Prion processing: a double-edged sword?

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Abstract

The events leading to the degradation of the endogenous PrP0 (normal cellular prion protein) have been the subject of numerous studies. Two cleavage processes, \( \alpha \)-cleavage and \( \beta \)-cleavage, are responsible for the main C- and N-terminal fragments produced from PrP0. Both cleavage processes occur within the N-terminus of PrP0, a region that is significant in terms of function. \( \alpha \)-Cleavage, an enzymatic event that occurs at amino acid residues 110 and 111 on PrP0, interferes with the conversion of PrP0 into the prion disease-associated isoform, PrPSc (abnormal disease-specific conformation of prion protein). This processing is seen as a positive event in terms of disease development. The study of \( \beta \)-cleavage has taken some surprising turns. \( \beta \)-Cleavage is brought about by ROS (reactive oxygen species). The C-terminal fragment produced, C2, may provide the seed for the abnormal conversion process, as it resembles in size the fragments isolated from prion-infected brains. There is, however, strong evidence that \( \beta \)-cleavage provides an essential process to reduce oxidative stress. \( \beta \)-Cleavage may act as a double-edged sword. By \( \beta \)-cleavage, PrP0 may try to balance the ROS levels produced during prion infection, but the C2 produced may provide a PrPSc seed that maintains the prion conversion process.

Introduction

As for numerous proteins, the endogenous PrP0 (normal cellular prion protein) undergoes a number of post-translational cleavage events during its life. The main cleavage processes are so specific, yet the function of these cleavage events and the fragments formed is only starting to be unravelled. It is accepted that PrP0 serves as a template for the generation of PrPSc (abnormal disease-specific conformation of prion protein), which is the abnormally folded PrP isoform associated with prion diseases. PrP0 is essential for prion disease development, and lack of PrP0 confers a resistance to prion infection [1–3] that can be reversed by the reintroduction of a PrP (prion protein) transgene [3–5]. PrP0 and PrPSc are structurally and biochemically distinct. Unlike PrP0, PrPSc has a proteinase-resistant core stemming from amino acid residues 90–231 [6,7]. For PrP0, the predominant cleavage events occur within the N-terminus of the protein, in regions that are significant for potential function and conversion into PrPSc. Whether the processing has positive or negative connotations for PrP0 has yet to be understood.

The PrP0 C-terminus is highly structured with a predominant \( \alpha \)-helical fold [8,9]. The distinction between PrP0 and the structure of PrPSc lies within the C-terminus: in this region, PrPSc has a much higher \( \beta \)-sheet content than PrP0; as a consequence, PrPSc is partially proteinase-resistant. In the absence of copper binding, the PrP0 N-terminus lacks any given structure [8,10]. Although the N-terminus of PrPSc, amino acid residues 23–89, is not required for prion propagation [11], the N-terminus can affect fibrillation [12,13]. In terms of functionality, the N-terminus of PrP0 has received a lot of attention.

Transgenic mice expressing PrP with deletions of amino acids 32–121, 32–134, 94–134 and 105–125 [14–16] exhibit ataxia with extensive neurodegeneration. The degenerative phenotypes were suppressed by co-expression of full-length PrP0. Recently it has been suggested that residues 23–31, KKRPKPGGW, the polybasic region, may also be responsible for the neurotoxic effects, as mice expressing \( \Delta \)23–134 PrP display no abnormalities [17], which is in contrast with the studies mentioned previously. This region, however, is essential for PrP0 internalization [18,19] and it has been linked with a ROS (reactive oxygen species)-reduction response [20]. The proline residues 26 and 28 may in part be responsible for the function. The proline residues appear to impart a small structural motif to the very end of PrP0 that may enable binding to glycosaminoglycans and their elimination produces a less rigid and potentially deleterious N-terminal fragment [20].

The PrP0 N-terminus is also involved in the binding of copper [21,22], binding occurs within the OR (octapeptide repeat) region, in which the sequence PHGGWGGQ is repeated four times. Copper binding in this region is cooperative [23,24] and, at low Cu2+ levels, it is redox-active. Cu2+ while bound to PrP0, at low levels, can be reduced to Cu1+ [25], which can then participate in the Fenton reaction. This region of the protein has significance for prion disease development. Prion disease is delayed in mice expressing PrP with the OR region deleted [26], and additional ORs are seen in certain cases of CJD (Creutzfeldt–Jakob disease) [27].
PrP<sup>C</sup> is subjected to a number of cleavage events within its N-terminus and the present review summarizes some of the cleavage events and their implications for PrP<sup>C</sup>.

**The cleavage events**

Two internal cleavage processes, α-cleavage and β-cleavage, are responsible for the main C- and N-terminal fragments seen in both cell culture and brain tissue (Figure 1). The first, α-cleavage, which occurs constitutively in 10–50% of PrP<sup>C</sup> [28–30], results in cleavage at amino acids 110 and 111 to produce a 17 kDa C-terminal fragment, C1, that is tethered to the plasma membrane and a corresponding 9 kDa N-terminal fragment, N1. C1, the major C-terminal fragment found in non-disease states, is produced by the ADAM (a disintegrin and metalloproteinase) 10 and ADAM17 members of the ADAM family [31]. A longer PrP C-terminal fragment of 21 kDa, C2, is found in amyloid fibrils in prion-diseased brains [28]. In the non-diseased state, C2 is only present in very small amounts. The cleavage leading to C2 generation, β-cleavage [32], occurs within or adjacent to the OR region. We identified that this cleavage results from self-cleavage by ROS that are generated locally on PrP<sup>C</sup> [33]. β-Cleavage is dependent on the OR region producing ROS; this occurs under conditions of copper binding to the histidine residues within the ORs and oxidative conditions, such as the presence of H<sub>2</sub>O<sub>2</sub>. Under these conditions, once copper is bound, it is reduced to Cu<sup>1+</sup>; tryptophan residues within the ORs are proposed to facilitate the reduction reaction [25]. ROS, which are then generated locally on the protein through the Fenton reaction, cleave alongside the protein backbone. This processing event is highly specific, occurring alongside the ORs.

**The cleavage fragments, what is their function?**

Both the α- and β-cleavage events incurred by PrP<sup>C</sup> are highly specific; this would indicate that they have potential function in terms of the fragments that are produced or in terms of the role of PrP<sup>C</sup> itself. α-Cleavage, with its products C1/N1, is generally seen as a positive processing event. It occurs in a hydrophobic domain, amino acids 106–126, and this area is considered to play a significant role in the pathological conversion. In addition, transgenic mice expressing C1 [PrP (Δ23–111)] display no signs of neurological disease, indicating that C1 is not itself neurotoxic. Mice expressing C1 [Tg(C1)/Prn-p0/0] are also resistant to scrapie infection [34].

The ability of PrP<sup>C</sup> to undergo α-cleavage has also been linked with a myelinotrophic function. CDP (chronic demyelinating polyneuropathy) has been observed in PrP<sup>C</sup>-deficient mice [35] at 60 weeks of age and has been linked with a lack of C1 fragment in the sciatic nerves. CDP can be rescued in mice expressing PrP that produced C1, but not by mutant PrP lacking C1 production. Bremer et al. [35] found that the peripheral myelin maintenance was dependent on the central domain of PrP<sup>C</sup>, amino acids 94–134, and the process was independent of the OR region. On the flip side, C1, unlike C2, is reported to have a p53-dependent pro-apoptotic function [36]. This may be modulated by N1, its partner product, as...
N1, but not N2, protected against pressure-induced ischaemia in models of rat retina, by modulating the p53 pathway [36].

β-Cleavage (C2/N2), however, requires a little more understanding. On the one hand, it is seen as a protective response to stress within the cell [37] and on the other, it may propel prion aggregation as the amyloidogenic core is left intact. Initially, we proposed ROS cleavage to be a negative event, as it produces a fragment similar in length to the C2 observed in CJD patients [28,38]. We also proposed that the C2 produced by ROS cleavage may itself impart toxicity in a similar manner to truncated PTP (Δ32–121/Δ32–134) expressed in mice [15]. In terms of a negative impact, β-cleavage/copper-oxidizing conditions aggregate and precipitate recombinant PrP [15] and produces PrP with physical characteristics associated with PrPSc [39]. In addition, recombinant PrP-(90–231), which mimics C2, can polymerize wild-type PrP [PrP-(23–231)] to a protease-resistant form in a copper-dependent manner [40]. Hypothetically, it should be possible for C2 to be cleaved further at the C1 site unless the β-cleavage induces folding events in C2. Sunyach et al. [36] found that C2 was not processed to C1, even when the C2 was completely degraded, which would indicate that it may not be possible for C2 to be processed to C1, and that C2 and C1 production are independent events. On conversion of PrPSc into PrPSc, the C1 cleavage site appears to become inaccessible to cleavage. Once produced, C2 may still have the ability to bind copper, but at His96 and His111 [41]; this is not the case for C1, and this may have implications for converting C2 into a PrPSc-like molecule. Jones et al. [42] identified that copper binding to the 109–122 region induces β-sheet folding in this domain. As indicated by Pushie and Vogel [41], it is possible that copper bridging of His96 and His111 may facilitate β-sheet formation that may propel misfolding once the OR region is removed by ROS.

β-Cleavage appears to have a dual role as the process also provides a critical protective mechanism for dealing with oxidative insult [37]. Cells lacking PrPSc are more susceptible to oxidative injury than those expressing full-length PrP capable of undergoing β-cleavage [37]. The same protective mechanism is not afforded by mutant PrPs (associated with inherited prion disease) that fail to undergo β-cleavage [37]. However, mutant PrP that fails to be processed by α-cleavage and only undergoes β-cleavage enhances cell viability to a greater extent than wild-type PrP [32]. ROS protection has been linked with the N-terminal product N2 and in particular the polybasic region and the proline residues 26 and 28 [20]. The work of Haigh et al. [20] would indicate that N2 is an active fragment, and that ROS reduction occurs through the polybasic region. β-Cleavage has also been seen to be protective in control ischaemia models [43]. The ischaemia response seen with PrPSc knockout (PrP Sc-/-) mice was rescued by transgenic mice that carried full-length wild-type PrPSc, but not by PrP that lacked the OR, suggesting a requirement for β-cleavage for protection against ischaemic insult [43].

**Conclusion**

Proteolytic processing of PrPSc is a switch; how and where it occurs at the precise locations on PrPSc may dictate the endpoint function of PrPSc. The impact of α-cleavage would appear clear-cut, its product interferes with the PrPSc–PrPSc conversion process [34]. But it also has signalling implications, and, surprisingly, C1 has a pro-apoptotic function that may be balanced by N1. The study of the impact of β-cleavage has taken some surprising turns. The C2 fragment from the β-cleavage/stress cleavage of PrPSc resembles in size the fragments isolated from prion-infected brains, and C2 may provide the seed for the abnormal conversion process. This would be consistent with the elevated stress levels observed in the prion system [44], which could elevate the level of β-cleavage. Increases in ROS levels seem to correlate with increases in the abnormal conversion process. Yet there is strong evidence that β-cleavage provides an essential process to reduce oxidative stress. In a non-infected system, PrPSc plays an important role in cell protection. In the infective system, it is possible that this protective β-cleavage acts as a double-edged sword. As prion infection propels and increases ROS levels, PrPSc may try to balance ROS levels through β-cleavage, but, in essence, its end product, C2, provides a PrPSc conversion seed that maintains the infection conversion process.

**References**


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