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Does the tail wag the dog? How the structure of a glycosylphosphatidylinositol anchor affects prion formation

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ABSTRACT

There is increasing interest in the role of the glycosylphosphatidylinositol (GPI) anchor attached to the cellular prion protein (PrP^C). Since GPI anchors can alter protein targeting, trafficking and cell signalling, our recent study examined how the structure of the GPI anchor affected prion formation. PrP^C containing a GPI anchor from which the sialic acid had been removed (desialylated PrP^C) was not converted to PrP^{Sc} in prion-infected neuronal cell lines and in scrapie-infected primary cortical neurons. In uninfected neurons desialylated PrP^C was associated with greater concentrations of gangliosides and cholesterol than PrP^C. In addition, the targeting of desialylated PrP^C to lipid rafts showed greater resistance to cholesterol depletion than PrP^C. The presence of desialylated PrP^C caused the dissociation of cytoplasmic phospholipase A_2 ($cPLA_2$) from PrP-containing lipid rafts, reduced the activation of $cPLA_2$ and inhibited PrP^{Sc} production. We conclude that the sialic acid moiety of the GPI attached to PrP^C modifies local membrane microenvironments that are important in PrP-mediated cell signalling and PrP^{Sc} formation.

Prion diseases occur following the conversion of a normal host protein designated the cellular prion protein (PrP^C) into disease-associated isoforms (PrP^{Sc}); the accumulation of which within the brain is associated with neurodegeneration and clinical symptoms. While much of the focus of prion research has been on protein structure, it is the role of the glycosylphosphatidylinositol (GPI) anchor, also described as the protein's "tail", that links most PrP^C molecules to membranes [1] which has maintained our interest. There is emerging evidence that the homogeneity of GPIs can alter the cellular targeting and the subsequent

trafficking of proteins, which has implications for prion formation. Our recent paper examined the effects of sialic acid, a rare modification of mammalian GPI anchors [2], upon the properties of PrP^{C} and consequently prion formation [3].

Although the presence of PrP^{C} is essential for prion formation [4], not all cells that express PrP^{C} are permissive for PrP^{Sc} replication and the reasons why these cells do not replicate PrP^{Sc} are not understood. Reports that efficient PrP^{Sc} formation occurs only when PrP^{C} is targeted to specific membrane microdomains [5] indicated that factors that affect the cellular targeting and intracellular trafficking of PrP^{C} are critical in regulating PrP^{Sc} formation. A seminal study showed that the presence of a GPI anchor targets PrP^{C} to lipid rafts and that these are required for efficient PrP^{Sc} formation [6]. It should be noted that the term lipid raft is somewhat simplistic as there exist many different rafts. The variety in their composition and function [7] raises the possibility that prion formation occurs only in a select number of rafts. The composition of the GPI anchor is one factor that contributes to the targeting of PrP^{C} to specific lipid rafts.

Neuraminidase digestion was used to produce a PrP^{C} with a GPI anchor lacking sialic acid (desialylated PrP^{C}), a modification that could not be achieved by genetic manipulation methods [8]. Our recent paper reported 4 major observations:

1) Desialylated PrP^{C} was not converted to PrP^{Sc}.
2) Desialylated PrP^{C} inhibited the conversion of PrP^{C} to PrP^{Sc}.
3) Desialylated PrP^{C} behaved differently from PrP^{C} with regards to its effects on membrane composition and cell signalling.
4) Desialylated PrP^{C} disrupted cell signalling mediated by PrP^{C}.

Initially $Prnp^{(0/0)}$ neurons pulsed with PrP^{Sc} were used to demonstrate that while exogenous PrP^{C} was converted to PrP^{Sc}, exogenous desialylated PrP^{C} was not. These experiments were supplemented with studies in wildtype neurons pulsed with PrP^{Sc}. In these cells the addition of PrP^{C} increased PrP^{Sc} formation whereas desialylated PrP^{C} significantly reduced the amount of PrP^{Sc} produced. The obvious question, “why do the effects of PrP^{C} and desialylated PrP^{C} differ so greatly?” was explored. Although both PrP^{C} and desialylated PrP^{C} were targeted to lipid rafts in recipient $Prnp^{(0/0)}$ neurons, sucrose density gradients suggested that they were targeted to different rafts; observations consistent with reports that lipid rafts are heterogeneous [7] and that the composition of the GPI anchor targets proteins to specific rafts.

The membrane surrounding GPI-anchored proteins is composed of specific phospholipids, glycolipids and cholesterol that constitute a lipid raft, the composition of which is dependent upon multiple interactions

between the protein, glycans and membrane lipids [9]. It is thought that a change in the composition of the GPI affects the composition of the surrounding raft. This hypothesis is supported by observations that the composition of GPIs attached to Thy-1 differs from those attached to PrP^{C} [2, 10] and that the membranes surrounding these molecules have different lipid compositions [11]. Therefore we hypothesised that the presence of sialic acid in the GPI has a direct effect upon the composition of the surrounding membrane.

When immunoprecipitation studies were used to isolate rafts surrounding PrP^{C} and desialylated PrP^{C} we found significantly higher concentrations of gangliosides and cholesterol associated with rafts containing desialylated PrP^{C} than rafts containing PrP^{C}. The significance of these observations was examined with a battery of functional tests. Firstly, desialylated PrP^{C} remained within rafts after cholesterol depletion, whereas PrP^{C} redistributed to the normal cell membrane. Secondly, desialylated PrP^{C} had a longer half-life than PrP^{C} in neurons. Finally, PrP^{C} is released from the surface of cells following activation of an endogenous GPI-phospholipase C (GPI-PLC) by glimepiride [12]. Glimepiride does not release all GPI-anchored proteins indicating that the GPI-PLC associates only with specific rafts. Our observation that treatment with glimepiride did not release desialylated PrP^{C} from cells indicated that desialylated PrP^{C} occupies different rafts to PrP^{C}. Although we do not know how sialic acid affects the composition of rafts it is possible that sialic acid contained within the GPI competes with gangliosides for sialic acid-binding proteins. If so then the removal of sialic acid from the GPI would allow greater incorporation of gangliosides into PrP^{C}-containing rafts. Gangliosides sequester cholesterol within the membrane which increases membrane rigidity and helps stabilize lipid rafts [13-15]. Thus the increased concentrations of gangliosides surrounding desialylated PrP^{C} would then explain the observed increased cholesterol density in rafts surrounding desialylated PrP^{C} and the increased resistance of desialylated PrP^{C} to cholesterol depletion. This hypothesis is compatible with reports the concentrations of gangliosides in rafts affects the expression and function of some GPI-anchored proteins [16, 17] including PrP^{C} [18].

Desialylated PrP^{C} also inhibited the conversion of PrP^{C} to PrP^{Sc} in primary cortical neurons and in 2 prion-infected cell lines, ScN2a and ScGT1 cells. Others have shown that the co-expression of mutant prion proteins had altered the cellular localisation of wild type PrP^{C} and portioning into lipid rafts [19]. Since the composition and hence the function of rafts is dynamic and controlled by an "induced fit" model [7] the concept that the binding of desialylated PrP^{C} to PrP^{Sc} modifies lipid rafts that are involved in PrP^{Sc} formation was explored [6]. More specifically, as the composition of membranes is affected by the glycan structure of GPIs [9] then the membrane surrounding PrP^{Sc}:PrP^{C} complexes would be expected to differ from membranes surrounding PrP^{Sc}:desialylated PrP^{C} complexes. We propose that the binding of desialylated PrP^{C} to PrP^{Sc}

changes the composition of the local membrane so that it is unfavourable for the conversion of PrP^{C} to PrP^{Sc}.

Studies of T cell receptor signalling show that the coalescence of outer membrane raft proteins affects the composition of the cytoplasmic leaflet and its association with signalling molecules [20, 21]. To explain how membrane composition could affect prion formation we hypothesized that the clustering of sialic acid-containing GPIs attached to PrP activates $cPLA_2$, a factor that promotes PrP^{Sc} formation [22] . This occurs naturally as a consequence of PrP^{Sc} self-aggregation and experimentally following cross-linkage of PrP^{C} by monoclonal antibodies. The cross-linkage of desialylated PrP^{C} did not activate $cPLA_2$ [8] and the presence of desialylated PrP^{C} reduced activation of $cPLA_2$ in prion-infected neurons. Surprisingly desialylated PrP^{C} did not inhibit the phospholipase A_2-activating peptide (PLAP)-induced activation of $cPLA_2$ indicating that it had an indirect effect upon this enzyme. The targeting of $cPLA_2$ to membranes containing their substrates can regulate the formation of second messengers such as platelet-activating factor that facilitate PrP^{Sc} formation[22] and in control ScGT1 cells activated $cPLA_2$ co-localised to PrP^{Sc}-containing rafts [23]. The addition of desialylated PrP^{C} to prion-infected cells caused the dissociation of $cPLA_2$ from rafts indicating that the density of sialic acid attached to GPIs is critical to the stabilisation and activation of $cPLA_2$. We hypothesize that the binding of desialylated PrP^{C} to PrP^{Sc} affects the composition of the underlying membrane so that it no longer captured and activated $cPLA_2$. This reduced the activation of $cPLA_2$ by existing PrP^{Sc} and hindered the conversion of PrP^{C} to PrP^{Sc}. It is noteworthy that desialylated PrP^{C} is surrounded by more gangliosides than PrP^{C}, which is consistent with reports that gangliosides inhibit the activation of $cPLA_2$ [24 25].

In conclusion we show that sialic acid attached to the GPI affects the properties of PrP^{C}, altering the surrounding cell membrane, PrP^{C}-induced cell signalling and the trafficking of PrP^{C}. Critically the presence of desialylated PrP^{C} reduced the activation of $cPLA_2$ and PrP^{Sc} formation in prion-infected cells. We propose that sialic acid on the GPI anchor attached to PrP^{C} affects its precise membrane targeting and the subsequent cell signalling that is conducive to its conversion to PrP^{Sc}.

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