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Characterization of the N-glycosylation of Recombinant IL-4 and IL-13 Proteins Using LC-MS/MS Analysis and the I-GPA Platform

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Abstract : Interleukin-4 (IL-4) and IL-13 are cytokines secreted by immune cells. Cytokines induce the proliferation of macrophages or promote the differentiation of secretory cells. The initiation and progression of allergic inflammatory diseases, such as asthma, are dependent on cytokines acting through related receptor complexes. IL-4 and IL-13 are N-glycoproteins. Glycan structures in glycoproteins play important roles in protein folding, protein stability, enzymatic function, inflammation, and cancer development. Therefore, the glycan structure of IL-4 and IL-13 needs to be elucidated in detail for the development of effective therapies. We report the first attempt to characterize the site-specific N-glycosylation of recombinant IL-4 and IL-13 via liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. The tandem mass spectra of intact N-glycopeptides were identified using the Integrated GlycoProteome Analyzer (I-GPA) platform, which can automatically and rapidly analyze multiple N-glycopeptides, including their glycan composition and amino acid sequences. The recombinant IL-4 and IL-13 were identified with amino acid sequence coverages of 84% and 96%, respectively. For IL-4, 52 glycoforms on one N-glycosylation site were identified and quantified. In IL-13, 232 N-glycopeptides from three N-glycosylation sites were characterized, with the site Asn52 being the most extensively glycosylated (~80%). The complex glycans were the most abundant glycan on IL-4 and IL-13 (~96% and 91%, respectively), and the biantennary glycans were the most abundant in both recombinant IL-4 and IL-13 proteins.

Keywords : site-specific N-glycosylation, LC-MS/MS, I-GPA

Introduction

Interleukin-4 (IL-4) and IL-13 were identified as structural and functional cytokines in the early 1980s. Both are related to immune function, fetal development, pregnancy, mammary development and lactation, and cognitive functions, such as memory and learning.[1] Their roles in the pathogenesis of asthma, atopy, pulmonary fibrosis and cancer are widely known. In immune and inflammatory responses, the interaction between cytokines and their receptors plays a key regulatory role in the complicated cellular interactions of hematopoietic cells, among other cell types. IL-4 and IL-13 are both T helper type 2 (Th2) cytokines, but have distinct functional roles. IL-4 is a critical cytokine of Th2-like lymphocytes[2] and acts to direct immune responses; however, the primary function of IL-13 is as an effector cytokine driving epithelial and fibrotic responses.[3]

Both IL-4 and IL-13 are glycoproteins. Glycosylation affects their biological activity and ability to induce certain effector functions. Li *et al*.[4] reported that glycosylated IL-4 in *Pichia pastoris* has less biological activity and stability compared to its non-glycosylated form. The IL-13 secreted in lymphocytes is more highly glycosylated than in bronchial smooth muscle cells (BSMCs).[5] These results indicate that the level and role of glycosylated IL-13 might differ among cell types.[6]

Glycosylation is one of the most important post-translational protein modifications in eukaryotic cells.[7] Glycan structures play important roles in protein stability,

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protein folding, enzymatic functions, inflammation, the immune response, and cancer.[8] The heterogeneity of glycosylation has led to a diverse range of protein functions.[9] Especially, the glycosylation for the recombinant proteins used as a drug is related to provide their consistent safety and efficacy. In order to strictly control the quality of protein drugs, site specific analysis of glycosylation in proteins is required.

The advanced high performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) is a useful tool to obtain information about glycosylation sites and glycan composition as well as peptide sequence and to map glycosylation patterns.[10] However, characterization of intact N-glycopeptides is challenging due to their low ionization efficiency, the microheterogeneity of glycans, and the complexity of tandem mass spectra (which reveal the fragmentation of moieties of both peptides and glycans). We had developed an MS-based high-throughput method for intact N-glycopeptides analysis without glycan releasing coupled with the Integrated GlycoProteome Analyzer (I-GPA), which allows for automatic identification and quantification of intact N-glycopeptide. Using I-GPA, we reported the largest number of site-specific N-glycopeptide isoforms in α-1-Acid Glycoprotein[10b] and developed fucosylated alpha-fetoprotein as a biomarker for early diagnosis of hepatocellular carcinoma (HCC).[11] We could determine core and outer fucosylation in N-glycopeptides as applying machine learning method to results from I-GPA.[12] In recent, I-GPA have been extended for O-glycopeptide identification.[13]

In this study, we characterized N-glycosylation of the recombinant IL-4 and IL-13 proteins using I-GPA with various tandem mass spectrometry methods, including collision-induced dissociation (CID), higher-energy C-trap dissociation (HCD), and electron-transfer/higher-energy collision dissociation (EThcD). These results will help to understand the role of IL-4 or IL-13 proteins as cytokines.

Experimental

Materials and samples

Iodoacetamide (IAA), 1,4-dithiothreitol (DTT), formic acid (FA), and ammonium bicarbonate (ABC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypsin, GluC, and chymotrypsin were obtained from Promega (Madison, WI, USA). Acetonitrile (ACN; HPLC grade) and water were obtained from Merck (Darmstadt, Germany). An AmiconUltra centrifugal filter (10K) was purchased from Millipore (Burlington, MA, USA).

Recombinant protein purification methods

Freestyle™ 293 (R79007; Thermo Fisher Scientific, Waltham, MA, USA) cells were grown in Erlenmeyer flasks (100 mL, 3.2×10^8 cells; Corning Inc., Corning, NY, USA) under 8% CO_2 at 37°C using an orbital shaker. The cells were transfected with pFUSE-hlgG2-Fc2 plasmids harboring a human IL-4 sequence or human IL-13 sequence. The ExpiFectamine™ (A14525; Thermo Fisher Scientific) transfection kit was used for the transfection. Supernatants of the transfected cell culture were collected individually on day 6 post-transfection and used for the purification. Recombinant human IL-4 and recombinant human IL-13 proteins were purified by Ni-NTA affinity chromatography and size exclusion column chromatography, respectively.

Digestion of samples

Recombinant IL-4 and IL-13 samples were prepared in 50 mM ABC buffer for digestion, after desalting by centrifugal filtration using a 10 kDa membrane filter (UFC501096; Millipore). In order to produce the appropriate size of N-glycopeptides in protein digestion, different enzymes were applied to IL-4 and IL-13. The protein solution was reduced by 10 mM DTT at 60°C for 1 h and alkylated by 25 mM IAA in a dark at room temperature, with a reaction time of 1 h. Alkylated IL-4 and IL-13 samples were digested with GluC and trypsin, respectively, at 37°C overnight (16 h). The IL-13 sample was divided into two equal-sized parts. One IL-13 sample was digested with peptide-N-glycosidase F (PNGase F) at 37°C overnight (16 h) to remove N-glycans attached to IL-13 protein. The other IL-13 sample was digested with GluC at 37°C overnight (16 h) followed by chymotrypsin at 37°C overnight (16 h). All digested samples were quenched in a 3% final concentration of FA. For the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, the solution was diluted by mobile phase A (99.9% water with 0.1% FA).

Nano LC-ESI-MSMS

Dissolved samples in mobile phase A were analyzed using an LC-MS/MS system consisting of an UltiMate 3000 RSLCnano system and an Orbitrap Eclipse Tribrid mass spectrometer (Thermo Scientific) equipped with a nano electrospray source. An autosampler was used to load the sample solutions into a C_{18} trap column (Acclaim PepMap™ 100, 75 μm × 2 cm; Thermo Fisher Scientific). The samples were desalted and concentrated on the trap column for 8 min at a flow rate of 3 μL/min. The trapped samples were then separated on a C_{18} analytical column (PepMap™ RSLC C18, 2 μm, 100 Å, 75 μm × 50 cm; Thermo Fisher Scientific). The mobile phases were composed of 99.9% water (A) and 99.9% ACN (B), and each contained 0.1% FA. The LC gradient started with 5% of B for 8 min, and was ramped to 20% of B for 80 min, 40% of B for 12 min, and 95% of B for 1 min; it was then held at 95% of B for 8.5 min and 5% of B for another 0.5 min. The column was re-equilibrated with 5% of B for 10 min before the next run. A voltage of 2,000 V was applied to produce the ions. During the chromatographic separation, the Orbitrap Eclipse Tribrid mass spectrometer

Table 1. Interleukin 4 (IL-4) and IL-13 sequence information obtained via LC-MS/MS analysis.

Protein	human recombinant protein sequences
human recombinant IL-4	HHHHHHHHHHLVPRGSHKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAASK**N**T TEKETFCRAATVLRQFYSHHEKDTRCLGATAQQFHRHKQLIRFLKRLDRNLWG LAGLNSCPVKEANQSTLENFLERLKTIMREKYSKCSSDYKDDDDK
human recombinant IL-13	HHHHHHHHHHHLVPRGSDYKDDDDKGPVPPSTALRELIEELVN**N**ITQNQKAPLCN GSMVWSI**N**LTAGMYCAALESLI**N**VSGCSAIEKTQRMLSGFCPHKVSAGQFSSL HVRDTKIEVAQFVKDLLLHLKKLFREGQFN

N, HHHHHHHHHH, LVPRGS and DYKDDDDK are N-glyco sites, 10X His, thrombin site and flag respectively for protein preparation. The underline sequences are identified by LC-MS/MS analysis. Expression vector was pFUSE-hIgG2-Fc2 in Expi293-F cells. IL-4 has one N-glycosylation site (Asn62). IL-13 has four N-glycosylation sites (Asn52, Asn 63, Asn71 and Asn86)

(Thermo Fisher Scientific) was operated in data-dependent mode, automatically switching between MS1 and MS2. Full-scan MS1 spectra (250–2,000 *m/z*) were acquired by the Orbitrap, with a maximum ion injection time of 100 ms at a resolution of 120,000 and automatic gain control (AGC) target value of 4.0e5. The MS2 spectra were acquired by the Orbitrap mass analyzer at a resolution of 15,000 with HCD (30% normalized collision energy, maximum ion injection time of 50 ms, AGC target value of 5.0e4), CID (35% normalized collision energy, maximum ion injection time of 50 ms, AGC target value of 5.0e4) and EThcD (15% SA collision energy, maximum ion injection time of 100 ms, AGC target value of 1.5e5). Previously fragmented ions were excluded for 30 s within 10 ppm.

Protein identification

The MS/MS spectra were analyzed using software and compiled into a database (Table 1). The reversed sequences of all proteins were added to the database and the false discovery rates (FDRs) were calculated. ProLucid[14] from the integrated proteomics pipeline (IP2; Integrated Proteomics Applications, Inc., San Diego, CA, USA) was used to identify the peptides, with a precursor mass error of 5 ppm and fragment ion mass error of 50 ppm. GluC (for IL-4) and trypsin/GluC/chymotrypsin (for IL-13) were selected as the enzymes, with one potential missed cleavage. Partial tryptic cleavage was allowed. Carbamidomethylation at cysteine and oxidation at methionine were chosen as static and variable modifications, respectively. For the IL-13 sample treated with peptide N-glycosidase F, deamidation at asparagine was set as a variable modification. The output data files were used to devise a protein list using DTASelect software[15] (The Scripps Research Institute, San Diego, CA, USA), with an FDR < 0.1.

Glycopeptide Identification

Site-specific N-glycopeptides of recombinant IL-4 and IL-13 proteins were automatically identified by I-GPA.[16] In I-GPA scoring entailed three steps: 1) Selection of *N*-glycopeptide from 15 glycan-specific oxonium ions using HCD-spectra; (M-score); 2) Selection of candidates by matching the isotope pattern to intact *N*-glycopeptides in the GPA-DataBase (S-score); and 3) Identification of *N*-glycopeptide by matching the experimental and theoretical CID and/or HCD fragment ions (Y-score) with FDR < 1%. When calculating the Y-score, Y ions (partially fragmented ions of glycan moieties), B ions (multiple monosaccharide fragments of the non-reducing ends of glycans), and b/y ions (from the amino acid sequences of peptides) were considered. GPA-DataBase were constructed for each sample, using the software GPA-DB-Builder, by combining possible enzymatic peptides of IL-4 and IL-13 and 226 N-linked glycans (retrosynthetic glycans from Kronewitter *et al.*[17] and glycans of the penta and hexa polylactosamine series from Ozohanics *et al.*[18]). The I-GPA search parameters were as follows: missed cleavages = 1; and "fixed modification" = carbamidomethyl cysteine/mammalian N-glycan. The mass tolerances of precursor and fragment ions were set to 0.02 Da for HCD, and 0.05 Da for CID and EThcD.

The nomenclature of the N-glycopeptide was based on the peptide sequence and numbers of hexoses (Hex), N-acetylglucosamines (GlcNAc), fucose (Fuc), and N-acetyl neuraminic acids (NeuAc) (#Hex_#HexNac_#Fuc_#NeuAc). The nomenclature of the glycan was based on a retrosynthetic glycans database [15]. For example, IFAASK***N***TTE _5_4 _1_2 N-glycopeptide was composed of IFAASK***N***TTE (the peptide sequence), 5 (the number of Hex), 4 (the number of N-GlcNAc), 1 (the number of Fuc), and 2 (the number of NeuAc). The italicized and underlined N (***N***) represents an N-glycosylation site.

Glycopeptide quantification

For quantitative analysis of N-glycopeptides, the q-GPA (quantitated-GPA) was used as the sum of three MS1 spectra using I-GPA software.[16] All N-glycopeptides from IL-4 and IL-13 were extracted and those with a coefficient of variation (CV) within 30% (three replicates) were quantitatively analyzed.

Results

Identification of recombinant IL-4 and IL-13 proteins

IL-4 and IL-13 are glycoproteins with one (Asn62) and four N-glycosylation sites (Asn52, Asn63, Asn71, and

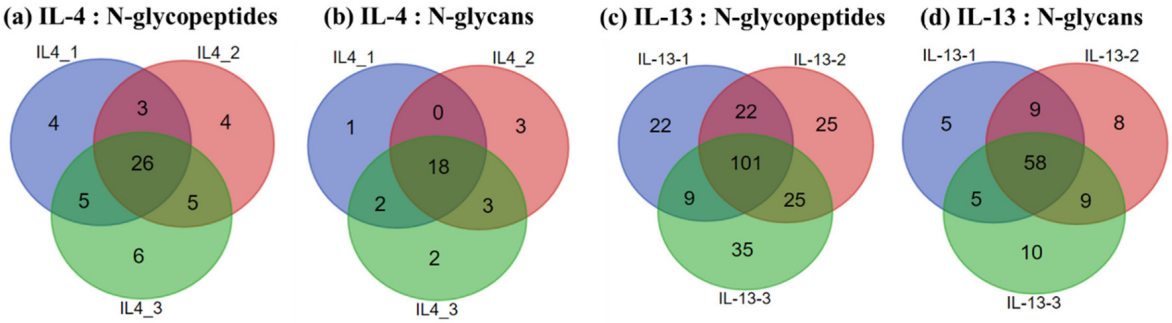


Figure 1. The numbers of N-glycopeptides and N-glycans identified from recombinant IL-4 and IL-13 glycoproteins.

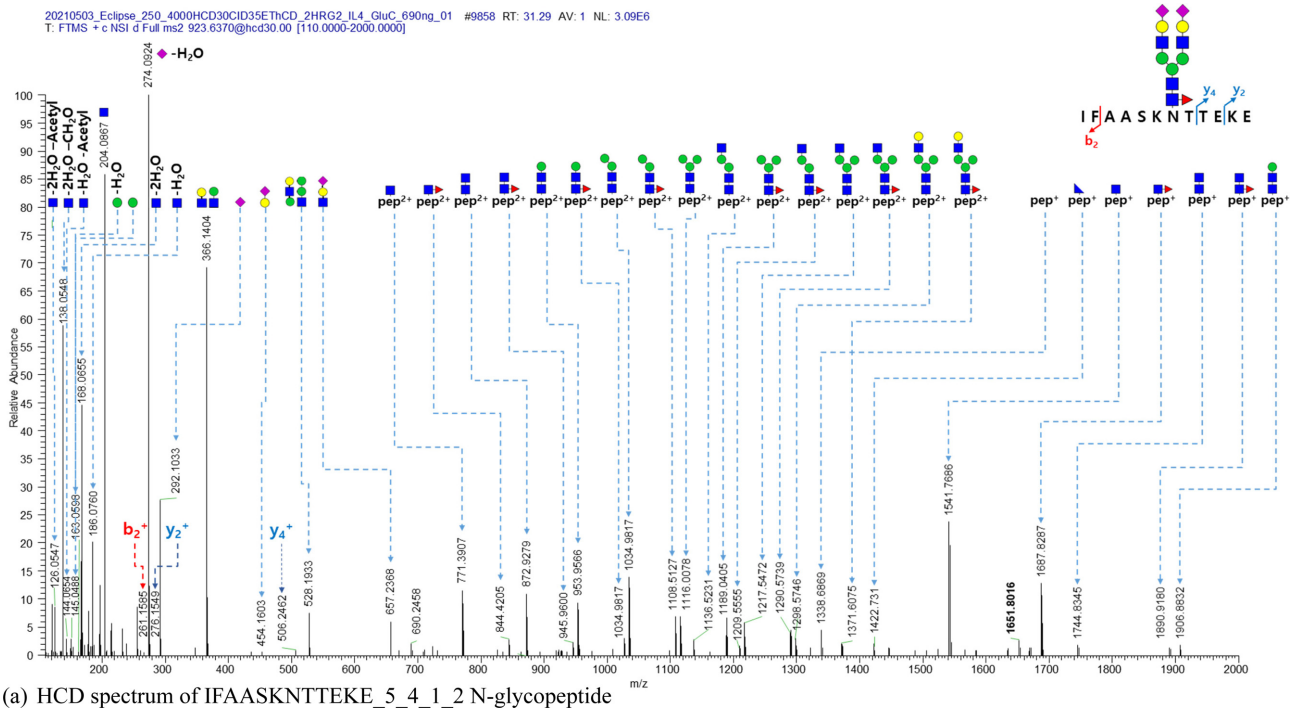
Asn86), respectively. The underlined sequences in Table 1 were analyzed by LC-MS/MS and identified by the IP2 search engine. The sequence coverage was over 84% for IL-4 with GluC digestion and over 96% for IL 13 with trypsin and PNGase F digestion. In the calculation of sequence coverage, only spectra with an Xcorr score > 2 were considered, where Xcorr represents the similarity between the experimentally obtained tandem mass spectrum and the data in a protein database.

Identification of N-glycopeptide from recombinant IL-4 and IL-13 proteins

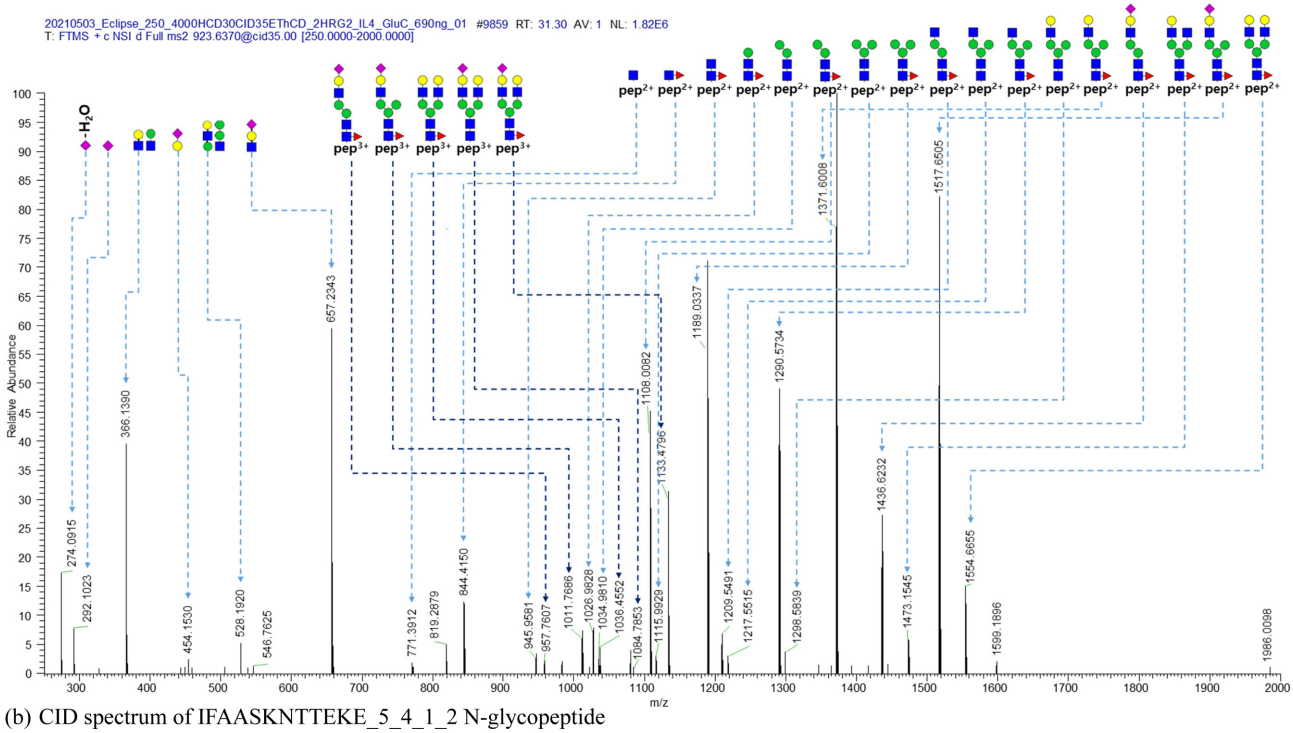
N-glycopeptides of recombinant IL-4 and IL-13 proteins were site-specifically and automatically identified by I-GPA. Detailed explanation was described in the "*Glycopeptide Identification*" of *"Experimental"* part.

Recombinant IL-4 was digested by GluC to generate a peptide of adequate length including the known N-glycosylation site of Asn62, and then analyzed by LC-MS/MS with three replicates. A total of 53 unique N-glycopeptides including the Asn62 N-glycosylation site were identified (average number = 39 ± 2, mode = 26) (Figure 1-(a)). A total of 29 unique N-glycans were identified (average number = 23 ± 2, mode = 18 being) (Figure 1-(b)).

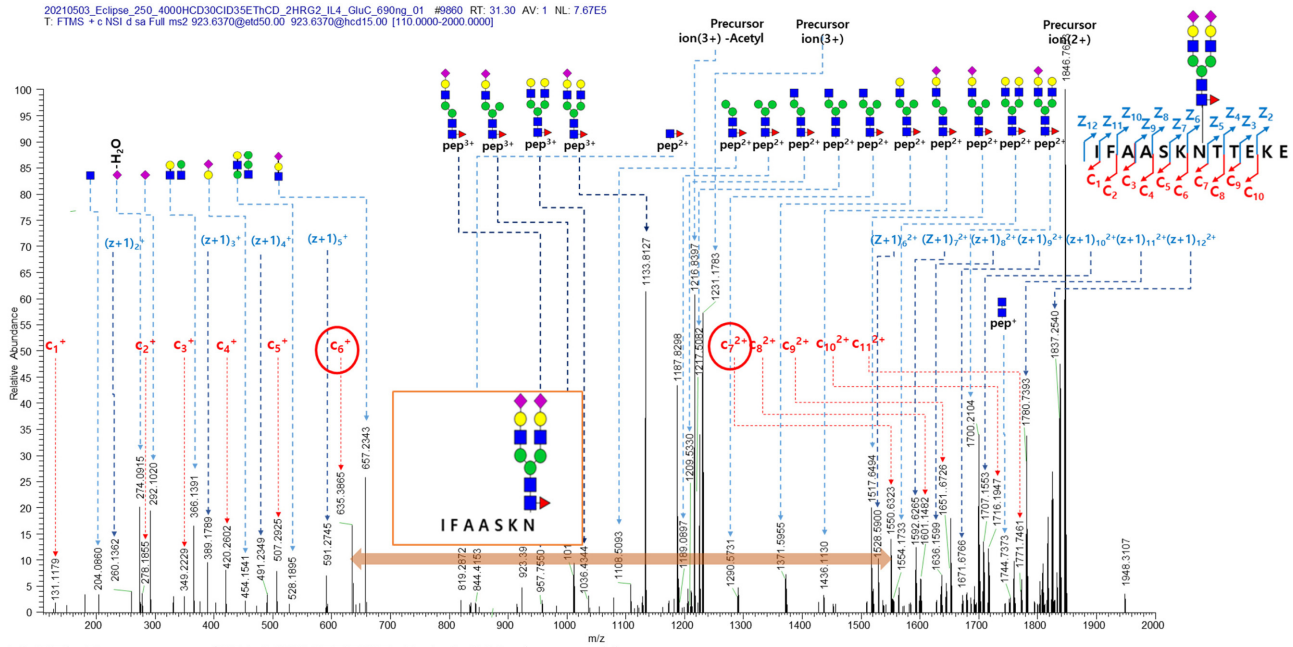
Because the recombinant IL-13 protein has four N-glycosylation sites (Asn52, Asn63, Asn71, and Asn86), it was sequentially treated with trypsin, GluC, and chymotrypsin to obtain peptides with a single N-glycosylation site. The total number of unique N-glycopeptides identified was 239 (average number = 166 ± 10, mode = 101). Except for Asn63, three N-glycosylation sites were confirmed (Figure 1-



(a) HCD spectrum of IFAASKNTTEKE_5_4_1_2 N-glycopeptide



(b) CID spectrum of IFAASKNTTEKE_5_4_1_2 N-glycopeptide



(c) EThcD spectrum of IFAASKNTTEKE_5_4_1_2 N-glycopeptide

Figure 2. (a) HCD, (b) CID, and (c) EThcD spectra for the IFAASKNTTEKE_5_4_1_2 N-glycopeptide identified from IL-4 via an I-GPA search.

(c)). There were 104 unique N-glycans (average number = 81 ± 4, mode = 58 being) (Figure 1-(d)). The supplementary Table 1 is showing the list of identified site-specific N-glycopeptides from IL-4 and IL-13.

Figure 2 shows representative tandem mass spectra for the N-glycopeptide IFAASKNTTEKE_5_4_1_2 of IL-4, which was identified by HCD, CID, and EThcD. The fragment ions included intact N-glycopeptide-like oxonium ions, glycan-cleaved glycopeptide fragment ions (B/Y), and peptide fragment ions (b/y and c/z). The HCD

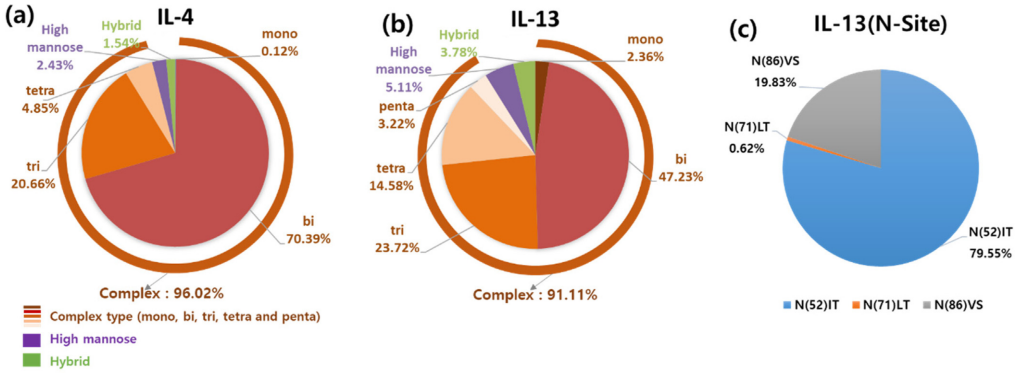


Figure 3. Distribution of glycan types for (a) IL-4 and (b) IL-13. (c) Distribution of the most abundant N-glycopeptides according to the N-sites of IL-13.

spectrum with various oxonium ions was very useful for glycopeptide identification. By revealing the b/y ions, amino acid sequence analysis was also possible. The CID spectrum contained more glycan-cleaved fragment ions (B/Y) and provided more information about glycan structures, including sialic acid (or neuraminic acid) and Fuc. The EThcD spectrum indicated the exact location of N-glycosylation. In Figure 1-(c), the c_7^{2+} to c z ions of m/z 1,550.6323 (1.35 ppm) revealed that a glycan consisting of five HEX, four hexosamines, one Fuc, and two sialic acids (5_4_1_2) was attached to Asn62.

Quantification of N-glycopeptides from recombinant IL-4 and IL-13 proteins

For quantitative analysis of N-glycopeptides, label-free quantification by I-GPA[9] was performed, in which the abundances were calculated based on the combined intensities of the top three isotope peaks at the three highest MS spectral points. If *N*-glycopeptide was not identified in a given run, we obtained the abundances of the corresponding *N*-glycopeptides with the same precursor ion (within a mass tolerance of 0.002 Da) and retention time (within 2 min) as the previously observed *N*-glycopeptide. N-glycopeptides with a CV within 30% (three replicates) were used in a quantitative analysis of IL-4 and IL-13. In total, 52 of 53 N-glycopeptides were identified for IL-4 protein and 232 of 239 N-glycopeptides for IL-13 protein.

The most abundant N-glycopeptide was IFAASKNTTE KE_5_4_1_2 for IL-4 and LVNITQNQK_5_4_1_1 for IL-13. In both of these proteins, most N-glycans were of the complex type with mono- to penta-antennary (96.02% for IL-4 and 91.11% for IL-13), followed by the high mannose type (2.43% for IL-4 and 5.11% for IL-13) and hybrid type (1.54% for IL-4 and 3.78% for IL-13) (Figure 3-(a) and (b)). For IL-13, the N-site having the most abundant N-glycopeptides was Asn52 (79.55%), followed by Asn86 (19.83%) and Asn71 (0.62%) (Figure 3-(c)).

Figure 4 shows the distribution of N-glycan types, and the relative abundance at three N-glycosylation sites from

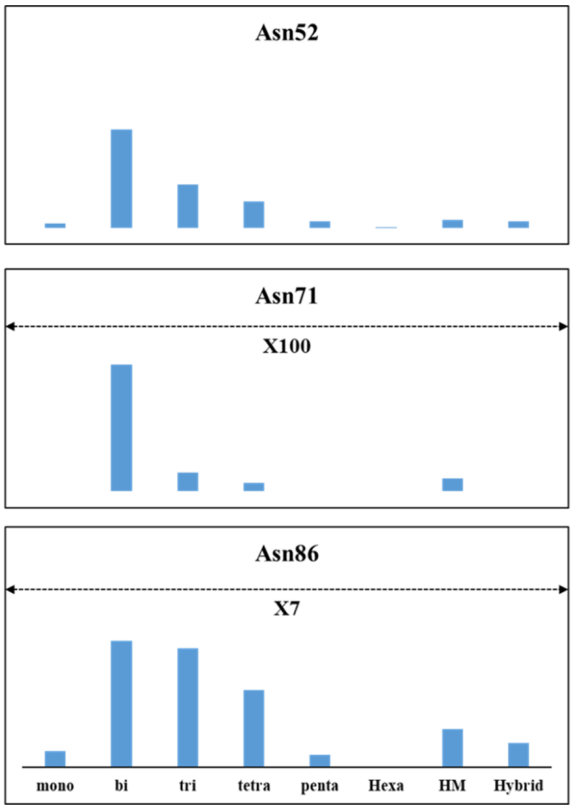
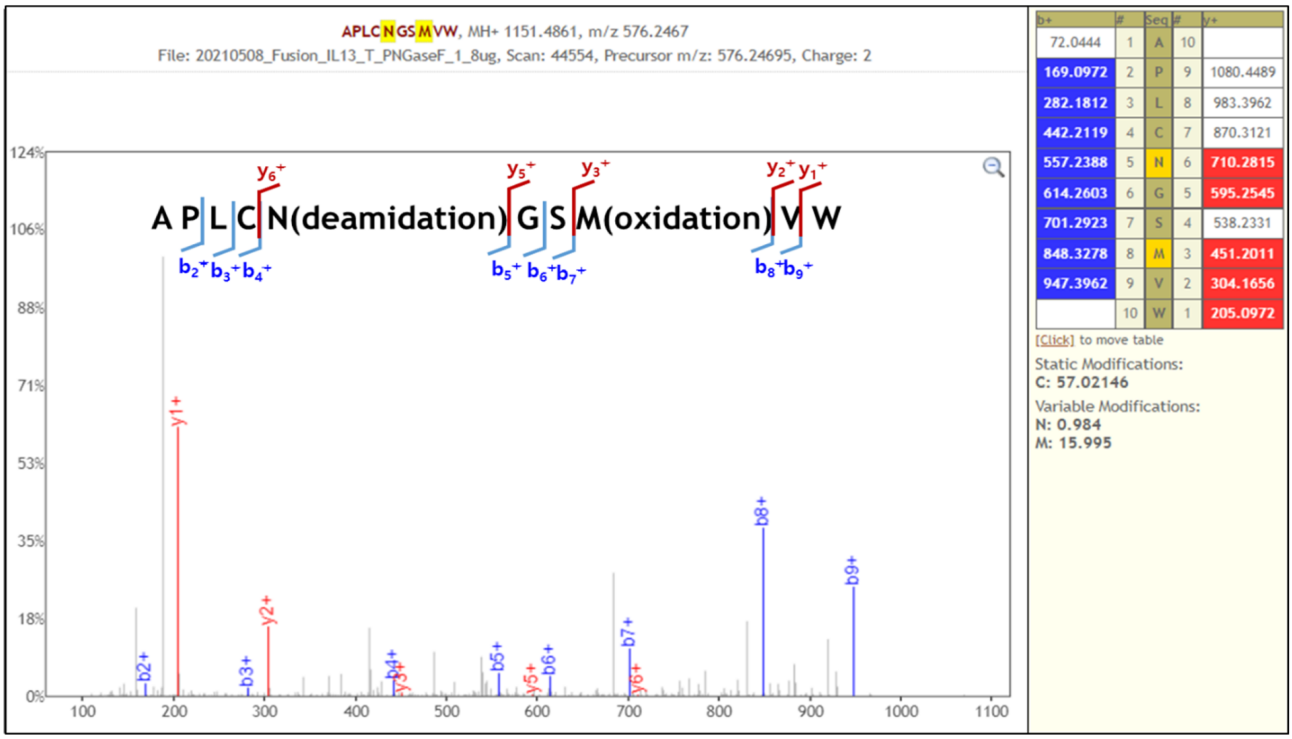


Figure 4. Quantitative analysis of 226 site-specific N-glycopeptides from recombinant IL-13 protein.

IL-13. The biantennary complex type was the major N-glycan at all sites; however, the percentages of highly branched antennary N-glycan of tri and tetra were relatively higher in the Asn86 N-glycosylation site than at other sites. This result could describe that the relative abundance of glycosylation and the detail information of attached glycan type are different according to the site-specific N-sites. The information of site-specifically

(a)



(b)

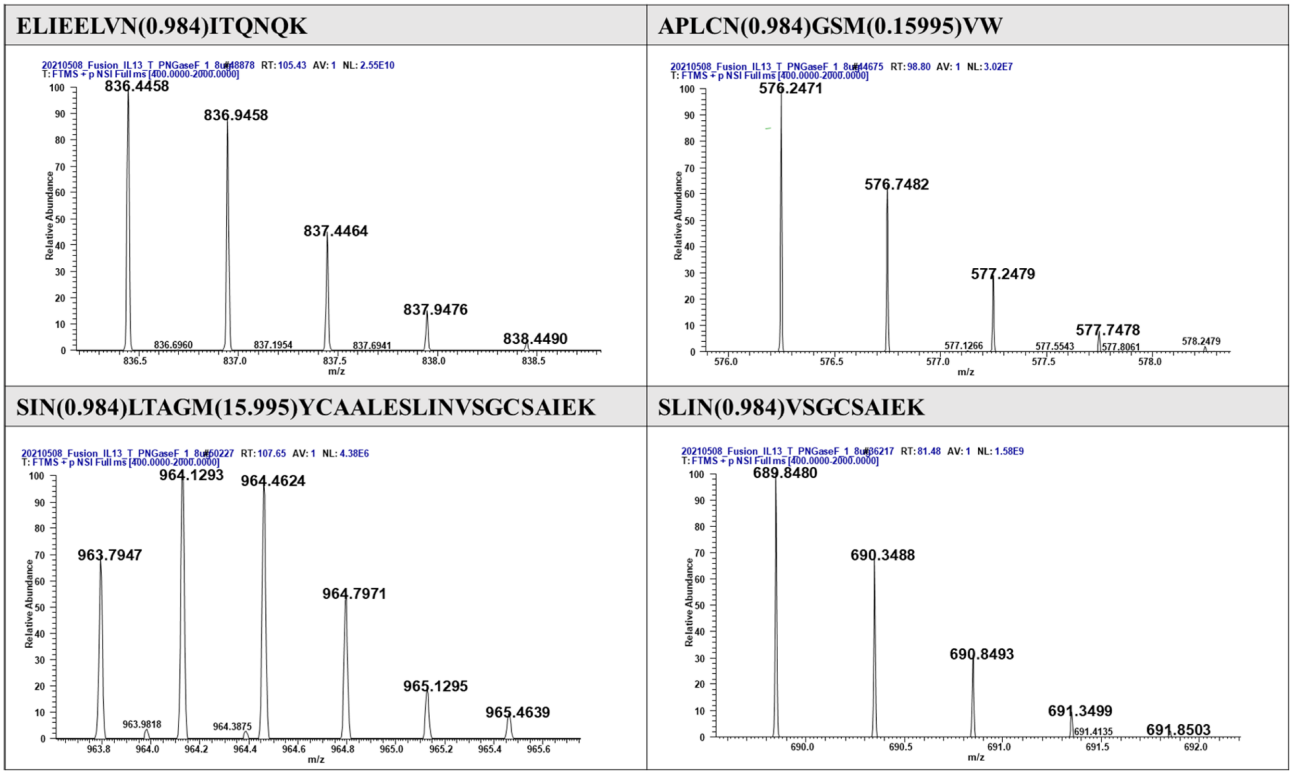


Figure 5. (a) the spectrum of the deamidated APLCNGSMVW peptide from the N-site (Asn63) in IL-13 revealed by an IP2 search and (b) the molecular ion spectrum of detected deamidated peptides from IL-13.

identified N-glycopeptides from IL-4 and IL-13 was listed up in the supplementary Table 1.

Discussion

We performed an LC-MS/MS analysis to characterize the N-glycosylation of recombinant IL-4 and IL-13 proteins, and confirmed the sites glycosylated by the various glycans. Biantennary complex type N-glycans were found to be the major form attached to proteins; however, the distribution of attached glycans differed among the various N-glycosylation sites.

In our study, I-GPA was applied for intact N-glycopeptide identification and quantification. It provided reproducible results, with 74~79% of N-glycopeptides characterized in two or more of three replicates, and 97% of N-glycopeptides having a CV within 30%.

Tandem mass spectra obtained by HCD, CID, and EThcD enabled successful identification of intact N-glycopeptide. Oxonium ions (indicating glycan attachment) and b/y ions (for amino acid sequencing) were identified in the HCD spectrum, while B/Y ions providing glycan structure information were observed in the CID spectrum, and c/z ions allowing precise localization were observed in the EThcD spectrum. To obtain a high-quality EThcD spectrum, it is important to first obtain a highly charged precursor ion during ionization. The N-glycopeptide IFAASKNTTEKE_5_4_1_2, which was generated from IL-4 by GluC digest, had two basic residues (lysine) and an N-terminal. Its glycosylation site was precisely assigned based on the c/z ions in the EThcD spectrum, and its four charged precursor ions were identified with high efficiency by EThcD (Figure 1-(c)).

Table 2. Spectral count of deamidated peptides from IL-13.

No.	N-site	Peptides containing N-site	# of identified spectral count	Xcorr score
1.	Asn52	R.ELIEELVN(0.984)ITQNQK.A	71	5.7772
2.	Asn63	K.APLCN(0.984)GSM(0.15995)VW.S*	6	2.1722
3.	Asn71	W.SIN(0.984)LTAGM(15.995)YCAALESLINVSGCSAIEK.T*	2	6.5955
4.	Asn86	E.SLIN(0.984)VSGCSAIEK.T*	35	3.8234

*partially tryptic peptides

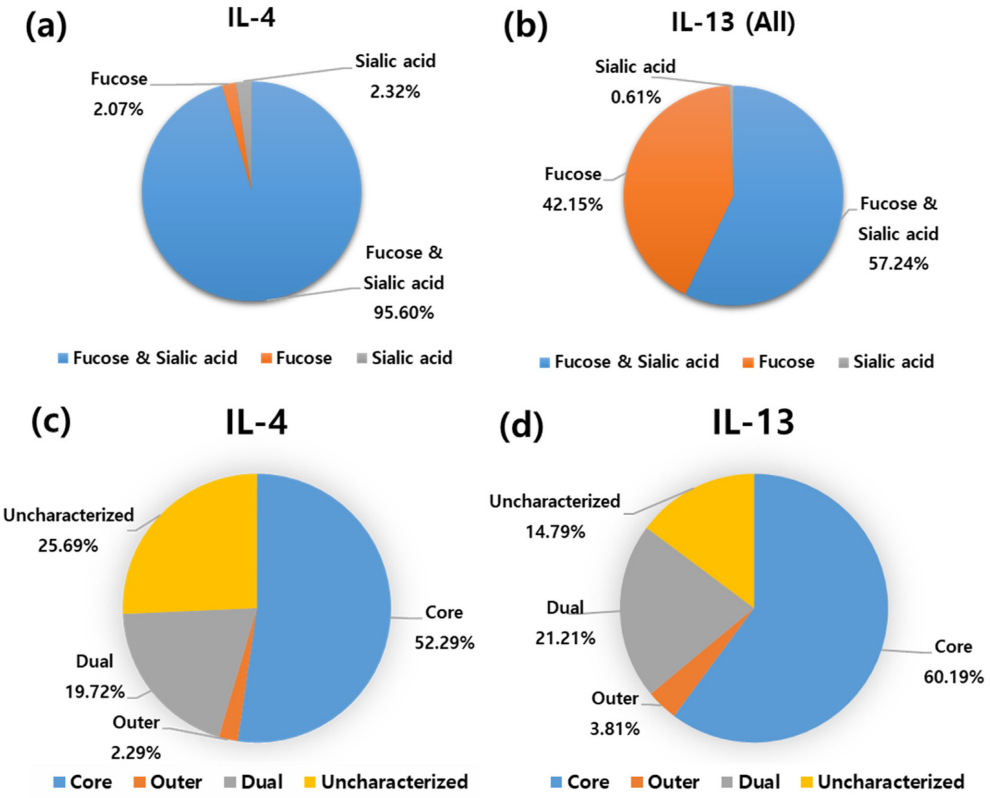


Figure 6. Distribution of complex types according to the inclusion of Fuc or sialic acid in (a) IL-4 and (b) IL-13, and the distribution Fuc types in (c) IL-4 and (d) IL-13.

In the analysis of IL-13, N-glycosylation at Asn63 of four known N-glycosylation sites was not identified. To determine whether N-glycans were attached to this site, IL-13 was digested by trypsin and then treated with PNGase F to remove the attached N-glycans. Finally, deamidated APLCN(0.984)GSLVW peptide (Figure 5- (a)) was identified at Asn63, as well as other deamidated peptides at Asn52, Asn71, and Asn86. When comparing the intensity (Figure 5-(b)) and the spectral count among the deamidated peptides (Table 2), it might be hard to detect intact N-glycopeptide forms including Asn63 because of poor detectability showing by low intensity and a few spectral counts.

The most abundant N-glycan in both IL-4 and IL-13 was the complex type. In Figure 3-(a) and (b), the N-glycan type can be determined according to the number of branches (mono-, bi-, tri- and ≥ tetra-antennary). The bi-antennary N-glycans were more abundant in IL-4 (70.39%) than IL-13 (47.23%). The overall abundance of tri-antennary and ≥ tetra-antennary N-glycans was 23.72% and 17.81%, respectively.

Complex glycans with both Fuc and sialic acid accounted for 95.60% of the total glycans in IL-4 and 57.24% in IL-13 (Figure 6-(a) and (b)).

In IL-13, 42.15% of complex N-glycans had Fuc without sialic acid. Generally, Fuc attachment to N-acetylglucosamine in N-glycan could be classified into core, outer, and dual fucosylation types according to the location of Fuc. Automatic classification using a machine learning method[12a] has been employed to predict core-fucosylated glycopeptides from N-glycopeptide spectra identified in both IL-4 and IL-13. Because sialylation and/or fucosylation play important roles in the structural stability and functions of N-glycoprotein,[19] our in-depth analysis of N-glycosylation in recombinant IL-4 and IL-13 could help determine the most important N-glycosylated cytokines for the development of immune disease treatments.

Conclusions

IL-4 and IL-13 are Th2 cytokines are used to stimulate allergies during the development of drugs for allergic diseases. In this study, we report site-specific N-glycosylation of recombinant IL-4 and IL-13 for the first time, which was achieved by LC-MS/MS analysis and use of the I-GPA platform. All experiments were performed with three replicates for identification and label-free quantification of intact N-glycopeptides. Over 97% of the identified N-glycopeptides had a CV within 30%. Finally, 52 and 232 intact N-glycopeptides were confirmed for IL-4 and IL-13, respectively. One N-glycosylation site was identified for IL-4, while four were identified for IL-13 (three formed by intact N-glycopeptides and one by a deamidated peptide). In recombinant IL-13, N-glycosylation at the Asn52 site was the most dominant. Biantennary complex type N-glycans were the most abundant glycans in both IL-4 and IL-13. In IL-13, tri- and tetra-antennary N-glycan were most abundant at the Asn86 site. About 92% of the N-glycopeptides in IL-4 contained both Fuc and sialic acid, while the corresponding value was 48% in IL-13. Core fucosylation was the dominant type of fucosylation.

Supplementary Information

Supplementary information is available at https://docs.google.com/spreadsheets/d/1ANF3xWB4_I7UtiVHH0Sl1171X4w6ARAA/edit?usp=sharing&ouid=111353140014732050956&rtpof=true&sd=true

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