spectrometry; Glycosylation; N-linked oligosaccharide; CDG; Glycoprotein

Contents

1. Introduction

Metabolic diseases are divided into two categories based on whether the defects are in synthesis or degradation. There are several metabolic disorders belonging to the latter category, mostly derived from defects in degradation enzymes. The "lysosomal disorders" form a large group, since lysosomes are the principal sites of intracellular degradation of macromolecules. As to the glycoproteins, α- and β-mannosidoses, sialidosis and fucosidosis are caused by defects of the lysosomal enzymes which break down their glycan moieties [1,2]. By contrast, disorders of glycoprotein glycan synthesis constitute only a small portion of metabolic disorders. This is apparently due to a lack of common clinical features, whereas lysosomal disorders have characteristic pathologies such as intracellular vacuoles or inclusion bodies and excessive urinary excretion of oligosaccharides that should be subjected to biochemical analysis. Alternatively, defects of synthesis may be likely to be prenatally lethal.

N-linked protein glycosylation takes place in two distinct cellular compartments: the endoplasmic reticulum (ER) and the Golgi apparatus [3,4]. In the ER, a core oligosaccharide

☆ This paper was presented at the 30th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry, Osaka, Japan, 8–9 September 2005.

* Tel.: +81 725 56 1220; fax: +81 725 57 3021.
E-mail address: waday@mch.pref.osaka.jp.

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.02.028

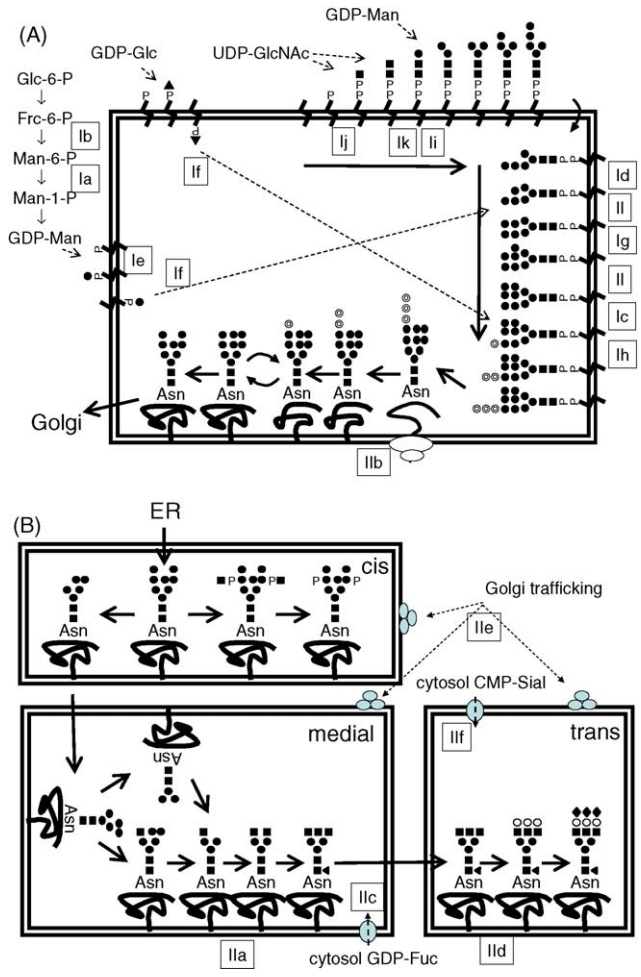

Fig. 1. N-linked oligosaccharide biosynthetic pathway and locations of known defects in various types of CDG. (A) ER pathway, (B) Golgi pathway. Symbols for monosaccharides: glucose (double circles); mannose (closed circles); *N*-acetylglucosamine (squares); galactose (open circles); fucose (triangles); *N*-acetylneuraminic acid (rectangles).

($Glc_3Man_9GlcNAc_2$) is assembled on a lipid carrier, dolichol, to form a membrane-anchored lipid-linked oligosaccharide (LLO) and is subsequently transferred en bloc to the target asparagine residue in a single enzymatic step. Further modifications which include trimming and addition of sugar molecules occur in the Golgi apparatus, depending on the protein (Fig. 1).

In yeast, various types of mutations in the genes for N-linked oligosaccharide synthesis have been identified, and most have defects in the early glycosylation step resulting in the accumulation of intermediate structures of LLO [5]. The modification of oligosaccharide structures in the Golgi apparatus is essential for the maturation of glycoproteins, especially in higher organisms.

2. Congenital disorders involving defective N-glycosylation of proteins

Approximately half of mammalian proteins are glycosylated. Therefore, clinical features vary enormously, making it difficult for physicians to recognize specific defects. The clinical features of CDG span a broad spectrum and affect many organs, e.g. the cerebellum and the gastrointestinal, hepatic, visual and immune systems [6,7]. The wide variety of symptoms explains why a group of disorders now called congenital disorders of glycosylation (CDG) was not recognized until the early 1980s.

The basis for the discovery of CDG was a report of abnormalities in multiple serum proteins in patients with decreased thyroxine-binding globulin and increased arylsulfatase A [8]. This led to a search for a defect in a feature common to these and other proteins, i.e. the glycan moiety, and substantial evidence for the molecular abnormality was obtained by demonstrating a sialic acid deficiency in serum transferrin, which has two disialo-biantennary *N*-glycans as the major oligosaccharide at Asn-432 and Asn-630, by isoelectric focusing (IEF) [9]. In those days, the IEF of transferrin was used to detect a change in the carbohydrate moieties of glycoproteins associated with certain diseases or conditions such as alcoholism [10], since the glycoform of transferrin is rather simple and composed mainly of two sialylated biantennary chains. As more patients with a common electrophoretic phenotype of transferrin and diverse multi-systemic symptoms were studied, the name “carbohydrate-deficient glycoprotein (CDG) syndrome” was introduced in 1991 [11], though the glycan defects were not characterized in detail. The abbreviation CDG was then changed and since 1999 has been taken to mean “congenital disorders of glycosylation” [12].

3. CDG-I and CDG-II

A simplified CDG nomenclature for N-linked oligosaccharide biosynthesis is currently used [6,13]. Group I CDG (CDG-I) defects are defined as those altering synthesis and transfer of the LLO to recipient proteins. Group II CDG (CDG-II) defects affect subsequent processing steps, mostly on N-linked sugar chains. Different types of disorders are indicated by a small letter code (a, b, c, etc.) consistent with the chronological order in which the defective gene was identified, and to date, 18 disorders have been documented (Fig. 1) [7]. The CDG with unknown genetic basis is named CDG-Ix or CDG-IIx.

Obviously, the CDG concept is not restricted to N-linked glycosylation. also covering other disorders affecting the biosynthesis of proteoglycans, O-linked oligosaccharides, or glycosylphosphatidylinositol anchors [14]. More importantly, the CDG group primarily affecting N-linked glycosylation is now expanding to include disorders for which the responsible genes are not glycosylation enzymes proper but other molecules involving intracellular trafficking, protein folding, other metabolic pathways, etc. [7,14–16].

4. Elucidation of the molecular abnormalities of classical CDG by mass spectrometry (MS)

Electrospray ionization (ESI) was developed in the mid 1980s [17,18], and was demonstrated to be effective for molecular mass measurement of large proteins in the early 1990s [19]. However, due to the broad distribution of isotopic clusters, measurements of large proteins exceeding 50 kDa were merely a technical challenge but not considered to be a practicable tool for defining

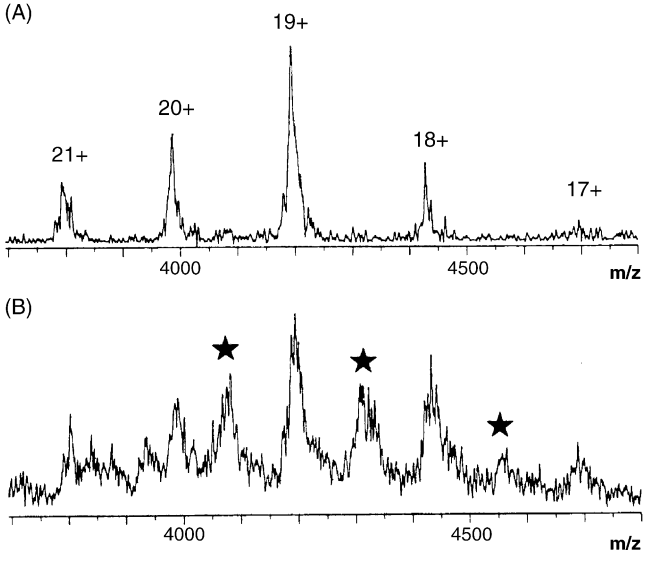

Fig. 2. ESI-MS of transferrin. (A) Control, (B) CDG-I. The peaks marked by asterisks are derived from a transferrin molecule lacking a whole oligosaccharide chain. Reproduced from Ref. [21] with permission.

molecular abnormalities. Meanwhile, the mass spectrometric approach to CDG was ready when ESI-MS of human transferrin was first reported in 1992 [20], but application to actual samples from patients was more difficult than that to model proteins.

The evidence of an early glycosylation defect in CDG-I was obtained in a study employing two different types of analysis of transferrin isolated from unrelated Japanese patients [21]. The first, chromatography of glycans released from transferrin, demonstrated no distinct abnormalities. The second, ESI-MS of transferrin, revealed the presence of transferrin lacking whole glycan chain(s) as well as normally glycosylated transferrin molecules. The mass spectrum is shown in Fig. 2. The masses of the (holo-)transferrin core structure and that of a single oligosaccharide (biantennary complex-type) chains are 75,181 and 2206 Da, respectively. The mass spectra of transferrin from patients showed abnormal peaks between the ions from the normal species, and the difference was calcultated to be 2200 Da. This and the chromatographic findings, taken in together, clearly indicated that glycans attached to transferrin molecules from patients to be normal structurally, while an entire glycan was missing from the molecule representing an abnormal band in IEF. The results confirmed the molecular pathology to be incomplete transfer of oligosaccharides to ER proteins due to insufficient synthesis of LLO or defective transfer processes.

5. ESI-MS for CDG screening

ESI-MS is currently used as a CDG-I screening method. A typical example is shown in Fig. 3; immunoaffinity purification followed by a 9-min run of LC–MS allows efficient diagnosis [22]. The deconvoluted spectra from the CDG patients clearly show the molecular abnormality (Fig. 4). The molecular weight of transferrin with two (fully sialylated) biantennary chains is 79,593 Da. The theoretical width at half the height of the signal for this size protein is approximately 20 Da. However, the best

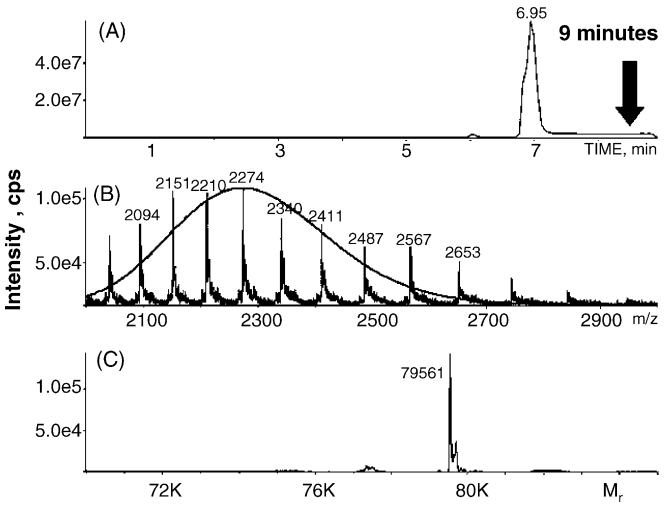

Fig. 3. LC–ESI-MS of immunoaffinity-purified transferrin. (A) Total-ion chromatogram of transferrin eluting at 7 min in a 9-min analysis. (B) Multiply-charged ion distribution of transferrin. (C) Mass data reconstructed by deconvolution. Reproduced from Ref. [22] with permission.

mass resolution of proteins exceeding 50 kDa is usually only a few hundred in the case of real samples measured by conventional mass spectrometers, due to heterogeneous modifications. Worse yet, alkali-metal is liable to attach to the glycan moiety of glycoproteins and thus increases the molecular (mass) heterogeneity of the ions and thereby broadens the signal. These issues point to a problem in Figs. 3 and 4. If the molecular masses of transferrin molecules in the sample are 79,561, 77,353, and 75,145 (these figures are not theoretical) for those with two (Tf-2OS), one (Tf-1OS) and no (Tf-0OS) glycan moieties, respectively, as shown in Fig. 4, the *m/z* values for the ions $[\text{Tf-2OS} + 36\text{H}]^{36+}$, $[\text{Tf-1OS} + 35\text{H}]^{35+}$ and $[\text{Tf-0OS} + 34\text{H}]^{34+}$ are very similar at 2211.028, 2211.086, and 2211.147, respectively. Obviously, these signals could never be discriminated from each other in the original (untransformed) mass spectrum. Anyhow, the algorithm for deconvolution simply transformed the mass spectrum into that shown in Fig. 4. It is readily recognized that a superior discrimination of these isoforms can be achieved in the higher mass region. This was why the measurement shown in Fig. 2 was fine-tuned so as to generate ions with

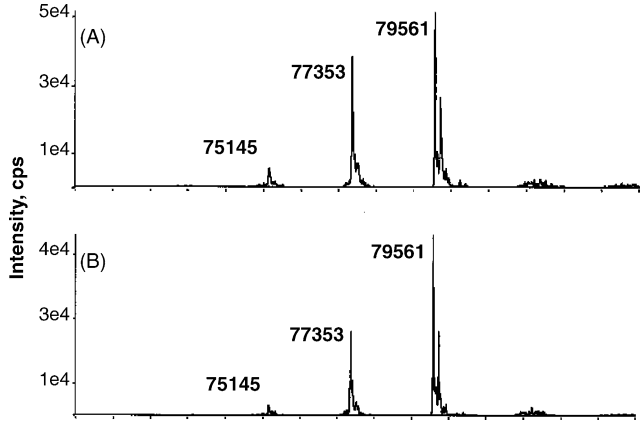

Fig. 4. LC–MS analysis of transferrin from CDG patients. Reproduced from Ref. [22] with permission.

a smaller number of charges, although the ions for Tf-2OS and Tf-0OS still overlapped [21].

The coupling of ESI with Fourier transform ion cyclotron resonance (FTICR) MS has provided unit resolving power even for large proteins, allowing resolution of sialic acid microheterogeneity in the $[M+51H]^{51+}$ ion of bovine transferrin, though no practical method has yet been established [23].

6. Matrix-assisted laser desorption/ionization (MALDI) for CDG

MALDI-MS predominantly generates singly-charged ions [24], and thus is free from the signal overlapping observed for a mixture of heterogeneous samples as with ESI-MS. The molecules exceeding 10 kDa are usually measured in linear mode in MALDI time-of-flight (TOF) MS. Vacuum MALDI using an ultraviolet laser causes loss of sialyl residues by fragmentation occurring in both the in- and post-source processes, and the majority of ions observed in the linear mode for sialylated glycoproteins correspond to metastable ions [25]. However, metastable decompositions during the flight do not reduce the intensity of a molecular ion signal, and thus the linear TOF analyzer is an ideal device for analysis of easily fragmenting and/or high mass analytes [26]. Due to the initial energy spread of ions, the resolving power of linear TOF instruments is only a few thousand even with the application of pulsed ion extraction. In addition, heterogeneities due to undefined modifications in the real sample impair resolution of the mass spectra of large molecules.

Nonetheless, low resolving power is adequate to demonstrate the absence of whole glycans in transferrin molecules of CDG-I. The first application of MALDI-TOF-MS of transferrin for CDG-I diagnosis was reported in 1994 [27]. A similar but independent study utilizing immunoprecipitation for quick sample preparation for MALDI-MS was reported shortly thereafter [28]. Typical MALDI mass spectra are presented in Fig. 5.

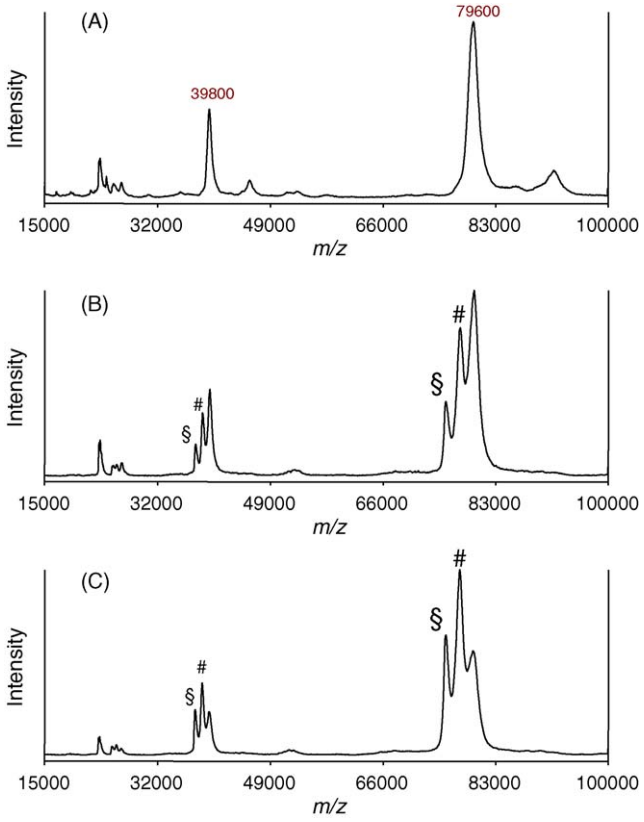

Fig. 5. MALDI-TOF mass spectra of transferrin. (A) Healthy individual, (B and C) CDG-I cases. The signal at *m/z* 39800 is the doubly-charged ion. Two isoforms lacking one (#) or two (§) oligosaccharide chains are indicated. The CDG samples were kindly provided by Dr. Isao Yuasa at Tottori University.

7. Problems in the detection and molecular diagnosis of CDG-II

The pathway related to CDG-I, or the biosynthesis and transfer of LLO from its lipid carrier to nascent proteins in the ER, requires 30–40 genes, while of the processing steps related to CDG-II probably involve more than 50 genes [29]. To date, only six distinct types of CDG-II have been identified, while twice that number, i.e. 12 types, of CDG-I have been documented. This appears to be attributable to the fact that the different types of CDG-II having diverse, or even no detectable, abnormalities in the *N*-glycan structures of the glycoproteins available for analysis, while CDG-I types have in common the hallmark of missing the entire *N*-glycan structure, making them readily detectable. The molecular abnormalities associated with CDG-II can be summarized as follows.

CDG-IIa is caused by the loss of *N*-acetylglucosaminyltransferase II enzymatic activity (Fig. 1) [30,31], which produces a characteristic transferrin molecule abnormality, in which each *N*-glycosylation site is occupied by a truncated monosialo-monoantennary *N*-glycan [32].

CDG-IIb is caused by a deficiency of glucosidase I, an enzyme that removes the terminal α1,2-linked glucose from the $Glc_3Man_9GlcNAc_2$ precursor just after its en bloc transfer from dolichyl diphosphate to a nascent protein in the endoplasmic reticulum [33]. To date, only one patient with CDG-IIb has been reported. The deficiency precludes further processing steps and the formation of complex type *N*-glycans is significantly impaired. However, the unprocessed *N*-glycans are not found in glycoproteins from the patient, most likely because they are cleaved by the endo-α1,2-mannosidase in an alternative biosynthetic pathway [33]. As a consequence, CDG-IIb shows a normal IEF profile of serum transferrin and cannot be detected by structural analysis of the patient's glycoproteins. Accumulation of the tetrasaccharide Glc_3Man_1 in the patient's urine was the clue which allowed detection of this disorder.

CDG-IIc has been called leukocyte adhesion deficiency II because it is a defect in sialyl-Lewis^x (sLe^x) expression. This disorder is caused by reduced transport of GDP-fucose into the Golgi [34–36], and the resulting decrease in fucosylated glycans manifests as a deficiency of core fucosylation of N-linked oligosaccharides of glycoproteins [37,38]. Since sialic acid is not involved, CDG-IIc shows a normal IEF profile of serum transferrin.

CDG-IId is caused by β1,4-galactosyltrasnferase I (Fig. 1). The defect results in a straightforward abnormality, that is loss of both galactose and sialic acid, in the *N*-glycan structures [39].

CDG-IIe results from impaired integrity of the Golgi trafficking machinery, i.e. the COG (conserved oligomeric Golgi) complex, due to a mutation encoding a COG-7 subunit, and decreased sialylation of glycoproteins has been reported in fibroblasts [40].

CDG-IIf is caused by a defect in the CMP-sialic acid transporter [41], resulting in a complete lack of sLe^x on leukocytes due to a deficiency in sialic acid. Interestingly, the IEF pattern of serum transferrin is normal, while apolipoprotein C-III (apoC-III), one of the few serum proteins with only *O*-glycans and which has 2,3-linked sialic acids, shows abnormal IEF and SDS-PAGE profiles [42].

These molecular features of glycoproteins indicate that elucidation of CDG-II requires detailed analysis of the glycan structures; e.g. the molecular diagnoses of CDG-IIa, IIc and IId could presumably be made based on the characteristic *N*-glycan structures. However, neither ESI-MS or MALDI-MS of intact transferrin can reliably define the precise changes at the sugar unit level. A solution is to analyze the glycans released from serum transferrin, other glycoproteins or whole serum. For example, the glycans are removed enzymatically from glycoproteins, fluorescence-labeled at their reducing termini by reductive amination with, e.g. 2-aminobenzamide, and examined by normal phase chromatography [32]. Alternatively, the released glycans are purified using a graphitized carbon column and analyzed by MS [43]. With either method, several glycan structures for serum transferrin and more than 10 structures from whole serum or plasma can be identified and quantified.

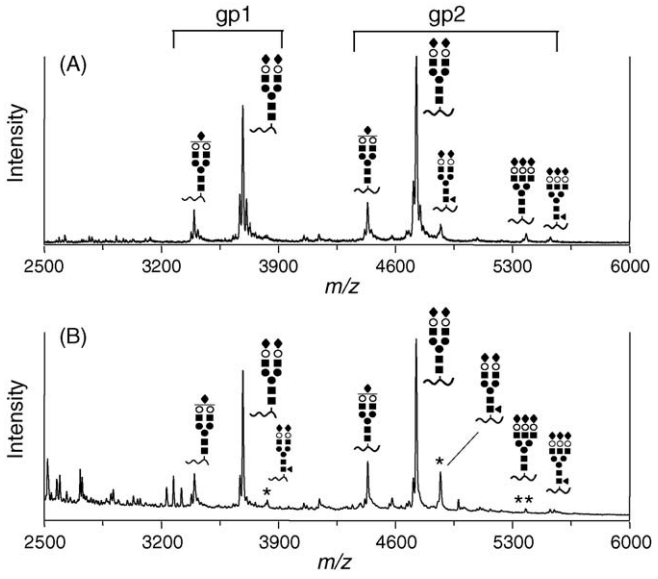

Fig. 6. MALDI mass spectra of transferrin glycopeptides. Tryptic peptides of carbamidomethylated transferrin were mixed with Sepharose CL4B in 1-butanol/ethoanol/water (4/1/1, v/v/v) solution, and the bound materials were eluted with 50% ethanol and subjected to MALDI-MS with 2,5-dihydroxybenzoic acid matrix. Site-specific glycoforms at Asn-432 (gp1) and Asn-630 (gp2) are indicated above the individual signals. (A) Control, (B) CDG-Ix. Marked signals are fucosylated (*) and triantennary (**) species. Symbols for monosaccharides: mannose (closed circles); *N*-acetylglucosamine (squares); galactose (open circles); (triangles) fucose; *N*-acetylneuraminic acid (rectangles).

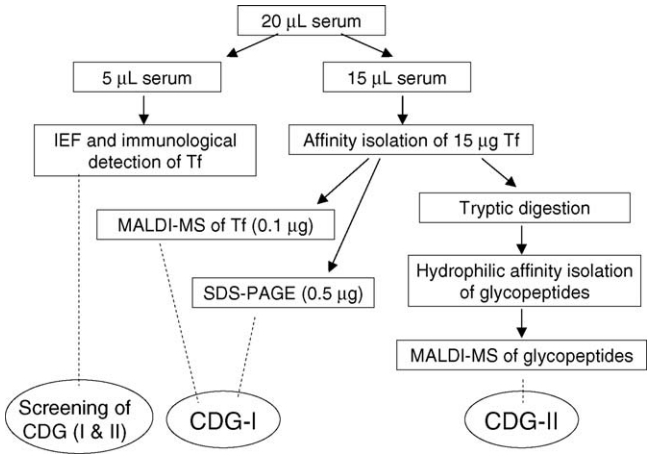

Fig. 7. Flow chart of the CDG screening program employed in Japan.

Another promising method of characterizing the glycan structures associated with CDG-II is glycopeptide analysis, which has become quite feasible with the development of a technique for isolating glycopeptides from the enzymatic digests of glycoproteins [44]. It utilizes hydrogen bonding between the oxygen atoms of glycopeptide glycans and carbohydrate-based gel matrices such as cellulose or Sepharose in the organic phase, allowing enrichment of glycopeptides from microgram amounts of transferrin. When combined with MALDI-MS, it allows the site-specific glycans of glycoproteins to be characterized as shown in Fig. 6. The mass spectrum of the glycopeptides derived from a tryptic digest of transferrin indicates that fucosylation and triantennary branching are much higher in glycopeptides containing the Asn-630 glycosylation site than in those containing Asn-432 (Fig. 6A). Increased fucosylation and decreased branching are reportedly common features of different types of CDG-I [45]. This was clearly shown in the mass spectrum of a CDG-Ix case (Fig. 6B). Interestingly, fucosylation of the glycans at Asn-432 is still minimal, suggesting that an analysis focusing on the Asn-630 glycosylation site would yield clearer results than that of global glycans released from transferrin. Obviously, this method will be effective for detecting

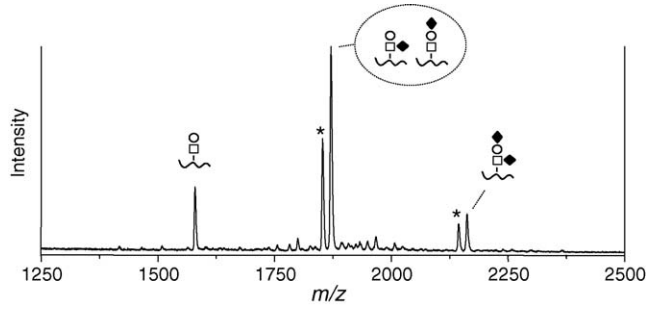

Fig. 8. MALDI mass spectrum of a glycopeptide from apoC-III. ApoC-III was digested with endoproteinase Asp-N and subjected to the method described in the caption of Fig. 6. The protonated glycopeptides bearing *O*-glycans at Thr-74 are observed in the mass spectrum. The ions indicated by asterisks are dehydrated species. Symbols for monosaccharides: *N*-acetylgalactosamine (open squares); galactose (open circles); *N*-acetylneuraminic acid (rectangles). Peptide sequence is DPEVRPTSAVAA (positions 68–79).

the decreased fucosylation of CDG-IIc and the defective glycan structures observed in CDGs-IIa, IId and IIe as well. Glycopeptide analysis has been incorporated into the screening system employed in Japan (Fig. 7).

The glycopeptide analysis is also effective for the glycoproteins bearing *O*-glycans [46]. As described above, electrophoresis of apoC-III, which is a small protein composed of 79 amino acid residues and carries a single core-1 mucin-type *O*-glycan with sialic acid, is useful for subdividing the unclassified CDG-IIx disorders [42]. The mass spectrum of the glycopeptide derived from endoproteinase Asp-N digestion is presented in Fig. 8, and it will be helpful in defining the molecular abnormality of CDG-IIx and for detecting other *O*-glycosylation defects.

8. Concluding remarks

Substantial numbers of CDG-II patients, with a diversity of unknown defects in the N- and occasionally the O-linked glycosylation pathways are now being described. Establishment of a screening method incorporating MS that allows analysis of glycoprotein glycans in a high-throughput and sensitive fashion is anticipated to expand our knowledge of CDG and the related abnormality caused by inherited or acquired disorders as well.

References

[1] M. Cantz, B. Ulrich-Bott, J. Inherit, Metab. Dis. 13 (1990) 523.
[2] J.C. Michalski, A. Klein, Biochim. Biophys. Acta 1455 (1999) 69.
[3] R. Kornfeld, S. Kornfeld, Annu. Rev. Biochem. 54 (1985) 631.
[4] P. Burda, M. Aebi, Biochim. Biophys. Acta 1426 (1999) 239.
[5] A. Herscovics, P. Orlean, FASEB J. 7 (1993) 540.
[6] S. Grünewald, G. Matthijs, J. Jaeken, Pediatr. Res. 52 (2002) 618.
[7] H.H. Freeze, M. Aebi, Curr. Opin. Struct. Biol. 15 (2005) 490.
[8] J. Jaeken, M. Vanderschueren-Lodewyckx, P. Casaer, L. Snoeck, L. Corbeel, E. Eggermont, R. Eeckels, Pediatr. Res. 14 (1980) 179.
[9] J. Jaeken, H.G. van Eijk, C. van der Heul, L. Corbeel, R. Eeckels, E. Eggermont, Clin. Chim. Acta 144 (1984) 245.
[10] H.G. van Eijk, W.L. van Noort, M.L. Dubelaar, C. van der Heul, Clin. Chim. Acta 132 (1983) 167.
[11] J. Jaeken, H. Stibler, B. Hagberg, Acta Paediatr. Scand. Suppl. 375 (1991) 1.
[12] M. Aebi, A. Helenius, B. Schenk, R. Barone, A. Fiumara, E.G. Berger, T. Hennet, T. Imbach, A. Stutz, C. Bjursell, A. Uller, J.G. Wahlstrom, P. Briones, E. Cardo, P. Clayton, B. Winchester, V. Cormier-Dalre, P. de Lonlay, M. Cuer, T. Dupre, N. Seta, T. de Koning, L. Dorland, F. de Loos, L. Kupers, L. Fabritz, M. Hasilik, T. Marquardt, R. Niehues, H. Freeze, S. Grünewald, L. Heykants, J. Jaeken, G. Matthijs, E. Schollen, G. Keir, S. Kjaergaard, M. Schwartz, F. Skovby, A. Klein, P. Roussel, C. Körner, T. Lübke, C. Thiel, K. von Figura, J. Koscielak, D. Krasnewich, L. Lehle, V. Peters, M. Raab, O. Saether, H. Schachter, E. Van Schaftingen, A. Verbert, A. Vilaseca, R. Wevers, K. Yamashita, Glycoconj. J. 16 (1999) 669.
[13] H.H. Freeze, Glycobiology 11 (2001) 129R.
[14] H. Schachter, Cell. Mol. Life Sci. 58 (2001) 1085.
[15] S. Wopereis, S. Grünewald, E. Morava, J.M. Penzien, P. Briones, M.T. Garcia-Silva, P.N. Demacker, K.M. Huijben, R.A. Wevers, Clin. Chem. 49 (2003) 1839.
[16] J. Jaeken, J. Inherit, Metab. Dis. 27 (2004) 423.
[17] C.M. Whitehouse, R.N. Dreyer, M. Yamashita, J.B. Fenn, Anal. Chem. 57 (1985) 675.
[18] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64.
[19] B.N. Green, R.W. Oliver, Biochem. Soc. Trans. 19 (1991) 929.
[20] Y. Wada, J. Tamura, B.D. Musselman, D.B. Kassel, T. Sakurai, T. Matsuo, Rapid Commun. Mass Spectrom. 6 (1992) 9.
[21] Y. Wada, A. Nishikawa, N. Okamoto, K. Inui, H. Tsukamoto, S. Okada, N. Taniguchi, Biochem. Biophys. Res. Commun. 189 (1992) 832.
[22] J.M. Lacey, H.R. Bergen, M.J. Magera, S. Naylor, J.F. O'Brien, Clin. Chem. 47 (2001) 513.
[23] M.V. Gorshkov, L. Paša Tolić, H.R. Udseth, G.A. Anderson, B.M. Huang, J.E. Bruce, D.C. Prior, S.A. Hofstadler, L. Tang, L.Z. Chen, J.A. Willett, A.L. Rockwood, M.S. Sherman, R.D. Smith, J. Am. Soc. Mass Spectrom. 9 (1998) 692.
[24] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[25] M.C. Huberty, J.E. Vath, W. Yu, S.A. Martin, Anal. Chem. 65 (1993) 2791.
[26] J. Zaia, Mass Spectrom. Rev. 23 (2004) 161.
[27] Y. Wada, J. Gu, N. Okamoto, K. Inui, Biol. Mass Spectrom. 23 (1994) 108.
[28] T. Nakanishi, N. Okamoto, K. Tanaka, A. Shimizu, Biol. Mass Spectrom. 23 (1994) 230.
[29] H.H. Freeze, Biochim. Biophys. Acta 1573 (2002) 388.
[30] J. Jaeken, H. Schachter, H. Carchon, P. De Cock, B. Coddeville, G. Spik, Arch. Dis. Child. 71 (1994) 123.
[31] H. Schachter, J. Jaeken, Biochim. Biophys. Acta 1455 (1999) 179.
[32] M. Butler, D. Quelhas, A.J. Critchley, H. Carchon, H.F. Hebestreit, R.G. Hibbert, L. Vilarinho, E. Teles, G. Matthijs, E. Schollen, P. Argibay, D.J. Harvey, R.A. Dwek, J. Jaeken, P.M. Rudd, Glycobiology 13 (2003) 601.
[33] C.M. De Praeter, G.J. Gerwig, E. Bause, L.K. Nuytinck, J.F. Vliegenthart, W. Breuer, J.P. Kamerling, M.F. Espeel, J.J. Martin, A.M. De Paepe, N.W. Chan, G.A. Dacremont, R.N. Van Coster, Am. J. Hum. Genet. 66 (2000) 1744.
[34] T. Lübke, T. Marquardt, K. von Figura, C. Körner, J. Biol. Chem. 274 (1999) 25986.
[35] K. Lühn, M.K. Wild, M. Eckhardt, R. Gerardy-Schahn, D. Vestweber, Nat. Genet. 28 (2001) 69.
[36] T. Lübke, T. Marquardt, A. Etzioni, E. Hartmann, K. von Figura, C. Körner, Nat. Genet. 28 (2001) 73.
[37] T. Marquardt, K. Lühn, G. Srikrishna, H.H. Freeze, E. Harms, D. Vestweber, Blood 94 (1999) 3976.
[38] L. Sturla, F. Fruscione, K. Noda, E. Miyoshi, N. Taniguchi, P. Contini, M. Tonetti, Glycobiology 15 (2005) 924.
[39] B. Hanßke, C. Thiel, T. Lübke, M. Hasilik, S. Höning, V. Peters, P.H. Heidemann, G.F. Hoffmann, E.G. Berger, K. von Figura, C. Körner, J. Clin. Invest. 109 (2002) 725.
[40] X. Wu, R.A. Steet, O. Bohorov, J. Bakker, J. Newell, M. Krieger, L. Spaapen, S. Kornfeld, H.H. Freeze, Nat. Med. 10 (2004) 518.
[41] I. Martinez-Duncker, T. Dupré, V. Piller, F. Piller, J.J. Candelier, C. Trichet, G. Tchernia, R. Oriol, R. Mollicone, Blood 105 (2005) 2671.
[42] S. Wopereis, E. Morava, S. Grünewald, M. Adamowicz, K.M. Huijben, D.J. Lefeber, R.A. Wevers, Glycobiology 15 (2005) 1312.
[43] P.B. Mills, K. Mills, N. Mian, B.G. Winchester, P.T. Clayton, J. Inherit, Metab. Dis. 26 (2003) 119.
[44] Y. Wada, M. Tajiri, S. Yoshida, Anal. Chem. 76 (2004) 6560.
[45] N. Callewaert, E. Schollen, A. Vanhecke, J. Jaeken, G. Matthijs, R. Contreras, Glycobiology 13 (2003) 367.
[46] M. Tajiri, S. Yoshida, Y. Wada, Glycobiology 15 (2005) 1332.