

Electrophoresis and Western Blot can Detect the Interaction of Proteins with the Pathogenic Prion Protein

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The amphipathic helix near the middle of the PrPc and the GPI anchor are the two sites which might allow it to interact with other proteins. PrPc binds to a family of heparin-like compounds and showed affinity for polyanions. Protein-protein interaction is necessary for signal transduction, transport across cell membranes, cell metabolism and muscle contraction. The creation of homo-oligomers or hetero-oligomers are examples of protein associations. Several enzymes, scaffolding proteins, carrier proteins and transcriptional regulatory factors carry out their functions as homo-oligomers. Covalent interactions represent the strongest association between proteins and are formed by disulphide bonds or electron sharing. Non-covalent bonds usually established during transient interactions and are weaker bonds such as ionic interactions, Van der Waals forces or hydrophobic bonds. Disruption of homo-oligomers in order to return to the initial individual monomers often requires denaturation of the complex. Aberrant protein-protein interactions are the basis of multiple aggregation-related diseases, such as Creutzfeldt-Jacob, Alzheimer's disease, and may lead to cancer.

In previous publications the results obtained in western blot and immune detection showed that the pathogenic prion protein interacts with the calix-arene [1], ions [2], guanidine containing chemicals [3-5], and monosaccharides and disaccharides carbohydrates [6] were reported. Also previously the prion protein interacted with the Triose Phosphate Isomerase had been published [7,8]. Here in the following experiments was added to proteinase K treated brain suspensions prepared from either bovine or ovine positive prion strains either one of the following proteins; polyarginine, polylysine, Concanaline A and triose phosphate isomerase.

The cellular prion protein (PrPc) is localized on the cell membrane and is highly conserved among mammals with a sequence similarity of about 85-97%. It possesses several physiological functions as ligand uptake, cell adhesion, signal transduction and a potential role in copper metabolism. PrPc and the pathogenic prion protein (PrPsc) possess the same amino acid composition, but vary in conformity. PrPc contain about 40% alpha-helix and less than 10% beta-sheet conformation where PrPsc contain about 50% as a beta-sheet. Therefore, infectivity gains and resistance to proteinase K is a consequence of conformational modification of PrPc by PrPsc [9].

Polyarginine and Polylysine Peptides

These peptides contain the positively charged amino acid arginine or lysine and has been used as antibacterial and anti-cancer therapeutic agents. In the following experiment 10 µl of proteinase K treated prion positive ovine brain suspension (PrPres) were added in Eppendorf tubes 2 to 8 then tris buffer PH 7.2 was added as 4 µl into the second tube, 3 µl into tube 3, 4, 2 µl into tubes 5 and 6 and 0 µl to 7 and 8. After dissolving the polyarginine or polylysine peptides in water at 1 M then both were added respectively as 1 µl in tubes 3 and 4, as 2 µl in 5 and 6 and as 4 µl in 7, 8. After preparation of the mixtures, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants which were loaded in lanes 2 to 8 respectively. The pellets were suspended in 10 µl of 50% v/v 8 M urea and Laemmli

denaturing buffer, vortexed, heated 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovered supernatants were loaded in lanes 9 to 15 respectively on 15% SDS PAGE, electrophoresis, transfer and immunodetection using monoclonal antibodies [3] (Figure 1).

The mode of interaction of these 2 peptides with the pathogenic prion protein is through precipitation of the prion protein as do the high positively charged inorganic salts. Thus a suggestion is to examine the inactivating activity of these peptides by injection intraperitoneally of mixtures with an adapted infectious prion strain in mice and if proved positive then by intracerebral injection of the peptide suspension in experimentally infected mice.

The concanavine A (Con A) is a lectin (carbohydrate-binding protein) known to bind with certain specific sugars, polysaccharides, glycoproteins and glycolipids and is homotetramer. Each subunit has a molecular weight of 26.5 Kd and 237 amino acids. The interaction between lectin and glycoprotein is not dependent on a specific sugar present in the glycoprotein nor on the nature of the glycoprotein therefore a novel purification method for immobilizing various glycoprotein was developed using con A sepharose chromatography columns [10].

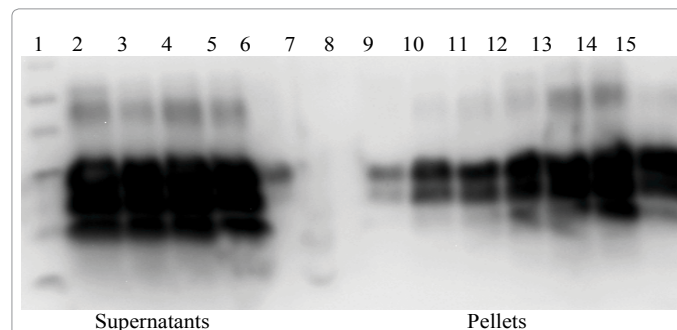


Figure 1: Showed the molecular weight marker lane 1, the PrPres control in lane 2, after adding 1 µl of either one of the 2 peptides revealed the presence of a great part of the prion protein in the supernatants at lanes 3 & 4 and a very little precipitation at lanes 10 & 11. By adding 2 µl a difference was evident as the lysine peptide precipitate almost of the PrPres lane 6 compared to the arginine peptide lane 5 which partially precipitate the PrPres. Most of the prion protein were precipitated by adding 4 µl of either one of the peptides as no PrPres was present in the supernatants lane 7 & 8 and is mostly present in the pellets lane 14 & 15.

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Received August 26, 2016; Accepted August 27, 2016; Published September 02, 2016

Citation: Moussa A (2016) Electrophoresis and Western Blot can Detect the Interaction of Proteins with the Pathogenic Prion Protein. J Chromatogr Sep Tech 7: e137. doi: [10.4172/2157-7064.1000e137](https://doi.org/10.4172/2157-7064.1000e137)

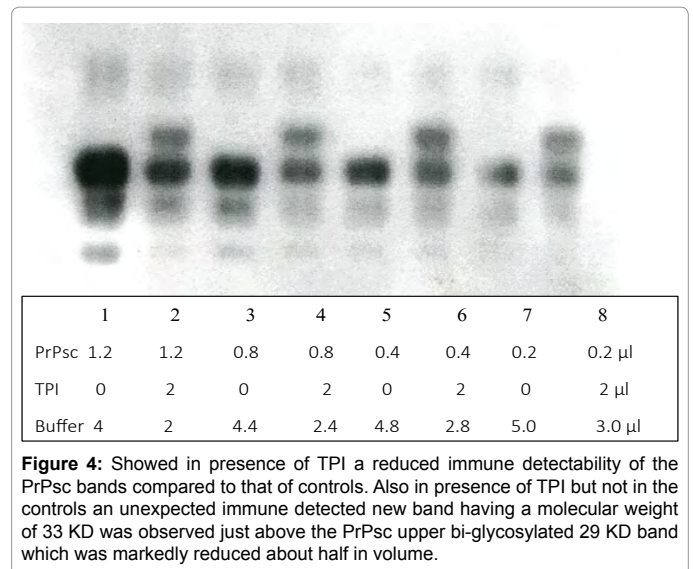
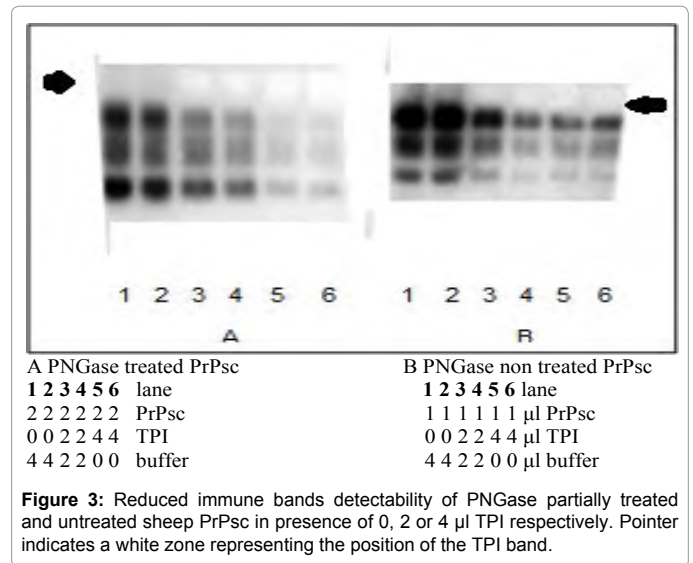
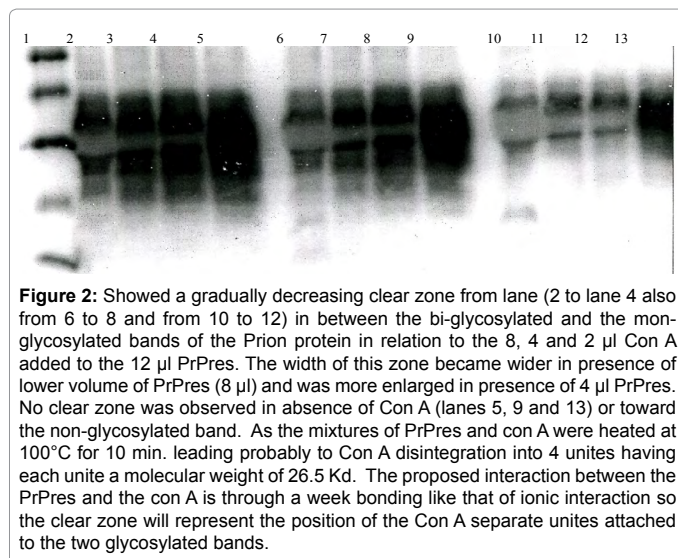
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In this experiment was added 12 µl PK treated ovine PrPres into Eppendorf tubes 2 to 5, 8 µl into 6 to 9 tubes and 4 µl in 10 to 13 tubes then 0, 4, 6 and 8 µl tris buffer PH 7.2 was added into the tubes 2 to 5 then 4, 6, 8 and 12 µl into tubes 6 to 9 and 8, 12, 14 and 16 µl into that from 10 to 13. Lastly Con A was added in a volume of 8, 4, 2 and 0 µl repeatedly to the tubes 2 to 4, 6 to 9 and 10 to 13 respectively. After vortexing the tubes were heated for 10 min. at 100°C then centrifuged for two min. at 10000 RPM. And the supernatants were collected and a constant volume from each was deposited and run on 15% SDS acrylamide gel, transfer on nitrocellulose membrane and immune blotted using an anti-PrP monoclonal antibody and peroxidase conjugated anti-mouse polyclonal serum (Figure 2).

Triose phosphate isomerase (TPI) a glycoprotein formed by a dimer of identical subunits, each of which is made up of about 250 amino acid residues with a molecular weight of 28 KD and is the key enzyme in cell metabolism controlling the glycolytic flow and energy production. The functional deficiency in TPI glycolytic enzyme activity is associated to neurodegeneration. In particular, inefficient glycolysis and ATP depletion which are also characteristic in Alzheimer's disease brains.

The results present in Figure 3 were obtained by adding in double, 0, 2 or 4 µl TPI and 4, 2 or 0 µl buffer to constant volume (2 µl) of PNGase partially treated (A) or (1 µl) of untreated (B) Sheep PrPsc, showed again a decreased bands intensity in parallel with the TPI volume added. Note a white zone (pointer) represent the position of the TPI band in the gel.

The results of a second experiment were observed after electrophoresis of the supernatant obtained after vortexing, heating and centrifugation of mixtures from two sets; one containing only either 1.2, 0.8, 0.4 or 0.2 µl of bovine PrPres alone and the other set contained beside the PrPres 2 µl TPI and the final volume of each tube of both sets was brought to 5.2 µl with Laemmli buffer. Electrophoresis was done on 15% SDS acrylamide gel, transfer on nitrocellulose membrane and a double immunoblotted using first a monoclonal anti-PrPsc antibody then a peroxidase conjugated anti-mouse polyclonal serum followed by washing in Phosphate Buffer Saline (PBS) PH 7.2 overnight at laboratory temperature. The second immunoblotting with done using a rabbit polyclonal anti PrPsc serum then a peroxidase conjugated anti-rabbit serum, The presence of a new unsuspected PrP immune detected protein band at 33 KD position (Figure 4) was not revealed



earlier during the previous immuno-blotting experiments using only one antibody as it could be hidden under the TPI band (Figure 3).

The bi-glycosylated band seemed to be divided into two parts, one interacting with the TPI and the other is present just below the TPI band and presenting the other part of the 27-29 Kd bi-glycosylated. This hidden band was revealed only after washing with Phosphate Buffer Saline buffer PH 7.2 overnight and re-immune detection by the anti-PrPsc rabbit hyper immune serum, washing and finally incubation with a peroxidase conjugated anti-rabbit polyclonal serum. Thus the mode of interaction between TPI and the prion protein can be through Vans der Waals attraction forces before washing and repulsion forces after washing.

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