REVIEW

Lectin-affinity chromatography brain glycoproteomics and Alzheimer disease: Insights into protein alterations consistent with the pathology and progression of this dementing disorder

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Alzheimer disease (AD) is a neurodegenerative disorder characterized pathologically by the accumulation of senile plaques and neurofibrillary tangles, and both these pathological hallmarks of AD are extensively modified by glycosylation. Mounting evidence shows that alterations in glycosylation patterns influence the pathogenesis and progression of AD, but the vast number of glycan motifs and potential glycosylation sites of glycoproteins has made the field of glycobiology difficult. However, the advent of glycoproteomics has produced major strides in glycoprotein identification and glycosylation site mapping. The use of lectins, proteins that have strong affinity for specific carbohydrate epitopes, to enrich glycoprotein fractions coupled with modern MS, have yielded techniques to elucidate the glycoproteome in AD. Proteomic studies have identified brain proteins in AD and arguably the earliest form of AD, mild cognitive impairment, with altered affinity for Concanavalin-A and wheat germ agglutinin lectins that are consistent with the pathology and progression of this disorder. This is a relatively nascent field of proteomics research in brain, so future studies of lectin-based brain protein separations may lead to additional insights into AD pathogenesis and progression.

1 Introduction

Alzheimer disease (AD), the seventh leading cause of death in the USA [1], is a neurodegenerative disorder character-

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Abbreviations: Asn, asparagines; **Aβ,** amyloid β-peptide; **Con A,** concanavalin A; **DSA,** *Daturastramonium*agglutinin; **GlcNAc,** N-acetylglucosamine; **HBP,** hexosamine biosynthetic pathway; **MCI,** mild cognitive impairment; **PNA,** peanutagglutinin; **p-tau,** hyperphosphorylated tau; **WGA,** wheat germ agglutinin

ized pathologically by the accumulation of senile plaques (SPs) and neurofibrillary tangles (NFTs). Alarmingly, while the rate of deaths due to illness that account for more deaths than AD, such as heart disease, stroke, and prostate cancer have been on the decline from 2000 to 2006, the rate of deaths attributed to AD have increased by 46% over the same time span [1]. By 2050, the number of annually diagnosed AD cases is projected to top 1 million people [1]. These imposing figures stress the need for increased research on AD pathogenesis and therapeutics to avert this arguably public health epidemic. The amyloid cascade hypothesis is currently the most popular explanation of AD pathogenesis and revolves around the 42 amino acid amyloid β-peptide (Aβ) that comprises the core of SP [2]. The amyloid cascade hypothesis states that the over-production and/or decreased clearance of Aβ results in the

formation of toxic Aβ oligomers triggering a multitude of events that lead to neuronal dysfunction, neuronal death, and the onset of AD [3, 4] (See Fig. 1). This hypothesis is supported by genetic studies examining missense mutations to the *amyloid precursor protein, presenilin 1,* and *presenilin 2* genes resulting in aberrant amyloid precursor protein metabolism and increased levels of amyloidgenic Aβ [5–12].

The amyloid hypothesis provides a compelling explanation of AD pathogenesis; however, the mechanisms associated with Aβ-induced neuronal dysfunction and death need further investigation. The field of proteomics is a powerful tool that analyzes the active cellular proteome under a myriad of experimental conditions designed to provide insight into disease mechanisms.

The scope of this review pertains to the observed changes in brain protein glycosylation patterns and the field of lectin-affinity-associated glycoproteomics in AD.

2 Proteomic methods for glycoprotein identification

2.1 Lectin-fractionation of glycoproteins

Protein glycosylation regulates many cellular processes including protein folding, protein regulation, targeting of proteins to appropriate organelles, and extracellular communication, and these processes are affected in AD [13, 14]. However, glycoproteomics studies are complicated by the mircoheterogeneity of glycoproteins in comparison to their non-glycosylated nascent forms [15]. The use of lectins, proteins that recognize specific carbohydrate motifs on the surface of glycoproteins, is the most commonly used technique to fractionate the glycoprotein sub-proteome [15]. Lectins that have been incorporated into AD glycoproteomics studies include concanavalin A (ConA), wheat germ

agglutinin (WGA), peanut agglutinin (PNA), *Datura stramonium* agglutinin, and Jacalin lectin. ConA has high affinity α-linked mannosyl and terminal glucosyl carbohydrate moieties that are the primary components of glycans associated with N-linked glycosylation [16–18]. However, it is important to note that ConA has a significant hydrophobic-binding domain and has been shown to bind proteins that do not fit the criteria of canonical N-linked glycosylation [19, 20]. WGA binds to N-acetylglucosamine (GlcNAc) and sialic acid residues that are commonly added to proteins during O-linked glycosylation [21]. PNA is specific for terminal galactosyl residues, while DSA targets β-linked GlcNAc that are found in N-linked glycans [22, 23]. Jacalin lectin has an affinity for galactosyl moieties coupled to N-acetyl-galactosamine in a β(1–3) linkage [24]. (See Table 1 for a summary of commonly used lectins and associated epitopes.) Lectin-affinity chromatography has the limitation of capturing only a subset of the glycoproteome associated with lectin epitope. However, the advent of multi-lectin columns targeted against common glycan motifs associated with specific tissues or bodily fluids have greatly enhanced the ability of lectins to comprehensibly capture the glycoproteome of the biological sample of choice. For instance, the studies of Yang and Hancock employed a multi-lectin column composed of ConA, WGA, and Jacalin for the fractionation of serum glycoproteins based on the high commonality of the aforementioned lectin epitopes for N-linked and O-linked glycans typical of serum proteins [25].

2.2 Enzymatic digestion and chemical modifications coupled to MS

Upon fractionation, glycoproteins are typically enzymatically digested and the resultant glycopeptides are further subjected to treatment with glucosidases to remove the
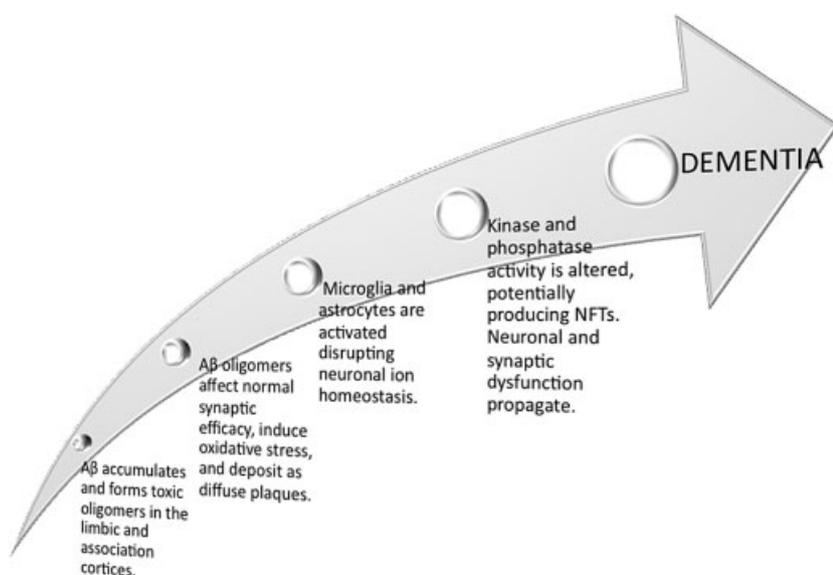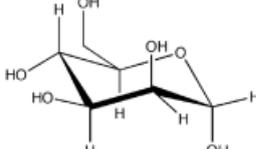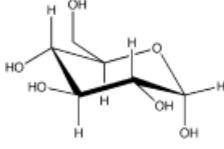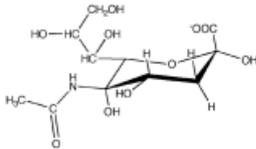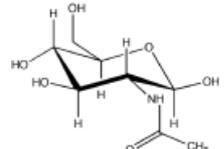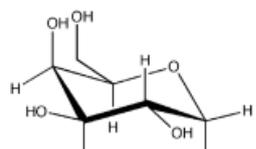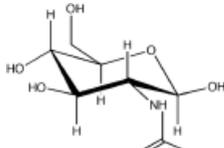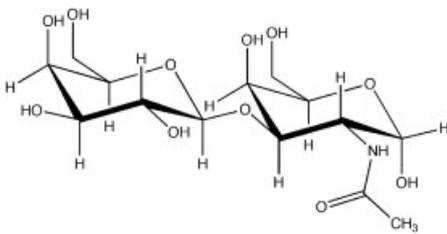

Figure 1. The amyloid cascade hypothesis of AD pathogenesis and progression.

Table 1. Commonly used lectins for glycoproteomics studies

Lectin	Carbohydrate epitope and structure
ConA	(i) α-Mannose
	(ii) Terminal glucose
WGA	(i) Sialic acid
	(ii) N-acetylglucosamine
PNA	Terminal galactose
DSA	N-acetylglucosamine (β-C1 position hydroxyl)
Jacalin lectin	β1–3-linked galactose to N-acetylgalactose amine

carbohydrate groups. The non-glycosylated parent peptides then are subjected to MS and the resultant mass spectrum is searched against protein databases for ultimate protein identification. This ''bottom-up'' proteomics approach is also used for site-mapping of specific glycosylation sites [26] (See Fig. 2). For example, the mapping of N-linked glycosylation sites is accomplished by using PNGase F to cleave the asparagine (Asn) glycan bond converting Asn into aspartic acid in the process [27]. The resultant mass shift of the apoprotein is 0.984 Da, and this method has been used to map N-linked glycosylation sites [27]. However, this technique does come with the caveat of requiring a highly sensitive instrument, such as a Fourier transform ion cyclotron resonance mass spectrometer, to detect the subtle 0.984 shift in m/z [27]. The mapping of N-linked glycosylation sites is aided by the canonical amino acid motif for glycosylation of Asn-*X*-Serine/Threonine (Ser/Thr), where *X* can be any amino acid but Proline (Pro).

O-linked glycosylation lacks a canonical amino acid sequence and is further complicated by cytosolic and nucleoplasmic O-linked glycosylation in addition to classical addition of O-linked glycans in the Golgi [28]. For the mapping of O-linked glycosylation sites Rademaker *et al.* and Hanisch *et al.* developed methods using chemical
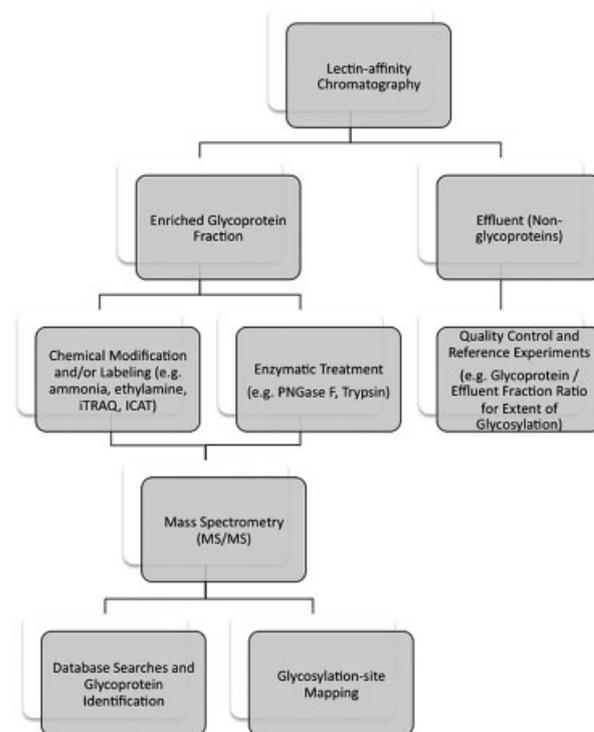

Figure 2. A general summary of ''bottom-up'' proteomics strategies to identify glycoproteins after lectin-affinity chromatography. The effluent of the chromatographic experiments provides data about the efficacy of the separation and potential data about glycosylated *versus* non-glycosylated forms of proteins.

modifications with ammonia and ethylamine [29, 30]. Glycopeptides were allowed to react with ammonia or ethylamine and exchange with the O-linked glycan resulting in m/z shifts of -1 for ammonia and $+27$ for ethylamine [29, 30]. Other commonly used labeling techniques include iTRAQ [31, 32] and ICAT [33, 34], which utilize isotopically labeled light and heavy tags, the N-terminus for isobaric tag for relative and absolute quantitation and cysteine residues for ICAT, to differentiate between control and experimental groups. In addition to chemical techniques, MS/MS fragmentation methods have been developed to first remove glycans and then fragment resultant peptides. The energy difference between removing the O-linked glycan and the energy required to further fragment the peptide has been used to simultaneously detect and identify O-GlcNAc-modified peptides [35].

3 Glycosylation alterations in AD

As noted above, the two classical pathological hallmarks of AD are SPs and NFTs. SPs are composed of a dense core of Aβ, central to the amyloid cascade hypothesis as discussed above, surrounded by dystrophic neurites. Other proteins, among which are clusterin, apoE, and α2-macroglobulin, and glycolipids are associated with SPs [36]. Proteins and glycolipids that compose SPs are highly sialylated, potentially masking the plaques from recognition by microglia [37]. NFTs are paired helical structures composed of hyperphosphorylated tau (p-tau), a microtubule-associated protein. Recent studies have shown that glycosylation may play a role in the formation and/or maintenance of NFTs [38–41]. The cytoplasmic addition of GlcNAc to serine/threonine residues (O-GlcNAc) has been to shown to regulate tau phosphorylation levels and suggests that O-GlcNAc affects NFT formation [39].

Extensive studies have shown that glucose utilization is disrupted in AD brain and precedes the appearance of clinical symptoms [42–46]. Impaired glucose metabolism also occurs in arguably the earliest form of AD, mild cognitive impairment brain (MCI) [47–53]. These findings suggest that the disruption of glucose metabolism is a causative agent for AD and not merely a consequence of the disease [40]. Global levels of O-GlcNAc modified proteins are decreased in AD brain, and work by Liu *et al.* showed that decreased O-GlcNAc levels of tau protein are inversely proportional to levels of p-tau [40]. Inhibition of the hexosamine biosynthetic pathway (HBP), the source of O-GlcNAc, in rat brain decreased the level of O-GlcNAc-modified tau and increased the level of p-tau [40]. On the other hand, the inhibition of protein phosphatase 2A had no effect on the levels of O-GlcNAc-modified tau [40]. In addition, rats that were subjected to decreased glucose metabolism mediated by fasting yielded similar findings as the HBP inhibition studies [40]. These experiments suggest that impaired glucose metabolism inhibits the HBP

pathway and increases p-tau levels, precipitating NFT formation.

Synapse loss is linked to learning and memory deficits in AD and has been shown to occur prior to SP and NFT formation [54, 55]. The O-GlcNAc-modified proteins, synapsin I and II, are enriched at synaptic terminals and regulate learning and memory in mice models [56]. Site-mapping studies using WGA lectin-enrichment coupled to MS show several proteins that are O-GlcNAc modified in human brain, including Bassoon, Piccolo, p25, Mek2, and RPN13/ADRM1 [57]. Piccolo and Bassoon have been shown to mediate scaffolding function during neurotransmission and are regulated by O-GlcNAc modification [58–61]. The protein p25 is also associated with protein scaffolding and promotes tubulin polymerization [62]. Mek2 is part of the Erk signaling cascade, which is a key regulator of synaptic plasticity and learning and memory [63], and RPN13/ADRM1 recruits and deubiquitinates the enzyme UCH37 in proteosomal degradation [64]. All the aforementioned proteins having O-GlcNAc modifications suggest that O-GlcNAc has a role in the maintenance and function of the synapse.

Our laboratory used ConA [20] and WGA (submitted for publication) in separate studies to identify lectin affinity-separated brain proteins from subjects with AD and amnestic mild cognitive impairment, arguably the earliest clinical form of AD (see Table 2). Identified proteins in the ConA study are not all glycoproteins, attesting to the above-mentioned hydrophobic-binding domain of this lectin. Interestingly however, most of the non-glycoproteins have a motif that is consistent with a primordial oligosaccharide attachment site.

Proteins identified in our ConA and WGA studies are involved in metabolism, cytoskeletal integrity and maintenance, synaptic function and neurotransmission, cell signaling, protein translation, and serve as chaperones. Each is consistent with the biochemistry, pathology, and/or clinical presentation of AD and/or MCI.

Insights into early changes in affinity of these brain proteins to lectins may provide clues about the progression of this dementing disorder and potential therapeutic targets to halt or slow this progression. As just three examples in MCI brain: (i) altered lectin affinity by GRP78 and PDI may reflect activation of an unfolded protein response in endoplasmic reticulum that leads to both positive and detrimental effects to the neuron. (ii) β-synuclein and DRP2, both of which are oxidatively damaged in AD brain [65], are involved in synaptic remodeling and neurite extension, respectively. Since learning and memory require synaptic remodeling, alterations in β-synuclein could be detrimental to this process. Moreover, DRP2 is involved in neurite extension, and alterations in this protein would be consistent with the known shortened neuritic length in AD, which would make interneuronal communication more difficult, an expectation of a memory disorder. (iii) WGA affinity alterations in neuronal-specific γ-enolase, also known to be

Table 2. Functional classes of brain proteins with altered lectin affinity for ConA and WGA in subjects with AD and MCI

Protein function	ConA		WGA	
	MCI	AD	MCI	AD
Metabolism		α-Enolase γ-Enolase GDH	γ-Enolase	γ-Enolase GDH
Chaperone	GRP78	HSP90	PDI	GP96
Cytoskeletal and cytoskeletal maintenance		TPM3	14-3-3 γ	14-3-3 ζ
	GFAP	GFAP	14-3-3 ε	TPM1
			TPM2	Gelsolin
				Calreticulin
Synaptic	DRP2	XAP4		
	β-Synuclein			β-Synuclein
Cell signaling	SDS-22			Calmodulin
Translation				DMGtRNAMT
Protease inhibitor				ACT

GDH, glutamate dehydrogenase; GRP78, glucose-regulated protein 78; HSP90, heat shock protein 90; PDI, protein disulfide isomerase; GFAP, glial fibrillary acidic protein; DRP2, dihydorpyrimdase 2; XAP4, Rab GDP-dissociation inhibitor XAP4; GP96, glucose-regulated protein 96; TPM, tropomyosin; SDS-22, protein phosphatase-related protein SDS22; DMGtRNAMT, dimethylguanosine tRNA methyltransferase; ACT, α1-antichymotrypsin.

oxidatively modified in AD and MCI brain [65], are consistent with the known decreased glucose utilization in MCI brain. However, enolase is also a multi-functional protein involved in, for example, ERK1/2-mediated pro-survival processes and in plasminogen-mediated clearance of Aβ [66]. Hence, alterations in enolase in MCI would be expected to be detrimental to neurons.

4   Concluding remarks

The field of glycobiology has been a difficult area to study in the past because of the broad range of proteins and types of glycans that occur in nature. However, glycoproteomics methods have advanced in the recent years by employing lectin-affinity chromatography coupled to MS. These techniques enrich and identify glycoproteins as well as map glycosylation sites. Changes in protein post-translational modifications and protein levels are well characterized in AD [65], however, studies of changes in glycosylation patterns as markers of AD progression in brain tissue, in spite of decreases in protein glycosylation such as O-GlcNAc, are few in number [40]. The future application of lectin-affinity chromatography coupled to MS reviewed in this manuscript will provide important platforms for future studies using brain tissue from subjects with MCI coupled with that from AD to provide temporal information to better understand AD pathogenesis and progression.

The authors have declared no conflict of interest.

5   References

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