

## Glycosylphosphatidylinositols: More than just an anchor?

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### ABSTRACT

There is increasing interest in the role of glycosylphosphatidylinositol (GPI) anchors that attach some proteins to cell membranes. Far from being biologically inert, GPIs influence the targeting, intracellular trafficking and function of the attached protein. Our recent paper demonstrated the role of sialic acid on the GPI of the cellular prion protein (PrP<sup>C</sup>). The “prion diseases” arise following the conversion of PrP<sup>C</sup> to a disease-associated isoform called PrP<sup>Sc</sup> or “prion”. Our paper showed that desialylated PrP<sup>C</sup> inhibited PrP<sup>Sc</sup> formation. Aggregated PrP<sup>Sc</sup> creates a signaling platform in the cell membrane incorporating and activating cytoplasmic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), an enzyme that regulates PrP<sup>C</sup> trafficking and hence PrP<sup>Sc</sup> formation. The presence of desialylated PrP<sup>C</sup> caused the dissociation of cPLA<sub>2</sub> from PrP-containing platforms, reduced the activation of cPLA<sub>2</sub> and inhibited PrP<sup>Sc</sup> production. We concluded that sialic acid contained within the GPI attached to PrP<sup>C</sup> modifies local membrane microenvironments that are important in PrP-mediated cell signaling and PrP<sup>Sc</sup> formation.

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Prion diseases occur following the conversion of a normal host protein (the cellular prion protein (PrP<sup>C</sup>)) into disease-associated isoforms (PrP<sup>Sc</sup>) or “prions” which accumulate within the brain causing neurodegeneration. Our recent paper examined the role of the glycosylphosphatidylinositol (GPI) anchor that links PrP<sup>C</sup> to cell membranes upon the properties of PrP<sup>C</sup> and consequently whether PrP<sup>C</sup> was converted to PrP<sup>Sc</sup>.<sup>1</sup> As efficient PrP<sup>Sc</sup> formation occurs only when PrP<sup>C</sup> is targeted to specific membrane micro-domains called lipid rafts,<sup>2</sup> the factors affecting the cellular targeting and intracellular trafficking of PrP<sup>C</sup> are critical in regulating PrP<sup>Sc</sup> formation. The GPI anchor targets PrP<sup>C</sup> to lipid rafts that are required for efficient PrP<sup>Sc</sup> formation.<sup>3</sup> Our recent paper showed that the targeting of PrP<sup>C</sup> to those lipid rafts involved in PrP<sup>Sc</sup> formation was dependent upon the composition of the GPI anchor, specifically the presence of sialic acid. We reported 3 major observations:

1. That desialylated PrP<sup>C</sup> behaved differently from PrP<sup>C</sup> with regards to protein targeting, intracellular trafficking, its effects on membrane composition,

cell signaling and critically, it was not converted to PrP<sup>Sc</sup>.

2. That desialylated PrP<sup>C</sup> inhibited the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>.
3. That desialylated PrP<sup>C</sup> disrupted cell signaling mediated by PrP<sup>Sc</sup>.

Although GPI-anchored proteins are targeted to lipid rafts, there exist many different, heterogeneous lipid rafts<sup>4</sup> and PrP<sup>Sc</sup> formation probably occurs in only a subset of these. The composition of lipid rafts surrounding GPI-anchored proteins is dependent upon multiple interactions between the protein, glycans and membrane lipids,<sup>5</sup> and consequently PrP<sup>C</sup> and desialylated PrP<sup>C</sup> were found within different lipid rafts. We hypothesized that sialic acid in the GPI has a direct effect upon the composition of the surrounding membrane. Immunoprecipitation and analysis of lipid rafts surrounding PrP proteins demonstrated higher concentrations of gangliosides and cholesterol associated with lipid rafts containing desialylated PrP<sup>C</sup> than with lipid rafts containing PrP<sup>C</sup>. The functional consequences of these changes were 2-fold; desialylated PrP<sup>C</sup> remained within lipid rafts

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after cholesterol depletion, whereas PrP<sup>C</sup> redistributed to the normal cell membrane, and that desialylated PrP<sup>C</sup> had a demonstrably longer half-life than PrP<sup>C</sup> in neurons. We speculated that if sialic acid contained within the GPI competes with gangliosides for sialic acid-binding proteins, then the removal of sialic acid would allow the incorporation of more gangliosides into PrP<sup>C</sup>-containing rafts. Gangliosides help sequester cholesterol that increases membrane rigidity and stabilize lipid rafts.<sup>6</sup> Thus the increased concentrations of gangliosides surrounding desialylated PrP<sup>C</sup> would explain the observed increased cholesterol concentration in lipid rafts surrounding desialylated PrP<sup>C</sup>. This hypothesis is compatible with reports that the concentrations of gangliosides in lipid rafts affects the expression of PrP<sup>C</sup>.<sup>7</sup>

When neurons from transgenic mice in which the PrP protein had been deleted (Prnp<sup>0/0</sup> neurons) were pulsed with PrP<sup>Sc</sup>, we found that PrP<sup>C</sup> was converted to PrP<sup>Sc</sup>, but desialylated PrP<sup>C</sup> was not. Perhaps of greater interest were observations that in wildtype neurons and neuronal cell lines the presence of desialylated PrP<sup>C</sup> significantly reduced the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>. While most potential therapeutics for prion diseases are targeted at the protein component of PrP, our work highlights the importance of the underlying cell membrane. Since the composition and hence the function of lipid rafts is controlled by an “induced fit” model<sup>4</sup> we hypothesized that the binding of desialylated PrP<sup>C</sup> to PrP<sup>Sc</sup> modified the lipid rafts involved in PrP<sup>Sc</sup> formation. Because the composition of lipid rafts is affected by the glycan composition of GPIs<sup>5</sup> we expected that the lipid rafts surrounding PrP<sup>Sc</sup>:PrP<sup>C</sup> complexes would differ from that of lipid rafts surrounding complexes of PrP<sup>Sc</sup>:desialylated PrP<sup>C</sup>. We proposed that the binding of desialylated PrP<sup>C</sup> to PrP<sup>Sc</sup> changes the composition of the lipid rafts so that it inhibits the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>.

The coalescence of outer membrane lipid raft proteins affects the composition of the cytoplasmic leaflet and its association with signaling molecules.<sup>8</sup> The clustering of sialic acid-containing GPIs attached to PrP proteins activates cPLA<sub>2</sub>,<sup>9</sup> an enzyme that promotes PrP<sup>Sc</sup> formation.<sup>10</sup> This occurs naturally as a consequence of PrP<sup>Sc</sup> self-aggregation, and cPLA<sub>2</sub> is concentrated within PrP<sup>Sc</sup>-containing lipid rafts.<sup>11</sup> The binding of desialylated PrP<sup>C</sup> to PrP<sup>Sc</sup> changed the underlying membrane so that it no longer captured and activated cPLA<sub>2</sub>. This reduced the activation of cPLA<sub>2</sub> by existing PrP<sup>Sc</sup> and hindered the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>. It is noteworthy that desialylated PrP<sup>C</sup> is surrounded by more gangliosides than PrP<sup>C</sup>, which is consistent with reports that gangliosides inhibit the activation of cPLA<sub>2</sub>.<sup>12</sup>

We concluded that sialic acid in the GPI anchors affects the properties of PrP<sup>C</sup>, altering the surrounding membrane; PrP-induced cell signaling and the trafficking of PrP<sup>C</sup>. Critically desialylated PrP<sup>C</sup> reduced the activation of cPLA<sub>2</sub> and PrP<sup>Sc</sup> formation in prion-infected cells. We propose that sialic acid on the GPI anchor attached to PrP<sup>C</sup> affects its precise membrane targeting and the subsequent cell signaling that is conducive to its conversion to PrP<sup>Sc</sup>.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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