

Detection of Prion Protein Intermolecular Interaction by Electrophoresis and Western Blot

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In biochemistry and pharmacology, a ligand is generally a small molecule 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. In general, high-affinity binding involves a longer residence time for the ligand at its receptor binding site than is the case for low-affinity binding. High-affinity binding of ligands is often physiologically important as some of the binding energy can be used to cause a conformational change. The hydrogen bond is often described as a strong electrostatic dipole-dipole interaction, has some covalent bonding features, usually involves a limited number of interaction partners and produces interatomic distances shorter than the sum of van der Waals radius.

Several detection methods for intermolecular interaction have been used as mass spectrometry, FRET imaging microscopy, high resolution NMR spectra, biosensor based magnetoresistance technology, fluorescence sensing ...etc. Here we used Polyacrylamide gel (PAGE) electrophoresis which is one of the techniques used for separation of macromolecules according to their electric charges and sizes and is mainly used in molecular biology of proteins and nucleic acids. Immune detection of proteins (western-blot) is usually done after electrophoresis and transfer using specific antibodies (WB).

Transmissible spongiform Encephalopathies (TSEs) are fatal neurodegenerative diseases including scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, chronic wasting disease in deer and Creutzfeldt-Jakob disease (CJD) in humans. This group of disorders is also called prion diseases because they are caused by non-conventional infectious agents mainly composed of a detergent-insoluble and protease-resistant isoform (PrP^{sc}) induced by conformational changes in the membrane bound cellular glycoprotein (PrP^c) [1,2].

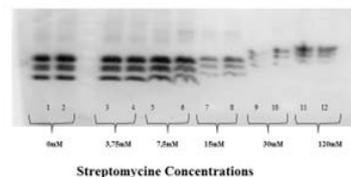
Several chemicals possessing guanidine group as streptomycin (dihydrostreptomycin, bis-3-aminoproylamine, guanidine hydrochloride, triethylene tetra mine and spermine tetrahydrochloride) were found interacting with the pathogenic prion protein (PrP^{sc}) and reproduced aggregation and precipitation of the prion protein. The interaction of streptomycin with proteins is optimum at alkaline PH and takes place through hydrogen bond transfer between the 2 guanidine groups on streptomycin and the amino-acids of one or several prion peptides ruling the possibility of a Schiff-base reaction [3]. Intramolecular hydrogen bonding is partly responsible for the secondary, tertiary, and quaternary structures of proteins. Ligand binding to protein alters its chemical conformation (three-dimensional shape). The conformational changes of a receptor protein will affect its functional state. The tendency or strength of binding is called affinity. Binding affinity is determined not only by direct interactions, but also by solvent effects that can play a dominant indirect role in driving non-covalent binding in solution (Figures 1 and 2). The interaction of the PrP^{sc} peptides and streptomycin is influenced by the quantity of streptomycin added, the PH and the presence of salts. The effect of some ions on the interaction of streptomycin with the prion protein was investigated. Adding first to constant 5 μ l volume of the ovine prion protein increasing volumes (1, 2, 4, 6 or 0 μ l) of 0.5 M sodium

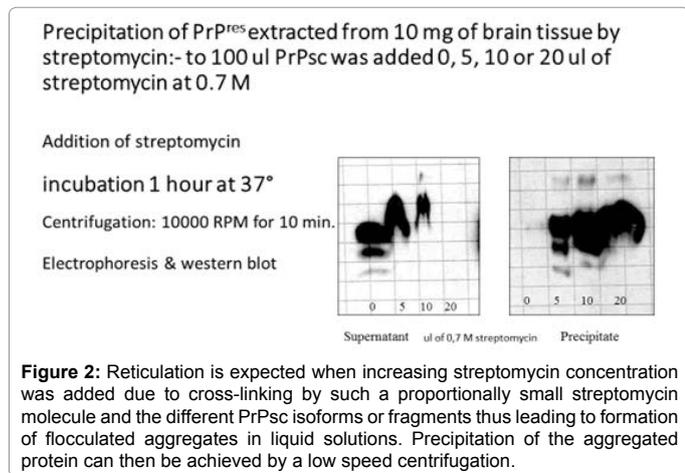
sulfate repeated for each volume 3 times followed by adding (2, 4 or 6 μ l) of 0.7 M streptomycin to each tube and finally bringing the total volume to 17 μ l by adding Laemmli buffer then loading and starting electrophoresis and WB. The results showed a higher increase of the molecular weight of the bands dependent on the quantity of sodium ions (4 and 6 μ l) and streptomycin (4 and 6 μ l) added (adjuvant effect).

When adding in each of three tubes either 1 or 2, 4, 6 or 0 μ l of 0.7 M streptomycin to constant 5 μ l volume of the ovine prion protein then adding 2, 4 or 6 μ l of 0.5 M sodium sulfate to each tube and completing the final volume of each tube to 17 μ l with Laemmli buffer then starting electrophoresis and WB. The result obtained showed no effect of adding sodium sulfate after adding the streptomycin and only a gradual increase of the molecular weight of each of the three PrP^{sc} bands in relation to the streptomycin volume added (Figure 3). Streptomycin had proved valuable for earlier and higher immunological detection of prions in clinical samples due to aggregation of the prion peptides as well as to a better attachment of antibodies to their epitopes through electric charge transfer on the protein surface. These changes of the surface electrostatic charges induced by streptomycin affect the prion protein stability leading to a reduced infectivity [4]. On the other hand the resistance to proteinase K digestion of the prion protein PrP^{sc} in presence of streptomycin was not affected proving that the structures controlling infectivity and PK resistance are different [5].

Use of electrophoresis and western blot for detection of prion protein streptomycin intermolecular interaction

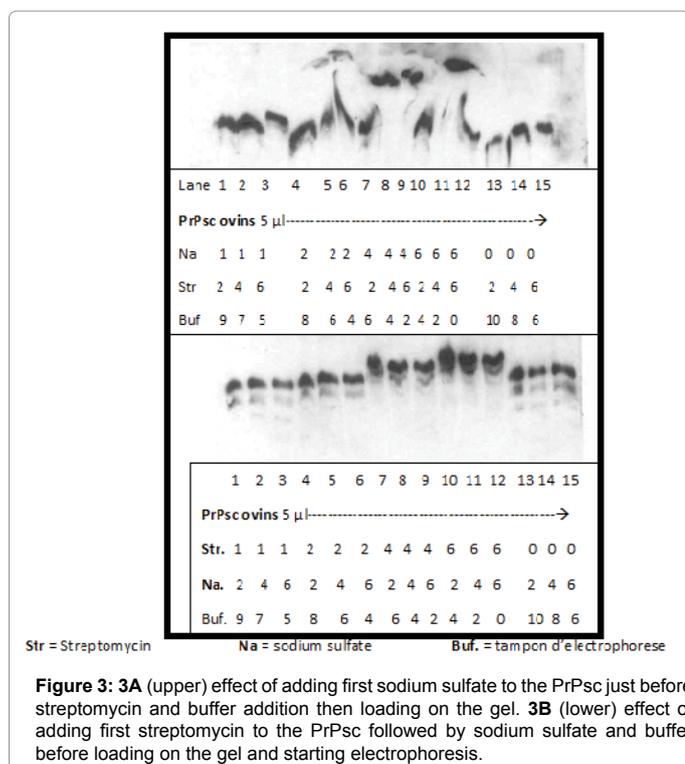
- Addition of variable concentration of streptomycin to constant amount of PrP^{sc} leads to a gradual increase of the molecular weight mass of the PrP^{sc} bands.





References

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