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The role of glycosylation in ionotropic glutamate receptor ligand binding, function, and trafficking

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Abstract. Members of the ionotropic glutamate receptor (iGluR) family have between 4 and 12 consensus asparagine (N)-linked glycosylation sites. They are localized on the extracellular N-termini, and the loop between the penultimate and last transmembrane domains. These regions also contain the essential elements for formation of the ligand binding site. N-linked glycosylation does not appear to be essential for formation of the ligand binding site per se, but there are demonstrated interactions between glycosylation state and ligand binding affinity, receptor physiology, susceptibility to allosteric modulation and, in some cases, trafficking. There is no indication of a general role for N-linked glycosylation in iGluRs; instead the effects of glycosylation vary among glutamate receptor subtypes and splice variants, with specific effects on structure or function with different subunits.

Key words. Glutamate; receptors; glycosylation; ionotropic; asparagine-linked.

Introduction

Most plasma membrane and secretory proteins are glycosylated, and glycosylation occurs at two types of amino acid sequences on proteins, as those sequences are translocated into the lumen of the endoplasmic reticulum (ER) or their extracellular domains are exposed to the lumen of the Golgi stacks. The primary type of glycosylation present in all forms of iGluRs is asparagine linked, and occurs at the universal consensus sequence QX(S/T), where Q = asparagine, X = any amino acid except proline and S/T = either serine or threonine. However, O-linked glycosylation, which occurs at S/T residues, may also be present [1]. N-linked glycosylation begins with the entirely formed *N*-acetylglucosamine (GlcNAc)2 Mannose9(Man) Glucose3-(Glc) moiety attached to the phospholipid dolicholphosphate. This initial segment is then transferred via oligosaccharyl transferase to the R group of consensus sequence asparagines to form an N-glycosidic bond, in many cases as the developing polypeptide chain is translocated into the lumen of the ER. An ordered process of trimming the oligosaccharide chains begins with glucosidases in the ER, and continues with sequential elimination of mannose residues by mannosidases through the medial Golgi, followed by variable addition of GlcNAc, galactose, sialic acid and fucose by glycosyltransferases in the Golgi compartments (for a review on N-linked oligosaccharide structure and processing, see [2]). Oligosaccharide side chains have been found to participate in or affect a wide variety of cellular and protein functions, such as formation-function of receptor ligand binding sites [3], correct assembly of receptor subunits [4], cell surface expression and membrane targeting [5, 6], intracellular transport [7], protection from proteases, cell-cell recognition and other protein-protein interactions (for overview, see [8]). Notably, however, the effects of carbohydrate chains on any given protein molecule vary widely, and there is no

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole propionic acid; ConA, concavalin A; CNQX, cyano-3-dihydro-7-nitrogluinoxaline-2-3-dione, DTT, dithiothreitol; ER, endoplasmic reticulum; Glc, glucose; GlcNAC, *N*-acetylglucosamine; iGluR, ionotropic glutamate receptor; MII, MIII, MIV, transmembrane domains II, III, and IV; NMDA, *N*-methyl-D-aspartate; TM, tunicamycin.

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rigorous structural-functional principle. Moreover, although the process of carbohydrate addition, trimming and readdition is quite ordered, carbohydrate chains are variously modified during the protein maturation process, and it is quite possible to find a number of side chains on the same protein, all modified differently after having passed through the Golgi.

In this review, we will discuss what is known about glycosylation of iGluR receptor subunits and how glycosylation interacts with ligand receptor binding, physiological function and trafficking. We will also describe other aspects of iGluR biology as they might relate to, or interact with glycosylation of this important category of receptor/channels.

Relevant properties of iGluRs

In the current model of transmembrane organization of the receptor subunits (fig. 1), each subunit has a relatively large N-terminal, extracellular domain three transmembrane domains, and a pore loop (M2) which extends into the pore from the intracellular side and constitutes its lining. The carboxyl-terminus of glutamate receptors is in the cytoplasmic domain, and several iGluR subunit C-termini have been shown to interact with proteins that restrict them to the synapse, via PDZ binding domains (for review, see [9]). In addition to restricting, or targeting glutamate receptors to synapses, these proteins may have some roles in controlling the trafficking of nascent receptors, and the turnover of synaptic populations of receptors. Additionally, the carboxyl-termini are the sites of modification by phosphorylation of many of the iGluR subunits, and of the association with other proteins involved in functional modulation and trafficking. Several subunits have been shown also to have their C-termini cleaved by calpain, a calcium-activated neutral protease [10, 11]. Both phosphorylation and cleavage by calpain could act to modulate insertion and internalization of receptors to synaptic sites and/or receptor physiological properties [12]. Moreover, interactions with various proteins, including PDZ domain-binding proteins, could alter relevant kinetic and physiological properties. For instance, in the case of the heteromeric receptor, mouse ε2/ζ1(rat NMDAR2B/NMDAR1), coexpressed in *Xenopus* oocytes with PSD-95, it appears that coexpression shifts the glutamate dose-response curve to the right, thus indicating a decrease in channel sensitivity to agonist [13]. In addition, calmodulin [14] and *N*-ethylmaleimide sensitive factor (NSF) have been shown to alter the physiological properties of iGluRs [15]. Thus, the appropriate receptor ligand binding site, kinetics and localization are dependent on receptor interactions with associated proteins.

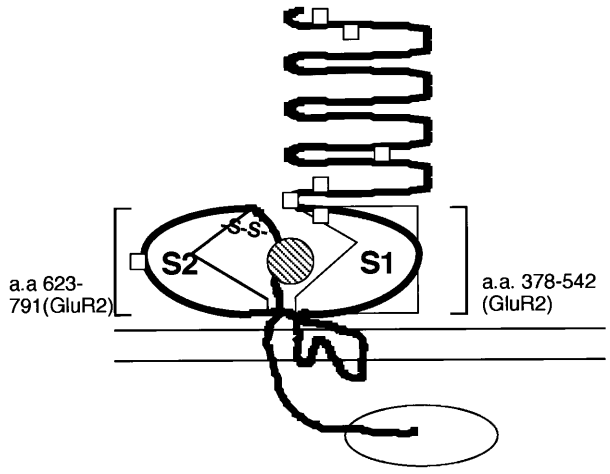

Figure 1. Transmembrane topology of iGluRs, and essential features of ligand binding sites. Glycosylation sites are indicated by open squares. The ligand binding domain includes regions S1, approximately 150 amino acids N-terminal to the first transmembrane domain, and S2, the extracellular loop between TMDIII and TMDIV.

Ligand-binding properties

^{3}H-AMPA binds to native AMPA receptors in brain as well as in AMPA receptors expressed in transfected cells. In the presence of KSCN (potassium thiocyanate), ^{3}H-AMPA recognizes two sites in rat brain membrane preparations, one with a high affinity ($K_d \cong 10–20$ nM) and a relatively small number of sites ($B_{max} \cong 0.5–2$ pmol/mg protein), and one with a lower affinity ($K_d \cong 500–1000$ nM), and a larger population of sites ($B_{max} \cong 10–20$ pmol/mg protein) [16, 17]. Further, autoradiographic studies of AMPA binding performed on brain sections with low concentrations of ^{3}H-AMPA (5–50 nM) indicate prominent binding in densely packed somatic fields in rat brain, such as the pyramidal, and granule cell layers of hippocampus, layer IV of rat cortex and piriform cortical somatic fields. With higher concentrations of AMPA, dendritic fields throughout the brain become more prominently labeled, and binding in cell body layers appears to be decreased [18]. CNQX, an antagonist of AMPA receptors which binds with apparent equal affinity to both sites of AMPA receptors [19], shows little relative binding in somatic layers at any concentration [18].

Several possibilities have been proposed to account for the different affinities of ^{3}H-AMPA binding sites [20]: (i) high- and low-affinity sites are interconvertible, and ligand binding negatively influences the affinity of the

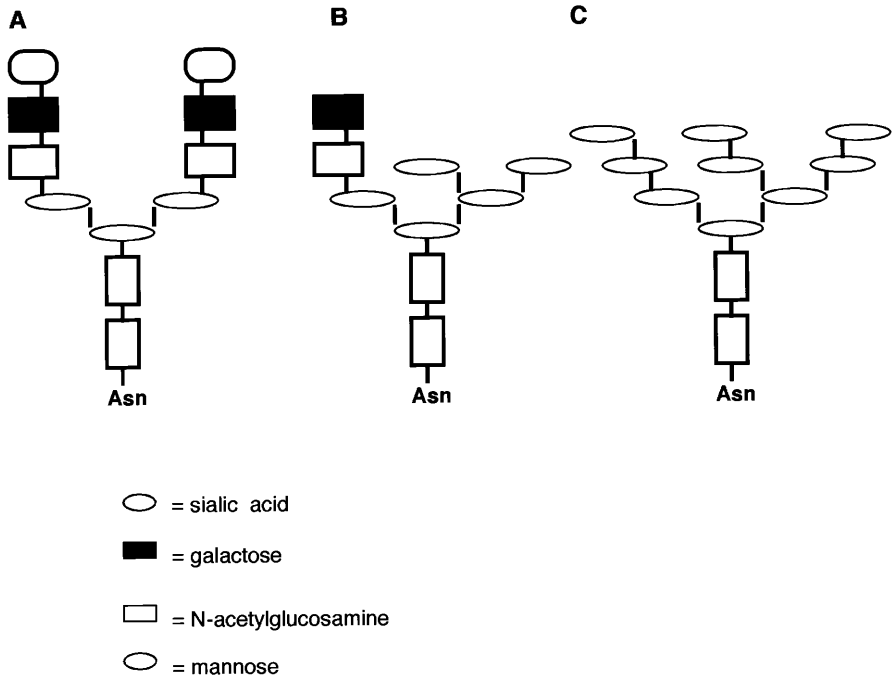

Figure 2. Classifications of the major types of asparagine-linked oligosaccharides. The three major classifications of oligosaccharide are complex (*A*), Hybrid (*B*), and simple (*C*). Open boxes represent *N*-acetylglucosamine (GlcNAc), open ovals represent mannose (Man), black boxes represent galactose (Gal) and dotted ovals represent sialic acid (SA). Below the oligosaccharides are indicated the enzymes used to determine glutamate receptor oligosaccharide structure, based on cleavage susceptibility. Some variations in sugar content of the pentasaccharide core do occur (adapted from review by [2]).

sites, (ii) posttranslational, covalent modification of the receptors produces a change in the affinity, (iii) noncovalent modifications, such as protein-protein interactions or protein-lipid interactions, influence the affinity of the receptors and (iv) high- and low-affinity sites are different receptors. It is important to observe that ^{3}H-AMPA binding in transfected cells only exhibits the high-affinity site [21]. This could be due either to differences in resultant carbohydrate structure across cell lines or the absence of an interacting protein normally found in neurons, but not in transfected cells.

The iGluR sequences involved in ligand binding have been the subject of extensive investigation. Fusion and expression of the S1 and S2 regions (see fig. 1) can, in specific cases (GluR2, GluR4) [22], reconstitute an appropriate ligand binding site, with a pharmacology similar to that of the transfected full-length version of the receptors. The S1 region is the last 150 amino acids of the N-terminal extracellular domain, and the S2 region represents the extracellular loop between MIII and MIV ([23]; fig. 1). These regions show high homology to Bacterial polar amino acid binding proteins [23–25]. The S1–S2 region of GluR2 has been crystallized, and its structure deduced using multiwavelength anomalous diffraction [26], and shows remarkable similarity to the bacterial glutamine binding protein (QBP). While not all iGluR S1–S2 fusion proteins reconstitute normal ligand binding characteristics, the sequence similarity, and presence of strictly conserved residues participating in agonist binding, support the structural and functional similarity. S1 and S2 exhibit two lobular domains that form a kidney-shaped ligand binding site. An intra-S2 disulfide bridge stabilizes an open conformation, and reduction/derivatization, or site-directed mutagenesis in NMDAR1 [27, 28], and GluR4 has demonstrated its pivotal role in determining agonist affinity [29]. Further, differential access to this disulfide bridge has been indicated to be the reason for the differential effects of redox agents on NMDA and AMPA receptor function. Thus, NMDA receptor function is potentiated by DTT, whereas this compound has no effect on AMPA receptors [27].

Glutamate receptor oligosaccharide types

There are generally three categories of carbohydrate chains based upon the types of sugars they are made up of: (i) high-mannose, or simple side chains, (ii) complex

side chains and (iii) hybrid chains (fig. 2). While it is possible, for instance, that oligosaccharides on iGluR receptors could vary widely, from a simple trimmed GlcNac2Man5 to branched and extensive complex oligosaccharides, the oligosaccharide type is generally the same at a particular site for a particular protein [30]. However, differences in species, tissue and cell types, and even culture conditions can produce heterogeneity in the final oligosaccharide forms within the same protein [31]. Different levels of expression and activity of glycosyltransferases in different Golgi compartments can produce variations in oligosaccharide form. Also, expression of the transfected protein itself or its level of expression can change the glycosylation pattern. Transfection studies with glutamate receptors have been performed in a number of cell types, and although not extensively described, changes in the glycosylation pattern between cell types probably exist. For instance, noticeable differences in the molecular weight and degree of shift resulting from asparigine-linked deglycosylation are apparent in AMPA/GluR and NMDAR1 receptors expressed in insect cells compared with receptors from rat brain [32, 33]. Differences in glycosylation state probably account for these changes. To date, few cell lines have been sufficiently characterized in terms of their glycosylation machinery to make good predictions regarding the pattern of glycosylation they can produce. None, at this point, could necessarily be relied upon to reproduce a native glycosylation state in a recombinant glutamate receptor [34]. Two types of heterogeneity in expression systems are possible. There are cases in which sites that are normally glycosylated are not. This is termed macroheterogeneity. On the other hand, more subtle changes in individual sugar residues produced by differences in activity, presence or specificity of glycosyltransferases, termed microheterogeneity, could also influence receptor function. Localization within the cell plays a key role in determining the oligosaccharide processing state as well. For instance, if a glycoprotein were to remain resident in the ER, it could be expected not to undergo further processing via the Golgi mannosidases, or glycosyltransferases. While the precise determinants of the final glycosylation state of any glycoprotein are not clear, polypeptide backbone conformation probably plays a key role. That is, the state or extent of oligosaccharide processing depends on the steric availability of the side chain that is being processed [31, 35]. Glycoprotein sensitivity to endoglycosidase H (Endo H), an enzyme that cleaves high-mannose forms of carbohydrate side chains has been used to reveal the role of protein folding in oligosaccharide processing. Under nondenaturing conditions, carbohydrate side chains available for cleavage are the more processed complex form, and thereby insensitive to Endo H. Under denaturing conditions, the side chains that are less processed are cleaved [35].

From site-directed mutagenesis of consensus asparagine residues in iGluR GluR1 subunits, it was demonstrated that the carbohydrate side chains are of variable sizes, which is consistent with differential processing of different side chains [36]. Additional observations indicate that AMPA receptors have mostly complex oligosaccharide forms, as they are relatively insensitive to treatment with Endo H [37] under denaturing conditions. However, a slight shift in molecular weight resulting from Endo H treatment may indicate the presence of at least one site that remains in high-mannose form. In addition to indicating that mature glutamate receptors have a complex form of oligosaccharide side chain, treatment with neuraminidase, an enzyme that cleaves sialic acid residues, suggests that iGluRs are not sialated [38]. Thus, side chains have a high mannose core, and neutral complex forms of sugar residues [39]. Other studies have indicated that the side chains on NMDA receptor subunits, in particular on the NMDAR1 subunit, remain in the simple, high-mannose form [40]. Whereas NMDAR1 has 12 consensus glycosylation sites, the most of any iGluR subunit, it is not clear how many of them are actually glycosylated. However, the glycine binding site of the NMDAR1 subunit consists of the characteristic bi-lobed binding site demonstrated for glutamate at the AMPA receptors. It is therefore somewhat curious that although its ligand binding site is similar in conformation, none of its side chains get trimmed or processed in the Golgi.

Glycosylation and ligand binding sites

AMPA receptor glycosylation, and its interaction with ligand binding and physiology has been best characterized among the iGluRs. The carbohydrate moieties are located primarily on the N-terminal domains of the receptor subunits (see fig. 3), although in a few kainate subunits, some are also located on the extracellular loop domain (see table 1; GluR5–7). The number of consensus glycosylation sites on AMPA receptor subunits ranges from 4 to 6 (table 1), with generally homologous positioning across subunits. An exception is GluR2, which does not have glycosylation sites at the distal, N-terminal end as the other subunits do. This property may be involved in regulating of the interactions of the receptors with lectins that alter their desensitization properties [41]. Whereas systematic elimination of glycosylation sites in GluR1 indicates that all six of its consensus glycosylation sites are indeed glycosylated [36], systematic verification of the individual sites for GluR2–4 has yet to be done. On the other hand, all ionotropic receptor subtypes are glycosylated, as indicated by a shift in the molecular weight of the proteins after *N*-glucosidase F treatment [42], or treatment with

tunicamycin (TM) [41]. In addition, inhibition of glycosylation by TM produces various changes in receptor physiology from the glycosylated forms, which suggests that receptors are normally glycosylated, and that glycosylation is important for iGluR receptor function [41].

Several types of experiments indicate that glycosylation interacts with ligand binding and function in glutamate receptors. It was recently demonstrated that the high-affinity ^{3}H-AMPA binding sites that appear prominently in cell body layers of rat brain frozen thawed tissue sections correspond to immature receptors, localized in the ER. Using variable preincubation temperatures, it was first demonstrated that these sites could be eliminated by preincubation at 37 °C [43]. Elimination of these sites was associated with a selective disappearance of structurally distinct forms of GluR1–3 subunits with molecular weights approximately 5 kDa smaller than full-length subunits [37]. Treatment of native receptors with *N*-glycopeptidase F indicated that the smaller form of the receptors differed by N-linked glycosylation state. Treatment with endoglycosidase H indicated that the smaller receptor species had a high-mannose oligosaccharide configuration, whereas that of the mature form was complex. Isolation of this population from rat brain via sucrose density gradients indicated that the population of receptors with the immature form exhibited a high affinity for ^{3}H-AMPA ($K_d \cong 14$ nM), whereas a mixed population of receptors from subcellular fractions containing post-ER elements exhibited a low affinity ($K_d \cong 200$ nM) [37]. Thus, while AMPA receptors can be converted to higher affinity by a number of means, so far the high-affinity AMPA receptors isolated in the ER represent the only purifiable form of high-affinity sites present in brain tissue.

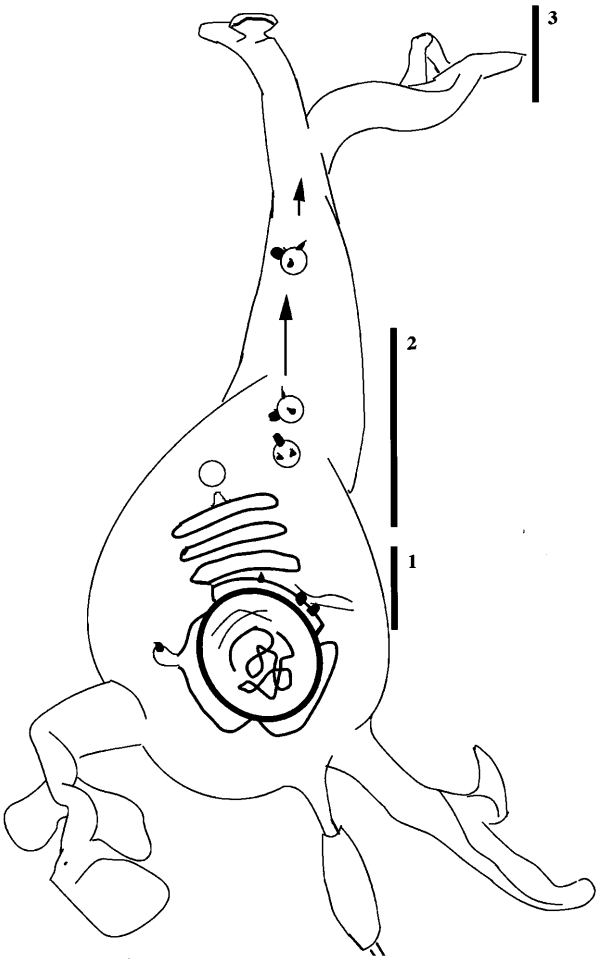

Figure 3. ^{3}H-AMPA binding sites in neurons. Lettered vertical bars adjacent to depiction of pyramidal cell indicate: (1) high-affinity ^{3}H-AMPA/GluR receptors are high-mannose, immature forms [37]; (2) Maturation of glycosylation state, or new protein-protein interactions along the secretory pathway convert high-affinity ^{3}H-AMPA receptors to low affinity and (3) synaptic receptors remain low affinity, but can be converted to high through PLA_2 activation [55].

The S1–S2 fusion protein of GluR4 has been expressed and characterized in *E. coli* as well as in insect cells. In insect cells, the secreted form of the receptor is likely glycosylated, although direct demonstration has not been done for the S1–S2 fusion protein. On the other hand, fusion proteins of GluR4 characterized in *E. coli* are not glycosylated and show similar ligand-binding characteristics to fusion proteins expressed in insect cells. The K_Ds and ligand displacement profiles for soluble S1–S2 fusion proteins both in bacteria and insect cells (K_Ds between 10 and 50 nM) are similar to those measured in full-length transfected proteins [22]. Other reports using mouse GluRα1 and GluRα2 full-length transcripts in insect *Spodoptera frugiperda* Sf 21 cells indicate that TM-treated cells lose AMPA binding sites, and it has been suggested that glycosylation is necessary for proper formation of ligand binding domains [32, 44]. No saturation kinetics was performed in insect cells in the presence and absence of TM; however, receptor levels determined by Western blots were found to be normal. Although these results appear contradictory, it is conceivable that TM may have a specific effect on the full length GluRs, and specifically on ^{3}H-AMPA binding. This explanation fits well with the apparent ligand binding in S1–S2 fusion proteins, and also with the apparent normal GluR1 and GluR2 functions in TM-treated *Xenopus* oocytes using kainate as an agonist [41]. It is also possible that the role of glycosylation in ligand binding varies among different types of receptors composed of different subunits. It should be noted that consensus glycosylation sites are localized proximally to amino acid residues critical in determining ligand interactions. Residues N385 and N393 of GluR2

Table 1. Effect of glycosylation on glutamate receptor ion channels.

Receptor type	Glycosylated sites (including signal peptide)	Accession number	Effect of Tunicamycin (TM)		
			Ligand binding	Physiology	Trafficking
GluR1	6 (a.a. 63, 249, 257, 363, 401, 406)	X17184	Abolishes ligand binding to 3H-AMPA in insect cells to full-length receptor.	Inhibits flop splice-variant response to kainate/increases flip variant, TM blocks concavalin A (Con A) effect.	—
GluR2	4 (256, 370, 406, 413)	M36419	Abolishes ligand binding to 3H-AMPA in insect cells to full-length receptor.	No effect of ConA with or without TM, but TM increases flip-variant response to kainate.	—
GluR3	5 (57, 260, 374, 409, 416)	M36420	— (not examined)	Inhibits both splice variants, TM blocks small ConA increase	—
GluR4	6 (52, 56, 258, 371, 407, 414)	M36421	3H-AMPA, glutamate, kainate bind S1–S2 fusion protein; glycosylation has no effect, as expression in insect cells and bacteria yield similar results.	Increases both splice-variant responses to kainate; TM blocks small ConA increase.	—
GluR5	9 (68, 74, 276, 379, 428, 439, 561, 446, 766)	Z11713	—	TM blocks ConA increase.	—
GluR6	9 (67, 73, 275, 378, 412, 423, 430, 546, 753)	Z11548	S1–S2 forms ligand binding site in insect cells, but not native characteristics.	TM blocks ConA increase.	—
GluR7	9 (39, 45, 247, 350, 384, 395, 402, 517, 721)	M83552	—	—	—
KA1	9 (158, 220, 272, 286, 323, 408, 415, 479, 736)	X59996	—	Increases GluR6(Q)/KA1 cotransfections; but not native characteristics.TM blocks ConA effect.	—
KA2	11 (15, 219, 271, 285, 322, 372, 394, 400, 407, 414, 478)	Z11581	—	Increases GluR6(Q)/KA2 contransfection; TM blocks ConA effect.	—
Delta-1	3 (200, 422, 498)	D10171	—	—	—
Delta-2	4 (294, 427)	Z17239	—	—	—
NMDAR1	12 (61, 203, 239, 276, 300, 350, 368, 440, 471, 491, 674, 771)	P35439	Large decreases number of glycine sites; S1–S2 forms ligand binding site in insect cells.	No response	Contrasting results regarding surface expression.
NMDAR2A	7 (75, 240, 380, 443, 446, 541, 687)	Q00959	Significant 3H-MK801 K_0 decrease in cotransfected cells.	No response	NR1 differentially degraded in contrasfected NR2B/NR1-1 cells
NMDAR2B	7 (74, 241, 348, 444, 491, 542, 688)	Q00960	—	No response	
NMDAR2C	5 (70, 337, 438, 539, 685)	Q00961	—	No response	—
NMDAR2C	7 (89, 349, 363, 464, 566, 712)	I58158	—	No response	—

(N406 and N413 in table 1) are 10 and 17 amino acids from E402, a residue critical in determining ligand affinity, specificity, and receptor kinetic properties [26]. Many iGluRs have glycosylation sites distributed near or within regions that constitute the critical aspects of the ligand binding domain (see table 1; fig. 1).

Several studies on the effects of glycosylation on NMDA receptors have been performed [33, 41, 45, 46]. When HEK 293 cells were cotransfected with NMDAR1 and NMDAR2A, and then exposed to TM, there was a significant change in the affinity of NMDA receptors for MK-801 ($K_d = 6 \pm 1.4$ nM; compared with 26 ± 5.8 nM) [46]. No differences in protein expression, or cell-surface expression, were observed. This increase in affinity is consistent with the decrease in EC_{50} for glutamate of heteromeric NMDA receptor complexes expressed in oocytes [41] when treated with TM; however, a change in B_{max} was also noted [41]. TM caused a rapid degradation of NMDAR1 but not NMDAR2 subunits in *Xenopus* oocytes, and also reduced cell surface expression of the heteromeric receptors. Also, single transfection of NMDAR1 subunits and treatment with TM in insect cells have indicated a loss in ^{3}H-2-dichloro-kynurenate (DCKA) binding sites [33]. Although these studies appear to be contradictory, differences in cell types may account for the differences in processing or transport of the transfected subunits. Taken together, these results indicate that glycosylation state affects trafficking, affinity of the ligand binding sites and stability of NMDA receptors.

Glycosylation and glutamate receptor physiology

Several early studies in which brain-extracted messenger RNA (mRNA) was injected into *Xenopus* oocytes had indicated a broad range of effects of TM on glutamate-evoked currents [47]. In a more recent study, site-directed mutagenesis of the glycosylation sites in GluR1, predominantly located in the amino acid sequences identified as necessary for ligand binding, produced a physiologically nonfunctional receptor. On the other hand, in the same study, treatment of oocytes producing wild-type GluR1 with TM gave functional responses, although the maximal kainate-evoked currents were reduced by $\sim 40-50\%$ [36]. Further characterization of the effects of glycosylation on glutamate receptor physiology indicated no strict dependence of functionality on glycosylation, although significant differences do exist in the physiology of unglycosylated receptors when compared with controls [41]. Further characterization of AMPA receptors has revealed that steady-state levels of glutamate-evoked current responses in TM-treated oocytes expressing GluR1-flop were approximately 11 % of control currents; however, in the case of the flip subunits, they were 198% of control. Differences in treated and nontreated for flip and flop splice variants were observed for GluR1, 2 and 4. Concavalin A (ConA), a mannose-associating lectin, has been shown to potentiate glutamate receptor responses by inhibiting desensitization [48, 49]. However, examination of the effect of ConA on GluR receptors in TM-treated oocytes predictably indicated that glycosylation was required for ConA-mediated inhibition of desensitization. It is important to note that the wild type GluR2 subunit was not affected by ConA. Introduction of a proximal N-terminal glycosylation site into GluR2, and another internal glycosylation site, however, reproduced the effects of ConA observed with other receptors [41].

The most profound effect of glycosylation is on NMDA receptor heteromers, where TM reduces current amplitudes to $< 1\%$ of control [41]. As indicated earlier, this appeared to be due to a selective loss or degradation of NMDAR1 subunits. In the absence of NMDAR1, it is unlikely that NMDAR2 subunits would be expressed on the cell surface [50]. Biochemical studies indicate that there is a large intracellular pool of rapidly degraded NMDAR1 subunits [40, 51, 52]. Some subunits of the NMDAR1 pool oligomerize with NMDAR2 subunits and are transported to the surface; however, in the presence of TM, the NMDAR1 pool is rapidly degraded even in the presence of NMDAR2 subunit expression [41]. This suggests that glycosylation of NMDAR1 subunits is essential for proper oligomerization with NMDAR2 subunits, and/or trafficking of the heterooligomers to the cell surface. The lack of ligand binding would seem to be a consequence of increased degradation.

The effect of glycosylation on kainate receptor ligand binding has not been studied in detail, whereas the role of N-glycosylation in the physiological function of these receptors has been well characterized. N-linked glycosylation serves a similar role in kainate receptors as in AMPA receptors. That is, glycosylation is necessary for modification of desensitization properties of kainate receptors [48, 49]. Normally, the effect of ConA on kainate receptors expressed in *Xenopus* oocytes is to produce the largest relative increase in kainate-evoked currents [41]. Whereas TM causes a modest potentiation of kainate-evoked responses, the effect of ConA is abolished after TM treatment. Further characterization of the nature of this interaction and the importance of particular glycosylation sites in GluR6 indicate that only one glycosylation site is required for the effect of ConA [53]. Further, ectopic sites could be introduced into the GluR6 receptor and result in the recovery of the ConA effect. Thus, for kainate receptors at least, extant glycosylation sites do not play any specific role in the dynamics of ConA interaction, but merely provide

for the inhibition of the bi-lobed binding site from closing and assuming a desensitized state. Interestingly, once again in KA2/GluR6 heteromeric receptors, it has also been demonstrated that SAP90/PSD-95 cotransfection strongly inhibits sensitization by association with the cytoplasmic tail [54].

Summary

Glycosylation plays a direct role in the ligand-binding affinity of some glutamate receptors, as it does in the sensitivity and dynamics of agonist-induced response. However, the large differences between in vitro and in vivo receptor ligand binding properties can be explained as either a glycosylation state that produces only one high-affinity site, or additional protein-protein interactions in vivo that decrease the affinity. Since receptors from brain can be transformed by treatment with enzymes such as PLA_2 to higher affinity, and cotransfection of proteins such as PSD-95/SAP90 can decrease desensitization [54, 55], and shift the EC_{50} [13] to greater agonist concentrations, it is likely that the large in vitro differences observed are due to additional protein-protein interactions encountered by glutamate receptors in neurons.

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