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Targeted Mass Spectrometry Approach Enabled Discovery of O-Glycosylated Insulin and Related Signaling Peptides in Mouse and Human Pancreatic Islets

Qing Yu[†], Alejandra Canales[‡], Matthew S. Glover[†], Rahul Das[‡], Xudong Shi[⊥], Yang Liu[§], Mark P. Keller[‡], Alan D. Attie[‡], and Lingjun Li^{*,§,†}

[†]School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53705, United States

[‡]Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, United States

[⊥]Department of Surgery, Wisconsin Institute for Medical Research, Madison, Wisconsin 53705, United States

[§]Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, United States

Abstract

O-Linked glycosylation often involves the covalent attachment of sugar moieties to the hydroxyl group of serine or threonine on proteins/peptides. Despite growing interest in glycoproteins, little attention has been directed to glycosylated signaling peptides, largely due to lack of enabling analytical tools. Here we explore the occurrence of naturally O-linked glycosylation on the signaling peptides extracted from mouse and human pancreatic islets using mass spectrometry (MS). A novel targeted MS-based method is developed to increase the likelihood of capturing these modified signaling peptides and to provide improved sequence coverage and accurate glycosite localization, enabling the first large-scale discovery of O-glycosylation on signaling peptides. Several glycosylated signaling peptides with multiple glycoforms are identified, including the first report of glycosylated insulin-B chain and insulin-C peptide and BigLEN. This discovery may reveal potential novel functions as glycosylation could influence their conformation and biostability. Given the importance of insulin and its related peptide hormones and previous studies of glycosylated insulin analogues, this natural glycosylation may provide important insights into diabetes research and therapeutic treatments.

Graphical Abstract

*Corresponding Author lingjun.li@wisc.edu. Phone: (608)265-8491. Fax: (608)262-5345.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b01926.

Mass spectra of identified glycosylated peptide hormones (PDF)

Identified O-glycosylated peptide hormones from mouse and human islets (XLSX)

Notes

The authors declare no competing financial interest.

Signaling peptides, including neuropeptides and peptide hormones, represent a major class of signaling molecules utilized by nervous and endocrine systems and are known to regulate a broad spectrum of physiological processes.^{1,2} Virtually all bioactive signaling peptides are produced from a larger, inactive molecular precursor which will undergo a series of enzymatic processing and post-translational modifications (PTMs) to finally render mature, bioactive forms.³ In addition to a peptide's primary sequence, PTMs also play a critical role in determining its structure and functionality.⁴ As mass spectrometry (MS) has become one of the most powerful and efficient tools for protein and peptide analysis, increasing attention has been directed to study the various PTMs occurring on peptides and proteins.^{5,6} Among all PTMs, glycosylation is one of the most challenging to study because of the variability and heterogeneity in the attached glycans, labile modification, and the isobaric nature of many of these glycans. While it has become routine to consider PTMs such as amidation and acetylation when sequencing signaling peptides, it is still difficult to identify glycosylation due to its extremely low abundance and a lack of enabling techniques.⁷⁻⁹ Structural characterization of glycosylated signaling peptides encompasses peptide identification, locations of glycan attachment sites, and evaluation of glycosylation site microheterogeneity. It is currently still challenging to collect all this information in a single mass spectrum, even though multiple fragmentation techniques have been developed. Peaks resulting from glycosidic bond cleavages dominate spectra generated by collision-induced dissociation with little knowledge of glycosylation sites and amino acid sequences, whereas c/z-ion series in electron-transfer dissociation (ETD) type of experiments yield the glycosylation site and peptide identity with little information on glycan side chain composition.^{10,11} The complex microheterogeneity of glycan and the often unpredictable nature of signaling peptide cleavage site also add to the problem by exponentially increasing the search space of any bioinformatics tools.^{2,12,13}

Pancreatic islets are endocrine cells that secrete various peptide hormones, including insulin, glucagon, and glicentin, to collectively control blood sugar level. Insulin has longestablished effects on the metabolism of carbohydrates, fats, and proteins by primarily promoting the absorption of glucose into cells.¹⁴ Mature insulin is derived from proinsulin and consists of two polypeptide chains, the A and B chains, linked together by disulfide bonds.^{15,16} Although most mammals have one insulin gene, mice and rats are unusual for having two nonallelic insulin genes.¹⁷ Insulin-2 is the murine homologue of the human insulin gene, and insulin-1 is thought to have evolved by a gene retroposition.¹⁷ Type 1 and 2 diabetes as well as a wide spectrum of diseases have been proven to be directly caused by or associated with

insufficient insulin supply or insulin resistance.^{18,19} In addition to its primary sequence, the characterization of its PTMs is very important because these modifications can alter molecular structure and bioactivity to various extents.^{20,21} Here for the first time we report the MS characterization of *O*-linked glycosylation on mouse insulin-1B and -2B chains, human insulin-B chain, and their corresponding C peptides as well as several other signaling peptides. A novel targeted MS-based method is developed, combining top-down, bottom-up MS methods and different fragmentation techniques to allow improved sequence coverage and accurate glycosylation site localization. Insulin and its related peptides are critical to homeostasis, and their dysregulation has been implicated in a wide spectrum of diseases. Previous studies with glycosylated insulin analogues also suggested altered bioactivity due to glycosylation.^{22–25} This discovery could provide another perspective toward diabetes research and potential therapeutic treatments.

EXPERIMENTAL SECTION

Isolation of Pancreatic Islets.

All animal studies were conducted at the University of Wisconsin, were preapproved by the University's Research Animal Resource Center, and were in compliance with all NIH animal welfare guidelines. Mouse islets were isolated using a collagenase digestion as previously described.²⁶ Briefly, the mouse pancreas was injected through the common bile duct with 0.6 mg/mL Type XI collagenase (Sigma-Aldrich) in HBSS with 0.35 g/L sodium bicarbonate and 0.02% RIA grade BSA. Following removal from the animal, the pancreas was incubated at 37 °C for 16 min with periodic shaking. The digested pancreas was then washed twice with HBSS before being passed through a 100 μ m mesh and subjected to a Ficoll gradient. Finally, islets were collected at room temperature using a stereomicroscope while maintained in Krebs–Ringer bicarbonate buffer (KRB) containing (in mM): 118.41 NaCl, 4.69 KCl, 2.52 CaCl₂, 1.18 MgSO₄, 1.18 KH₂PO₄, 25 NaHCO₃, and 5 HEPES supplemented with 0.2% BSA and 16.7 mM glucose. Islets were washed twice with phosphate buffered saline (PBS) and centrifuged at 170g, for 2 min each. The PBS supernatant was removed, and the islet pellet was stored at –80 °C until lysis for mass spectrometry.

All human islets were received through the Integrated Islet Distribution Program (IIDP). Upon arrival, human islets were cultured overnight in RPMI containing 8 mM glucose, supplemented with penicillin (100 μ g/mL) and streptomycin (100 μ g/mL) (Pen/Strep), and 10% heat-inactivated FBS. Islets were then stored at –80 °C until lysis for mass spectrometry.

Islet Peptide Extraction.

Microcon YM-30 cutoff filters were rinsed with 2 $\times$ 80 μ L 20%/30%/50% ACN/MeOH/H₂O and centrifuged (2 $\times$ 15 min, 13 000g). Mouse and human islets were sonicated at 4 °C with a sonic dismembrator (3 $\times$ 8 s) (Thermo Fisher Scientific) in 100 μ L of acidified methanol (MeOH/H₂O/HAc = 90/9/1, v/v/v). Extract was centrifuged at 20 000g, 10 min, 4 °C. Supernatant was transferred to the YM-30 filter and spun through (20 min, 14 000g at 4 °C).

Reduction, Alkylation, and Trypsin Digestion.

Peptide extract was resuspended in 100 μL of 50 mM ammonium bicarbonate buffer, reduced (5 mM DTT, 1 h at room temperature), and alkylated (15 mM IAA, 30 min at room temperature in the dark). Alkylation was capped by incubation in 5 mM DTT for 5 min at room temperature. For top-down analysis, samples were desalted with C18 solid-phase extraction and dried down under vacuum. For bottom-up analysis, each sample was digested with 1 μL of trypsin for 6 h at 37 $^{\circ}\text{C}$, desalted with C18 solid-phase extraction, and dried down under vacuum.

LC-MS/MS Method and Label-Free Quantitation.

All LC-MS experiments were performed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific) interfaced with a Dionex Ultimate 3000 UPLC system (Thermo Fisher Scientific). A binary solvent system composed of H_2O containing 0.1% formic acid (A) and ACN containing 0.1% formic acid (B) was used for all analysis. Peptides were loaded and separated on a 75 $\mu\text{m} \times 15\text{ cm}$ self-fabricated column packed with 1.7 μm , 150 $\text{\AA}$, BEH C18 material obtained from a Waters UPLC column (part no. 186004661). Samples were loaded with 3% solvent B, and solvent B was linearly ramped to 30% in 90 min, ramped to 75% in another 20 min. For the Orbitrap Fusion Lumos analysis, the full MS spectra were acquired in an Orbitrap at a resolution of 120 000. The Top Speed method was enabled to ensure full MS spectra were acquired every 3 s. Most abundant precursor ions were selected for data-dependent HCD at a resolution of 30 000 and a normalized collision energy of 35%. If peaks at m/z 138.0545 (HexNAc oxonium fragment ions), m/z 204.0867 (HexNAc oxonium ions), or m/z 366.14 (HexHexNAc oxonium ions) ($\pm m/z$ 0.01) were within the top 30 most abundant peaks, a subsequent electron-transfer/higher-energy collision dissociation (EThcD) MS/MS scan of the precursor ion in the Orbitrap at a resolution of 60 000 was triggered.^{7,27} These glycan oxonium ions were chosen as *N*-linked glycosylation is almost inevitably initiated by *N*-acetylglucosamine (GlcNAc), and the vast majority of *O*-glycosylation is also initiated by *N*-acetylgalactosamine (GalNAc) or GlcNAc. Though in rare cases an *O*-glycosylation can start with fucose or glucose, they are very likely to be extended by addition of these GalNAc and GlcNAc.²⁸ Therefore, this provides a very general method for researchers to look for these modified molecules in a targeted fashion.

Data Processing.

Peptide identification was performed with Byonic software. EThcD spectra were searched against a human or mouse neuropeptide database (<http://isyslab.info/NeuroPep/>).²⁹ A precursor tolerance of 10 ppm and a fragment ion tolerance of 0.02 Da were allowed. Carbamidomethylation of cysteine residues was set as static modification. Oxidation of methionine residues was set as rare dynamic modification. Common dynamic modifications consisted of C-terminal amidation and *O*-linked glycosylation. Label-free quantitation was performed in Skyline, and MS1 peak area for each peptide was normalized to total ion chromatography.³⁰

RESULTS AND DISCUSSION

O-Linked glycosylation has been widely recognized as a critical factor in controlling protein folding and functionality.³¹ Most of the previous studies have been primarily focused on glycoproteins.²⁸ Small signaling peptides represent another major class of molecules responsible for intercellular communication,^{2,32} yet their glycosylation study has largely been ignored due to a lack of enabling analytical techniques. However, a few existing reports did indicate that such modification could also occur on these small signaling peptides and could significantly change its biological property.^{8,33} Since signaling peptides often exist in extremely low abundance, it becomes even harder to perform any type of enrichment on this unique modification. To avoid signal suppression from unmodified peptides and improve instrument sensitivity, a targeted MS approach is needed. We took advantage of diagnostic oxonium ions produced with HCD and only allow an additional hybrid EThcD^{7,34} scan to be performed on those potentially glycosylated precursors that produced these signature oxonium ions (Figure 1).²⁷ This novel method enabled us to perform the first large-scale identification of *O*-glycosylated signaling peptides and discovered several biologically important peptides exhibiting such modification.

Due to our targeted MS approach, improved sensitivity and selectivity were achieved enabling the large-scale discovery of naturally occurred *O*-linked glycosylation on insulin and several other signaling peptides. Table 1 lists all glycoforms identified on mouse insulin-B chains and -C peptides. Top-down and bottom-up MS approaches coupled with HCD and EThcD provided confident localization and identified multiple glycan compositions, containing up to four monosaccharides on the threonine toward the C-terminus. Similar glycosylation patterns also exist in human islets as revealed by applying the same strategy (Table 1). Briefly, mouse or human islets were isolated, and peptide extraction was performed with acidified methanol following previously described protocols.^{6,35} Homogenate was centrifuged and filtered through a Microcon YM-30 cutoff filter. Peptides were then reduced (5 mM DTT) and alkylated (15 mM IAA). Samples for top-down analysis were desalted with C18 solid-phase extraction, and samples for bottom-up analysis were digested with trypsin and desalted. MS spectra were collected on an Orbitrap Fusion Lumos instrument (Thermo Fisher Scientific) with a HCD-triggered EThcD method. Data was first analyzed with a Byonic¹² software package which provided glycopeptide sequencing capability and further manually annotated.

Illustrated in Figure 2 are annotated mass spectra of one glycoform from mouse insulin-1B chain. Intact *O*-glycosylated peptide was captured and characterized by both HCD and EThcD. The EThcD spectrum (Figure 2A) was processed with Xtract (Thermo Fisher Scientific) to deconvolute multiply charged ions (Figure 2C) which allowed for manual annotation of additional product ions. EThcD provided both b/y- and c/z-ion series to achieve nearly complete sequence coverage and confident glycosite localization. Although HCD-generated b and y ions containing threonine residue did not usually retain the glycan side chains, the modifications were well-preserved on the peptide backbone during radical-induced fragmentation with ETD, indicated by c- and z-ions containing the threonine residue with intact glycans attached.^{7,34,36,37} As indicated by c28 and z4 ions in Figure 2C and c6 and z4 ions in Figure 2D, the 3-residue glycan side chain was mapped to Thr27. It is also

worth pointing out that, in the tryptic peptide, the missed cleavage at lysine might be caused by the nearby glycan side chain blocking access of trypsin.³⁸ In addition, diagnostic glycan oxonium ions at m/z 186, 204, and 366 Da suggested the presence of *N*-acetylhexoseamine (HexNAc) and hexose (Hex) while product ions at m/z 274 and 292 suggested the presence of *N*-acetylneuraminic acid (NeuAc). Corresponding neutral loss from its precursor also corroborated this glycan composition.^{39,40} Another critical piece of information is differentiation of isobaric monosaccharide species, namely, GalNAc and GlcNAc. *O*-GlcNAc generally occurs within the nuclear and cytoplasmic compartments and is often not extended to form complex structures.²⁸ GalNAc-initiated *O*-glycosylation tends to occur on extracellular proteins along the secretory pathway. We took advantage of the difference in oxonium ion fragmentation profiles between GalNAc and GlcNAc and assigned insulin to be GalNAc based on the GlcNAc/GalNAc ratio.⁴¹ According to Halim et al., GlcNAc and GalNAc generate distinctive HCD fragmentation profiles that can be utilized to calculate GlcNAc/GalNAc ratio, with a ratio below 1 indicating the presence of GalNAc and a ratio above 1 indicating GlcNAc. Ratios for all identified glycosylated insulins are listed in Table 1, and with one exception for the human insulin-B chain that has core 2 glycan structure with both GalNAc and GlcNAc (ratio = 1.2), all other ratios suggest the presence of GalNAc instead of GlcNAc. Along with the fact that *O*-GlcNAc is primarily found within the nuclear and cytoplasmic compartments of the cell without signal sequences and not elongated or modified to form complex structures, insulin, which possesses signal peptide, needs to be secreted, and has several complex glycoforms, is likely modified by GalNAc rather than GlcNAc along the secretory pathway.^{28,42}

Attachment of sugar molecules to insulin could significantly change its property and functional activities. Several previous studies attempted to generate insulin derivatives with sugar attachment and demonstrated altered receptor affinity and bioactivity.^{22,24,43} Glycosylated insulin also has been explored to develop a self-regulating delivery system based on the competitive binding nature of blood glucose and glycosylated insulin to lectin.^{44–46} In addition, it is intriguing to observe quantitative level changes of these glycosylated peptides in diabetic mice compared to healthy controls. Therefore, natural *O*-glycosylation on signaling peptides might possess a yet unexplored regulatory mechanism of these chemical messengers in various biological systems.

In addition to insulin-B chains, we found several other signaling peptides that also possessed *O*-linked glycosylation from mouse and human islets (Table 2 and Table S1). Thr21 on insulin-1C peptide and Thr23 on insulin-2C peptide can be glycosylated in mouse, whereas at a similar position, Ser20 in the insulin-C peptide can be glycosylated in human as there is no Thr in the sequence (Table 1). Islet amyloid polypeptide (IAPP) is a 37-residue peptide hormone, one of the major products of β -cells of the pancreatic islets that is cosecreted with insulin.⁴⁷ Our results confirm findings reported in previous studies that identified *O*-glycosylated threonines at positions 6 and 9.^{48,49} Furthermore, we identified a novel glycosite of IAPP at Thr30 from mouse islets. As IAPP has a regulatory role in insulin, secretion and glycosylation may affect its conformation and binding property; glycosylation on multiple sites may indicate different modulatory effects on IAPP activity. Conserved glycosylation was observed in BigLEN in both mouse and human islets, which has been proposed to function as a neuropeptide involved in regulating body weight.⁵⁰ Another

interesting discovery was glycosylation on somatostatin. Even though it was previously reported that somatostatin-22 from catfish could be glycosylated,⁵¹ mammalian somatostatin-14 or -28 has never been reported to have such modification. Somatostatin is a potent inhibitor of insulin secretion, and therefore, any modification may indicate an altered regulatory effect.^{52,53} In addition to glycosylation on mature and intact peptide hormones, multiple truncated sequences from large peptide hormones, including glicentin and chromogranin-A, with flanking dibasic residues were identified (Table S1). The presence of characteristic dibasic residues (KR, KK, or RR) was often considered as potential endoproteolytic cleavage sites to generate neuropeptides or peptide hormones.^{3,54,55}

It was also important to quantitatively characterize these glycopeptides. Most of these peptides have been implicated in diabetes, with insulin being a key player. We chose to use normal BTBR^{+/2} mice and BTBR^{ob/ob} mice with type 2 diabetes (T2D), to study how these peptides would change with diabetes. Label-free quantitation was performed with biological triplicates, and peptide peak areas were normalized to total ion chromatogram. As illustrated in Figure 3A, unmodified insulin had larger peak areas, indicating higher expression levels in BTBR^{+/2} mice compared to BTBR^{ob/ob}. Student's *t*-test also suggested significant changes with a *p*-value <0.05. Glycosylated insulins produced signals with several orders of magnitude lower than their unmodified counterparts. However, this does not imply that glycosylated insulin is present at several orders of magnitude lower abundance. Simply comparing signal intensities between modified and unmodified peptides is not a valid quantitative approach as they could have dramatically different ionization efficiency and signal response due to the modification. More work needs to be done in order to determine what portion of insulin is glycosylated and its biological implications. Comparison of peak areas revealed the same decreasing trend in diabetic mice (Figure 3B). The most dramatic change occurred on mouse insulin-2B chain linked to HexNAc(1)Hex(1)NeuAc(1). Although it had an intense LC peak in BTBR^{+/2} mice islet extract, it only produced close-to-noise response in BTBR^{ob/ob}, representing significant downregulation and negligible existence in diabetic mice.

Quantitation was also performed on IAPP and BigLEN (Figure 3C). IAPP contributes to the progression of diabetes by aggregating into pancreatic islet amyloid deposits, which have a proven impact on the pathogenesis of type 2 diabetes.⁴⁷ Therefore, it was not surprising that IAPP was up-regulated in BTBR^{ob/ob} mice with T2D. Similarly, four out of five glycosylated IAPPs exhibited up-regulation with T2D. Even though there is no direct evidence relating BigLEN to diabetes, it is a ProSAAS-derived neuropeptide closely associated with food intake and body weight.⁵⁶ Its overexpression can result in overweight characteristics, and ProSAAS-derived peptides are known to affect protein convertase activity, which may be related to pro-insulin processing.^{56,57}

CONCLUSIONS

In conclusion, we have developed a novel and efficient method to discover and characterize glycosylated peptide hormones and neuropeptides. This targeted MS approach involves an initial quick HCD scan to specifically look for peptides that can produce diagnostic glycan oxonium ions and only trigger a subsequent EThcD scan on those potentially glycosylated

peptides. Because typical EThcD experiments require extra time for electron-transfer reaction, this new approach enables saving instrument time and allows targeted discovery of modified peptides. Furthermore, as signaling peptides often exist in very low abundance and their modified counterparts could be present at even lower levels, this targeted MS strategy avoids spending too much instrument time on unmodified peptides unnecessarily and reduces the risk of missing detection of those low-abundance modified signaling peptides. Additionally, these pilot HCD scans generate unique product ion patterns, which can be utilized to help differentiate GalNAc from GlcNAc due to their distinct HCD fragmentation profiles. Unlike tryptic peptides, signaling peptides can have a wide range of amino acid sequence lengths, which could be difficult to fully characterize with MS. The combination of top-down and bottom-up strategies takes advantage of both techniques and produces a more complete picture of these important signaling peptides in biological systems. The application of this novel targeted MS approach has allowed identification of glycosylated insulin-B chain, insulin-C peptide, and related peptide hormones in mouse and human pancreatic islets for the first time. Semiquantitative analysis of multiple glycoforms revealed differential regulation of both unmodified and modified peptides between healthy and T2D mice. The existence of these glycosylated peptide hormones may have a variety of functional implications. For example, glycosylation may alter insulin conformation and therefore its binding property and biostability. These possibilities are currently being investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Workflow for identifying endogenously *O*-glycosylated signaling peptides. Endogenous peptides from mouse or human pancreatic islets were extracted by homogenization in an acidified methanol and filtered through a 30 kDa molecular weight cutoff filter. Top-down and bottom-up analyses were carried out using a high-energy collisional dissociation (HCD) product ion-triggered electron-transfer high-energy collisional dissociation (EThcD) approach.

Figure 2.

Spectra of one mouse insulin-1B chain with *O*-linked GalNAc(1)Hex(1)NeuAc(1) on Thr27. Top-down (A) mass spectrum of the intact insulin-1B chain was acquired with EThcD and deconvoluted to reveal more product ions (C). Bottom-up spectrum of the tryptic peptide containing the glycosylation site (D) was also acquired with EThcD. HCD fragmentation was performed on the intact form, and its low mass region was enlarged (B). Dotted red lines denote b/y ions that were formed with glycans detached, and all annotated c- and z-ions containing the threonine residue have intact glycans attached, as denoted by the solid red lines (D).

Figure 3.

Label-free relative quantification of regular insulin (A), glycosylated insulin (B), and IAPP and BigLEN (C). For each peptide, the peak area was calculated and log 10 transformed. Bar graph represents mean + standard deviation. Asterisk indicates a statistically significant difference ($p < 0.05$) when compared to BTBR⁺². Superscript a, b, and c denote glycosylation sites as labeled in Tables 1 and 2.

Table 1.

Detected Glycosylated Insulin-B Chains from Mouse and Human Islets

	glycan masses	M + H	GlcNAc/GalNAc
Mouse (<i>Mus musculus</i>) Insulin-1B Chain			
-FVVKQHLC* ^q GPHLVEALYLVC* ^q GERGFFYT ^q PKS.-	0	3552.7918	
^q HexNAc(1)Hex(1)	365	3917.924	0.82
^q HexNAc(1)Hex(1)NeuAc(1)	656	4209.0914	0.67
Mouse (<i>Mus musculus</i>) Insulin-2B Chain			
-FVVKQHLC* ^q GSHLVEALYLVC* ^q GERGFFYT ^q PMS.-	0	3545.7166	
^q HexNAc(1)Hex(1)NeuAc(1)	656	4201.9442	0.58
^q HexNAc(1)Hex(1)NeuAc(2)	947	4493.0396	0.54
Mouse (<i>Mus musculus</i>) Insulin-1C Peptide			
-EVEDPQVEQLGSGPGDLQT ^q LAEVARQ.-	0	3120.5655	
^q HexNAc(1)Hex(1)NeuAc(2)	947	4067.8988	0.53
^q HexNAc(1)Hex(1)NeuGc(1)NeuAc(1)	963	4083.8919	0.49
Mouse (<i>Mus musculus</i>) Insulin-2C Peptide			
-EVEDPQVAQLELGGPGAGDLQT ^q LAEVAQQ.-	0	3132.5678	
^q HexNAc(1)Hex(1)NeuAc(2)	947	4079.897	0.61
Human (<i>Homo sapiens</i>) Insulin-B Chain			
-FVNQHLC* ^q GSHLVEALYLVC* ^q GERGFFYT ^q PKT.-	0	3542.7347	
^q HexNAc(1)	203	3745.8141	0.71
^q HexNAc(1)Hex(1)	365	3907.8669	0.66
^q HexNAc(2)Hex(1)	568	4110.9463	1.21
^q HexNAc(1)Hex(1)NeuAc(1)	656	4198.9623	0.62
^q HexNAc(1)Hex(1)NeuAc(2)	947	4490.0577	0.62
Human (<i>Homo sapiens</i>) Insulin-C Peptide			

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	glycan masses	M + H	GlcNAc/GalNAc
-EAEDLQVGQVELGGPGAGSLQPLALEGSLQ-	0	3019.5195	
EDLQVGQVELGGPGAGS ^d LQPLALEGSLQ-	656	3346.6238	0.56
^d HexNAc(1)Hex(1)NeuAc(1)			
L.GGGPGAGS ^d LQPLALEGSLQ-			
^d HexNAc(1)Hex(1)NeuAc(2)	947	2656.2119	0.59

^dDenotes glycosylation. C
* denotes carbamidomethylation.

Table 2.

Intact Glycosylated Peptide Hormones Identified from Mouse Islets

	glycan nominal mass	GlcNAc/GalNAc
IAPP		
-.KC*NTAT ^a C*AT ^a QRLANFLVRSSNNLGPVLPPT ^c NVGSNTY.-	947	0.62
^a HexNAc(1)Hex(1)NeuAc(2)		
^a HexNAc(1)Hex(1)NeuAc(1)	656	0.6
^b HexNAc(1)Hex(1)NeuAc(1)	656	0.72
^c HexNAc(1)Hex(1)NeuAc(2)	947	0.57
^c HexNAc(1)Hex(1)NeuAc(1)	656	0.65
BigLEN		
-.LENPS ^a PQAPARRLLPP.-		
^a HexNAc(1)Hex(1)NeuAc(1)	656	0.8

^aDifferent glycosylation site.

^bDifferent glycosylation site.

^cDifferent glycosylation site.