Inhibition of Scrapie-Associated PrP Accumulation

Probing the Role of Glycosaminoglycans in Amyloidogenesis

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Abstract

Accumulation of an abnormal, protease-resistant form of an endogenous protein, PrP, is a characteristic feature of scrapie and related transmissible spongiform encephalopathies. This abnormal isoform is also present in the amyloid plaques that are often observed in these diseases. In mouse neuroblastoma cells persistently infected with scrapie, the abnormal protease-resistant isoform of PrP is derived from an operationally normal protease-sensitive precursor. Conversion of PrP to the protease-resistant state occurs either on the plasma membrane or along an endocytic pathway by an unknown mechanism. Inhibitors of protease-resistant PrP accumulation have been identified, and these include the amyloid-binding dye Congo red and certain sulfated glycans. The similarity of these compounds to sulfated glycosaminoglycans, which are components of all natural amyloids, has led to the hypothesis that the inhibitors act by competitively blocking an interaction between endogenous glycosaminoglycan(s) and PrP that is critical for amyloidogenic PrP accumulation. The proven prophylactic effect of these sulfated glycans in animal models of scrapie suggests that they represent a group of compounds that might interfere with the pathogenic formation of amyloid in a variety of diseases, such as Alzheimer’s disease.

Index Entries: Scrapie; PrP; amyloid; Congo red; glycosaminoglycans; Alzheimer’s disease.

Introduction

Scrapie is a transmissible, neurodegenerative disease of unknown etiology that was first recognized in sheep, but can also be transmitted to other mammals. Mouse and hamster models of scrapie have become experimental prototypes for a group of diseases known as the transmissible spongiform encephalopathies (TSEs). Prominent examples of TSEs include kuru, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler Scheinker disease of humans and bovine spongiform encephalopathy of cattle. One of the characteristic features of the TSEs is the accumulation, sometimes in the form of amyloid plaques, of an abnormally proteinase K-resistant isoform of a host protein, PrP (1–5). The fact that the
protease-resistant PrP isoform (PrP-res) copurifies with infectivity, yet does not appear to be associated with any scrapie-specific nucleic acid, has led to the hypothesis that PrP-res is the infectious scrapie agent (6). Although this hypothesis is still speculative (7–10), it is clear that PrP plays an important role in TSE pathogenesis.

Normally PrP is expressed in a protease-sensitive form (PrP-sen) in brain and other tissues (5,11–16). Although PrP is developmentally regulated (17–19) and has been implicated in lymphocyte activation (20), its normal function is unclear. During scrapie pathogenesis, PrP-res accumulates in the central nervous system and other tissues (1,2,12,21–23). PrP-sen and PrP-res are encoded by the same host gene (24) with no apparent difference appearing at either the level of the mRNA (4,5) or primary protein sequence (13,25). Thus, the scrapie-specific modification of PrP is believed to arise posttranslationally, and this has been borne out by biosynthetic studies (26,27). The mechanism by which PrP is converted to the TSE specific form is not known, but a clear understanding of this process is essential, since it appears to play a crucial role in the pathogenesis and transmission of the TSEs.

Utilizing tissue culture cells persistently infected with the scrapie agent, the biosynthesis of both PrP-sen and PrP-res and their metabolic relationship to each other have been studied (26–30). The recent discovery of a class of compounds that specifically inhibits the accumulation of PrP-res in these cultures has provided insights at a molecular level into a possible mechanism by which PrP becomes protease-resistant and amyloidogenic (31,32). In this article, we will briefly review what is known about the biosynthesis of PrP-sen and PrP-res, and discuss in greater detail studies that define inhibitors of PrP-res accumulation, the effect these inhibitors have in vivo, and insights these inhibitors provide into mechanisms of amyloidogenesis. It is important to note that PrP-res is not always observed in the form of amyloid fibrils, so to equate PrP-res formation with amyloidogenesis is an oversimplification. The process is perhaps more accurately described as an abnormal metabolic stabilization and aggregation of PrP that can ultimately result in the formation of classic amyloid deposits. Nonetheless, the metabolic stabilization of amyloidogenic precursor proteins must occur in all amyloidoses in order for amyloid to accumulate. Therefore, one can regard all the steps of PrP-res formation as potentially relevant to the general understanding of amyloidogenesis.

In Vitro Tissue-Culture Models of Scrapie

Although many diverse tissue-culture cell lines express PrP (5,33–35), few have proven to be susceptible to persistent scrapie infection. The rat pheochromocytoma cell line PC12 can reliably accumulate infectivity to relatively high specific infectivities when differentiated in the presence of nerve growth factor (36–39), but little is known about the biosynthesis of PrP in these cells. Most analyses of PrP biosynthesis in vitro have been performed in scrapie-infected (Sc‘MNB) or uninfected (MNB) murine neuroblastoma cells (35,40,41), or in scrapie-infected hamster brain cells (42). Sc‘MNB cells remain persistently infected, divide, replicate scrapie agent, and accumulate PrP-res, providing an easily manipulated system for studying the biosynthesis of both PrP-sen and PrP-res.

Biosynthesis of Normal and Scrapie-Associated PrP

Pulse-chase experiments using both 35S-methionine-labeled MNB cells and Sc‘MNB cells have led to an understanding of the basic biosynthesis and processing of both PrP-sen and PrP-res and their precursor–product relationship (26–28,30,43). These studies have shown that although the synthesis of PrP-sen in uninfected and scrapie-infected MNBs appears the same, the biosynthesis of PrP-res differs dramatically from that of PrP-sen in Sc‘MNBs.

Biosynthesis of Normal PrP

Nascent PrP is loaded cotranslationally into the endoplasmic reticulum where several events occur: An N-terminal signal peptide is cleaved (13,44,45), a glycosphatidylinositol (GPI) anchor is attached, and high-mannose glycans are added to one or two potential N-linked glycosylation sites (28). PrP is then translocated into the Golgi apparatus where the high-mannose glycans are converted to complex or hybrid glycans (28). Translocation to the cell surface follows, and PrP is anchored to the plasma membrane via the GPI anchor (28,46,47). The majority of the cell-surface PrP is phospholipase-sensitive, although a small proportion appears to be resistant to phospholipase treatment (28,29). Once on the cell surface, PrP has a half-life of 3–6 h; over time, most PrP appears to be degraded, but some is released into the tissue culture medium (26–28,34). Soluble forms of PrP have also been found in vivo in human cerebral spinal fluid (48) and in MNB...