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Current Topics

Prion Glycoprotein: Structure, Dynamics, and Roles for the Sugars

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ABSTRACT: The prion protein contains two N-linked glycosylation sites and a glycosylphosphatidylinositol (GPI) anchor. The large size of the N-linked sugars, together with their dynamic properties, enables them to shield two orthogonal faces of the protein almost completely. Thus, the sugars can protect large regions of the protein surface from proteases and from nonspecific protein−protein interactions. Immunoprecipitation of prion protein with calnexin suggests that in the ER the oligosaccharides may provide a route for protein folding via the calnexin pathway. Major questions relate to the relevance of the glycoform distribution (as defined by glycan site occupancy) to strain type and disease transmission. Glycan analysis has shown that prion protein contains at least 52 different sugars, that these consist of a subset of brain sugars, and that there is site specific glycan processing. PrP^{Sc} from the brains of Syrian hamsters contains the same set of glycans as PrP^{C}, but a higher proportion of tri- and tetra-antennary sugars. This may be attributed to a decrease in the activity of GnTIII. The GPI anchor, which is modified with sialic acid, may allow the prion protein to be mobile in the lipid bilayer. Potentially, this provides a possible means for translocating the prions from one cell to another.

Spongiform encephalopathies are a group of neurodegenerative diseases that affect both humans and animals. Most (80%) arise sporadically; some (19%) are genetically induced, and a small proportion (1%) can be transmitted between mammals by inoculation with, or dietary exposure to, infected tissues. Transmission of the disease appears to depend solely on a structural change in a neuronal glycoprotein, the prion protein (*1*, *2*) which contains two N-glycosylation sequons (AsnXaaThr) in its primary structure. In disease, the normal cellular form (PrP^{C})[1] is converted into the form first identified in fractions isolated from the brains

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[1] Abbreviations: aa, amino acid; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt-Jakob disease; nvCJD, new variant Creutzfeldt-Jakob disease; Clx, calnexin; DTT, dithiothreitol; ER, endoplasmic reticulum; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; GnT, *N*-acetylglucosaminyl transferase; GPI, glycosylphosphatidylinositol; Man, mannose; N-glycan, asparagine (Asn)-linked glycan; NMR, nuclear magnetic resonance; O-glycan, serine/threonine (Ser/Thr)-linked glycan; PDI, protein disulfide isomerase; PIPLC, phosphatidylinositol phospholipase C; PNGase F, peptide N-glycosidase F; PrP, prion protein; PrP^{C}, cellular isoform of the prion protein (precursor of PrP^{Sc}); PrP^{Sc}, scrapie-like or disease-causing isoform of the prion protein; PrP 27−30, protein that results from limited digestion of PrP^{Sc} with proteinase K by truncation of the N-terminus; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SHa, Syrian hamster; Tg, transgenic; TSE, transmissible spongiform encephalopathy; uPA, urokinase plasminogen activator. The nomenclature for describing oligosaccharide structures

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of Syrian hamsters (SHas) with experimental scrapie, known as PrP^{Sc}, which contains an increased proportion of β-sheet (*3*) and a protease-resistant core. In human prion diseases, which include new variant Creutzfeldt-Jakob disease (nvCJD), while the conversion of PrP^{C} to PrP^{Sc} seems to be a central event in disease transmission, other aspects of PrP expression, topology, folding, and trafficking may be important in the pathological processes which ultimately result in disease. The functions of the prion protein are not yet clear, although possible roles in copper transport, synaptic function, circadian rhythm, promoting genetic diversity, and signal transduction have been suggested (*4*−*8*).

Architecture of the Prion Glycoprotein

The normal cellular prion protein (PrP^{C}) is a glycoprotein (Prio_Human; P04156) containing 209 amino acids in which α-helices are the predominant feature of the secondary structure (*3*). PrP^{C} is normally attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (*9*). A series of molecular models for the region of PrP^{C}, corresponding to the protease-resistant core of PrP^{Sc} (amino acid residues 90−231), is shown in Figure 1a−d. The models are based on the solution structure of Liu et al. (*10*). In this structure, the C-terminal helix extends to residue 227, leaving only the last four residues without structure. In contrast to this, the structure determined by Riek et al. (*11*) shows that the last 14 amino acids are flexible. These data suggest that at least the last four residues form a flexible linkage to the GPI anchor. The linkage between the C-terminus of the protein (the backbone C of Ser231) and the ethanolamine phosphate bridge attached to the trimannosyl core of the GPI anchor is also flexible. Two orientations of PrP^{C} with respect to the cell membrane are shown in Figure 1a. In the first, the protein is extended as far as possible from the membrane, and in the other, the positively charged face of the protein is oriented toward the membrane as proposed by Riek et al. (*11*). On the basis of data from a simulation study, with which the figures presented here are largely consistent, Zuegg and Gready (*12*) have proposed that the protein can make contact with the membrane.

The two potential N-glycosylation sites (Asn181IleThr and Asn197PheThr) are located in the region of the protein with the best defined secondary structure, that is the disulfide (Cys179−Cys214)-bridged helix−loop−helix motif at the C-terminus (Figure 1b). The N-glycan sites are variably occupied, and at least in the Syrian hamster (*13*, *14*) and the mouse (*15*), a heterogeneous range of oligosaccharides is attached to the molecule.

Oligosaccharides have dynamic properties. In addition, the N-glycosidic linkage to asparagine is flexible and, as a result, the sugars shield large regions of the surface of the prion protein. The two glycans cover orthogonal faces of the protein (Figure 1c), sterically hindering either intermolecular protein−protein interactions or intramolecular interactions involving residues 1−90. The glycan at Asn181 is attached to an α-helix, while that at Asn197 is located on a loop. In the helix, the side chains of His177, Asn181, and Lys185 are exposed, providing potential binding sites which are blocked when the sugar is attached. The membrane distal faces of the protein carry a net negative charge (*11*), and consequently, the negatively charged glycans are unlikely to be involved in long-range interactions with the protein surface.

Inspection of the models indicates that the proposed binding site for protein X, a molecule which has been proposed to mediate the conversion of PrP^{C} to PrP^{Sc} (*16*, *17*), is not obstructed by the sugars (Figure 1d). Potential sites for O-glycosylation are indicated in Figure 2. O-Glycosylation is initiated in the Golgi apparatus through the transfer of *N*-acetylgalactosamine (GalNAc) to Ser or Thr residues in the fully folded protein. Although a number of sites appear to be accessible for O-glycosylation, no O-linked glycans have been detected either in PrP^{C} isolated from normal Syrian hamster brains or in PrP 27−30 isolated from the brains of scrapie-infected hamsters (P. M. Rudd, D. Groth, H. Serban, S. B. Prusiner, and R. A. Dwek, unpublished data).

Comparison of the Prion Protein and Thy-1 GPI Anchors

The prion protein is usually, although not invariably (*18*, *19*), attached to the neuronal cell membrane via a GPI anchor at Ser231 (*9*). Inspection of the molecular model indicates that the polar C-terminus, which links the protein to the GPI anchor, makes very few noncovalent interactions between the protein and the anchor. This provides the protein with considerable dynamic freedom relative to the membrane. In contrast, in another neuronal glycoprotein, Thy-1, there are extensive interactions between the protein domain and the GPI anchor glycan such that the protein is in direct contact with the membrane (Figure 3). These interactions are possible in Thy-1 because the anchor is attached to a cysteine residue involved in an intramolecular disulfide bond (*20*).

At present, only limited data on the acyl chain composition of the GPI anchors are available (Figure 4). Stahl et al. (*9*) showed that the GPI anchor of the prion protein contained stearic acid; this saturated C18:0 fatty acid would be expected to promote lipid order in the membrane. The lipid composition of the GPI anchor of a subfraction of brain Thy-1 (that is resistant to hydrolysis by phospholipase C) has equimolar amounts of the fully saturated palmitic (C16:0) and stearic acids, with small amounts of longer chain (C20−24) lipids, and unsaturated octadecanoic acid (C18:1) (E. Hounsell, personal communication).

Brain Thy-1 (rat) and PrP (SHa) have differently modified GPI anchor glycans (Figure 4). Although all proteins containing a GPI signal sequence which are expressed in the same cell initially receive the same anchor, GPI anchors are frequently modified during transit to the cell surface. In Thy-1, the common trimannose core is modified by the addition of β-1−4 GalNAc to Man1 and α1−2 mannose to Man3 (*20*), while preliminary studies of PrP^{C} showed that there are five populations of glycans and that 15% contain sialic acid (Figure 4) (*21*). These data indicate that the core glycans of PrP^{C} are more accessible than those of Thy-1, consistent with the finding that the Thy-1 protein makes extensive interactions with the anchor glycan, keeping it close to the cell membrane during transit both inside the cell and on the cell surface. Addition of sialic acid is a relatively

is as follows: A(*X*) indicates the number (*X*) of antennae linked to the trimannosyl core, G(*X*) the number (*X*) of terminal galactose residues in the structure, F core fucose, B bisecting *N*-acetylglucosamine (GlcNAc), SA sialic acid, G or Gal galactose, and M or Man mannose.

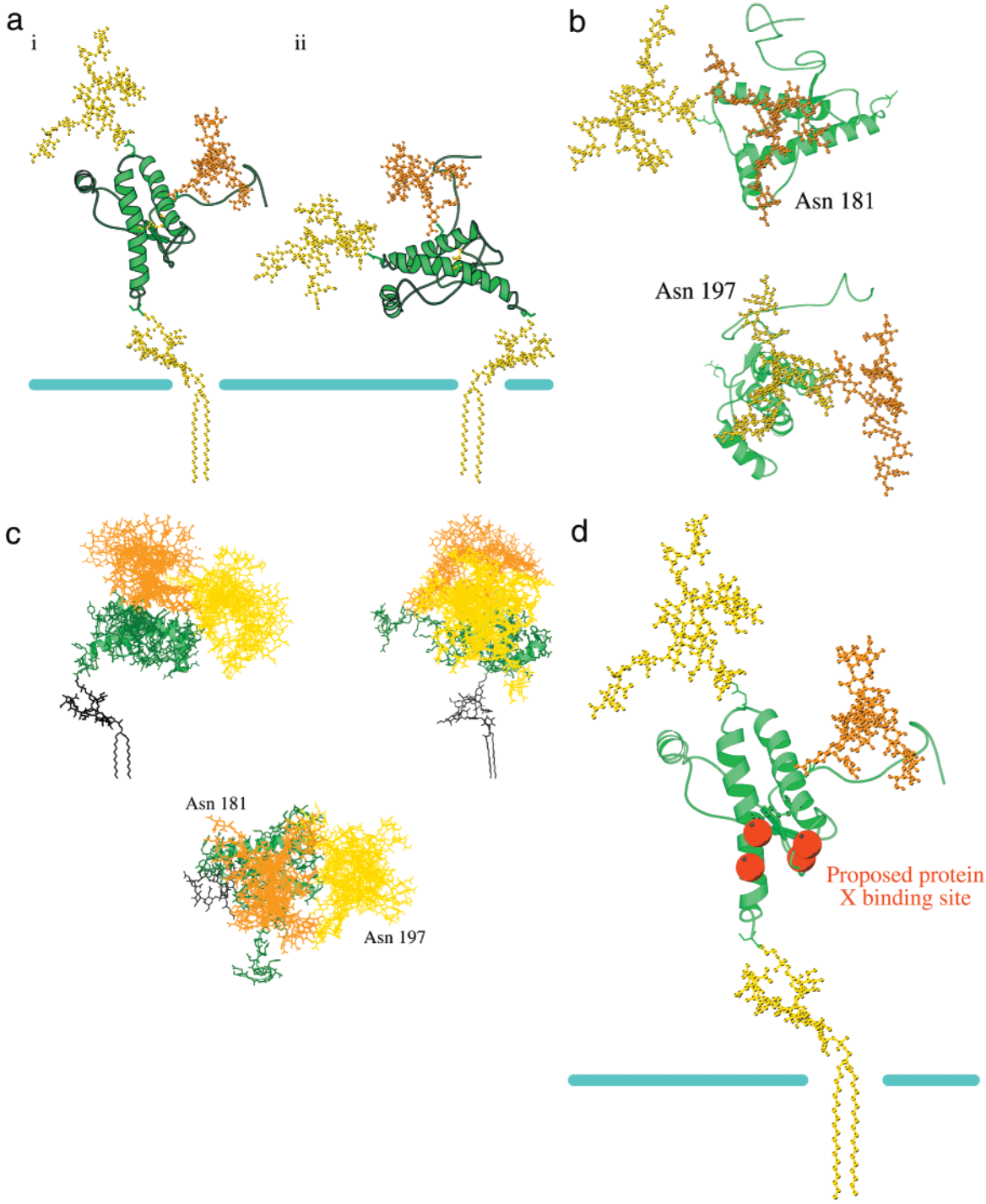


FIGURE 1: Molecular model of PrP^C, residues 90−231, based on one of the NMR structures of the recombinant protein (*10*, *52*), the sequence of the GPI anchor (*21*), and the N-glycan analysis (*14*). The glycan structures (A3G3FBSA3 at site 181 and A4G4FBSA4 at site 197) were built using the database of average crystallographic linkages (*53*). The torsion angles around the Asn Cα−Cβ and Cβ−Cγ bonds were adjusted to eliminate unfavorable steric interactions between the glycans and the protein surface. Nomenclature for describing oligosaccharide structures: A(3,4) indicates the number of antennae linked to the trimannosyl core, G(3,4) the number of terminal galactose residues in the structure, F core fucose, B bisecting *N*-acetylglucosamine (GlcNAc), and SA(3,4) the number of terminal sialic acid residues in the structure. (a) The GPI anchor is shown in two orientations with respect to the protein (i) with the GPI extending directly away from the protein maximizing the distance between the negatively charged glycans and the membrane surface and (ii) with the positively charged face of the protein oriented toward the membrane surface [as proposed by Riek et al. (*11*)]. There is likely to be considerable dynamic freedom at all the attachment points for the glycans. (b) Two orthogonal views showing the areas of the protein surface covered by the glycans at Asn181 (orange) and Asn197 (yellow). (c) Three orthogonal views showing the range of conformational space available to the glycans at Asn181 (orange) and Asn197 (yellow). The structures show an overlay of different positions of the glycans relative to the protein as a result of altering the conformation of the Asn side chain. The range of allowed N−Cα−Cβ−Cγ and Cα−Cβ−Cγ−Oγ torsion angles of the Asn side chain are based on the observed range of crystallographic torsion angles (*54*), limited by steric interactions between the glycan and the protein. (d) The molecular model of PrP^C, residues 90−231, shown in Figure 1a, highlighting the residues implicated in protein X binding (*17*).

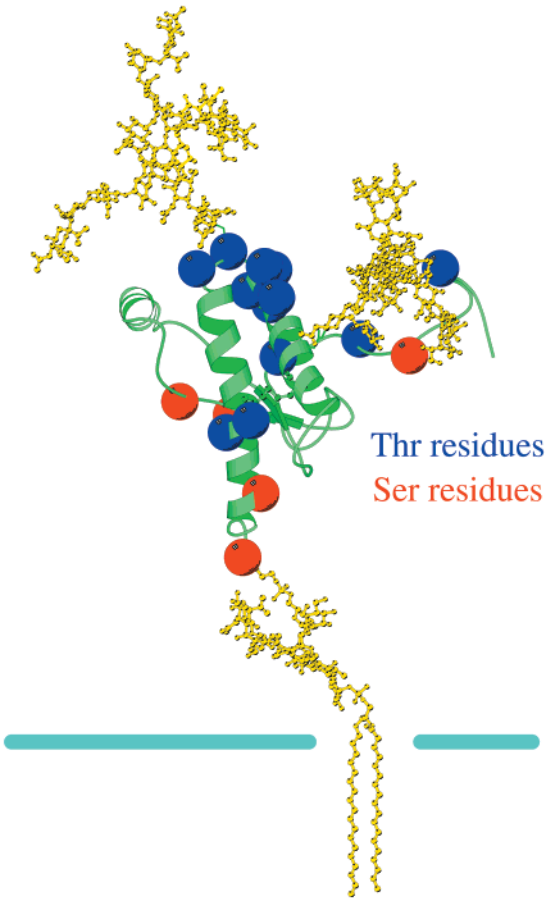


FIGURE 2: Molecular model of PrP^C, residues 90−231, shown in Figure 1a, highlighting the positions of the Ser (red) and Thr (blue) residues. All the Ser residues are available as potential O-glycosylation sites. However, a large number of the Thr residues, including a cluster of seven, are unavailable for glycosylation either through the tertiary structure of the protein or through protection by the N-glycans.

unusual modification of mammalian anchors, and no role for this feature has yet been proposed. To date, only a small number of proteins, including pig membrane dipeptidase (*22*), are known to contain such a sialic acid modification to their GPI anchors.

Location of PrP^C and Thy-1 on the Neuronal Cell Surface

Thy-1 and PrP^C occupy different domains on the neuronal cell surface (Figure 5) (*23*). PrP^C is concentrated within discrete sphingolipid−sterol "rafts". These rafts appear to form a boundary of intermediate lipid order (and so detergent insolubility) between the fluid glycerolipid regions composed of unsaturated kinked lipids and the highly insoluble, fully ordered domains occupied by Thy-1 (*23*). The small, high-density patches of PrP^C found on the neuronal surface (particularly at the cell body of sensory neurons) contrast with the distribution of the considerably more abundant Thy-1 that is dispersed at lower density over much larger areas of the neuronal (especially axonal) surface (*23*).

The different solubility of the lipid environments occupied by PrP and Thy-1 might reflect differences in the degree of saturation and chain length of the acyl fatty acids as these are properties which contribute to the degree of lipid order found in sphingolipid−sterol domains (*24*). Current data suggest that these differences are not great; therefore, the

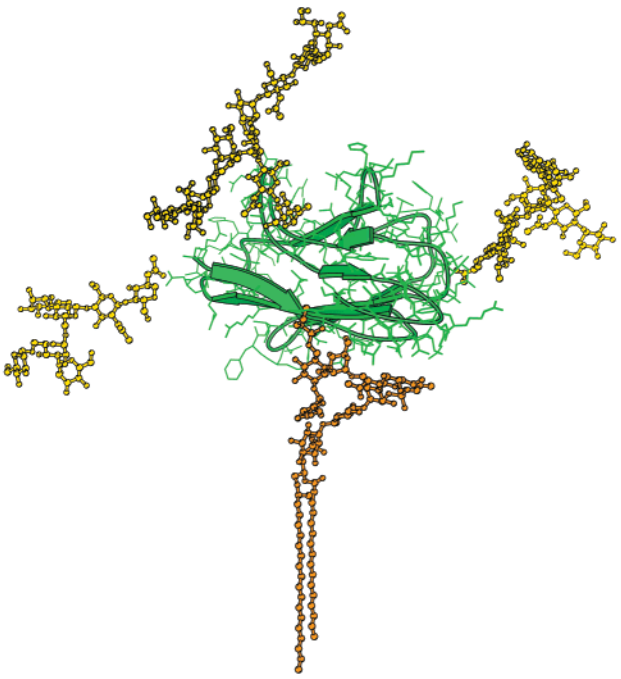


FIGURE 3: Molecular model of rat brain Thy-1 based on the N-glycan analysis (*55*) and the sequence of the GPI anchor (*9*, *21*). The GPI anchor is very close to the protein surface because the attachment residue is involved in an intramolecular disulfide bond.

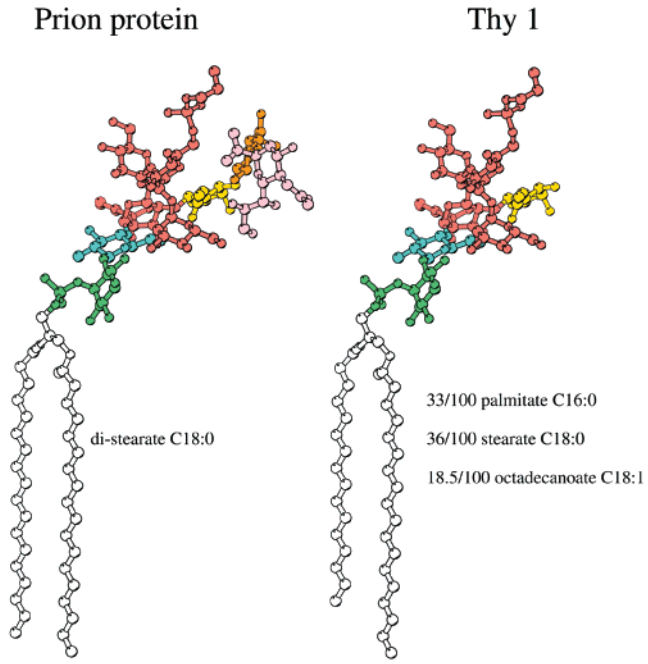


FIGURE 4: Comparison of the GPI anchors from the prion protein [left (*9*, *21*)] and Thy-1 [right (ref *20* and a personal communication from E. M. Hounsell)]. Sugar residues are colored as follows: green, inositol; blue, glucosamine; brown, mannose; yellow, *N*-acetylgalactosamine; orange, galactose; and pink, *N*-acetylneuraminic acid.

clustering of PrP^C into distinct membrane patches may rather be due to the association of PrP^C with transmembrane proteins responsible for the trafficking of PrP^C into endocytic pathways. Whether transmembrane proteins participate in the pathological conversion of PrP^C into PrP^{Sc} remains to be established (*23*).

In contrast to Thy-1, PrP undergoes rapid endocytosis. This function is normally mediated by trafficking motifs within the cytoplasmic domain of the protein. Since GPI anchors

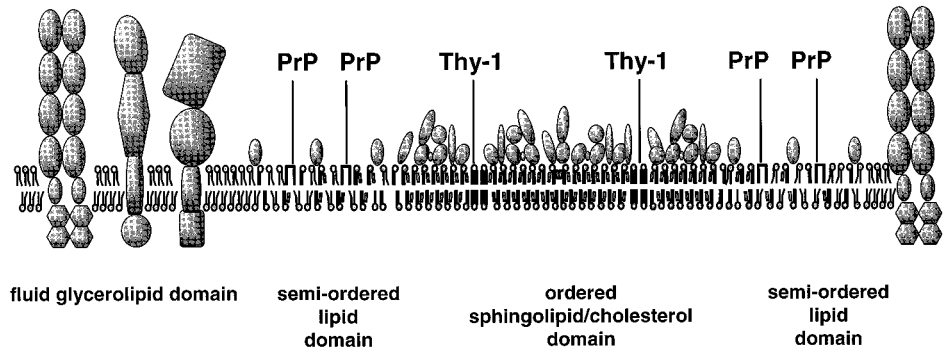


FIGURE 5: Prion protein and Thy-1 are located in different cell surface lipid domains. PrP^C is concentrated within discrete sphingolipid−sterol rafts. These rafts appear to form a boundary of intermediate lipid order (and so detergent insolubility) between the fluid glycerolipid regions composed of unsaturated kinked lipids and the highly insoluble, fully ordered domains occupied by Thy-1 (*23*).

are inserted into the top leaflet of the lipid bilayer, glycoproteins which use such anchors, such as the uPA receptor, endocytose by binding to an endocytosing transmembrane receptor (*25*). Such a requirement may also account for the location of PrP in a semiordered domain. PrP has been located both in coated pits (*23*) and in caveolae-like domains (*26*). PrP^{Sc} formation appears to be restricted to caveolae-like domains (*17*), consistent with the finding that there are no widespread differences in glycosylation of the normal and diseased forms of the prion protein (*14*). In contrast to PrP^C, PrP^{Sc} is partially resistant to proteases in the endosomal pathway and is deposited in the lysosome. An unglycosylated mutant has been shown to have a shorter half-life than either the wild-type prion protein or a monoglycosylated mutant and is resistant to degradation in the endosome (*27*).

Does the GPI Anchor Enable the Prion Protein to Translocate?

There is substantial evidence that GPI-anchored proteins are clustered in sphingolipid−sterol microdomains or rafts (review in ref *28*). GPI anchors extend only into the top leaflet of the lipid bilayer with the result that such proteins are relatively mobile on cell membranes. Several GPI-anchored proteins, including Thy-1, transfer spontaneously to plasma membranes (*29*). Such a mechanism may allow the prion protein to translocate between cells or to leave the cell surface in lipid-associated vesicles which can then integrate with other cell membranes. Translocation or vesicle formation provides a possible mechanism for the formation of disease-associated plaques and for the transport of both normal and infectious prion proteins within the neuron and from cell to cell.

Conversion of PrP^C to PrP^{Sc}

In general, glycosylation improves the stability of proteins (*30*). On this basis, the stability of the diglycosylated prion protein would be expected to exceed that of the mono- or unglycosylated forms. Intuitively, it follows that the greater the number of sugars, the higher the free energy barrier between the ground state and the transition state in which the prion protein could convert from the α-helical structure to the β-sheet conformation. In the α-helical structure, stability depends on precise docking of side chains. In contrast, the β-sheet form can be locked by intermolecular association with other β-conformers, suggesting that initiation of a self-propagating conversion reaction could lead to the accumulation of PrP^{Sc}. Alternatively, PrP^{Sc} formation may occur through a template-assisted process whereby the conformation of PrP^{Sc} is imposed on PrP^C as it is converted into a nascent molecule of PrP^{Sc} (*31*). PrP^{Sc} was produced more rapidly when scrapie-infected cells were treated with tunicamycin to inhibit N-glycosylation (*32*). Blocking N-glycosylation also promoted acquisition of scrapie-like properties by the prion protein in transfected Chinese hamster ovary cells in culture (*33*). The same effect was noted when the glycosylation sites were deleted, although these data should be treated with caution since this result may be a consequence of the amino acid substitutions.

In proteinase K-treated PrP^{Sc} from nvCJD, the diglycosylated protein was the most abundant and there was relatively little unglycosylated PrP compared with other strains of CJD (*34*). Therefore, in nvCJD the relative instability of the unglycosylated protein may not be relevant.

Strain Type and Glycosylation

Strain type is characterized by such factors as the incubation time and patterns of disease pathogenesis. Strains can also be distinguished by differences in the fragments produced by protease cleavage of accumulated PrP^{Sc}. The differences in the fragments reflect differences in the structure of the amino termini (*16*, *35*) or possibly a structural change in the protein or sugars which alter the accessibility of the enzymes to potential cleavage sites.

Prion strain variation appears to be characterized by the banding pattern of the proteinase K digests of PrP^{Sc} on an SDS−PAGE gel (*34*, *36*, *37*). In such digests, some bands may represent clipping of the protein, either at the level of the gene or by proteases. However, if all the bands can be reduced to a single species on treatment with PNGase F, an enzyme which removes N-linked glycans from proteins, the intensity of the staining of the original bands reflects the relative occupancy of the glycosylation sites.

A totally unexpected finding is that when disease is passed from infected animals to a new host the banding pattern reflecting the glycosylation site occupancy of the newly diseased prions (PrP^{Sc}) reflects that of the prions from the original infected animal, not that of the cellular prion protein (PrP^C) of the recipient. For example, nvCJD in humans has a glycosylation pattern similar to that of BSE in cattle. The number and location of the sugar chains may correlate with or direct the location of the prion protein to particular regions

of the neuron and to specific regions of the brain (*38*). These data suggest that normal PrP^{C} and PrP^{Sc} may migrate to specific regions of the brain depending on their glycosylation status. If this is the case, infectious prions may then convert the host PrP^{C} which, since it is in the same location, has a similar glycosylation status. Alternatively, this may be a stochastic process in which the specificity is created by "homologous" binding of similarly glycosylated proteins. The role of glycosylation may therefore be a causal one in propagating strain variation in that PrP variant glycoforms can be targeted to distinctive subpopulations of neurons in the brain (*38*, *39*).

In addition, some preparations of PrP 27−30 spontaneously crystallize in the presence of uranyl acetate ions (*40*). The dark stains which can be seen within the crystals using electron microscopy are consistent with the presence of negatively charged sialic acid residues bound to uranyl acetate. Although this has not been shown to be physiological, the finding may at least provide a potential structure for prion protein aggregates.

N-Linked Glycan Site Occupancy May Influence the Folding Pathway of the Prion Protein

Glycosylated PrP^{C} was immunoprecipitated with antibodies to binding protein, grp94, protein disulfide isomerase (PDI), calnexin (Clx), and calreticulin, while the unglycosylated form associated principally with grp94 (*27*). These proteins and chaperones may direct the prion protein to different folding pathways or selectively influence the folding of different regions of the protein. While these data point to a role for sugars and chaperones in folding, it is interesting to note that in vitro the prion protein can fold spontaneously.

Several factors are known to control glycosylation site occupancy. These include alterations of the primary structure close to the AsnXaaSer/Thr acceptor sequon which influence the efficiency with which the $Glc_3Man_9GlcNAc_2$ oligosaccharide precursor is transferred to the protein (*41*, *42*). This may be relevant to some diseases where there are points of mutation close to the glycosylation sites (Figure 6). Additionally, Capellari et al. (*27*) demonstrated that prion protein glycosylation can be controlled by changing the redox balance of the cell.

Detailed Analysis of the N-Glycans Attached to the Prion Protein

$SHaPrP^{C}$ and $SHaPrP^{Sc}$ glycoforms contain at least 52 different sugars at the two glycosylation sites (*13*, *14*). There is some site specific processing, and in mouse PrP^{C}, Asn197 contains a higher proportion of tri- and tetra-antennary glycans than Asn184 (*15*). Although the same range of sugars is present in the scrapie form (PrP^{Sc}), there is an increase in the level of bi- and triantennary glycans.

In mammalian cells, the GlcNAc transferases are a family of five enzymes that first initiate the conversion of oligomannose to complex or hybrid glycans by attaching GlcNAc in a β1−2 linkage to the $Man_3GlcNAc_2$-Asn core (GnTI). This step is essential for the action of further processing enzymes, including that of GnTII, -IV, and -V which lead to the formation of bi-, tri-, and tetra-antennary glycans, respectively. GnTV cannot operate until GnTII has added a GlcNAc residue to the 2 position of the mannose (Man1)

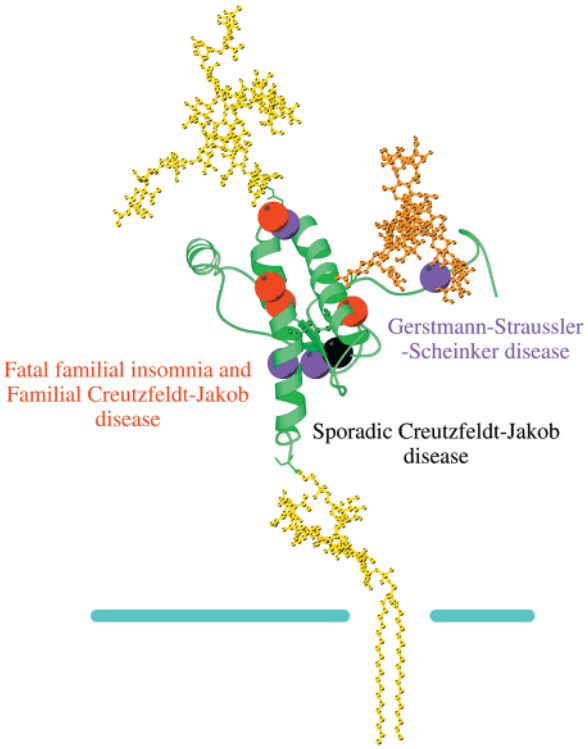
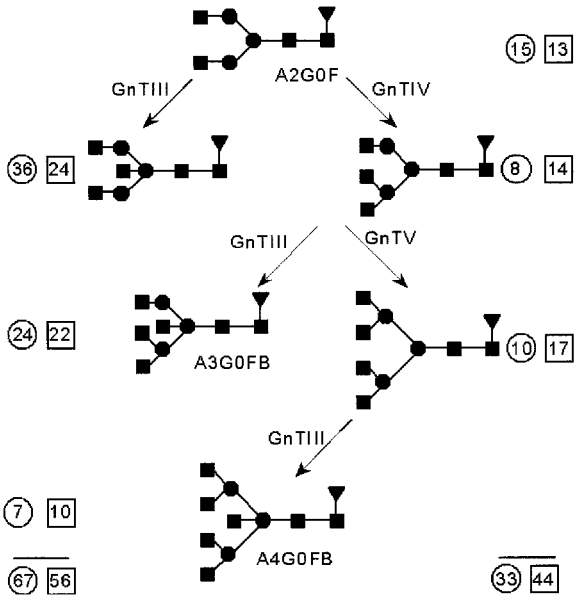


FIGURE 6: Molecular model of PrP^{C}, residues 90−231, highlighting the points of mutation associated with various disease states. Amino acid residues at which mutations are associated with (a) familial fatal insomnia and familial Creutzfeldt-Jacob disease (red) (178, 200, 208, and 210), (b) Gerstmann-Straussler-Scheinker disease (purple) (102, 117, 198, and 217), and (c) sporadic Creutzfeld-Jakob disease (black) (129). In particular, the D178N, V180L, T183A, F198S, and E200K mutations are very close to Asn181 and Asn197 and might be expected to alter the efficiency of glycosylation.

residue linked α1,6 to the core (Figure 7). GnTIII links a GlcNAc residue to position 4 of the Man at the bisect of the conserved pentasaccharide core. This substitution, which can occur at any point in the pathway, inhibits further branching of the sugars by preventing processing by GnTII, -IV, or -V. The increase in the level of bi- and triantennary glycans in PrP^{Sc} can most simply be explained by a decrease in the levels of GnTIII in the cells on which PrP^{Sc} is expressed (*14*). A decrease in GnTIII activity may reflect a downregulation at the level of the gene which has multiple promoters. In this study, PrP^{C} and PrP 27−30 were derived from whole brain. If only a subset of neurons are affected, the significance of this result could be much greater. Another explanation for these findings may be that a certain subset of PrP^{C} glycoforms are predisposed to form PrP^{Sc} aggregates, so self-assembly rather than (or as well as) biosynthesis could drive the subtle glycosylation differences between PrP^{C} and PrP^{Sc}.

These glycosylation changes may not necessarily be involved in disease pathogenesis, but may reflect disease-associated processes, such as inflammation. In the advanced stages of prion disease, a conspicuous atypical inflammatory response is indicated by the presence of activated microglia in regions of the brain which also show vacuolation and PrP^{Sc} amyloid deposition. This effect, together with the recruitment of CD8+ T-lymphocytes to the site of pathology, has been noted in the brains of scrapie-affected mice 8 weeks postinjection (*43*, *44*) and in human disease (*45*, *46*). The inflammatory response in the brain is distinguished from the



	%Bi-	%Tri-	%Tetra
PrP^C	51	32	17
PrP^{Sc}	37	36	27

FIGURE 7: Relative proportions of cores containing bisecting GlcNAc in PrP^C and PrP 27−30 in the context of the N-linked glycosylation pathway. The percentages of different classes of glycans containing bisected and nonbisected cores in PrP^C (numbers in circles) and PrP 27−30 (numbers in squares). The ratio of bisected to nonbisected glycans in PrP 27−30 was 1.3:1 compared with 2:1 in PrP^C. *N*-acetylglucosamine (■), Man (●), and core Fuc (▼).

classical inflammatory response in that it is dominated by the recruitment of mononuclear cells and by the presence of activated microglia (*47*).

Prion Proteins Contain a Subset of Brain Sugars

Prion proteins (irrespective of differences between normal and pathogenic isoforms) contain a particular subset of the N-glycans present in whole brain tissue (*48*).

In rat and mouse brain tissue, 75% of N-glycans are acidic and half of these carry sialic acid as the sole anionic charge. The remainder carry other or additional charges such as sulfate, glucuronic acid, and phosphate. In contrast, in the prion protein all charged glycans carry sialic acid as the only anionic charge. This indicates that N-glycan processing of the prion proteins is somewhat simplified at the later stages, relative to brain N-glycosylation in general.

No oligomannose structures were detected on the prion proteins. This is in stark contrast to the abundance of oligomannosidic structures detected in brain tissue generally (*49*), but particularly on synaptic membrane preparations and on a number of defined synaptic glycoproteins (*50*) and on the abundant neural glycoprotein Thy-1 (*51*). The N-glycans in the prion proteins are exclusively of the complex bi-, tri-, and tetra-antennary type. The relative content of biantennary N-glycans is twice as high in the prion protein as in total brain tissue. Core and outer arm fucose and bisecting GlcNAc levels were also higher in the prion protein. The presence of sialyl $Lewis^x$ structures suggests that the sugars may function as recognition epitopes.

Conclusion

Many potential roles for the N-linked glycans on the prion protein can be attributed to the large size of the N-linked sugars with respect to the protein. The large sugars coupled with their dynamic properties and the flexibility of the oligosaccharide chain around the N-glycosidic linkage enable the sugars to protect extensive regions of the surface of the protein. Specific sugars attached to the protein in the ER ($GlcNAc_2Man_9Glc_1$) may provide a route for protein folding by allowing the protein to interact with calnexin. While glycan site occupancy appears to relate to strain type and disease transmission, the basis for this finding remains unclear. Glycan analysis has shown that the prion protein contains a subset of brain sugars and that there is site specific glycan processing. $SHaPrP^{Sc}$ contains the same set of 52 glycans as PrP^C, but a higher proportion of tri- and tetra-antennary sugars. This increase in the level of multiantennary structures may be attributed to a decrease in the activity of GnTIII. Whether this finding is significant in terms of pathogenesis or reflects some part of the disease process is also a question which remains to be answered. The prion protein is attached to the membrane via a GPI anchor. The flexibility of the prion protein around the GPI anchor attachment site may allow the protein to be mobile with respect to the lipid bilayer, although this has not been established experimentally. The lipid chains, which penetrate only the top leaflet of the bilayer, may potentially provide a possible means for translocating the prions on the cell surface or from one cell to another.

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