Addressing the glycan complexity by using mass spectrometry: In the pursuit of decoding glycologic

Yoshimi Kanie and Osamu Kanie*

*Correspondence: kanie@tokai-u.jp

CrossMark

← Click for updates

Institute of Advanced Biosciences, Department of Applied Biochemistry, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan.

Abstract

Biomolecules often contain carbohydrates. Such molecules, namely glycoconjugates, play central roles in cell adhesion, tumor migration, and attachment of pathogens. The conjugation of “natural products” with glycans is not a template-dependent process but is achieved by multiple enzymatic reactions. Because of the nature of the synthetic process, glycans exist as a complex mixture creating a glycoform and thus, the analysis becomes inevitably difficult. Mass spectrometry is one of the most informative, and thus important, tools for the structural analysis of glycans. The obvious advantage of mass spectrometry is the high sensitivity at low femtomole detection levels. A variety of ions can be obtained depending on the adducted cationic species. In order to obtain sequential information, the gas-phase dissociation reaction is used. Among the various techniques, collision-induced dissociation (CID) has been frequently used in the past. Although the technique is proven useful, there are cases that normal CID process is not suitable. This review highlights an emerging technique that focuses on the activation-energy difference between the isomeric glycans, and will complement the current methods. Furthermore, the possible source and the methodology for obtaining useful structural information are discussed.

Keywords: Mass spectrometry, collision-induced dissociation, energy-resolved mass spectrometry, combinatorial library

Introduction

Nucleotides, amino acids, carbohydrates, and lipids are the essential elementary molecular units constituting biological systems. Among these, polymers and/or their conjugates directly drive biological events. Glycans, the oligomers of carbohydrates, linked to lipids and proteins, play pivotal roles in many biological phenomena. Since glycan synthesis is non-template dependent, the structural investigation is inevitable. The fact that the human glycan is composed of nine key monosaccharides (Figure 1) does not give the impression that the structural analysis is difficult. The actual analysis, however, is far from easy, involving problems associated with sample amounts, and more importantly, the structural complexity arising from the presence of isomeric structures.

Mass spectrometry (MS) is advantageous in handling a minute amount of sample and in obtaining structure-related information. Therefore, it is one of the important techniques used in the structural analysis of glycan. Advanced MS equipment is becoming more accessible and is supporting research

on glycomics and glycoproteomics. The position of glycan attachment could be obtained by a sophisticated method using stable isotope labeling during *N*-glycanase digestion, but the glycan structure cannot be elucidated [1,2]. Therefore, the glycan structure must be addressed independently after cleavage from the protein, or as a glycopeptide after proteolytic digestion [3-5].

MS provides the mass-to-charge ratio (m/z value), while further detailed structural information is obtained from MS/MS experiments. Collision-induced dissociation (CID) is the most frequently used technique to obtain fragmentation information [6-16]. The low-energy collision method is most often carried out using triple quadrupole (TQ) and quadrupole ion trap (QIT), which results in fewer fragment ions as a result of the cleavage of relatively labile chemical bonds. Generally, it is very difficult to gather systematic information about the ions obtained from isomeric compounds because of the limited availability of reference compounds, but such ions can at least be distinguished from one another.

Despite the difficulties in obtaining structural information in a systematical manner, it is possible to distinguish the isomeric glycan structures, including the linkage positions, and more importantly, the anomeric configurations [3,17-19]. Diastereomeric ions such as anomeric isomers can be easily distinguished by low-energy CID methods, which will be discussed in a later section. However, in this case, although the linkage position can be determined by analyzing the fragment ions obtained during CID experiments, the anomeric configuration cannot be determined, unless the observer has a reference material of the exact structure, which is not practical.

In order to achieve “inner cleavages” of stable carbon-carbon linkages, high-energy CID is preferred [20-23]. The high-energy collision method provides a large variety of fragments, which is often used in shotgun proteomics research, typically using the time of flight (TOF)-TOF equipment [24,25]. However, the acetal structures present in glycans are prone to dissociation at rather low energy, and the database of the obtained complex MS/MS spectra has not been well developed; this has hampered research on glycoproteomics.

Review

Chemical aspects of glycan structure

The “diversity” of glycans might be described as follows. Among various biomolecules, the glycans contain the largest amount of information in their structure within a given volume. The information is defined by three factors: 1) the combination of the chiral centers present in a constituting unit structure, 2) the functional groups in a unit, and 3) the linkage position between the units. These represent the chiral centers present at C-2–C-5 in individual monosaccharides such as glucose, mannose, and galactose, and the anomeric configurations of the glycosidic (α - and β -) linkage between individual sugar units; the functional groups at each carbon atom, such as hydroxyl, *N*-acetyl, and sulfate; and the glycosidic linkage position of individual sugar units, respectively (See Figure 1). We now consider the amount of information possibly contained in an *n*-mer of carbohydrate units. If we assign one “bit” to a single chiral center, hexoses such as glucose and mannose contain 2^5 bits of information. Thus, an *n*-mer of a

randomly selected hexose contains $2^5 \cdot n$ bits. This theoretical number includes D- and L-sugars as well. Furthermore, we should take into account the structural diversity caused by the linkage position, which adds an extra factor of $4 \cdot (n-1)$ to the theoretical number. Generally, the glycan function is observed in molecules up to pentasaccharides including branched structures, making the calculation of the amount of information more complex (Figure 2) [26]. On the other hand, the number for the peptide is based on the chirality of amino acid (2), the number of different side chains (19) and glycine without chirality, and the number of units (*n*).

The theoretical number gives the possible number of configurations and only some of them were synthesized and used by organisms. Life has evolved by using a small fraction of this capability space, but this can be interpreted that life has infinite possibility for the future taking advantage of the numerous amount of information that can be incorporated into the glycan structures. It is easy to imagine that such complex and diverse glycan structures can be utilized in a variety of interactions among the other biomolecules. Glycans attached to proteins and lipids play pivotal roles in many biological phenomena such as malignant transformation, immunity, differentiation, and infectious diseases [27-31]. Therefore,

glyco-related compounds are the candidates for drug seed and functional materials, which impart interfacing capability to cells and tissues. The glycans themselves are susceptible to endogenous hydrolases, and thus, they might not be suitable as drug candidates. However, replacement of the acetal oxygen atom with sulfur atom or methylene group results in resistance to hydrolyzing enzymes [32,33]. Furthermore, drug discovery based on the identified pharmacophore seems more promising [34,35]. The importance of utilizing combinatorial library is also recognized in the glycan research field, which is still extremely challenging [27-29,31,32,34,36-50].

The glyco-related library will be utilized as seeds to find new therapeutic drug candidates. Despite the difficulties in accessing the glyco-related library, it is considered that such a library is extremely useful for structural investigation because structural information is contained in a logically arranged series of compounds. We will discuss this aspect in Combinatorial library as the primary information source section.

Dissociation methods in mass spectrometry focusing on the energy difference between structural isomers

A series of fragments, product ions, are produced from a precursor ion under the CID conditions. This means that the fragment possesses some structural features of the precursor. Therefore, it is possible to observe the dissociation of the precursor ion or the formation of the product ions.

For example, a singly positively charged linear glycan mainly produces a series of products as a result of the cleavage of relatively labile glycosidic linkages under low-energy CID conditions (Figure 3). The spectrum providing the m/z values does not give any information regarding the presence or absence of structural isomers in the sample and the probable isomer

when it is a pure sample. The structurally isomeric glycans produce very similar spectra consisting of a series of ions with the same m/z values. However, the heights of some of the signals are often different, making it possible to distinguish the isomeric compounds by comparing the spectra (Figure 4) [51,52]. The spectral matching method relies on the fact that the different chemical bonds in isomeric compounds require different energies to dissociate, producing a distinguishable pair of spectra. For determining the exact structure in addition to distinguishing, it is necessary to have a variety of reference compounds. Importantly, the product ions can also be used as references in the spectral matching by performing further CIDs in the MSⁿ stages [53-55].

The key to spectral matching is the availability of the right reference for comparison. However, it is practically not possible to obtain all the reference samples or their information. In such instances, it is of extreme importance to extract as much information as possible using the available glycans.

Let us consider a pair of synthetic disaccharides consisting of an L-fucose (Fuc) and a D-galactose (Gal) (Figure 5). The CID of the sodiated α - and β -fucosyl galactosides of ROH (α - and β -Fuc-(1 $\rightarrow$ 2)- α -Gal-OR) results in the preferential formation of Y-ions as product ions (see Figure 3 for assignment). The Y-ion, [Gal-OR+Na]⁺ in this case, is proven to be pure enough to be used as a reference for comparing the other ions produced from different precursor ions (see the following section) [56]. Therefore, this type of ion species can be suitably used as a reference in spectral matching. From such information, the isomeric structure is elucidated. However, dissociation of Fuc units in both isomeric compounds is the sole event in the CID experiments, which makes distinguishing spectra impossible. Fucose belongs to the family of deoxy sugars. Absence of electron withdrawing substituent group nearby the acetal position stabilizes the oxocarbenium intermediate, and making a glycosidic linkage of such sugar more labile. Therefore, cautions have to be paid when analyzing the structure of glycans containing deoxy sugars. Another important carbohydrate is sialic acid, which is also a deoxy sugar. This particular monosaccharide is deeply involved in a number of biological events; therefore, its structural elucidation is important [57-59]. Unlike the other deoxy sugars, sialic acid has a unique structure with nine carbon atoms, a carboxyl group at the C-1 position, and a carbonyl group at C-2 forming a glycosidic linkage. (Figure 1) Although this particular linkage is as labile as those of the other deoxy sugars, it becomes as stable as those of the other ordinary monosaccharides after esterification [60,61] or amidation [62,63] of a carboxyl group. Another important feature of sialic acid is that if linked to 3-position of adjacent galactose could form lactone structure, which can be used to distinguish the positional isomers of this particular monosaccharide [64].

Suitable ion species for the structural analysis

The most important issue in the analysis of glycans, although

not often discussed, is whether the reference data being used are reliable. The data would be unreliable if obtained from the CID of impure ions. A pure reference compound is easily obtained by careful purification using techniques such as HPLC, and its structure can be determined by NMR. Here, it is difficult to decide if the spectral data obtained from a product ion under the CID conditions are reliable. Authenticity of data is imperative in accurate spectral matching, although this aspect has seldom been the topic of discussion. The C-ion species produced from a glycoside has a hemiacetal structure that might anomerize under CID conditions, judging by an analogy of behavior of hemiacetals in aqueous medium. To ascertain this issue, a new concept, stage-discriminated correlation of energy-resolved mass spectrometry, was introduced [56]. After a statistical evaluation of the MS/MS data obtained from the above series of ions, the A- and B-ions were found to be mixtures of various structural isomers, in principle. On the other hand, the C- and Y-ions were suitable precursors for further MS/MS experiments. Interestingly, the hemiacetal structure (C-ion) does not undergo anomerization under low-energy CID conditions [65,66]. The C- and Y-ion species are often found in the MS/MS spectrum; hence, they are considered to be suitable reference ions. Furthermore, the anomeric configuration of a glycosidic linkage can be determined by comparing the MS/MS data of C-ion as a precursor. Taking advantage of synthetic chemistry, it was reported that the carbohydrate C-ions could be generated stereo-specifically in gas phase, from the corresponding 4-aminobutylglycosides [67,68]. This particular glycoside with an amino group could also be utilized in preparing the required conjugates for biological investigations [69].

New parameter for use in structural analysis

Is there any structural information other than the m/z values and relative signal heights useful in glycan analysis? Relatively weak chemical bonds tend to dissociate considerably faster compared to others. For example, glycosidic linkages are labile; hence, we often observe product ions associated to their cleavage. Among them, the glycosidic linkages of fucose (Fuc) and sialic acid (Sia) are more labile and often dissociate prior to the other glycosidic linkages as discussed above. In the dissociation of the individual ions, α Fuc-(1 $\rightarrow$ 2)- α Gal-OR and β Fuc-(1 $\rightarrow$ 2)- α Gal-OR, fucosyl cleavage is the sole event (Figure 5). In such a case, the spectral matching method cannot be applied. Hence, a new parameter is required to address the cases involving of an ion containing labile chemical bond. Usually, CID is carried out at a fixed collision energy. Varying the energy leads to changes the signal heights of the product ions. A method that collects a series of spectra over a range of collision-energies is the energy-resolved mass spectrometry (ERMS), which provides information regarding the activation energy of a gas-phase dissociation reaction [70]. The dissociation of Fuc residues from a pair of precursor ions in the above case can be identified by ERMS (Figure 6). Since the method is

very sensitive to the structural differences, it has been used to distinguish structurally closely related glycans [71-75].

ERMS is often carried out on the QIT MS apparatus, but is not limited to a particular equipment. In the QIT system, the series of product ions produced by MS/MS are not further subjected to collisional excitation because the product ions are just contained inside the Q-field while the precursor ion only is activated. As a result, the product ions remain without any further dissociation, which is an advantage over the ERMS carried out in the TQ system. However, the TQ system is also used to analyze the energy dependence of a precursor ion under the CID conditions. In such a case, the product ions are subjected to further collisional excitation since all the ions drift through the Q-field, which results in "peak-shaped" growth-and-break down curves unlike the "growth curves" usually observed in the QIT system. Based on the observation of individual product ions, it was reported that structural discrimination was possible in the LC-TQ MS system [76]. Further increase in the CID energy results in the breaking down of the backbone peptide linkage and enables the peptide sequence analysis in an ERMS experiment cycle [77]. Therefore, ERMS is a quite useful technique in the glycoproteomics research as well. The advantages of ERMS carried out on the TQ-MS system are as follows: 1) the isomeric glycan structures can be distinguished, 2) the site of glycan attachment is identified, and 3) the sequence of the peptide portion is obtained at the same time.

One disadvantage of ERMS on TQ-system could be the limitation of the stage of MS/MS to be MS^2 due to its specification, although it is not limited, in theory, in QIT system. However, it was shown that mathematical treatment of the ERMS data obtained in the TQ system enabled the analysis of the relationships between the product ions. It is considered that the obtained data are equivalent to those obtained by the MS^n stage in the QIT system [78].

Other methods useful in glycan analysis

So far, we highlighted the collision-induced dissociation

techniques used to obtain useful information in the glycan analysis. Other important techniques are available for glycan-involving analysis, a few of which are briefly summarized in this section.

Electron-transfer dissociation

Electron-transfer dissociation (ETD) is a promising fragmentation technique because a radical anion transfers an electron to a peptide precursor cation, causing radical-based fragmentation at the *N*-C α bond on a peptide. This process is orthogonal to the vibrational excitation process in the CID-based fragmentation. The availability of suitable analytical equipment is also a critical factor for the successful application of ETD. An instrument equipped with both ETD and CID units is available and is advantageous for the analysis of the glycopeptides obtained after proteolytic digestion [7,8,13,14,79,80].

Infrared photodissociation

The mechanism of CID is based on the vibrational activation of ions of biomolecules, triggered by repeated collisions with inert gas molecules and the consequent achievement of the transition state to form various product ions. A similar activation of ions might be possible by irradiating a resonant infrared (IR) beam on the trapped ion cloud. Although the observed dissociation events have not been explained, it is possible to distinguish the isomeric oligosaccharides by this method [81-84].

Ion-mobility mass spectrometry

Ion-mobility mass spectrometry (IMS/MS) has garnered much attention in the isomeric glycan analysis, in conjunction with MS/MS techniques. This technique is executed in a hybrid system with an ion-mobility separation functionality and the MS system. The first unit in this mass spectrometer is the IMS unit, where the ions travel through an electric field filled with inert gas. The unit does not provide sufficient energy for the ion dissociation but affects the traveling speed of the ion depending on the ion cross section. The ions of isomeric glycans differ in their molecular structures, and in turn, their cross sections. Therefore, the ions show different mobilities in an electric field. The technique can be advantageous in analyzing the isomeric glycans and their mixtures [85,86].

Combinatorial library as the primary information source

Glycans are composed of multiple carbohydrate units, and an aspect of diversity of that family of molecules can be described by a combinatorial number calculated for an oligomer as discussed in Chemical aspect of glycan structure section. Glycans are synthesized by the sequential reactions of enzymes; hence, only some parts of the combinatorial numbers can exist. This fact indicates that the number of reference compounds is limited and forces us to investigate a glycan structure using limited information. This might seem sufficient when one focus on molecules obtained from a particular organism, but

it cannot be applied to the analysis of molecules from a wide range of natural sources. To address this issue, two methodologies can be considered: 1) establishing a method for the determination of the absolute configuration of a chiral center or 2) fulfilling all the possible structural space for determining the absolute glycan structure.

Further research is needed before we can establish a method for the absolute configuration, but we might be able to adopt the second method by utilizing a combinatorial oligosaccharide library. As already discussed, a great deal of effort has been devoted to generate a combinatorial library, although the target is to find drug seeds. Such a library is considered a "seed" of the glycan structural information as well. A pioneering study in this direction used a series of synthetic trisaccharides and discussed the energy differences among them toward glycosyl dissociation [87]. The expansion of obtainable information not only generates more reference data, but also enables abstracting structural information even the library is not complete. This might be possible because a combinatorial library consists of a logical series of molecules. Owing to the logical arrangement of structures, some missing data belonging to the absent compounds might be estimated. Several approaches have been undertaken to estimate the missing data.

One of the approaches focusing on the glycosyl dissociation using a small combinatorial library analyzed the effect of anomeric configurations on the dissociation. All the possible anomeric combinations of the sequence, Fuc-Gal-Glc-OR, were analyzed using ERMS, showing that anomeric configurations could be resolved [73]. Furthermore, investigation of all the combinations of anomers and linkage isomers of Fuc-Gal-OR has been reported, with the activation energies for all the 16 isomeric disaccharides being distinguishable, reflecting their structural features [71].

Here, an important finding is that there exists a correlation between the structure and the information obtained from the MS/MS experiments, which indicates that the assumption that some missing data might be estimated using a well-designed logical set of library compounds is reasonable.

Conclusion

MS has contributed to the investigation of glycans with diverse structures. In most cases, the amount of the diverse glycan related compounds isolated from natural sources is limited. This poses difficulties in the structural analysis. MS is advantageous in such instances because of its high sensitivity. Techniques capable of isolating such small amounts of samples are required, in addition to new preparation methods. Although not included in this review, the recent advances in deriving glycan samples from glycoproteins and glycolipids and their use in the biological investigations are also worth mentioning [88,89].

Furthermore, vast amounts of information cannot be handled without informatics [90-94]. In order to obtain the

product ions, a certain form of energy is required for exciting the precursor ion. Research on glycan analysis is certainly gaining momentum, suggesting that more products are being formed. With some impetus, structural analysis of glycans may reveal more important products.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	YK	OK
Research concept and design	--	--
Collection and/or assembly of data	✓	✓
Data analysis and interpretation	--	--
Writing the article	--	✓
Critical revision of the article	✓	✓
Final approval of article	--	✓
Statistical analysis	--	--

Acknowledgement

This work was supported by JSPS KAKENHI 15K14399 for Challenging Exploratory Research.

Publication history

Editor: Mariusz Skwarczynski, University of Queensland, Australia.
Received: 15-Jan-2017 Final Revised: 30-Mar-2017
Accepted: 13-Apr-2017 Published: 10-May-2017

References

- Kaji H, Saito H, Yamauchi Y, Shinkawa T, Taoka M, Hirabayashi J, Kasai K, Takahashi N and Isobe T. **Lectin affinity capture, isotope-coded tagging and mass spectrometry to identify N-linked glycoproteins.** *Nat Biotechnol.* 2003; **21**:667-72. | [Article](#) | [PubMed](#)
- Kaji H, Yamauchi Y, Takahashi N and Isobe T. **Mass spectrometric identification of N-linked glycopeptides using lectin-mediated affinity capture and glycosylation site-specific stable isotope tagging.** *Nat Protoc.* 2006; **1**:3019-27. | [Article](#) | [PubMed](#)
- Dell A and Morris HR. **Glycoprotein structure determination by mass spectrometry.** *Science.* 2001; **291**:2351-6. | [Article](#) | [PubMed](#)
- Cancilla MT, Penn SG, Carroll JA and Lebrilla CB. **Coordination of alkali metals to oligosaccharides dictates fragmentation behavior in matrix assisted laser desorption/ionization/fourier transform mass spectrometry.** *J Am Chem Soc.* 1996; **118**:6736-45. | [Article](#)
- Sheeley DM and Reinhold VN. **Structural characterization of carbohydrate sequence, linkage, and branching in a quadrupole ion trap mass spectrometer: neutral oligosaccharides and N-linked glycans.** *Anal Chem.* 1998; **70**:3053-9. | [PubMed](#)
- Domon B and Costello CE. **A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates.** *Glycoconj J.* 1988; **5**:397-409. | [Article](#)
- Hogan JM, Pitteri SJ, Chrisman PA and McLuckey SA. **Complementary structural information from a tryptic N-linked glycopeptide via electron transfer ion/ion reactions and collision-induced dissociation.** *J Proteome Res.* 2005; **4**:628-32. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Wu SL, Huhmer AF, Hao Z and Karger BL. **On-line LC-MS approach combining collision-induced dissociation (CID), electron-transfer dissociation (ETD), and CID of an isolated charge-reduced species for the trace-level characterization of proteins with post-translational modifications.** *J Proteome Res.* 2007; **6**:4230-44. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Zhang Z and Shah B. **Prediction of collision-induced dissociation spectra of common N-glycopeptides for glycoform identification.** *Anal Chem.* 2010; **82**:10194-202. | [Article](#) | [PubMed](#)
- North SJ, Hitchen PG, Haslam SM and Dell A. **Mass spectrometry in the analysis of N-linked and O-linked glycans.** *Curr Opin Struct Biol.* 2009; **19**:498-506. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Demelbauer UM, Zehl M, Plematl A, Allmaier G and Rizzi A. **Determination of glycopeptide structures by multistage mass spectrometry with low-energy collision-induced dissociation: comparison of electrospray ionization quadrupole ion trap and matrix-assisted laser desorption/ionization quadrupole ion trap reflectron time-of-flight approaches.** *Rapid Commun Mass Spectrom.* 2004; **18**:1575-82. | [Article](#) | [PubMed](#)
- Zaia J. **Mass spectrometry and glycomics.** *OMICS.* 2010; **14**:401-18. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Molina H, Matthiesen R, Kandasamy K and Pandey A. **Comprehensive comparison of collision induced dissociation and electron transfer dissociation.** *Anal Chem.* 2008; **80**:4825-35. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Wang D, Hincapie M, Rejtar T and Karger BL. **Ultrasensitive characterization of site-specific glycosylation of affinity-purified haptoglobin from lung cancer patient plasma using 10 μm i.d. porous layer open tubular liquid chromatography-linear ion trap collision-induced dissociation/electron transfer dissociation mass spectrometry.** *Anal Chem.* 2011; **83**:2029-37. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Zaia J. **Mass spectrometry and the emerging field of glycomics.** *Chem Biol.* 2008; **15**:881-92. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- An HJ, Froehlich JW and Lebrilla CB. **Determination of glycosylation sites and site-specific heterogeneity in glycoproteins.** *Curr Opin Chem Biol.* 2009; **13**:421-6. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- An HJ and Lebrilla CB. **Structure elucidation of native N- and O-linked glycans by tandem mass spectrometry (tutorial).** *Mass Spectrom Rev.* 2011; **30**:560-78. | [Article](#) | [PubMed](#)
- Bursey MM and Nystrom JA. **Survey of reaction types in low-energy collisional activation of protonated methyl alkyl ketones.** *Anal Chem Acta.* 1984; **159**:265-74. | [Article](#)
- Reinhold VN, Reinhold BB and Costello CE. **Carbohydrate molecular weight profiling, sequence, linkage, and branching data: ES-MS and CID.** *Anal Chem.* 1995; **67**:1772-84. | [PubMed](#)
- Tang H, Mechref Y and Novotny MV. **Automated interpretation of MS/MS spectra of oligosaccharides.** *Bioinformatics.* 2005; **21** Suppl 1:i431-9. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Mechref Y, Novotny MV and Krishnan C. **Structural characterization of oligosaccharides using MALDI-TOF/TOF tandem mass spectrometry.** *Anal Chem.* 2003; **75**:4895-903. | [PubMed](#)
- Spina E, Sturiale L, Romeo D, Impallomeni G, Garozzo D, Waidelich D and Glueckmann M. **New fragmentation mechanisms in matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry of carbohydrates.** *Rapid Commun Mass Spectrom.* 2004; **18**:392-8. | [Article](#) | [PubMed](#)
- Kuster B, Naven TJ and Harvey DJ. **Effect of the reducing-terminal substituents on the high energy collision-induced dissociation matrix-assisted laser desorption/ionization mass spectra of oligosaccharides.** *Rapid Commun Mass Spectrom.* 1996; **10**:1645-51. | [Article](#) | [PubMed](#)
- Pan S, Chen R, Aebersold R and Brentnall TA. **Mass spectrometry based glycoproteomics--from a proteomics perspective.** *Mol Cell Proteomics.* 2011; **10**:R110 003251. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Gillece-Castro BL and Burlingame AL. **Oligosaccharide characterization with high-energy collision-induced dissociation mass spectrometry.** *Methods Enzymol.* 1990; **193**:689-712. | [Article](#) | [PubMed](#)
- Laine RA. **A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05 x 10¹² structures for a reducing hexasaccharide: the Isomer Barrier to development of single-method saccharide sequencing or synthesis systems.** *Glycobiology.* 1994; **4**:759-67. | [PubMed](#)
- Dennis JW, Laferte S, Waghorne C, Breitman ML and Kerbel RS. **Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with**

- metastasis. *Science*. 1987; **236**:582-5. | [Article](#) | [PubMed](#)
28. Ladisch S, Becker H and Ulsh L. Immunosuppression by human gangliosides: I. Relationship of carbohydrate structure to the inhibition of T cell responses. *Biochim Biophys Acta*. 1992; **1125**:180-8. | [Article](#) | [PubMed](#)
29. Karlsson KA. Animal glycosphingolipids as membrane attachment sites for bacteria. *Annu Rev Biochem*. 1989; **58**:309-50. | [Article](#) | [PubMed](#)
30. Suzuki Y. Gangliosides as influenza virus receptors. Variation of influenza viruses and their recognition of the receptor sialo-sugar chains. *Prog Lipid Res*. 1994; **33**:429-57. | [Article](#) | [PubMed](#)
31. Ragupathi G, Slovin S, Adluri S, Sames D, Kiml-J, Kim HM, Spassova M, Bornmann WG, Lloyd KO, Scher HI, Livingston PO and Danishefsky SJ. A fully synthetic Globo H carbohydrate vaccine induces a focused humoral response in prostate cancer patients: A proof of principle. *Angew Chem Int Ed*. 1999; **38**:563-6. | [Article](#)
32. Hummel G and Hindsgaul O. Solid-phase synthesis of thio-oligosaccharides. *Angew Chem Int Ed*. 1999; **38**:1782-4. | [Article](#)
33. Baytas SN, Wang Q, Karst NA, Dordick JS and Linhardt RJ. Solid-phase chemoenzymatic synthesis of C-sialosides. *J Org Chem*. 2004; **69**:6900-3. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
34. Kogan TP, Dupre B, Keller KM, Scott IL, Bui H, Market RV, Beck PJ, Voytus JA, Revelle BM and Scott D. Rational design and synthesis of small molecule, non-oligosaccharide selectin inhibitors: (alpha-D-mannopyranosyloxy)biphenyl-substituted carboxylic acids. *J Med Chem*. 1995; **38**:4976-84. | [PubMed](#)
35. Ernst B and Magnani JL. From carbohydrate leads to glycomimetic drugs. *Nat Rev Drug Discov*. 2009; **8**:661-77. | [Article](#) | [PubMed](#)
36. Ding Y, Labbe J, Kanie O and Hindsgaul O. Towards oligosaccharide libraries: a study of the random galactosylation of unprotected N-acetylglucosamine. *Bioorg Med Chem*. 1996; **4**:683-92. | [Article](#) | [PubMed](#)
37. Ye XS and Wong CH. Anomeric reactivity-based one-pot oligosaccharide synthesis: a rapid route to oligosaccharide libraries. *J Org Chem*. 2000; **65**:2410-31. | [Article](#) | [PubMed](#)
38. Takahashi T, Adachi M, Matsuda A and Doi T. Combinatorial synthesis of trisaccharides via solution-phase one-pot glycosylation. *Tetrahedron Lett*. 2000; **41**:2599-603. | [Article](#)
39. Kanie O, Ohtsuka I, Ako T, Daikoku S, Kanie Y and Kato R. Orthogonal glycosylation reactions on solid phase and synthesis of a library consisting of a complete set of fucosyl galactose isomers. *Angew Chem Int Ed Engl*. 2006; **45**:3851-4. | [Article](#) | [PubMed](#)
40. Ohtsuka I, Ako T, Kato R, Daikoku S, Koroghi S, Kanemitsu T and Kanie O. Synthesis of a library of fucopyranosyl-galactopyranosides consisting of a complete set of anomeric configurations and linkage positions. *Carbohydr Res*. 2006; **341**:1476-87. | [Article](#) | [PubMed](#)
41. Ako T, Daikoku S, Ohtsuka I, Kato R and Kanie O. A method of orthogonal oligosaccharide synthesis leading to a combinatorial library based on stationary solid-phase reaction. *Chem Asian J*. 2006; **1**:798-813. | [Article](#) | [PubMed](#)
42. Shintate K, Kitaoka M, Kim YK and Hayashi K. Enzymatic synthesis of a library of beta-(1→4) hetero- D-glucose and D-xylose-based oligosaccharides employing celloextrin phosphorylase. *Carbohydr Res*. 2003; **338**:1981-90. | [Article](#) | [PubMed](#)
43. Yamago S, Yamada T, Ito H, Hara O, Mino Y and Yoshida J. Combinatorial synthesis of an oligosaccharide library by using beta-bromoglycoside-mediated iterative glycosylation of selenoglycosides: rapid expansion of molecular diversity with simple building blocks. *Chemistry*. 2005; **11**:6159-74. | [Article](#) | [PubMed](#)
44. Liang R, Yan L, Loebach J, Ge M, Uozumi Y, Sekanina K, Horan N, Gildersleeve J, Thompson C, Smith A, Biswas K, Still WC and Kahne D. Parallel synthesis and screening of a solid phase carbohydrate library. *Science*. 1996; **274**:1520-2. | [Article](#) | [PubMed](#)
45. Liu L, Ping Li C, Cochran S and Ferro V. Application of the four-component Ugi condensation for the preparation of sulfated glycoconjugate libraries. *Bioorg Med Chem Lett*. 2004; **14**:2221-6. | [Article](#) | [PubMed](#)
46. Ng ES, Yang F, Kameyama A, Palcic MM, Hindsgaul O and Schriemer DC. High-throughput screening for enzyme inhibitors using frontal affinity chromatography with liquid chromatography and mass spectrometry. *Anal Chem*. 2005; **77**:6125-33. | [Article](#) | [PubMed](#)
47. Halkes KM, St Hilaire PM, Crocker PR and Meldal M. Glycopeptides as oligosaccharide mimics: high affinity sialopeptide ligands for sialoadhesin from combinatorial libraries. *J Comb Chem*. 2003; **5**:18-27. | [Article](#) | [PubMed](#)
48. Ying L, Liu R, Zhang J, Lam K, Lebrilla CB and Gervay-Hague J. A topologically segregated one-bead-one-compound combinatorial glycopeptide library for identification of lectin ligands. *J Comb Chem*. 2005; **7**:372-84. | [Article](#) | [PubMed](#)
49. Fumoto M, Hinou H, Ohta T, Ito T, Yamada K, Takimoto A, Kondo H, Shimizu H, Inazu T, Nakahara Y and Nishimura S. Combinatorial synthesis of MUC1 glycopeptides: polymer blotting facilitates chemical and enzymatic synthesis of highly complicated mucin glycopeptides. *J Am Chem Soc*. 2005; **127**:11804-18. | [Article](#) | [PubMed](#)
50. Hotchkiss T, Kramer HB, Doores KJ, Gambin DP, Oldham NJ and Davis BG. Ligand amplification in a dynamic combinatorial glycopeptides library. *Chem Commun*. 2005; **14**:4264-6. | [Article](#)
51. Kameyama A, Kikuchi N, Nakaya S, Ito H, Sato T, Shikanai T, Takahashi Y, Takahashi K and Narimatsu H. A strategy for identification of oligosaccharide structures using observational multistage mass spectral library. *Anal Chem*. 2005; **77**:4719-25. | [Article](#) | [PubMed](#)
52. Ito H, Takegawa Y, Deguchi K, Nagai S, Nakagawa H, Shinohara Y and Nishimura S. Direct structural assignment of neutral and sialylated N-glycans of glycopeptides using collision-induced dissociation MSn spectral matching. *Rapid Commun Mass Spectrom*. 2006; **20**:3557-65. | [Article](#) | [PubMed](#)
53. Garozzo D, Giorgio M and Impallomeni G. Determination of linkage position and identification of the reducing end in linear oligosaccharides by negative ion fast atom bombardment mass spectrometry. *Anal Chem*. 1990; **62**:279-86. | [Article](#)
54. Pfenninger A, Karas M, Finke B and Stahl B. Structural analysis of underivatized neutral human milk oligosaccharides in the negative ion mode by nano-electrospray MSⁿ (part 2: application to isomeric mixtures). *J Am Soc Mass Spectrom*. 2002; **13**:1341-8. | [Article](#) | [PubMed](#)
55. Yamagaki T, Suzuki H and Tachibana K. In-source and postsource decay in negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of neutral oligosaccharides. *Anal Chem*. 2005; **77**:1701-7. | [Article](#) | [PubMed](#)
56. Daikoku S, Kurimoto A, Mutsuga S, Ako T, Kanemitsu T, Shioiri Y, Ohtake A, Kato R, Saotome C, Ohtsuka I, Koroghi S, Sarkar SK, Tobe A, Adachi S, Suzuki K and Kanie O. Ion-trap mass spectrometry unveils the presence of isomeric oligosaccharides in an analyte: stage-discriminated correlation of energy-resolved mass spectrometry. *Carbohydr Res*. 2009; **344**:384-94. | [Article](#) | [PubMed](#)
57. Varki A. Sialic acids as ligands in recognition phenomena. *FASEB J*. 1997; **11**:248-55. | [Article](#) | [PubMed](#)
58. Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S and Varki A. Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. *Nat Biotechnol*. 2010; **28**:863-7. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
59. Schauer R. Sialic acids as regulators of molecular and cellular interactions. *Curr Opin Struct Biol*. 2009; **19**:507-14. | [Article](#) | [PubMed](#)
60. Handa S and Nakamura K. Modification of sialic acid carboxyl group of ganglioside. *J Biochem*. 1984; **95**:1323-9. | [Article](#) | [PubMed](#)
61. Miura Y, Shinohara Y, Furukawa J, Nagahori N and Nishimura S. Rapid and simple solid-phase esterification of sialic acid residues for quantitative glycomics by mass spectrometry. *Chemistry*. 2007; **13**:4797-804. | [Article](#) | [PubMed](#)
62. Sekiya S, Wada Y and Tanaka K. Derivatization for stabilizing sialic acids in MALDI-MS. *Anal Chem*. 2005; **77**:4962-8. | [Article](#) | [PubMed](#)
63. Liu X, Qiu H, Lee RK, Chen W and Li J. Methylamidation for sialoglycomics by MALDI-MS: a facile derivatization strategy for both α2,3- and α2,6-linked sialic acids. *Anal Chem*. 2010; **82**:8300-6. | [Article](#) | [PubMed](#)

64. Holst S, Heijs B, de Haan N, van Zeijl RJ, Briaire-de Bruijn IH, van Pelt GW, Mehta AS, Angel PM, Mesker WE, Tollenaar RA, Drake RR, Bovee JV, McDonnell LA and Wührer M. **Linkage-Specific in Situ Sialic Acid Derivatization for N-Glycan Mass Spectrometry Imaging of Formalin-Fixed Paraffin-Embedded Tissues.** *Anal Chem.* 2016; **88**:5904-13. | [Article](#) | [PubMed](#)
65. Kanie O, Kurimoto A, Kanie Y, Daikoku S, Ohtake A and Suzuki K. **Analysis of behavior of sodiated sugar hemiacetals under low-energy collision-induced dissociation conditions and application to investigating mutarotation and mechanism of a glycosidase.** *Proc Jpn Acad Ser B Phys Biol Sci.* 2009; **85**:204-15. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
66. Shioiri Y, Suzuki K, Daikoku S, Kurimoto A, Ito Y and Kanie O. **Stereospecific generation and analysis of α - and β -hemiacetals of monosaccharides in gas phase.** *Carbohydr Res.* 2013; **382**:43-51. | [Article](#) | [PubMed](#)
67. Shioiri Y, Kurimoto A, Ako T, Daikoku S, Ohtake A, Ishida H, Kiso M, Suzuki K and Kanie O. **Energy-resolved structural details obtained from gangliosides.** *Anal Chem.* 2009; **81**:139-45. | [Article](#) | [PubMed](#)
68. Shioiri Y, Suzuki K and Kanie O. **Mechanism of a gas-phase dissociation reaction of 4-aminobutyl glycosides under CID MS/MS conditions.** *J Mass Spectrom.* 2008; **43**:1132-9. | [Article](#) | [PubMed](#)
69. Suzuki K, Tobe A, Adachi S, Daikoku S, Hasegawa Y, Shioiri Y, Kobayashi M and Kanie O. **N-Hexyl-4-aminobutyl glycosides for investigating structures and biological functions of carbohydrates.** *Org Biomol Chem.* 2009; **7**:4726-33. | [Article](#) | [PubMed](#)
70. Kurimoto A, Daikoku S, Mutsuga S and Kanie O. **Analysis of energy-resolved mass spectra at MSn in a pursuit to characterize structural isomers of oligosaccharides.** *Anal Chem.* 2006; **78**:3461-6. | [Article](#) | [PubMed](#)
71. Daikoku S, Ako T, Kato R, Ohtsuka I and Kanie O. **Discrimination of 16 structural isomers of fucosyl galactoside based on energy-resolved mass spectrometry.** *J Am Soc Mass Spectrom.* 2007; **18**:1873-9. | [Article](#) | [PubMed](#)
72. Kurimoto A and Kanie O. **Distinguishing isomeric pyridylaminated high-mannose (Man7) oligosaccharides based on energy-resolved mass spectra.** *Rapid Commun Mass Spectrom.* 2007; **21**:2770-8. | [Article](#) | [PubMed](#)
73. Daikoku S, Ako T, Kurimoto A and Kanie O. **Anomeric information obtained from a series of synthetic trisaccharides using energy resolved mass spectra.** *J Mass Spectrom.* 2007; **42**:714-23. | [Article](#) | [PubMed](#)
74. Shioiri Y, Kurimoto A, Ako T, Daikoku S, Ohtake A, Ishida H, Kiso M, Suzuki K and Kanie O. **Energy-resolved structural details obtained from gangliosides.** *Anal Chem.* 2009; **81**:139-45. | [Article](#) | [PubMed](#)
75. Daikoku S, Widmalm G and Kanie O. **Analysis of a series of isomeric oligosaccharides by energy-resolved mass spectrometry: a challenge on homobranch trisaccharides.** *Rapid Commun Mass Spectrom.* 2009; **23**:3713-9. | [Article](#) | [PubMed](#)
76. Toyama A, Nakagawa H, Matsuda K, Sato TA, Nakamura Y and Ueda K. **Quantitative structural characterization of local N-glycan microheterogeneity in therapeutic antibodies by energy-resolved oxonium ion monitoring.** *Anal Chem.* 2012; **84**:9655-62. | [Article](#) | [PubMed](#)
77. Kolli V and Dodds ED. **Energy-resolved collision-induced dissociation pathways of model N-linked glycopeptides: implications for capturing glycan connectivity and peptide sequence in a single experiment.** *Analyst.* 2014; **139**:2144-53. | [Article](#) | [PubMed](#)
78. Kanie O, Kanie Y, Daikoku S, Shioiri Y, Kurimoto A, Mutsuga S, Goto S, Ito Y and Suzuki K. **Multi-stage mass spectrometric information obtained by deconvolution of energy-resolved spectra acquired by triple-quadrupole mass spectrometry.** *Rapid Commun Mass Spectrom.* 2011; **25**:1617-24. | [Article](#) | [PubMed](#)
79. Coon JJ, Syka JEP, Schwartz JC, Shabanowitz J and Kelleher NL. **Anion dependence in the partitioning between proton and electron transfer in ion/ion reactions.** *Int J Mass Spectrom.* 2004; **236**:33-42. | [Article](#)
80. Alley WR, Jr., Mechref Y and Novotny MV. **Characterization of glycopeptides by combining collision-induced dissociation and electron-transfer dissociation mass spectrometry data.** *Rapid Commun Mass Spectrom.* 2009; **23**:161-70. | [Article](#) | [PubMed](#)
81. Xie Y and Lebrilla CB. **Infrared multiphoton dissociation of alkali metal-coordinated oligosaccharides.** *Anal Chem.* 2003; **75**:1590-8. | [PubMed](#)
82. Polfer NC, Valle JJ, Moore DT, Oomens J, Eyler JR and Bendiak B. **Differentiation of isomers by wavelength-tunable infrared multiple-photon dissociation-mass spectrometry: application to glucose-containing disaccharides.** *Anal Chem.* 2006; **78**:670-9. | [Article](#) | [PubMed](#)
83. Stefan SE and Eyler JR. **Differentiation of glucose-containing disaccharides by infrared multiple photon dissociation with a tunable CO₂ laser and Fourier transform ion cyclotron resonance mass spectrometry.** *Int J Mass Spectrom.* 2010; **297**:96-101. | [Article](#)
84. Tan Y and Polfer NC. **Linkage and anomeric differentiation in trisaccharides by sequential fragmentation and variable-wavelength infrared photodissociation.** *J Am Soc Mass Spectrom.* 2015; **26**:359-68. | [Article](#) | [PubMed](#)
85. Gabryelski W and Froese KL. **Rapid and sensitive differentiation of anomers, linkage, and position isomers of disaccharides using High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS).** *J Am Soc Mass Spectrom.* 2003; **14**:265-77. | [Article](#) | [PubMed](#)
86. Hofmann J, Hahm HS, Seeberger PH and Pagel K. **Identification of carbohydrate anomers using ion mobility-mass spectrometry.** *Nature.* 2015; **526**:241-4. | [Article](#) | [PubMed](#)
87. Lain R, Pamidimukkala KM, French AD, Hall RW, Abbas S, Jain R and Matta KL. **Linkage position in oligosaccharides by fast atom bombardment ionization, collision-activated dissociation, tandem mass spectrometry and molecular modeling.** *L-Fucosylp-(α 1 $\rightarrow$ X)-N-acetyl-D-glucosaminylp-(β 1 $\rightarrow$ 3)-D-galactosylp-(β 1-O-methyl) where X = 3, 4, or 6.* *J Am Chem Soc.* 1988; **110**:6931-9. | [Article](#)
88. Song X, Lasanajak Y, Xia B, Heimbürg-Molinario J, Rhea JM, Ju H, Zhao C, Molinaro RJ, Cummings RD and Smith DF. **Shotgun glycomics: a microarray strategy for functional glycomics.** *Nat Methods.* 2011; **8**:85-90. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
89. Song X, Ju H, Lasanajak Y, Kudelka MR, Smith DF and Cummings RD. **Oxidative release of natural glycans for functional glycomics.** *Nat Methods.* 2016; **13**:528-34. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
90. Raman R, Raguram S, Venkataraman G, Paulson JC and Sasisekharan R. **Glycomics: an integrated systems approach to structure-function relationships of glycans.** *Nat Methods.* 2005; **2**:817-24. | [Article](#) | [PubMed](#)
91. von der Lieth CW, Lutteke T and Frank M. **The role of informatics in glycobiology research with special emphasis on automatic interpretation of MS spectra.** *Biochim Biophys Acta.* 2006; **1760**:568-77. | [Article](#) | [PubMed](#)
92. Aoki-Kinoshita KF. **An introduction to bioinformatics for glycomics research.** *PLoS Comput Biol.* 2008; **4**:e1000075. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
93. Eavenson M, Kochut KJ, Miller JA, Ranzinger R, Tiemeyer M, Aoki K and York WS. **Qrator: a web-based curation tool for glycan structures.** *Glycobiology.* 2015; **25**:66-73. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
94. Walsh I, Zhao S, Campbell M, Taron CH and Rudd PM. **Quantitative profiling of glycans and glycopeptides: an informatics' perspective.** *Curr Opin Struct Biol.* 2016; **40**:70-80. | [Article](#) | [PubMed](#)

Citation:

Kanie Y and Kanie O. **Addressing the glycan complexity by using mass spectrometry: In the pursuit of decoding glycologic.** *Bio Chem Comp.* 2017; **5**:3.
http://dx.doi.org/10.7243/2052-9341-5-3