

## Mass spectrometry in the detection and diagnosis of congenital disorders of glycosylation

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Mass spectrometry (MS) of large molecules such as proteins and oligosaccharides has not been employed in clinical practices, while that of small metabolites is widely used for the screening and diagnosis of various congenital diseases. Congenital disorders of glycosylation (CDG) is a newly recognized group of diseases derived from defects in the biosynthetic pathway of protein glycosylation and the patients are never decisively diagnosed unless the glycoprotein molecules are analyzed. We have constructed a diagnostic system where MS of glycoproteins and glycopeptides identifies abnormalities in their glycan moieties. This program is anticipated to reveal the prevalence of CDG and to demonstrate the essential role of MS in the emerging field of medicine, disease glycomics and glycoproteomics.

**Keywords:** glycoproteins, congenital disorders of glycosylation, glycomics, proteome

A typical contribution of mass spectrometry (MS) in the clinical field has been the quantitation of metabolites to diagnose inborn errors of metabolism. In contrast, the MS of disease-related proteins or large biomolecules has been performed on the qualitative basis since our first report on the structural characterization of protein mutations by MS at the International Mass Spectrometry Conference in 1982.<sup>1</sup> The approach was, however, surpassed by the genetic analysis that leapt forward in the late 1980s, at the same time as soft ionization was developed. Ten years later, in 1992, MS recorded a remarkable achievement in the study of the disorders affecting post-translational modification of proteins.<sup>2</sup> The group of diseases is now called congenital disorders of glycosylation (CDG). The classical type of CDG, or CDG-I, results from deficiencies in the early *N*-glycosylation pathway, in which 30–40 genes are involved, for biosynthesis of the precursor lipid-linked oligosaccharide and its transfer to proteins in the endoplasmic reticulum. The CDG-II diseases are caused by defects in the subsequent glycoprotein *N*-glycan processing steps, conducted by more than 50 gene products. To date, 12 and six different types of CDG-I and II, respectively, have been identified.<sup>3</sup>

Approximately half of mammalian proteins are glycosylated and, in the CDG patients, most of the glycoproteins have varying levels of abnormality in their

glycan moiety. Therefore, the clinical features of CDG span a broad spectrum and affect many organs, for example, the cerebellum and the gastrointestinal, hepatic, visual and immune systems. The wide variety of symptoms explains why a group of disorders now called CDG was not recognized until the early 1980s. The patients are never decisively diagnosed unless the glycoprotein molecules are analyzed. The best analyte is transferrin, bearing less heterogeneous oligosaccharides, and the hallmark of the molecular abnormality in CDG-I is a defect of oligosaccharide chains that causes a drastic change in the molecular mass of transferrin by 2200 Da per glycan set (Figure 1). In most clinical laboratories, the detection is performed by isoelectric focusing, in which the loss of oligosaccharide chain, including terminal sialic acids, gives distinct extra bands that are not found in healthy situations. In some laboratories, electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) MS has been used for the screening of CDG-I.<sup>4,5</sup> An advantage of using MS is that it is applicable to the dried serum spotted on a filter paper, making the delivery of specimen easy (our unpublished observation).

In CDG-II, a sugar-unit or branch level of change is a typical phenotype and, thus, requires detailed analysis of the glycan structures for detection or diagnosis. However,

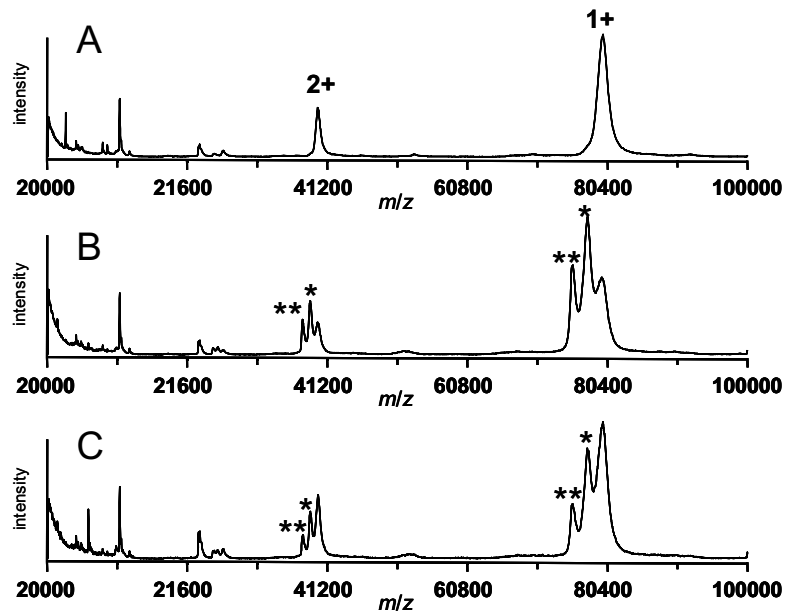


Figure 1. MALDI TOF mass spectra of transferrin. (A) Healthy individual. (B and C) CDG-I cases. The signals marked by asterisks are the isoforms lacking one (\*) or two (\*\*) oligosaccharide chains.

neither ESI-MS or MALDI-MS of intact transferrin can reliably define the precise changes at the sugar unit level, unless the size of analytes is reduced. A solution is to analyze the glycans released from glycoproteins or whole serum; previous reports identified and quantified more than ten structures from serum glycoproteins.<sup>6,7</sup> Another promising method of characterizing the glycan structures associated with CDG-II is glycopeptide analysis, which has become feasible with the development of a technique for isolating glycopeptides from the enzymatic digests of glycoproteins.<sup>8,9</sup> It utilizes hydrogen bonding between the oxygen atoms of glycopeptide glycans and carbohydrate-based gel matrices such as cellulose in the organic phase, allowing enrichment of glycopeptides from microgram amounts of transferrin. When combined with MALDI-MS measured

in a linear time-of-flight mode, it allows the glycan heterogeneity to be characterized, as shown in Figure 2. The protonation of glycopeptides occurs on the peptide backbone. Therefore, the signal intensity for the molecules with the same peptide sequence is well correlated with the relative abundance of the different glycans attached to the peptide, although a small amount of sialic acid is lost by “prompt fragmentation” in the MALDI ion source. In fact, ratios of the species with or without fucosylation in the biantennary oligosaccharide can be measured with fair CVs (coefficient of variations) of 5–17%, suggesting the glycopeptide approach is effective in detecting the reduced fucosylation levels caused by impaired fucose supply in CDG-IIc. Glycopeptide analysis has been incorporated into the CDG screening system employed in Japan (Scheme 1).

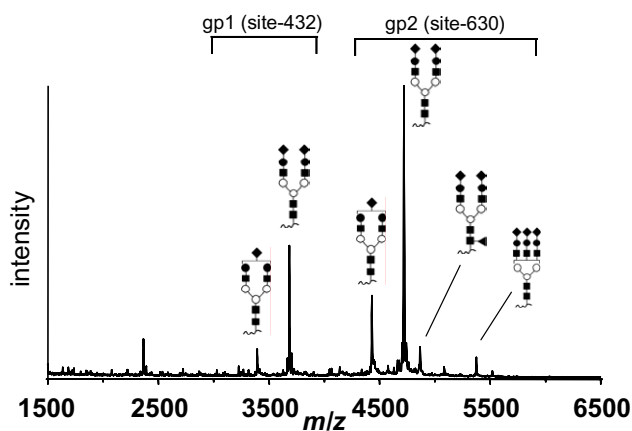
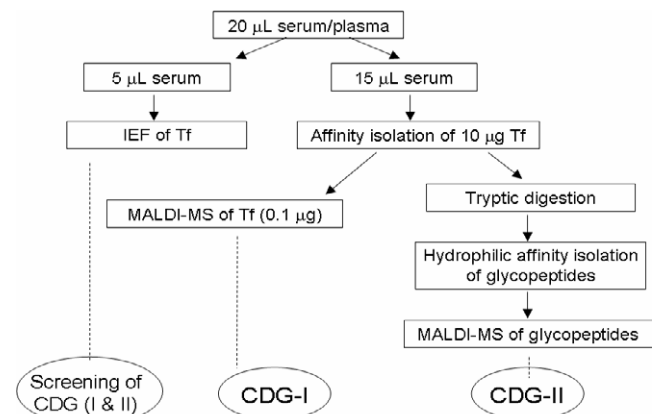


Figure 2. MALDI TOF mass spectrum of transferrin glycopeptides. Site-specific glycoforms at Asn-432 (gp1) and Asn-630 (gp2) are indicated above the individual signals.



Scheme 1. Flow chart of the analysis for CDG molecular diagnosis.

Basically, MS requires internal standards for quantitation, but the reliability, as well as limitations, of the relative quantitation by MS has not been discussed in sufficient detail. With respect to glycomics, the relative but not absolute quantitation is requested and is reliably achieved by MALDI or ESI-MS as demonstrated by a recent multi-institutional study on transferrin and immunoglobulin-G glycans.<sup>10</sup> In conclusion, the quantitation by MS is expected to play a central role in molecular diagnosis in the emerging field of medicine, disease glycomics and glycoproteomics.

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