Journal of Chromatography
Separation Techniques

Moussa, J Chromatogr Sep Tech 2016, 7:3
DOI: 10.4172/2157-7064.1000e133

Editorial Open Access

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

Aly Moussa*

National Agency for Sanitary Enviromental, Laboratoire de Lyon, France

Most of the publication studying the effect of cations on the cellular prion protein (PrPc) used either a recombinant PrPc, extracted PrPc from animal organs or in Neuronal cells cultures. Heir is investigated the effect of ions on the pathogenic prion protein proteinase K treated (PrPres) using electrophoresis and western blot.

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 possess several physiological functions as ligand uptake, cell adhesion, signal transduction and a potential role in cupper metabolism. PrPc and the pathogenic prion protein (PrPsc) possess the same amino acid composition, but vary in conformity. PrPc contain about 40% alfa-helix and less than 10% beta-sheet conformation where PrPsc contain about 50% as a beta-sheet. Therefore infectivity gain is a consequence of conformational modification of PrPc by PrPsc [1].

Ions such as cations and anions are specifically recognized by several proteins where they play a structural role as either cofactors activating or stabilizing their binding partners or being involved in enzymatic reactions. They induce significant conformational changes on their binding proteins leading to their complete folding or can trigger oligomerization. Cation binding proteins can discriminate between very similar cations as calcium and magnesium.

In 1995 it was suggested that PrPc is implicated in copper regulation. Binding copper stimulated PrPc expression, protect cells against apoptosis and oxidative stress and stimulate nerve growth. Copper binding was found through the 4 to 5 copies of the octarepeat region. PrPc endocytosis was induced by both copper and zinc. Later it was found that copper binding can be also through histidine 96 and 111 and at histidines within the folded C terminal domain PrP (125-231) [2].

In the following experiments was added different salt concentration of either one of the six sulfate or chloride salts of sodium, magnesium, ammonium, zinc, copper or cobalt to proteinase K treated brain suspensions prepared from either bovine or ovine prion strain. Electrophoresis and western Blot were carried after preparation of the mixtures, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants, loading, on 15% SDS PAGE, electrophoresis, transfer and immunodetection using monoclonal antibodies [3].

The results presented in Figure 1 were obtained by pipetting in each of the 15 Eppendorf tubes 5 µl of PK treated ovine PrPsc and adding into the first tube 6 µl Laemmli buffer, to the tubes from 2 to 5 were added 1, 2, 4 and 6 µl of 0.5 M Na_2SO_4 and 5, 4, 2 and 0 µl Laemmli buffer respectively, to the tubes from 7 to 10 were added 1, 2, 4 and 6 µl of 0.5 M $MgSO_4$ and 5, 4, 2 and 0 µl Laemmli buffer respectively and to the tubes from 12 to 15 were added 1, 2, 4 and 6 µl of 0.5 M $(NH_4)_2$ SO_4 and 5, 4, 2 and 0 µl Laemmli buffer respectively.

In Figure 2, 5 µl of PK treated ovine PrPsc were distributed into each of 15 Eppendorf tubes then was added to the first tube 6 µl Laemmli buffer, to the tubes from 2 to 5 were added 1, 2, 4 and 6 µl of 0.2M $ZnSO_4$ and 5, 4, 2 and 0 µl Laemmli buffer respectively, to the tubes from 7 to 10 were added 1, 2, 4 and 6 µl of 0.2M $CuSO_4$ and 5, 4, 2 and 0 µl Laemmli buffer respectively and to the tubes from 12 to 15 were added 1, 2, 4 and 6 µl of 0.2 M $CoSO_4$ and 5, 4, 2 and 0 µl Laemmli buffer respectively.

In Figure 3, 5 µl of PK treated ovine PrPsc were distributed into each of 15 Eppendorf tubes then to each of 3 adjacent tubes was added either 0, 1, 2, 4 or 6 µl of 0.5 M Na_2SO_4. Then $MgSO_4$ at 0.5M was repeatedly distributed as 4, 2 and 1 µl into each of 3 adjacent tubes respectively and finally 6, 5, 4, 2 and 0 µl Laemmli buffer were added into each of the 3 adjacent tubes respectively.

When adding (Figure 4a) in each of three adjacent tubes either 1 or 2, 4, 6 or 0 µl of 0.5 M sodium sulfate to constant 5 µl volume of

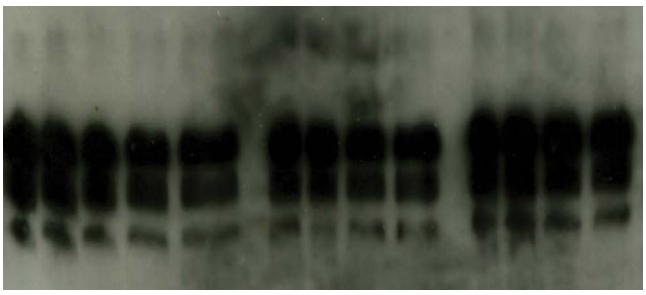

Figure 1: By adding gradually increased volumes of sodium sulfate, magnesium sulfate or ammonium sulfate the PrPres bands became gradually wider at the higher added volumes.

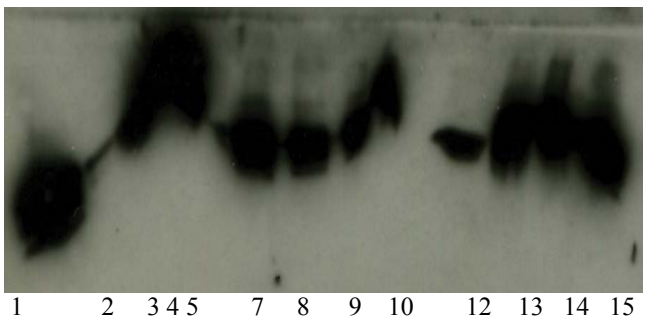

Figure 2: A doubling of the molecular masse of the prion protein bands (oligomerization) were observed after the addition of either Zn, Cu or Co sulfate to the PrPres compared to that of the control (lane 1).

***Corresponding author:** Aly Moussa, National Agency for Sanitary Enviromental, Laboratoire de Lyon, France, Tel: 336-174-609-52; E-mail: moussa69@club-internet.fr

Received May 16, 2016; **Accepted** May 17, 2016; **Published** May 23, 2016

Citation: Moussa A (2016) Electrophoresis and Western Blot can Detect the Interaction of Ion Ligand with the Pathogenic Prion Protein. J Chromatogr Sep Tech 7: e133. doi:10.4172/2157-7064.1000e133

PK treated ovine prion protein then adding either 2, 4, 6 or 0 µl of 0.7 M streptomycin to each of 3 adjacent tubes and completing the final volume of each tube to 17 µl with Laemmli buffer, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants, loading, on 15% SDS PAGE, electrophoresis, transfer and immunodetection using monoclonal antibodies.

Where after adding (Figure 4b) in each of three successive tubes either 1 or 2, 4, 6 or 0 µl of 0.7 M streptomycin to a constant 5 µl volume of a PK treated ovine prion protein then adding 2, 4 or 6 µl of 0.5 M sodium sulfate to each of 3 adjacent tubes and completing the final volume of each tube to 17 µl with Laemmli buffer then vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants, loading, on 15% SDS PAGE, electrophoresis, transfer and immunodetection using monoclonal antibodies [3].

The results in Figure 5 were obtained by distributing in 8 Eppendorf tubes 5 µl of bovine PK treated PrPsc then adding to 1st tube 1 µl of 1 M NaCl and 5 µl Laemmli buffer, to the 2nd tube 6 µl of 1 M NaCl, to the 3th tube 6 µl Laemmli buffer, to the 4th tube 1 µl of 1 M $MgCl_2$ and 5 µl Laemmli, to the 5th tube 6 µl of 1 M $MgCl_2$, to the 6th tube 6 µl Laemmli buffer, to the 7th tube 1 µl of 1 M NH_4Cl and 5 µl Laemmli buffer and to the 8th tube 6 µl of 1 M NH_4Cl.

The results in Figure 6 were obtained by distributing in 8 Eppendorf tubes 5 µl of bovine PK treated PrPsc then adding to the 1st tube 1 µl of 1 M $ZnCl_2$ and 5 µl Laemmli buffer, to the 2nd tube 6 µl of 1 M $ZnCl_2$, to the 3th tube 6 µl Laemmli buffer, to the 4th tube 1 µl of 1 M $CuCl_2$ and 5 µl Laemmli, to the 5th tube 6 µl of 1 M $CuCl_2$, to the 6th tube 6 µl Laemmli buffer, to the 7th tube 1 µl of 1 M $CoCl_2$ and 5 µl Laemmli buffer and to the 8th tube 6 µl of 1 $MCOCl_2$.

In biochemistry and pharmacology, a ligand is generally a small molecules that forms a complex with a biomolecule to serve a biological purpose. The binding occurs by intermolecular forces, such as ionic bonds, hydrogen bonds and van der Waals forces [4]. The results obtained (Figure 1) showed that Na_2, Mg and $(NH_4)_2$ sulfate can interact with the PrPres inducing an increase of the band width proportional to the added volumes of ions. Addition of increased volumes of first Na_2SO_4 then $MgSO_4$ to the PrPres resulted in a continuous increase of the molecular weight of the prion protein bands in relation to the volume added of both salts and leading to protein aggregation (Figure 3).

The addition of increased volumes of either one of different volumes of 0.2 M of Zinc, Copper or Cobalt sulfate to the proteinase K treated PrPsc resulted into the appearance of oligomerizations of the prion protein (Figure 2) confirming that not only the copper but also Zinc and Cobalt sulfate can bind with the 90-231 protein region. Also proving that not only the octarepeat region but also the histidine and probably another amino acids present within the region 90-231 in the C-terminal domain can also interact with these ions [2]. A decrease in the reactivity was observed when adding different volumes of 1M of Zinc, Copper or Cobalt chloride to the PrPres compared to the sulfate salts of the same ions (Figure 6).

The comparison between the reactivity of 0.5 M Na_2, Mg or $(NH_4)_2$ and 0.2 M of Mg, Cu or CO sulfate and that of 1M of Na, Mg, NH_4, Mg, Cu or CO chloride salts revealed that the nature of the Kosmotropic ion sulfate is superior to chloride in binding with proteinase K treated prion protein (Figures 1, 2, 5 and 6). So the sulfate salts has not to be administered in case of neurodegenerative disease as they can do conformational changes in normal peptide chains and also induce aggregation and oligomerization of amyloid proteins leading to their accumulation within cells and ending with degeneration and death of these cells.

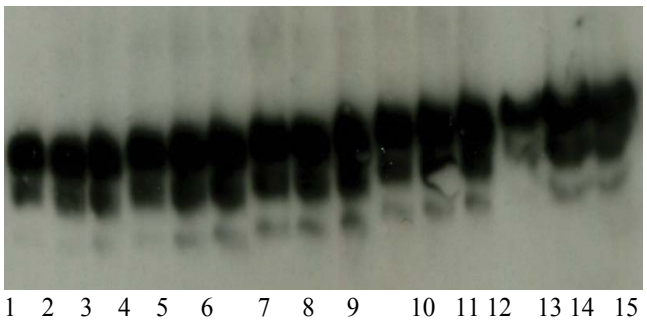

Figure 3: A gradual increase of the molecular weight of the prion protein after duel addition of increasing volumes of 0.5 M Na_2SO_4 and 0.5 M $MgSO_4$ compared to controls obtained in presence of only $MgSO_4$ (lane 1 to 3).

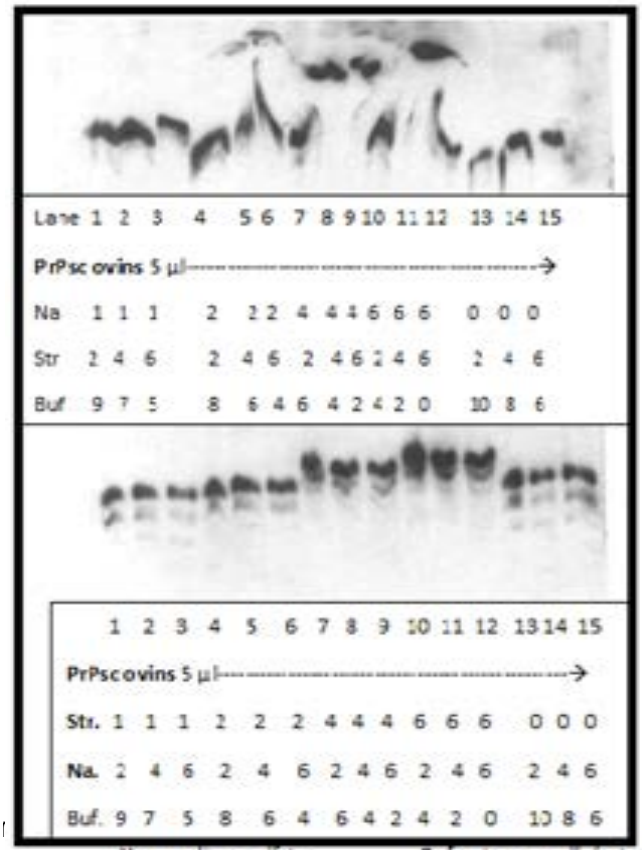

Figure 4: A) Upper: Oligomerization of the prion protein were observed after adding increasing doses of the first sodium sulfate molecule and then of streptomycin. **B)** Lower: Adding first streptomycin to the PrPres followed by sodium sulfate and buffer showed only the usual gradual increase of the molecular weight of each of the three PrPsc bands in relation to the streptomycin volume added.

Figure 5: No increase of the molecular weight was observed after adding either one of sodium, magnesium or ammonium chloride.

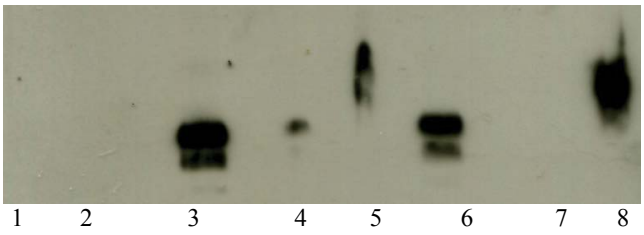

Figure 6: Precipitation occurred on adding the Zinc chloride as well when adding the low volume of copper or cobalt chloride. After adding only the 6 µl of either the copper or cobalt chloride salts an increase of the molecular weight of the prion protein bands became evident compared to the control.

As previously reported [4] if the sodium sulfate was added before adding streptomycin oligomerization of the prion protein was observed where when added after streptomycin only aggregation of the prion protein occurred. These result revealed an adjuvant action of both ions when sodium sulfate was added first and no combined interaction occurred when adding streptomycin first (Figure 4A and 4B).

References

1. Prusiner SB (1998) Prions. Proc Natl Acad Sci USA 95: 13363-13383.
2. Walter ED, Stevens DJ, Spevacek AR, Visconte MP, Dei Rossi A, et al. (2009) Copper Binding Extrinsic to the Octarepeat Region in the Prion Protein. Curr Protein Pept Sci 10: 529-535.
3. Moussa A, Coleman AW, Bencsik A, Leclere E, Perret F, et al. (2006) Use of streptomycin for precipitation and detection of proteinase K resistant prion protein (PrPsc) in biological samples. Chem Commun (Camb) 9: 973-975.
4. Moussa A (2015) Detection of Prion Protein Intermolecular Interaction by Electrophoresis and Western Blot. J Chromatogr Sep Tech 6: e131.