*N*型糖ペプチドの負イオンフラグメンテーションの特徴

Fragmentation characteristics of *N*-linked glycopeptides in negative-ion mode

○西風隆司、川畑慎一郎、田中耕一　（株式会社島津製作所　田中最先端研究所）

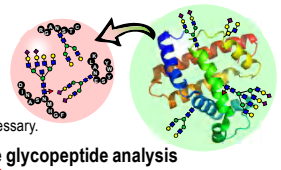
○ Takashi Nishikaze, Shin-ichirou Kawabata, Koichi Tanaka　(Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation)

SHIMADZU

MSSJ2014 2P-42

ms3d FIRST Program

Introduction



Glycopeptide analysis

- Glycan profiling + Glycosite analysis
  = **Site-specific glycoprofiling**
- Structural analysis using MS/MS is necessary.

Common approach: **Positive-ion mode glycopeptide analysis**

- CID: Glycosidic bond cleavages → ○ verification of the glycan composition
- CID and ExD (ECD or ETD) are available for peptide identification.
  - Preferential glycosidic bond cleavages
  - Glycan rearrangements
  - Ionization efficiency largely depends on the presence of basic amino acid (A.A.) residues.

} ✕ In-depth glycan structural analysis

Alternative approach: **Negative-ion mode glycopeptide analysis**

- Potentially useful for detecting glycopeptide signals
- MS/MS may result in additional structural information

Nwosu, C. et al. *Anal. Chem.* 2010, 82, 9654-9662.

However, glycopeptide fragmentation behavior in neg.-in mode mostly remains unclear.

Purpose of this study: Understanding the fragmentation behavior of *N*-linked glycopeptides in negative-ion mode

Methods

Analytes: well-characterized glycoproteins

- RNaseB, IgG, Fetuin (Fet), Transferrin (Tf), Lactoferrin (LF), AGP

Enzymes: Trypsin, Thermolysin, PronaseE, etc.

Glycopeptide enrichment

- Hydrophilic affinity enrichment (cellulose microtip)

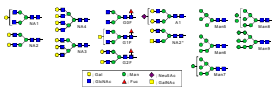
MALDI-QIT-TOFMS (*AXIMA-Resonance*™)

- CID in ion trap, collision gas: Ar

Tested glycopeptides

- >200 glycopeptides

Peptide Sequence	Peptide Mass (Da)	Glyco-protein	Major Glycoform	Enzyme
N	132.1	RNaseB	Man5-9	pronase E
NL	246.2	RNaseB	Man5-9	pronase E
RN	288.2	RNaseB	Man5-9	pronase E
NLT	346.2	RNaseB	Man5-9	pronase E
SRN	375.2	RNaseB	Man5-9	pronase E
RNLT	502.3	RNaseB	Man5-9	trypsin
NLTK	474.3	RNaseB	Man5-9	pronase E
NLTKD	589.3	RNaseB	Man5-9	pronase E
SRNLTK	717.4	RNaseB	Man5-9	Lys-C
NLTKDR	745.4	RNaseB	Man5-9	pronase E
FN	279.1	IgG	G0F, G1F, G2F	thermolysin
YN	295.1	IgG	G0F, G1F, G2F	thermolysin
NGT	320.1	IgG	G0F, G1F, G2F	pronase E
FNGT	467.2	IgG	G0F, G1F, G2F	thermolysin
EEQYNST	869.3	IgG	G0F, G1F, G2F	pronase E
TKPREEQYN	1163.6	IgG	G0F, G1F, G2F	thermolysin
EEQYNSTYR	1188.5	IgG	G0F, G1F, G2F	trypsin
TKPREEQYNST	1351.6	IgG	G0F, G1F, G2F	pronase E
AKTKPREEQYN	1362.7	IgG	G0F, G1F, G2F	thermolysin
NAKTKPREEQYNST	1664.8	IgG	G0F, G1F, G2F	pronase E
TKPREEQYNSTYR	1670.8	IgG	G0F, G1F, G2F	pronase E
N	132.1	TF	NA2	pronase E
NK	260.1	TF	NA2	pronase E
YN	295.1	TF	NA2	pronase E
NKS	347.2	TF	NA2	pronase E
NYNKS	624.3	TF	NA2	pronase E
NYNKSD	739.3	TF	NA2	Glu-C
NKSDNCE	865.3	TF	NA2	pronase E
NKSDNCEETPE	1307.5	TF	NA2	pronase E
CGLVPVLAENYNK	1475.7	TF	NA2	trypsin
GSN	276.1	TF	NA2	pronase E
GSNV	375.2	TF	NA2	pronase E
GSNVT	476.2	TF	NA2	pronase E
GSNVTDC	751.3	TF	NA2	pronase E
FGSNVTDC	898.2	TF	NA2	pronase E

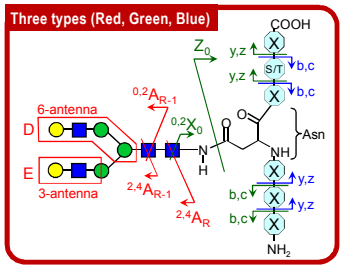


Peptide Sequence	Peptide Mass (Da)	Glyco-protein	Major Glycoform	Enzyme
ANCS	450.2	Fet	NA2, NA3	pronase E
LANCS	563.2	Fet	NA2, NA3	thermolysin
ANCSV	549.2	Fet	NA2, NA3	pronase E
NDS	334.1	Fet	NA2, NA3	pronase E
LNDSR	603.3	Fet	NA2, NA3	thermolysin
LNDSRVV	702.4	Fet	NA2, NA3	thermolysin
NDSRVV	688.4	Fet	NA2, NA3	pronase E
SNGS	363.1	Fet	NA2, NA3	pronase E
SNGSY	526.2	Fet	NA2, NA3	pronase E
AESNGS	563.2	Fet	NA2, NA3	pronase E
AESNGSY	726.3	Fet	NA2, NA3	thermolysin
NAESNGS	677.3	Fet	NA2, NA3	pronase E
NAESNGSY	840.2	Fet	NA2, NA3	pronase E
NNS	333.1	LF	Man7, Man8, Man9	pronase E
LNNS	446.2	LF	Man7, Man8, Man9	pronase E
NVT	332.2	LF	Man5, Man6, Man7	pronase E
SQGN	404.2	LF	Man5, Man6, Man7	pronase E
SQGNVT	604.3	LF	Man5, Man6, Man7	pronase E
VNQT	460.2	LF	Man5, Man6, NA1, NA2, NA2*, A1	pronase E
NVNQT	573.3	LF	Man5, Man6, NA1, NA2, NA2*, A1	pronase E
GLNVNQT	743.4	LF	Man5, Man6, NA1, NA2, NA2*, A1	pronase E
NDT	348.1	LF	Man7, Man8, Man9	pronase E
NDTVW	633.3	LF	Man7, Man8, Man9	pronase E
NDTVWEN	876.4	LF	Man7, Man8, Man9	pronase E
NDTVWENT	977.4	LF	Man7, Man8, Man9	pronase E
VNDTVWEN	1103.5	LF	Man7, Man8, Man9	pronase E
VNDTVWENT	1204.6	LF	Man7, Man8, Man9	pronase E
ITN	346.2	AGP	NA2, NA3	thermolysin
TNKT	405.2	AGP	NA2, NA3	pronase E
VPVPITNAT	910.5	AGP	NA2, NA3	pronase E
LVPVPITNATLD	1251.7	AGP	NA2, NA3	pronase E
EYN	424.2	AGP	NA2, NA3	pronase E
EYNKS	639.3	AGP	NA2, NA3	pronase E
RNEEYN	823.3	AGP	NA2, NA3	pronase E
FRNEEYNKS	1185.5	AGP	NA2, NA3	thermolysin
NEEYNKSVQEI	1351.6	AGP	NA2, NA3	pronase E
TPNKT	599.3	AGP	NA2, NA3, NA4	pronase E
FTPNKT	706.4	AGP	NA2, NA3, NA4	pronase E
FTPNKTEDT	1051.5	AGP	NA2, NA3, NA4	thermolysin
FTPNKTEDTIF	1311.6	AGP	NA2, NA3, NA4	thermolysin
IYN	408.2	AGP	NA2, NA3, NA4	thermolysin
FYN	442.2	AGP	NA2, NA3, NA4	thermolysin
FYNSSY	779.3	AGP	NA2, NA3, NA4	thermolysin
NTT	334.1	AGP	NA2, NA3, NA4	pronase E
VQRENGT	802.4	AGP	NA3, NA4	thermolysin

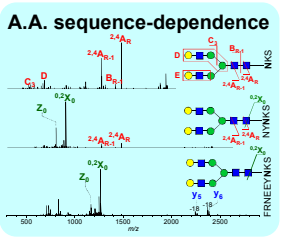
Results and Discussion

The fragment species are variable depending on their A.A. sequence, and can be classified into three types.

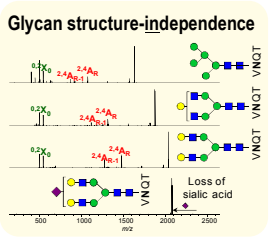
Three types (Red, Green, Blue)



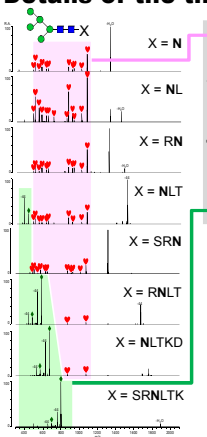
A.A. sequence-dependence



Glycan structure-independence



Details of the three types of fragment ions

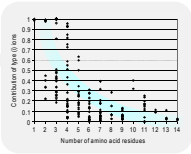


Neg.-ion CID spectra of Man5-type glycopeptides with increasing number of A.A.residues

complementary

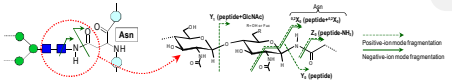
Type (i): glycan fragments ♥

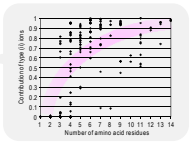
- Glycopeptides with short A.A. sequences preferentially yield this type of ions. The contribution of type (i) ions **negatively** correlates with the number of A.A. residues.
- These are same fragment ion species with neg.-ion CID of (released) *N*-glycan; therefore, they are potentially useful for characterizing in-depth glycan structure.



Type (ii): glycan-lost fragments ♦

- Glycopeptides with medium or long A.A. sequences preferentially yield this type of ions. The contribution of type (ii) ions **positively** correlates with the number of A.A. residues.
- Two fragment ion species (i.e., $^{0,2}X_0$ and Z_0) are formed. These ions are useful for the confirmation of peptide mass.





- These signals are often accompanied by specific neutral losses caused by fragmentation within the side-chain of A.A. residues.

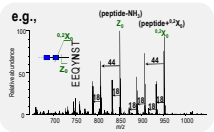
Characteristic neutral losses

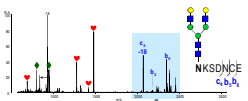
Ser: -30 (CH_2O)　　Asp & Glu: -18 (H_2O)

Thr: -44 (CH_3CHO)　　Asn & Gln: -17 (NH_3)

Carbamidomethylated Cys: -91 (NH_2COCH_2SH)

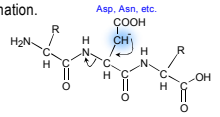
Bowie, JH. et al. *Mass Spectrom. Rev.* 2002, 21, 87-107.



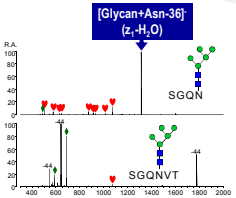


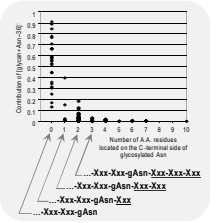
Type (iii): Fragment ions with intact *N*-glycan moiety ♠

- This type of ions is formed by direct and specific peptide backbone cleavages of N-C□ bonds. (N-terminal sides of Asp, Asn, Glu, and Gln).
- c/z ions are formed.
- These signals are often accompanied by specific neutral losses described above.
- Rationalized by the mechanism via enolate anion formation.

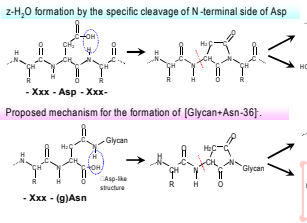


- When a glycosylated Asn (gAsn) is located on the C-terminus, **[glycan+Asn-36]⁻ ion (z_1-H_2O)** is preferentially formed. **(glycosite-dependence)**





- The formation can be rationalized the same way with the z-H_2O formation in deprotonated peptide fragmentation.



Conclusions & Summary

Purpose: Understanding the fragmentation behavior of *N*-linked glycopeptides in neg.-ion mode

Methods: >200 glycopeptides
MALDI-generated singly-deprotonated ions
Trap-type low-energy CID

Results: Fragment species depends on their A. A. sequences, and can be classified into **three types**;
(i) Glycan fragments ♥
(ii) Glycan-lost fragments ♦
(iii) Fragment ions with intact *N*-glycan moiety ♠

Glycopeptides with short A.A. sequence…♥ > ♦
Glycopeptide with long A.A. sequence……♥ < ♦
N-terminal sides of Asp, Asn, Glu, (Gln)……..+ ♠
Glycosylation near the C-terminus
…… **[Glycan+Asn-36]⁻(z_1-H_2O)**

Concluding remarks:

- Observed fragments are reasonably explained by a combination of existing fragmentation rules suggested for *N*-glycans and peptides in neg.-ion mode.
- Neg.-ion CID of deprotonated glycopeptides potentially offers in-depth structural information of the glycan moiety. (type (i))
- Glycopeptide detection and characterization are now possible in neg.-ion mode.

Acknowledgements

This research is supported by the Japan Society for the Promotion of Science (JSPS) through its "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program).

Publication

Nishikaze, T. et al. *JASMS*, 2014, 25, 988-998.