Reactive oxygen species (ROS)-mediated β-cleavage of the prion protein in the mechanism of the cellular response to oxidative stress

N.T. Watt and N.M. Hooper

Proteolysis Research Group, School of Biochemistry and Microbiology, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds LS2 9JT, U.K.

Abstract

The PrP^C [cellular isoform of PrP (prion protein)] can undergo a conformational conversion to produce a proteinase-resistant form PrP^Sc (scrapie isoform of PrP), a step critical for the development of prion disease. Although essential for disease progression, the normal cellular function of PrP^C remains unknown. Suggestions to date have centred on a protective role against oxidative stress. We have demonstrated that ROS (reactive oxygen species)-mediated β-cleavage of PrP^C occurs at the cell surface, can be inhibited following hydroxyl radical quenching and has a prerequisite for the octarepeat region in the N-terminus of the protein. Significantly, two disease-associated mutants of PrP, namely PG14 and A116V (Ala^116→Val), were unable to undergo β-cleavage and this lack of proteolysis was accompanied by functional consequences in cells expressing these mutant proteins. The cells were found to be less viable following exposure to copper and H_2O_2, had reduced levels of glutathione peroxidase and increased amounts of intracellular oxygen radicals. These results suggest that β-cleavage of PrP^C is an initial consequence following exposure to ROS in the extracellular environment contributing to a pathway involved in antioxidant protection of neuronal cells.

Introduction

Prion diseases occur when a conformational conversion in the normal PrP^C [cellular isoform of PrP (prion protein)] produces a proteinase-resistant form PrP^Sc (scrapie isoform of PrP). Examples of such neurodegenerative disorders include scrapie in sheep, bovine spongiform encephalopathy in cattle and Creutzfeldt–Jakob disease in humans [1]. Although an essential prerequisite in the development of disease by providing the substrate for conversion [2,3], the physiological role of PrP^C is not clear. It remains to be established whether prion diseases are, in part, due to the loss of a normal neuroprotective function of PrP^C [4].

PrP^C is a glycosylphosphatidylinositol-anchored glycoprotein that undergoes several proteolysis steps. α-Cleavage occurs at amino acids 110/111, possibly by a member of the ADAM (a disintegrin and metalloproteinase) family of zinc metalloproteinases, to produce a 17 kDa C-terminal fragment C1 and an N-terminal fragment N1 [5,6]. Cleavage close to, or within, the octarepeat region has been termed β-cleavage and appears to be mediated by ROS (reactive oxygen species). This generates a 21 kDa C-terminal fragment C2 and a corresponding N-terminal fragment N2 [5,7–9]. Further understanding of the significance of these proteolysis steps and the role of the fragments they generate is crucial to appreciate the biological function of PrP and may provide insights into novel therapeutic approaches.

ROS-mediated β-cleavage of PrP^C occurs rapidly at the cell surface

Murine PrP containing the 3F4 epitope stably expressed in the human neuroblastoma SH-SY5Y cell line undergoes both α- and β-cleavages as shown by the production of C1 and C2 respectively. Exposure of the cells to Cu^{2+} and H_2O_2 increased the production of C2 via β-cleavage, but had no effect on α-cleavage or the levels of full-length PrP^C. The hydroxy radical quencher DMSO caused a dose-dependent reduction in the production of C2 consistent with β-cleavage being a ROS-mediated process.

To determine whether cell-surface PrP^C was subject to ROS-mediated β-cleavage, cells expressing PrP^C were surface-biotinylated before treatment with 100 μM H_2O_2 and 10 μM Cu^{2+}. Following immunoprecipitation of PrP with antibody 3F4, biotinylated full-length PrP and C2 were visualized by immunoblotting with peroxidase-conjugated streptavidin. Immediately following biotinylation, negligible biotinylated C2 was detected in the cell lysate, although significant amounts of biotinylated full-length PrP were present. However, an increase in the level of biotinylated C2
fragment was evident following incubation of the cells with H$_2$O$_2$ and Cu$^{2+}$ for 10 min, indicating that C2 is formed from PrP$^{\text{C}}$ exposed at the cell surface.

The octapeptide repeats are required for the ROS-mediated $\beta$-cleavage of PrP$^{\text{C}}$

To determine whether cleavage required the octapeptide repeats, we examined the ROS-mediated processing of PrP$^{\Delta \text{oct}}$ that lacks the copper-binding octapeptide repeat region. Lysates from cells expressing PrP$^{\Delta \text{oct}}$ were subjected to immunoblot analysis with antibodies 3F4, SAF32 (which recognizes an epitope within the octapeptide repeats) and 6H4 (which recognizes an epitope in the C-terminal half of the protein). SAF32 failed to detect PrP$^{\Delta \text{oct}}$, but this construct was detected by 3F4 and 6H4. Although antibody 6H4 clearly detected the C1 fragment in cells expressing PrP$^{\Delta \text{oct}}$, neither 6H4 nor 3F4 detected the C2 fragment. These results indicate that the octapeptide repeats are required for PrP$^{\text{C}}$ to undergo $\beta$-cleavage.

ROS-mediated $\beta$-cleavage is defective in two disease-associated mutants of PrP

The proteolytic processing of two disease-associated mutants of PrP was examined. PG14 contains an extra nine copies of the octapeptide repeat and is associated with familial human prion disease and A116V, in which Ala$^{116}$ (murine PrP numbering, equivalent to Ala$^{117}$ in human PrP) is mutated to Val, is associated with Gerstmann–Sträussler–Scheinker disease. Lysates from cells expressing the two mutants were subjected to immunoblot analysis with antibodies SAF32, 3F4 and 6H4. Although all three antibodies detected full-length protein in cells expressing either PG14 or A116V and 6H4 detected the C1 fragment, there was no detection of the C2 fragment in either cell line even after prolonged exposure of the immunoblots or prolonged exposure of the cells to Cu$^{2+}$ and H$_2$O$_2$. These results indicate that in cells expressing PG14 or A116V, C2 is not formed upon exposure of the cells to ROS, whilst C1 is formed normally.

Failure to undergo ROS-mediated $\beta$-cleavage has functional consequences

To determine whether inability to undergo ROS-mediated $\beta$-cleavage affected a biological function of PrP$^{\text{C}}$, the resistance to oxidative stress of cells expressing PrP$^{\Delta \text{oct}}$, PG14 and A116V was examined. The viability of cells expressing the various mutants of PrP was assessed by measurement of cell number using Hoechst 33342 staining. Cells expressing PrP$^{\Delta \text{oct}}$, PG14 or A116V all displayed significantly reduced viability when challenged with H$_2$O$_2$ and Cu$^{2+}$ as compared with cells expressing wtPrP (wild-type PrP) ($P < 0.001$). Measurement of intracellular free radical generation was made using the fluorescent dye dihydrodichlorofluorescein diacetate following exposure of the cells to 10 $\mu$M Cu$^{2+}$ and 100 $\mu$M H$_2$O$_2$. Whereas there was a significant decrease in radical formation in the wtPrP-expressing cells as compared with the untransfected cells, cells expressing PrP$^{\Delta \text{oct}}$, PG14 or A116V had a similar level of radical formation as the untransfected cells. Glutathione peroxidase is a key component of an important antioxidant pathway in neurons, detoxifying H$_2$O$_2$ upon glutathione oxidation. The wtPrP-expressing cells had a higher level of glutathione peroxidase activity than the untransfected cells, whereas the cells expressing PrP$^{\Delta \text{oct}}$, PG14 or A116V all had significantly decreased glutathione peroxidase activity ($P < 0.001$). Together, these results indicate that cells expressing PrP$^{\Delta \text{oct}}$, PG14 or A116V, none of which undergo ROS-mediated $\beta$-cleavage, were not protected against oxidative stress in the same way that cells expressing wtPrP were protected.

To examine further the relationship between ROS-mediated $\beta$-cleavage and the resistance of cells to oxidative stress, we sought to block $\beta$-cleavage of wtPrP and then assess cell viability upon exposure to ROS. We reasoned that the antibody SAF32 that binds to the octapeptide repeats may prevent $\beta$-cleavage. Cells expressing wtPrP were incubated in the presence of either antibody SAF32 or antibody 3F4 as control before exposure to Cu$^{2+}$ and H$_2$O$_2$. Although the formation of C2 still occurred in cells incubated with 3F4, its production was significantly reduced by SAF32. Furthermore, cells incubated with SAF32 had a significantly lower viability when exposed to Cu$^{2+}$ and H$_2$O$_2$ than cells exposed to either 3F4 or no antibody, providing further evidence that $\beta$-cleavage is involved in the cellular response to oxidative stress.
Conclusion
It is well documented that PrP−/− cells are more susceptible to oxidative damage and toxicity caused by ROS, implicating PrPC in the cellular response to oxidative stress [10,11]. However, the mechanism by which this is mediated is not known. Our observation that surface-biotinylated PrPC rapidly undergoes β-cleavage upon exposure of cells to ROS and that a failure of β-cleavage correlates with increased sensitivity of cells to oxidative stress provides the first direct evidence that β-cleavage may be a critical first step whereby PrPC protects cells against external oxidative stress [12]. Consistent with this, cells expressing wtPrP have increased viability and glutathione peroxidase activity, with reduced intracellular free radicals when exposed to ROS compared with untransfected cells [12]. These protective responses to ROS are not observed in the cells expressing PrPΔoct, which fails to undergo β-cleavage, or in cells expressing wtPrP, where β-cleavage is blocked by the binding of antibody SAF32 to the octapeptide repeats [12]. The observation that ROS-mediated β-cleavage of PrP is defective in two disease-associated mutants, PG14 and A116V, adds further weight to the argument that prion diseases are, in part, due to the loss of a normal function of PrPC. If PrPC is not available to undergo ROS-mediated β-cleavage due to mutation for example, such as in PG14 and A116V, the normal cellular response to oxidative stress is compromised (Figure 1), which may in turn contribute to the neurodegeneration.

References

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