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Detection, quantification, and glycotyping of prion protein in specifically activated enzyme-linked immunosorbent assay plates

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Abstract

The conversion of a normal glycoprotein, prion protein (PrP^{C}), to its abnormal protease-resistant isoform (PrP^{Sc}) seems to be one of the main factors underlying the pathogenesis of spongiform encephalopathies. There are many studies indicating that PrP interacts with glycosaminoglycans, and we exploited this interaction to develop a sensitive solid phase assay for detection of both PrP forms. Glycosaminoglycans, such as chondroitin sulfate and heparin, were immobilized by their negative charge to enzyme-linked immunosorbent assay (ELISA) plate wells activated by glutaraldehyde and spermine. PrP in the samples examined (recombinant PrP or tissue homogenate) was allowed to interact with glycans. The interaction of recombinant PrP was more efficient against immobilized chondroitin sulfate of type A, and a linear correlation with concentration was demonstrated. From this curve, the concentration of each one of the PrP isoforms in biological samples can be determined. In addition, and taking into account that glycosylation of prion protein is species specific, we used similarly activated ELISA plate wells to determine different PrP glycoforms. A monoclonal antibody against PrP was immobilized, and PrP present in the samples (brain homogenates) was bound and visualized by various lectins. The most interesting outcome of the study is the differential binding of ricinus communis agglutinin I to the normal and scrapie brain homogenates. Dattura stramonium lectin and wheat germ agglutinin seem to bind almost equally to both samples, and all three have an increased sensitivity to PrP^{Sc} after proteinase K digestion.

Keywords: Chondroitin sulfate; Heparin; Scrapie; BSE; Tonsils; Lectin

Prions are infectious pathogens causing a group of invariably fatal neurodegenerative diseases by an entirely novel mechanism that remains poorly understood even today [1]. Scrapie, bovine spongiform encephalopathy (BSE),[1] Creutzfeldt–Jakob disease (CJD), kuru, Gerstmann–Sträussler syndrome (GSS), and fatal familial insomnia (FFI) are among the most common prion diseases in animals and humans. The major component of infectious prion particles is a protein denoted PrP^{Sc} rich in β-pleated sheet conformation. PrP^{Sc} is a post-translationally modified isoform of the normal prion protein, denoted PrP^{C}, a glycolipid-anchored plasma membrane protein with a predominant α-helix conformation that is widely expressed in neurons and glia in the central nervous system [2]. PrP contains two potential *N*-glycosylation sequences (Asn-Xaa-Thr), and both, one, or none can be glycosylated [3,4]. It has been shown that a three-band pattern is obtained by Western blot, representing the high, low, and nonglycosylated forms maintained after digestion of PrP^{Sc} with proteinase K. The extent of glycosylation at each site is characteristic for each species [5,6]. Glycan analysis has shown that PrP contains at least 52 different sugars, that these consist

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[1] *Abbreviations used:* BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler syndrome; FFI, fatal familial insomnia; PrP^{C}, normal prion protein; TSE, transmissible spongiform encephalopathy; GAG, glycosaminoglycan; ELISA, enzyme-linked immunosorbent assay; CSA, chondroitin sulfate A; CSE, chondroitin sulfate E; CSB, chondroitin sulfate B; CSC, chondroitin sulfate C; GA, glutaraldehyde; BSA, bovine serum albumin; *o*-PD, *o*-phenylenediamine; DSL, datura stramonium lectin; WGA, wheat germ agglutinin; RCA, ricinus communis agglutinin I; PBS, phosphate buffer; PMSF, phenylmethylsulfonyl fluoride; PBS–T, phosphate buffer containing 0.1% Tween 20; rPrP, recombinant prion protein.

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of a subset of brain sugars, and that there is site-specific glycan processing [7]. The size of carbohydrate chains present in the protein differs in PrP from different strains. These differences have been used to identify at least five different types of CJD, by a method called glycotyping [8]. Identification of sugar moieties can be performed by using lectins that specifically bind (or crosslink) carbohydrates, as is well known in spite of our ignorance about the function of lectins in nature. This unique group of proteins has provided researchers with a powerful tool to probe the biological structures and processes of carbohydrates.

Heparan sulfate and heparin proteoglycans have been found to be associated with amyloid deposits in a number of diseases, including the cerebral amyloid plaques of Alzheimer's disease and the transmissible spongiform encephalopathies (TSEs). The role of those molecules in amyloid formation and the neurodegenerative pathology of these diseases have not been established [9]. These findings led scientists to believe that there is a common mechanism by which heparan sulfate proteoglycans play a role in the pathogenesis of amyloidosis in prion diseases [10,11]. Ultrastructural analyses of brain tissues from patients with Alzheimer's disease showed that the normal staining pattern of heparan sulfate proteoglycans is disrupted on the endothelial surface of the vascular basement membrane [12]. In addition, proteoglycans isolated from skin fibroblast from patients with Alzheimer's disease showed increased sulfate incorporation relative to that from normal controls. Moreover, analysis of the disaccharides, which were produced by treatment with heparinase and heparitinase, showed differences in composition between controls and patients with Alzheimer's disease [13].

On the other hand, it has been shown that Congo red, a disulfonated amyloid-binding dye, and glycans such as pentosan sulfate and dextran sulfate inhibit prion replication and PrP^{Sc} accumulation in scrapie-infected neuroblastoma cells [14–16]. The mechanism by which inhibition is achieved is based on alteration of the cellular localization of PrP^{C}, the noninfectious precursor of PrP^{Sc}, from the plasma membrane to the cell interior [2]. Similar results have been obtained using heparin and heparin-like molecules of low molecular mass. Inhibition of PrP^{Sc} synthesis in scrapie-infected cells, and even reversion of their infectious phenotype to normal, was observed [17]. In this case, it is considered that PrP^{C} and PrP^{Sc} are bound to endogenous glycosaminoglycan (GAG) chains as an essential step for accumulation of PrP^{Sc} in a protease-resistant, potentially amyloidogenic state [15,18].

Prion diseases are usually diagnosed clinically and confirmed by postmortem histopathological examination of brain tissue. The only reliable molecular marker for prion diseases is PrP^{Sc} that accumulates in the central nervous system and, to a lesser extent, in lymphoreticular tissues. Currently, there is no diagnostic test that detects prion diseases in live animals and humans [19], although some approaches of enzyme-linked immunosorbent assay (ELISA) and Western blotting have been proposed by various laboratories. The aim of the current study was to establish an ELISA method for the detection and quantification of prion protein in brain and in tissues easily obtained from biopsies such as tonsils. Direct analysis of prion protein from one-step tissue homogenates may be difficult because other macromolecules may interfere and so the results might not be correct. For this reason, analysis of prion protein has been achieved by employing GAGs immobilized onto solid phase and exploiting their selective interaction with prion protein present in tissue homogenates. The finding that sulfated proteoglycans have been detected [20,21] in scrapie amyloid plaques prompted us to undertake this project. These macromolecules, which lack hydrophobic nature, were first immobilized on activated microplate wells [22–24]. Prion protein was then detected immunochemically using the suitable antibodies. The distinction between normal and pathological isoform was performed by using degradation of the sample with proteinase K. Similarly activated ELISA plate wells were also applied for the qualitative identification of the sugar moieties present in the glycosylation sequences in different TSE and normal samples. The type of glycosylation was examined by using various lectins that recognize different types of sugars present in prion protein.

Materials and methods

Materials

ELISA plates were obtained from Kima (Italy). Chondroitin sulfate A (CSA) and chondroitin sulfate E (CSE) were prepared as described previously [25,26]. Chondroitin sulfate B (CSB, dermatan sulfate) and chondroitin sulfate C (CSC) were obtained from Seikagaku America. Glutaraldehyde (GA), *N*-lauroylsarcosine, Triton X-100, and Tween 20 were obtained from Serva (Germany). Bovine serum albumin (BSA), spermine, *o*-phenylenediamine (*o*-PD), heparin, and proteinase K were obtained from Sigma Chemical (USA). An enhanced chemiluminescence kit (SuperSignal West Femto Chemiluminescent Substrate) was purchased from Pierce (USA). Secondary antibodies conjugated with peroxidase, recombinant bovine PrP, polyclonal antibody against human PrP (epitope: amino acids 79–97) were obtained from Chemicon (USA). A monoclonal antibody against scrapie PrP (epitope: amino acids 142–160) was purchased from SPIbio (SAF 70) (USA), and another monoclonal antibody against PrP (66-98a3) was a gift from J. Langeveld (Lelystad, Holland). Biotinylated lectins, datura stramonium lectin (DSL), wheat germ agglutinin (WGA), and ricinus communis agglutinin I (RCA) were obtained from Vector Labs (USA). Streptavidin and biotin conjugated with alkaline phosphatase were obtained from New England Biolabs (USA).

Tissue samples

Brainstems from sheep with natural scrapie and normal sheep and tonsils from sheep were obtained from the