Biochemistry II

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Prion Disease

Proteins can be found throughout the body in many different conformations. Some proteins are capable of altering the shape of other proteins. These proteins are known as prions and have the ability to become infectious to an organism. Diseases can be caused by prions, commonly known as spongiform diseases. The name spongiform comes from the sponge like development found on brain tissue, producing holes in infected individuals. This rare degenerative brain disease is the product of a misfolded protein. Although these proteins are detected throughout the brain, symptoms can spread leading to unpredictable behavior, weight loss, loss of cerebral function, problems with coordination, sense of balance, and posture.

The prion disease that has been studied in depth and is best known is bovine spongiform encephalopathy (BSE) also known as mad cow disease. Creutzfeldt-Jakob disease is similar to BSE but is found in humans, scrapie found in sheep and chronic wasting disease can be found in elk and deer. Infections arise when the functional prion alters its conformation. Infectious prions have the ability to alter the shape of any prion it comes into contact with. This continuous change causes the progressive deterioration of the brain. Unfortunately, there are no medications or therapies for spongiform diseases, resulting in termination of the infected organism. Recently, research has supported the claims of earliest clinical signs associated with transmissible spongiform encephalopathy infections in order to provide a better understanding of the pathology.

In 2004, a group of scientists utilized Fourier transform infrared (FT-IR) spectroscopy to distinguish the difference between the pathological prion protein PrP27–30 causing Bovine

Spongiform Encephalopathy, scrapie, and Creutzfeldt-Jakob disease. FT-IR showed each disease presented unique characteristics in the secondary structure of the prion protein. Western blotting was used to detect PrP27–30 in a sample of brain tissue homogenate. SDS PAGE was used to separate proteins by length of the polypeptide (Thomzig 2004). The proteins were transferred to a polyvinylidene (PVDF) membrane where they were stained with biotinylated goat anti-mouse IgG antibodies. Antibodies binding to prion protein could be seen using a mixture of blue tetrazolium and 5-bromo-4-chloro-3-indolylaphosphate (Thomzig 2004). In order to determine the secondary structure, PrP27-30 extracts were examined. All samples were suspended in D_2O in order prevent the protons in the solvent to show a signal on the NMR. A strong band was seen at 1616 cm^{-1} to 1640 cm^{-1}. This band is due to the different β sheet structures. A peak can be seen at 1671 cm^{-1} resulting from a turn connected to the β sheets. BSE and scrapie strains revealed an absorption band of varying intensity at 1677 cm^{-1} and 1679 cm^{-1}. The α helical structures presented an absorption band at 1656 cm^{-1} to 1659 cm^{-1}, all varying intensity (Thomzig 2004). The data collected from the spectra patterns indicates variations in β sheets and other structural components. This allows different transmissible spongiform encephalopathy agents to be separated from each other. When PrP27–30 undergoes FT-IR, the data provides a tool used for capturing molecular strains without the need for restriction enzymes and antibodies that are specific to transmissible spongiform encephalopathies.

"Prion Strain-Dependent Differences in Conversion of Mutant Prion Proteins in Cell Culture," is an experiment published in 2006 that focuses on mutations induced in a normal prion protein. A single residue of glutamine was replaced with other amino acids, including arginine, lysine, phenylalanine, glutamic acid and histidine. After manipulating the molecular structure, the ability to convert to an abnormal prion protein was compared using N2a58 neuronal cells contaminated with Chandler or 22L mouse adapted scrapie strain (Atarashi 2006).

Using infrared spectroscopy, these strains provided abnormal prion protein with the same sequence but different conformation. When substitutions occurred on codons 97, 167, 171 and 216 the prion proteins opposed conversion in cells infected by both Chandler and 22L mouse adapted scrapie. Substitutions on codons 185 and 218 created abnormal prion protein in cells infected by Chandler but not in 22L mouse adapted scrapie (Atarashi 2006). These substitutions were detected using IR spectra. This data is important in order to gain a better understanding of the structure of the abnormal prion protein and the process of its formation. In turn, this will give insight to the molecular foundation of transmissible spongiform encephalopathy strains.

In March of 2013 a group a scientists published the paper, "*Enhanced Virulence of Sheep-Passaged Bovine Spongiform Encephalopathy Agent Is Revealed by Decreased Polymorphism Barriers in Prion Protein Conversion Studies"*. This article focuses on the conversion reaction of homologous protein misfolding cyclic amplification (PMCA) in ovine scrapie with prion protein genotypes VRQ/ VRQ and BSE (Priem 2013). The product after proteinase K digestion, pseudo intensity colors are displayed in a typical three banding patterns represented by a western blot. The three bands are composed of glycoform prion protein variants, di-, mono-, and nonglycosylated. The first lane, F, is before treatment, second lane, I, is after incubation but without sonication, and last lane, S, is after PMCA which is incubated and sonication (Priem 2013). Protein recognition was achieved with monoclonal 9A2 antibody. The difference in molecular mass of Bovine BSE and sheep scrapie which are most evident in the fraction of nonglycosylated prion protein (Priem 2013). These results show a single round of PMCA of homologous conversions demonstrating saturation of a reaction caused by a depletion of normal cellular prion only after 40 hours. Scrapie and BSE share a near linear relationship between conversion and time efficiency (Priem 2013). This study focuses on PMCA experiments

regulated at 24 hours in order to stay abundantly in a linear range. This is important because a linear range will allow sufficient data of the conversion reactions.

In 2014, *"Conformational Stability of Mammalian Prion Protein Amyloid Fibrils Is Dictated by a Packing Polymorphism within the Core Region,"* was published. This study examines the conformational stability of the abnormal prion protein identified by the resistance to chemical and thermal denaturation. In order to differentiate the structural basis of stability, two strains of recombinant human prion protein showing dramatic differences have been characterized. Experiments involving hydrogen deuterium exchange of backbone amide revealed infectious amyloid forms of Sup35, a yeast prion protein (Cobb). Stability differences between mammalian prion protein amyloid result from packing arrangements within the core of amyloid. Some packing arrangement include steric zipper interfaces and can be observed by x-ray fiber diffraction and hydrogen deuterium exchange involving histidine side chains within the core (Cobb). The stability of strain specific differences in prion protein amyloid are determined by polymorphism packing. Although this study focuses on prion protein amyloid fibrils, this study is important because the structural basis is similar to brain derived abnormal prion protein. These findings allow for a greater understanding of the structure of prion strains.

Human Molecular Genetics published, *"In Vitro Screen of Prion Disease Susceptibility Genes Using the Scrapie Cell,"* in 2014. This article uses an in vitro screening assay that unites the scrapie cell assay in neuroblastoma derived cells that are vulnerable to prion formation by overexpressing or silencing of certain genes (Brown). Neuroblastoma derived cells are capable of propagating prions at an alarming rate indefinitely. When neuroblastoma cells are combined with prions, the cells divide and prion numbers increase. Each cell was infected with prion proteins for three weeks before the assay begun. A total of 18,000 cells were plated and exposed to RML brain homogenate (Brown). Non infected brain homogenate was used as a control. Cells

were then treated with protease K. Using ICSM18 anti-prion antibodies protease K resistant prions were detected. Next, alkaline phosphatase-linked anti IgG1 antiserum was used and able to be seen due to alkaline phosphatase conjugate substrate (Brown). Using an enzyme linked immune spot assay, immune detection of abnormal prion protein was quantified by calculating the number of cells infected (Brown). Stable cell lines are generated allowing for detection of effects by genes. These gene effects include propagation, uptake and accumulation of abnormal prion proteins.

Scientists from New York University School of Medicine published the paper, *"Mucosal Immunization with An Attenuated Salmonella Vaccine Partially Protects White-tailed Deer from Chronic Wasting Disease."* This study involved infecting white tailed deer with attenuated Salmonella that express prion protein and a control of non vaccinated white tailed deer. After a mucosal response was confirmed, the immunized animals were orally and locally boosted by applying polymerized recombinant prion protein on both the rectal mucosa and tonsils (Goni). Equally, the control animals and vaccinated animals were orally challenged with chronic wasting disease infected brain homogenate. Three years after the chronic wasting disease was challenged orally, each member of the control group developed clinical signs of chronic wasting disease. (Goni). A prolongation of the development period in the vaccinated animals was noted. One deer remained free of chronic wasting disease. This was determined by clinical signs and by recto-anal mucosa associated lymphoid tissue, which assessed the presence of abnormal prion protein by immunohistochemistry. Ig from an infected deer and purified from plasma and compared to the plasma from a control deer (Goni). Western blots were used to show that immunoglobulins obtained from the vaccinate respond to abnormal prion protein. This data provided is important because this is the first partial vaccination success against a prion disease in a species that is in danger.

In June of 2014"*Preclinical Detection of Variant CJD and BSE Prions in Blood,*" was published by with the help of many scientists. In this study, the detection of preclinical signs of variant Creutzfeldt Jakob Disease and bovine spongiform encephalopathy prions were observed in the blood of infected individuals. In vitro amplification of Creutzfeldt Jakob Disease and bovine spongiform encephalopathy agents were acquired using Protein Misfolding Cyclic Amplification (PMCA) (Lacroux). Primate and sheep models of the disease were utilized in order to provide an understanding on a molecular level for the barriers of transmission for transmissible spongiform encephalopathies. Primates were transfused with blood that came from primates in the terminal stage of Creutzfeldt Jakob Disease (Lacroux). Each sample collected completed six rounds of amplification. These products were observed for the presence of abnormal prion proteins by Western blot (Lacroux). The results show the conversion between two strains of prion protein molecules of the same sequence modulates the intraspecies or interspecies ability to transmit transmissible spongiform encephalopathy agents (Lacroux). This data is important because of the possibility of blood borne transmission is a serious concern to the public. Knowing how the infection spreads allows us to minimize or possibly eliminate the number of individuals affected.

On March 10th, 2015, *"Changes in Retinal Function and Morphology Are Early Clinical Signs of Disease in Cattle with Bovine Spongiform Encephalopathy"* was published. In this paper, a group of scientists observed the earliest clinical signs of bovine spongiform encephalopathy. This infection included changes in retinal morphology and function. A non-invasive method to generate a cross section of the retina in vivo known as optical coherence tomography was used in this experiment (Greenlee). This method revealed any changes in thickness of the retina associated with bovine spongiform encephalopathy infection. At terminal stages of this infection a thinning of the retina was noted. Twelve months post inoculation,

retinal thinning is able to be detected (Greenlee). It was also discovered the intensity of the clinical signs is directly related to the amount of abnormal prion protein accrued in the retina. Optical Coherence Tomography was used to measure the thickness of the dorsocentral retina, in vivo. Each animal had at least 10 measurements recorded each time (Greenlee). This ensured the average thickness was accurate. After inoculating animals with BSE, retinal thickness considerably decreased. Western blot was used on the brainstem and homogenized at room temperature. The sample was then digested with proteinase K at 48 °C for 40 minutes (Greenlee). The homogenate was loaded into sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. Next the sample was transferred to PVDF membrane and developed in either monoclonal antibody P4 or monoclonal antibody 6H4 (Greenlee). A secondary antibody, biotinylated sheep anti-mouse, was added. Chemifluorescent detection method was used and detected by a multimode scanner. The average time for onset of spongiform encephalopathy in cattle to occur was 22.8 months (Greenlee). This prion accumulation is responsible for the inflammatory response noticed in the tissue. This experiment shows the ability to detect spongiform encephalopathy at least five months before obvious signs of the disease have been presented (Greenlee). This data is significant because knowledge of the first clinical signs of a fatal infection provides a better understanding for the pathology and therapeutic methods needed in the future.

This rare degenerative brain disease is the product of a misfolded protein. The theory of a protein transmitting a disease seemed unrealistic since it lacked a nucleic acid intermediate. Infectious prions have the ability to alter the shape of any prion it comes into contact with. This continuous change causes the progressive deterioration of the brain. Unfortunately, there are no medications or therapies for spongiform diseases. Due to the work of many scientists, the earliest clinical signs associated with transmissible spongiform encephalopathy infections are surfacing.

This provides a better understanding of the pathology and can possibly lead to treatments instead of a certain death.

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