

Tyrosine O-GalNAc Alters the Conformation and Proteolytic Susceptibility of APP Model Glycopeptides

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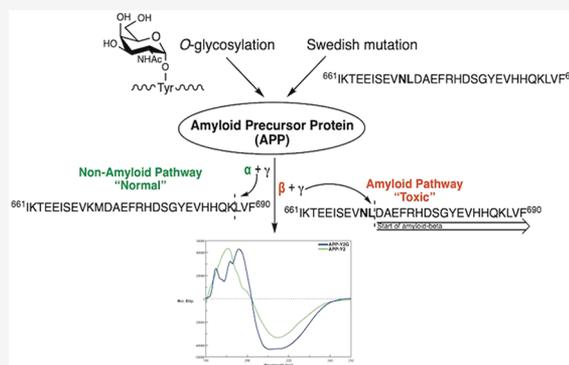
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ABSTRACT: The amyloid- β precursor protein (APP) undergoes proteolytic cleavage by α -, β -, and γ -secretases, to determine its fate in Alzheimer's disease (AD) pathogenesis. Recent findings suggest a possible role of O-glycosylation in APP's proteolytic processing. Therefore, we synthesized native and Swedish-double-mutated APP (glyco)peptides with Tyr⁶⁸¹-O-GalNAc. We studied conformational changes and proteolytic processing using circular dichroism (CD) spectroscopy and enzyme cleavage assay, respectively. CD analysis was carried out in four solvent systems to evaluate peptide environment and O-glycosylation induced conformational changes. The Swedish mutation and Tyr⁶⁸¹-O-GalNAc were the key factors driving conformational changes. Furthermore, the level of α - and β -secretase activity was increased by the presence of mutation and this effect was more pronounced for its glycosylated analogues. Our results suggest that O-glycosylation of Tyr⁶⁸¹

can induce a conformational change in APP and affect its proteolytic processing fate toward the amyloidogenic pathway.

KEYWORDS: Alzheimer's disease (AD), APP, O-glycosylation, tyrosine, CD analysis, proteolytic cleavage



Glycosylation is a frequent and heterogeneous post-translational protein modification occurring in all domains of life.¹ It is estimated that >50% of all proteins are glycosylated and serve several important brain functions such as memory and learning along with protein folding and cell–cell interactions.² Several studies have indicated that altered protein glycosylation can also lead to the onset and progression of diseases, including Alzheimer's disease (AD).³ AD is caused by extracellular deposits consisting of amyloid β -peptide ($A\beta$) forming fibrils, which are derived from amyloid precursor protein (APP), a type 1 transmembrane glycoprotein, during amyloidogenic processing by β - and γ -secretases.⁴ In contrast, the cleavage of APP by α - and γ -secretases results in the nonamyloidogenic pathway that protects the brain from β -amyloid deposition.^{5,6} A small percent of AD is also caused by single genetic mutations that are passed down through families known as familial AD (FAD). The Swedish double mutation (Lys670Asn/Met671Leu), located near the N-terminus region of $A\beta$, results in a major increase in the total output of $A\beta$ 40 and $A\beta$ 42 by providing a better substrate for the β -secretase enzyme.^{7,8}

APP undergoes both N- and O-glycosylation prior to processing and $A\beta$ production.⁹ Two potential N-glycan sites in APP have been identified at Asn467 and Asn496, and their importance in the sorting and secretion of APP has been reported.¹⁰ The identification and the effect of O-glycans on APP function and its subsequent role in AD pathogenesis remain challenging. Two types of O-glycan structures have

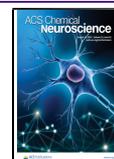
been identified in APP, the complex O-GalNAc glycans, also known as mucin type O-glycans,^{11–14} and cytoplasmic O-GlcNAcylation, which involves an attachment of single GlcNAc to serine and threonine residues.^{15,16} It has been proposed that increased O-GlcNAcylation can affect trafficking and final subcellular localization of APP.^{17,18} Considering the distinct effects of mucin type O-glycosylation on the proteolytic activity of many enzymes,^{19,20} O-GalNAcylation may affect the proteolytic cleavage of APP and/or its secretion.^{21,22}

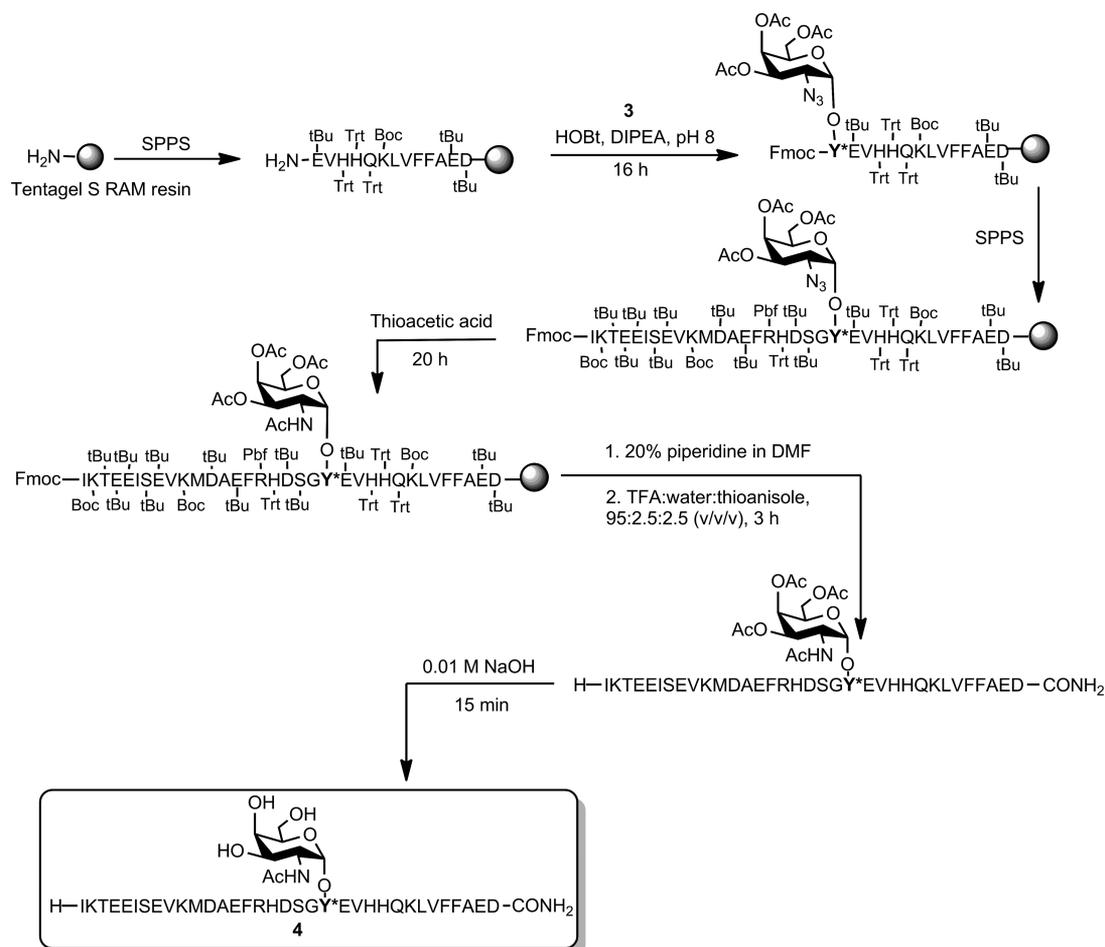
Intrigued by the number of mucin-type O-glycan sites that have been reported for APP, we decided to focus on a unique type of O-glycosylation, where APP is modified with an O-GalNAc residue on the phenolic hydroxyl group of the only tyrosine (Tyr⁶⁸¹) present in the $A\beta$ 42 sequence at position 10. The O-glycans linked to Tyr⁶⁸¹ resemble sialylated core-1 mucin-type (Neu5Ac)_{1–2}Gal β 3(Neu5Ac)GalNAc α 1-O-glycans.^{11,13,23} This modification was first discovered in 2011 in a set of short glycosylated $A\beta$ fragments, 15–20 amino acids in length from cerebrospinal fluid (CSF) of AD patients.¹¹ We

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Scheme 1. Stepwise Synthesis of APP Tyr⁶⁸¹-O-glycopeptide 4Table 1. Characterization of APP (Glyco)peptides 4–7 by Analytical RP-HPLC and MALDI-MS^a

APP-Tyr-(glyco)peptides	sequence	RP-HPLC	MALDI-TOF MS (M + H) ⁺	
		t _R (min)	calcd (Da)	obsd (Da)
APP-Y1G (4)	IKTEEISEVKM~DAEFRHDSGY*EVHHQK~LVFFAED	17.1	4265.14	4265.41
APP-Y1 (5)	IKTEEISEVKM~DAEFRHDSGYEVHHQK~LVFFAED	17.6	4062.09	4062.27
APP-Y2G (6)	IKTEEISEVNL~DAEFRHDSGY*EVHHQK~LVFFAED	17.8	4231.99	4232.71
APP-Y2 (7)	IKTEEISEVNL~DAEFRHDSGYEVHHQK~LVFFAED	18.2	4030.02	4029.37

^aY* = Tyr⁶⁸¹-O-GalNAc, NL = Swedish mutation, M~D and L~D = β -secretase cleavage sites, and K~L = α -secretase cleavage site. RP-HPLC conditions as described in [Methods](#). Retention times (t_R) are given in minutes.

hypothesized that Tyr⁶⁸¹-O-glycosylation and the Swedish mutation can influence conformation and proteolysis of A β (glyco)peptides by α - and β -secretases.

In this study, we synthesized an α -linked GalNAc-Tyr⁶⁸¹ building block, which was incorporated in A β glycopeptide analogues with extended N-terminal domain to include the β -secretase cleavage site with or without the Swedish mutation. Next, these analogues were analyzed for their secondary structure content using CD spectroscopy. Lastly, the analogues were studied in an enzyme cleavage assay for their proteolytic susceptibility toward α - and β -secretase. Collectively, our results demonstrate that the conformation of the Tyr⁶⁸¹-O-glycosylated analogues bearing the Swedish mutation is complex, with ratios of β -structure, α -helix, and random coil being strongly dependent on solvent environment. Moreover, the site-specific Tyr⁶⁸¹-O-glycosylation and Swedish mutation accelerate cleavage by both secretases and provide the first

evidence of a direct correlation between GalNAc-Tyr⁶⁸¹ modified glycopeptides and APP's structural and cleavage properties.

Preparation of GalNAc-Tyr⁶⁸¹ Building Block and APP Model (Glyco)peptides. The assembly of Tyr⁶⁸¹-O-glycosylated peptides bearing “mucin-type” glycosylation, α -N-acetylgalactosamine (GalNAc), was performed using standard Fmoc-based automated solid-phase peptide chemistry. The building block approach was used for the incorporation of the O-glycosylated Tyr 3 into an APP 661–694 region of the protein ([Scheme 1](#)). We used Koenigs–Knorr activation conditions for the O-glycoside formation.^{24,25} The procedure involved coupling of the azido chloride derivative 2 with the pentafluorophenyl ester of Fmoc-protected Tyr to form Fmoc-Tyr(2-azido-1- α -D-Gal)-OPfp 3 (see the [Supporting Information](#), [Scheme S1](#), pages [S2–S6](#)). The purity of the Tyr building

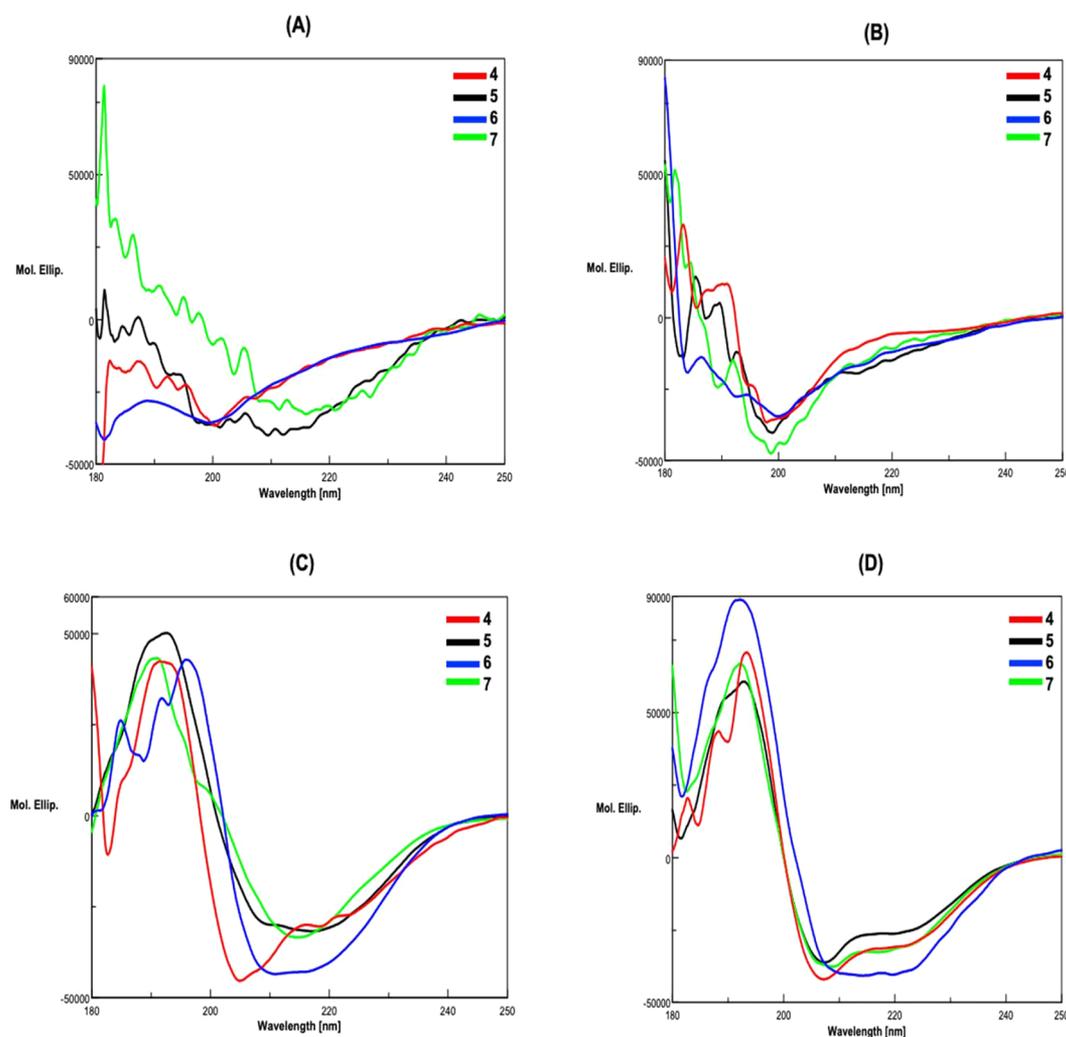


Figure 1. Circular dichroism spectra of APP Tyr (glyco)peptides 4–7 in (A) water, (B) 10 mM sodium phosphate buffer, pH 7.4, (C) TFE/water = 1:1 (v/v), and (D) 100% TFE at 25 °C.

block 3 was confirmed by RP-HPLC and MALDI-MS. NMR spectra ascertained the α -linkage.

(Glyco)peptides were prepared using an automated solid phase peptide synthesis (SPPS) approach on Tentagel S RAM resin. The exception was the glycosylated building block 3, that was coupled manually at the desired site of glycosylation of the growing peptide chain. After completion of the peptide sequence, the resin was treated with thioacetic acid for direct one-pot reductive acetylation of the azido group on the sugar moiety. Glycopeptide cleavage from the resin was performed using trifluoroacetic acid (TFA) with thioanisole and water as scavengers, followed by deprotection of acetyl groups from the sugar moiety under basic conditions to obtain the final deacetylated glycopeptides 4 (Scheme 1) and 6. The corresponding nonglycosylated peptides 5 and 7 were also cleaved from the resin in a similar manner. (Glyco)peptides 4–7 were obtained in high purity, as indicated by their RP-HPLC elution profiles and MALDI-TOF MS analysis (Table 1 and see the Supporting Information, pages S7–S10).

The amino acid sequences of the four (glyco)peptide analogues are part of the APP 661–694 region and include the $A\beta$ -(1–23) fragment containing the α -secretase cleavage site (K~L). In order to study the enzyme activity by β -secretase, the sequences were extended at the *N*-terminus with either

IKTEEISEVKM or IKTEEISEVNL to incorporate its cleavage site (M~D or L~D). The RP-HPLC analysis revealed that the nonglycosylated peptide APP-Y2 (7) bearing the Swedish mutation exhibited a 0.6 min longer retention time (t_R) compared to the mutation-free APP-Y1 peptide 5. This difference in the t_R suggests the increased hydrophobicity of the peptide sequence containing the Swedish mutation.²⁶ As expected, upon the addition of the GalNAc moiety at Tyr⁶⁸¹, the overall hydrophilicity of the peptide APP-Y1G (4) and APP-Y2G (6) increased, resulting in a decrease in t_R by 0.5 and 0.4 min, respectively (Table 1).

Secondary Structure Analysis. Circular dichroism (CD) was used to study the role of Tyr⁶⁸¹-*O*-glycosylation on the conformation of (glyco)peptides in four different solvents, water, sodium phosphate buffer (10 mM, pH 7.4), 50% trifluoroethanol (TFE) in water (v/v), and 100% TFE (Figure 1). The percentages of secondary structures were calculated for all CD spectra using the BeStSel method (Table 2). This method uses an algorithm that allows quantitative estimation of β -sheet and distinguishes between parallel and antiparallel β -sheets, along with β -turns.²⁷

In water, the addition of *N*-terminal fragment, IKTEEISEVKM, to $A\beta$ -(1–23) in 5 resulted in disruption of the typical β -sheet secondary structure (Figure 1A) commonly associated

Table 2. Summary of the Secondary Content (%) Present in APP-Tyr-(Glyco)peptides 4–7 Determined by BeStSel for CD Spectra Obtained in (A) Water, (B) 10 mM Sodium Phosphate Buffer, pH 7.4, (C) TFE/Water = 1:1 (v/v), and (D) 100% TFE (v/v)^a

(A)					
APP-Tyr- (glyco) peptides	α -H (%)	β -AP (%)	β -P (%)	β -T (%)	RC (%)
APP-Y1G (4)	0.0	3.3	2.5	0.0	94.2
APP-Y1 (5)	0.0	3.1	10.5	0.0	86.4
APP-Y2G (6)	0.5	11.5	0.0	2.2	85.9
APP-Y2 (7)	31.1	19.2	17.4	15.9	16.3
(B)					
APP-Tyr- (glyco) peptides	α -H (%)	β -AP (%)	β -P (%)	β -T (%)	RC (%)
APP-Y1G (4)	6.0	27.4	0.0	14.5	52.1
APP-Y1 (5)	4.4	9.3	0.0	25.2	61.1
APP-Y2G (6)	26.7	0.0	0.0	34.7	38.6
APP-Y2 (7)	14.2	12.6	0.0	13.8	59.4
(C)					
APP-Tyr- (glyco) peptides	α -H (%)	β -AP (%)	β -P (%)	β -T (%)	RC (%)
APP-Y1G (4)	35.6	0.0	0.0	0.8	63.6
APP-Y1 (5)	44.3	0.0	13.2	0.1	42.4
APP-Y2G (6)	51.9	0.0	22.2	0.0	26.0
APP-Y2 (7)	23.2	4.6	23.2	0.0	49.0
(D)					
APP-Tyr- (glyco) peptides	α -H (%)	β -AP (%)	β -P (%)	β -T (%)	RC (%)
APP-Y1G (4)	58.2	0.0	0.0	0.0	41.8
APP-Y1 (5)	56.6	0.0	0.0	0.7	42.7
APP-Y2G (6)	86.4	0.8	10.3	1.1	1.4
APP-Y2 (7)	72.3	0.0	0.0	6.1	21.6

^aThe content is divided into α -helix (α -H), antiparallel β -sheet (β -AP), parallel β -sheet (β -P), β -turn (β -T), and random coil (RC).

with the CD spectrum for A β variants in water.^{28,29} Deconvolution of the spectra revealed that peptide 5 was 86.4% random coil and 13.6% β -sheet (Table 2A). Hence, the overall hydrophilicity of A β -(1–23) increased and the conformation of peptide 5 become largely disordered by the presence of polar and charged amino acids in the sequence. In contrast, the presence of a double-mutation K670N and M671L in peptide 7 led to a rise in α -helix, β -sheet, and β -turn content (31.1%, 36.6%, and 17.4%, respectively). The distribution of the α -helix and β -structure (parallel and antiparallel β -sheet and β -turn) content in 7 is similar to other A β mutant variants reported.²⁹ Site-specific O-glycosylation of Tyr⁶⁸¹ induced a change in the secondary structure of 4 by decreasing β -sheet and increasing random coil (5.8% and 94.2%, respectively) compared to its nonglycosylated counterpart 5. This effect is more pronounced for the Swedish-mutation containing peptide 6, where α -helix along with the β -structure content is significantly reduced (0.5% and 11.5%, respectively) compared to its nonglycosylated counterpart 7.

The spectra recorded in sodium phosphate buffer (10 mM, pH 7.4), mimicking physiological conditions relevant for α -secretase and β -secretase cleavage, showed a prominent presence of random coil (40–60%) and increase in β -turns (14–35%) compared to water (Figure 1B and Table 2B). It is not surprising that (glyco)peptides 4–7 are partially

disordered in a low ionic strength buffer such as 10 mM sodium phosphate.^{30,31} The synthesized (glyco)peptides carry a net negative charge at pH 7.4, thereby causing negatively charged phosphate anions in the buffer to contribute toward electrostatic interactions and create repulsion with the peptide backbone.³² The native glycopeptide 4 had the highest amount of antiparallel β -sheet and the Swedish-mutated glycopeptide 6 had the highest amount of β -turn in this solvent system. Evidently, modifications such as the Swedish mutation and O-glycosylation of Tyr⁶⁸¹ in APP can induce small perturbations in the conformational equilibrium.

For a better understanding of the changes that the microenvironment has on the secondary structure of the (glyco)peptides, the membrane-mimicking solvent TFE was used to probe helix formation and assess their structure-forming potential.³³ The spectra recorded in the mixture of TFE and water (1:1, v/v) showed characteristics of α -helix, β -sheet, and random coil (Figure 1C). The highest β -sheet content was found for peptide 7 carrying the Swedish mutation (Table 2C). Glycosylation disrupted β -sheet and slightly decreased α -helix content for the native sequence 4. However, there was no change in β -sheet content, and a significant increase in stabilization of α -helix was observed with glycopeptide 6. By increasing the ratio of TFE to 100%, the amount of α -helix content increased even further regardless of the sequence, 57% for peptide 5 and 72% for peptide 7, respectively (Table 2D). In addition, the loss of β sheet content was observed. Glycosylation had a minor effect on the native sequence 4, the ratio of α -helix and random coil was almost unchanged. On the contrary, the effect of glycosylation was more pronounced for the peptide with the Swedish mutation, where stabilization of the α -helix structure and regain of the β -sheet structure (10%) were observed. These findings suggest that even though the environment affected secondary structure, the Swedish mutation and Tyr⁶⁸¹-O-GalNAc were the key factors driving that effect.

Proteolysis by BACE1 and ADAM10. To gain insight into the role of Tyr⁶⁸¹-O-glycosylation on the proteolytic susceptibility of APP model peptides, enzyme cleavage assays were performed with β -secretase (BACE1) and α -secretase (ADAM10), respectively. Each enzyme produced two fragments upon cleavage, for which yields were determined after 24 h treatment. The yields of intact peptide and fragments were evaluated by the RP-HPLC peaks (Table 3, and the Supporting Information, pages S15–S26).

The native sequence 5 was not affected by BACE1 (100% peptide recovery), and 88.6% of peptide was recovered upon treatment with ADAM10. On the other hand, peptide 7, that

Table 3. Proteolytic Cleavage of APP-Tyr-(Glyco)peptides 4–7 upon Treatment with BACE1 and ADAM10 Enzymes^a

APP-Tyr-(glyco) peptides	BACE1		ADAM10	
	recovered (%)	cleaved (%)	recovered (%)	cleaved (%)
APP-Y1G (4)	100	0.0	86.5	13.4
APP-Y1 (5)	100	0.0	88.6	11.4
APP-Y2G (6)	57.4	42.4	64.2	35.7
APP-Y2 (7)	86.8	13.1	72.8	27.1

^aThe values were calculated as described in Methods with SD < 6%, and identity of fragments was determined by RP-HPLC and MALDI-TOF analysis (see the Supporting Information, pages S15–S26).

carries the Swedish mutation, was cleaved to a greater extent by both enzymes. However, the cleavage by ADAM10 (nonamyloidogenic pathway) was more efficient; 27.1% of the peptide was cleaved compared to 13.1% cleaved by BACE1 (amyloidogenic pathway). Glycosylation did not affect the proteolytic cleavage of the native sequence 4 by BACE1, but a small increase in the cleavage products was observed for ADAM10, 13.4% compared to 11.4% for its nonglycosylated counterpart 5. However, glycosylation had a more significant effect on the sequence carrying the Swedish mutation. BACE1 treatment resulted in 42.4% cleaved glycopeptide 6, whereas after ADAM10 treatment, 35.7% cleaved product was observed. Considering that a more significant increase in the proteolytic susceptibility of the glycopeptide 6 was detected for the BACE1 enzyme (29.4%) compared to the ADAM10 (8.6%), the BACE1 driven amyloid pathway could potentially offset the benefits of the increased shedding by ADAM10 to the nonamyloidogenic pathway. Thus, although the Swedish mutation is an important factor for BACE1 enhanced activity, the presence of the site-specific *O*-glycosylation of Tyr⁶⁸¹ is another key factor that can alter cleavage rates toward the amyloidogenic pathway, and potentially affect the subcellular localization of APP.

In summary, we have shown that the APP site-specific glycosylation on Tyr⁶⁸¹ with mucin-type glycosylation (α -GalNAc) and the Swedish mutation induces a conformational change and subsequently affects its proteolytic processing fate. The (glyco)peptides in this study exist in a conformational equilibrium between random coil, β -sheet, or α -helical structures, which is impacted by solvent variables such as buffer ions, polarity, etc. as well as internal modifications of the analogues such as mutation and glycosylation. In agreement with the recent findings, where fragments of Tyr *O*-glycosylation have been detected predominantly in CSF samples of AD patients,¹¹ our results show that the rate at which β -secretase cleaved glycopeptide 6 was significantly higher than that for α -secretase activity when compared to its nonglycosylated counterpart 7. Therefore, Tyr⁶⁸¹-*O*-glycosylation can shift the fate of APP toward the amyloidogenic pathway in the presence of the Swedish mutation. We believe that our studies, although at the current stage basic in nature, may pave the way for a better understanding of the role of glycosylation in APP processing and thus the development of novel AD-modifying therapies.

METHODS

Reagents. Tentagel S RAM resin was obtained from Advanced ChemTech (Louisville, KY). Fmoc-protected amino acids and coupling reagents for peptide synthesis were purchased from Chem-Impex (Wood Dale, IL). *N,N'*-Diisopropylethylamine (DIPEA), thioacetic acid, trifluoroacetic acid, and solvents (DCM, DMF, acetonitrile, and water) were of HPLC grade and were purchased from Fisher Scientific (Atlanta, GA) or Sigma-Aldrich (St. Louis, MO). Sodium phosphate buffer (0.01 M, pH 7.4) was prepared using sodium phosphate (mono- and dibasic) from Fisher Scientific (Atlanta, GA). The *O*-GalNAcylated building block of Tyr for glycopeptide synthesis was prepared and characterized as outlined in the Supporting Information, pages S2–S3. Recombinant human BACE-1 (rhBACE-1) and BACE-1 fluorogenic peptide substrate IV (MCA-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(DPN)-Arg-Arg-NH₂) were from R&D Systems (catalog #ES004 and #931-AS, respectively). Recombinant human ADAM10 and ADAM10 substrate (Mca-KPLGL-Dpa-AR-NH₂) were from R&D Systems (catalog #936-AD and #ES010, respectively).

Synthesis of APP (Glyco)peptides. Standard Fmoc chemistry was employed to synthesize all peptide analogues on a PS3 automated peptide synthesizer (Protein Technologies Inc., Tucson, AZ). The amino acid couplings were done using a 4-fold excess of amino acids, HOBt, and HCTU in the presence of 0.4 M *N*-methylmorpholine (NMM) in DMF. The Fmoc protecting group was removed using 20% piperidine in DMF. For glycopeptides, at the desired site of glycosylation, the Fmoc-protected pentafluorophenyl ester of *O*-glycosylated Tyr (3) (Supporting Information, page S2) was coupled manually using a 1.5-fold excess in the presence of DIPEA (pH 8) for 16 h. The completion of coupling was confirmed using the ninhydrin test. The on-resin reduction and acetylation of azide to *N*-acetyl was carried out using 4 mL of thioacetic acid for 20 h and cleaved from the resin using a TFA/thioanisole/water acid mixture in a 95:2.5:2.5 ratio for 3 h. The cleavage solution was then precipitated in cold methyl-*tert*-butyl-ether (MTBE) to yield the crude acetylated APP glycopeptides. Deprotection of the *O*-acetyl groups on the glycan of the glycopeptide sequence was done using a 0.01 M NaOH solution for 15 min to yield the final crude deacetylated glycopeptides.

Purification and Characterization of APP (Glyco)peptides. Peptide purification and analysis were performed on an Agilent Technologies 1260 Infinity system. The analytical RP-HPLC method uses an Aeris Peptide C18 column (250 × 4.6 mm, 5 μ m, 120 Å) at 0.8 mL/min flow rate, with 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B) as the eluents. The elution gradient for analytical RP-HPLC purification was 0 to 60% B over 30 min. The preparative RP-HPLC method uses the Grace Vydac monomeric C18 column (250 × 22 mm, 15–20 μ m, 300 Å) at 10 mL/min flow rate, with 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B) as the eluents. The elution gradient for preparative RP-HPLC purification was 0 to 50% B over 110 min. The peptide analogues were detected at 214 nm by using a UV-vis detector (Agilent 1260 Infinity DAD). Purified peptides were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Bruker Microflex system using α -cyano-4-hydroxycinnamic acid as matrix.

Circular Dichroism (CD) Spectroscopy. CD spectra of the APP (glyco)peptides were recorded in four solvent systems: water, 10 mM sodium phosphate buffer, 50% trifluoroethanol (TFE) in water (v/v), and 100% TFE. A quartz cell of 1 mm optical path length was used, and the spectra were measured over a wavelength range of 180–250 nm with a scanning speed of 100 nm/min and a response time of 4 s at 25 °C. The concentration of the (glyco)peptides was 0.065 mg/mL and was determined using RP-HPLC. All spectra were baseline-corrected to account for the signal contribution from solvent and then converted into molar ellipticity (deg cm² dmol⁻¹).³⁴ The percentages of secondary structures were determined using the BeStSel method.²⁷ All spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD).

Proteolysis. All APP-based substrates were prepared as 10 mM stocks in DMSO. Before proteolysis, the activity of BACE1 and ADAM10 was verified by the reaction with the fluorogenic BACE1 substrate Mca-SEVNLDAEFRK(Dnp)RR-NH₂³⁵ and fluorogenic ADAM10 substrate Mca-KPLGL-Dpa-AR-NH₂³⁶ respectively, as per the manufacturer's instructions. For the proteolysis assay, APP-based substrates were diluted in BACE1 activity buffer (0.1 M sodium acetate, pH 4.0) or ADAM10 activity buffer (10 mM HEPES, 0.001% Brij-35, pH 7.5) to the final assay concentration of 100 μ M. BACE1 was diluted to 50 nM final concentration. ADAM10 was diluted to 10 nM final concentration. Reactions were incubated overnight at 37 °C in the dark.

Analysis of BACE1 and ADAM10 Cleavage Products. After 24 h of incubation, the enzyme reaction solutions containing the APP (glyco)peptide with BACE1 or ADAM10 were analyzed using analytical RP-HPLC on the Aeris C18 column with 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B) as the eluent. The elution gradient was 0 to 60% B over 30 min with a flow rate of 0.8 mL/min. Detection was at λ = 214 nm. The percentages of intact and cleaved (*N*- and *C*-terminal) fragments in the absence and presence of secretases were evaluated by the integration of the RP-HPLC peaks.

Integrations were averaged from two injections, and product identification was achieved by MALDI-TOF.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acschemneuro.1c00387>.

Copies of NMR, RP-HPLC and MALDI-TOF spectra for 3–7, BeStSel secondary structure predictions, and characterization of enzyme cleavage products for 4–7 (PDF)

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Author Contributions

Y.S. and M.C. wrote the manuscript. D.O. conducted the synthesis of the glycosylated Tyr building block. Synthesis of glycopeptides and their CD analysis was done by Y.S. and A.M. Proteolytic cleavage assay was conducted by D.M. and Y.S.

Notes

The authors declare no competing financial interest.

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