COMMENTARY

Prions in control of cell glycosylation?

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Prion proteins that are normal cellular components or involved in pathology can vary little or not at all in primary amino acid sequence, but their glycosylation is different, e.g. in scrapie versus normal forms; in mouse strain-specific isolates; and in BSE (bovine spongiform encephalopathy) and variant CJD (Creutzfeldt–Jakob disease) versus classical CJD. The results of Nielsen et al. published in this issue of the *Biochemical Journal* show that changes in glycosylation are not restricted to the prion. The paper

comprehensively characterizes a decrease in the glycosylation of the insulin receptor in scrapie-infected neuroblastoma cells, but no change in glycosylation of the insulin-like growth factor-1 receptor. Thus the scrapie prion can influence glycosylation, not only of itself, but also of other selected cell glycoproteins.

Key words: insulin-like growth factor receptor, insulin receptor, prion, protein glycosylation, scrapie, spongiform encephalopathy.

In the central nervous system, insulin acts as an important neurotrophic factor promoting cell growth and differentiation of neuronal cells during brain development, and is critical for normal brain function, including spatial and emotional learning and memory [1]. *In vitro*, insulin induces neuroblastoma cells to proliferate and protects various neuronal cells from apoptosis [2,3]. Insulin acts via the transmembrane IR (insulin receptor), which is a glycoprotein having 18 asparagine residues which can accept N-linked glycosylation and also some serine/threonine amino acids that are O-linked glycosylated [4]. The IR found in the brain and peripheral neurons differs from the corresponding receptor in peripheral tissues (liver, muscle and adipocytes) structurally and biochemically. The lower molecular mass on SDS/PAGE is believed to be caused primarily by alterations in glycosylation. The paper by Nielsen et al. [4] in this issue of the *Biochemical Journal* concentrates on the analysis of IR glycosylation and finds in neuroblastoma cells infected with scrapie prion an overall reduction in glycosylation, which correlates with a reduction in affinity for insulin binding. This is the first time that the glycosylation of other proteins besides the prion itself has been shown to vary in scrapie-infected cells. For the prion, differences in glycosylation have been characterized in both glycosylation structure and size, and in site occupancy (there are two possible asparagine residues that can receive N-linked glycosylation). The glycosylation pattern of prion proteins differs in scrapie pathological forms, in mouse strain-specific isolates and in BSE (bovine spongiform encephalopathy) and variant CJD (Creutzfeldt–Jakob disease) versus classical CJD [5]. Nielsen et al. [4] did not characterize the prion glycosylation in their paper, but they did study the IGF-1R (insulin-like growth factor-1 receptor), which has a similar structure and function to IR. Unlike IR, the glycosylation of the IGF-1R did not change on scrapie infection of neuroblastoma cells. Thus the scrapie prion seems to influence glycosylation, not only of itself, but also of selected other cell glycoproteins.

There has been much speculation as to how N-linked site occupancy can be controlled in a specific way. This occurs at the level of protein biosynthesis in the ER (endoplasmic reticulum). Nascent peptides are co-translationally modified by the transfer from lipid (dolichol phosphate) of oligosaccharides containing

14 monosaccharides. This transfer occurs on to asparagine residues that are in the sequon Asn-Xaa-Thr/Ser. Linkage to the N of asparagine designates these as N-linked glycosylation. Three of the terminal monosaccharides are cleaved off in an ordered manner during protein maturation at the ER in a process that seems to be involved in quality control of the finished glycoprotein, so that it has the correct conformation, for example. The prion is unique amongst proteins that cause protein-folding disorders in that it is embedded in membranes via a GPI (glycosyl-phosphatidylinositol) anchor. The addition of the anchor occurs at the end of protein synthesis when a particular amino acid signalling sequence at the C-terminus is cleaved off and the GPI is added. The GPI would normally dictate the membrane transport through intracellular pathways, positioning in the plasma membrane and recycling. In the case of the prion, there is evidence that structures at the N-terminal end of the molecule also affect where it travels. It can thus be speculated that the prion has particular access to the membrane areas, for example where glycosylation is taking place. In addition to such activity at the ER, throughout transport of all glycoproteins through the Golgi apparatus, the N-glycosylation is being altered until the different patterns found in the mature glycoprotein are achieved. O-glycosylation occurs at the later stages of Golgi transport, and there are additional changes possible at the cell surface and in recycling (such as loss of the then terminal neuraminic acid monosaccharide residues). For the prion, both site occupancy (e.g. in mouse strains and in BSE versus classical CJD) and the final glycosylation pattern (in scrapie versus the normal cellular form) can be changed. For the IR as reported by Nielsen et al. [4], the latter class of changes were characterized, such as alterations in the levels of neuraminic acid. These authors comprehensively studied the glycosylation changes and excluded other reasons for decreased molecular mass of the IR in scrapie-infected neuroblastoma cells, such as protease digestion, different hybrid forms (a complex area discussed in the paper), alternative splicing and phosphorylation. Interestingly, they do quote that a decrease in the IR β chain due to proteolytic degradation has been observed in several midbrain regions of Parkinsonism patients [6].

Of further note (reviewed in [4] from these authors' previous findings) is that scrapie infection of neuroblastoma cell lines

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induces 2- and 4-fold increases in IR and IGF-1R protein levels respectively without a corresponding increase in specific insulin- and IGF-binding sites or cell growth in insulin- or IGF-1-containing medium. For IGF-1R, this was shown to be caused by a 7-fold decrease in IGF-1R affinity. However, for IR, the decrease in insulin binding was found when related to the increase in immunoreactive IR, and the receptor binding affinity overall was unchanged. This indicated "the presence of two populations of IRs [in scrapie-infected neuroblastoma cells]: one receptor population that is normally expressed, processed and functional, as determined by an unchanged receptor binding affinity and another receptor population that does not recognize insulin, but is detected by the anti-IR β antibody" that the authors used in immunoreactive detection [4]. The IR β -chain showed an apparent decrease of molecular mass to approx. 85 kDa compared with 95 kDa in non-infected cells.

Questions remain as to the significance of altered glycosylation. Do prions magnify their pathological effects by altering cell glycosylation? If prions affect many levels of intracellular membrane signalling, including the machinery for glycosylation, why the selectivity? Is the change in glycosylation of the IR a reversion to its state on peripheral tissues rather than neural cells (as suggested), which in some way alters the prion effect on its function?

Are changes in glycosylation primary or secondary to alterations in IR biochemistry and prion conformational change?

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