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Interaction of the Pathogenic Prion Protein with Iron Salts

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Copper, zinc and iron play an important role in neurodegenerative diseases and have an impact on both protein structure (misfolding) and oxidative stress. Homeostasis of these metal ions usually involves a great number of proteins which regulate the proper metal biology. Iron is involved in multiple biological processes within the human brain, including neurotransmitter function, myelin synthesis along with energy production via its ability to change valence states. Also, labile iron is free to participate in the Fenton reaction resulting in the generation of reactive oxygen species (ROS) which go on to induce oxidative stress and neuronal damage. A link between increased levels of iron in brain areas of Alzheimer's disease (AD) and Parkinson disease (PD) has been recognized. Also, was observed during the evolution of Alzheimer's disease (AD), Parkinson disease (PD) and Creuztfeldt-Jacob disease (CJD) an elevated levels of non-enzymatic protein glycation and formation of crosslinks via stable advanced glycation end products (AGEs) [1].

Neurodegenerative disorders of the central nervous system are slowly developing, insidious conditions that contribute first to neuronal cell degeneration and later cell death. During the development of the spongiform encephalopathies in human and animals the surface cellular glycoprotein (PrPc) undergoes a misfolding conformation, become resistant to proteinase K and in detergents it accumulates as insoluble β-sheet containing aggregates [2,3]. As, the interaction product between the pathogenic prion protein and iron *in vitro* remain unknown, therefor in this paper experiments were planned to elucidate the interaction product produced after adding to the proteinase K treated prion protein (PrPres) either iron oxide, iron II sulfate heptahydrate or iron III citrate monohydrate.

The following experiment was done to show the interaction of iron oxide [Fe_2O_3] with the pathogenic prion protein treated with proteinase K. The samples deposited in lane 2 to 7 and 9 to 14 were prepared as follows; 10 µl of distilled water were dropped in each of 6 Eppendorf tubes, into the first tube was added 10 µl of 1 M iron oxide, successively I/2 dilution were done till 1/64 at the 6th tube from which 10 µl were discarded and 5 µl PrPres were added to each of the 6 tubes. After preparation of the mixtures, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants (S) which were loaded in lanes 2 to 7 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 (P) were loaded in lanes 9 to 14 respectively. Also In lane 1 and 15 was added the molecular weight marker and in lane 8 was added 5 µl PrPres and 10 µl distilled water, after vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovered supernatants were loaded on 15% SDS PAGE, electrophoresis, transfer and immunodetection using monoclonal antibodies [4] (Figure 1).

The results in Figure 1 showed that a fraction of the PrPres were present in the supernatant non-interacting with the iron oxide as no increase of its molecular weight was evident where most of the prion protein were heavily precipitated by the iron oxide compared to PrPres control lane 8.

The results in Figure 2a were observed after electrophoresis of the supernatant (S) lane 1 to 6 obtained after vortexing, heating and centrifugation of mixtures prepared by distribution of 10 µl of tris buffer in 6 tubes, adding 10 µl of 4 M iron II sulfate heptahydrate [$Fe_2(SO_4)_3$ $6H_2O$] to the first tube, doing 1/2 dilutions till 1/ 64 at the 6th tube then discarding 10 µl from this last tube and finally adding 10 µl PrPres to the tubes 1 to 6. The PrPres control tube contained 10 µl tris buffer and 10 µl PrPres and were deposited at lane 7 and 15. After preparation of the mixtures, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants (S) which were loaded in lanes 1 to 6 respectively. Then 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 (P) were loaded in lanes 9 to 14 respectively. In lane 8 was deposited the molecular weight marker. Electrophoresis was done on 15% SDS acrylamide gel, transfer on nitrocellose membrane and a immunoblotted using a monoclonal anti-PrPsc antibody then a peroxidase conjugated anti-mouse polyclonal serum.

The results in Figure 2a showed a very faint presence of a PrPres band at lanes 1 and 2 indicating that almost the PrPres was precipitated at this higher iron sulfate concentration. With decreased iron sulfate content, non-reacting PrPres bands appeared at lanes 3 to 6. In the precipitating zone (lanes 9 to 14) the first two iron sulfate dilutions 1/2 and 1/4 presented unexpected lowered precipitation zone and instead exist a diffused black curved area which is better seen in Figure 2b. Lanes 11 to 14 showed decreasing PrPres band sizes in correspondence with the iron sulfate concentration.

Figure 2b present the re-suspended iron sulfate-PrPres precipitate showing degradation products induced at the higher concentration lane 9 and gradually decreased thereafter in the precipitation zone and at the dilution 1/8 to 1/16 in the supernatant zone.

The interaction of 2 M iron III citrate monohydrate with the pathogenic prion protein treated with proteinase K is presented in Figures 3a and 3b. The samples deposited in lane 1 to 6 and 9 to 14 were prepared as follows; 10 µl of distilled water were dropped in each of 6 Eppendorf tubes, into the first tube was added 10 µl of 2 M iron citrate, successively I/2 dilution were done till 1/64 at the 6th tube from which 10 µl were discarded then 5 µl PrPres were added to each of the 6 tubes. After preparation of the mixtures, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants (S) which were loaded in lanes 1 to 6 respectively. Then the pellets were suspended in 10 µl of 50% v/v 8 M urea and Laemmli

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denaturing buffer, vortexed, heated 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovered supernatants (P) were loaded in lanes 9 to 14 respectively. The PrPres control were loaded in lane 8 and 15 and were prepared by added 5 µl PrPres and 10 µl distilled water, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes and recovering the supernatants. After loading the collected supernatants on 15% SDS PAGE, then electrophoresis, transfer and immunodetection were done using monoclonal antibodies [4] (Figure 3a).

The results in Figure 3a presented a zone phenomenon where the supernatants of the first dilutions of iron citrate induced an increase of the molecular weight of the PrPres bands (lane 1 and 2) indication an interaction of the iron citrate with the prion protein then a drop of the detected bands sizes at the dilution 1/8 and 1/16 (lanes 3 and 4) and thereafter reappeared the normal PrPres bands (lanes 5 and 6). Iron citrate induced also degradation of the precipitated PrPres bands at the dilution 1:2 to the dilution 1:8 then the degradation decreased thereafter.

Figure 3b showed a degradation area due the interaction at the higher iron citrate concentration and the PrPres at lanes 1 and 2 and no more after. Where in the precipitation zone (9 to 13) existed a degradation zone due to the iron citrate-PrPres interaction from the dilution 1:2 and lesser degradation from lanes 10 to 13.

The results obtained showed that the three iron salts interacted each one with the PrPres resulting in the precipitation of this amyloid protein. The precipitation capacity varied from week precipitation as do the iron oxide, to high precipitation capacity solon the concentration employed as do the iron sulfate and finally a higher precipitation capacity with a zone phenomenon as produced with the iron citrate. This precipitation activity was probably due to an ion exchange between the amino acids of the PrPres peptide and the iron salt.

Also, was observed a chemical reduction of the iron sulfate and the iron citrate to a lower iron grade content as evidenced by the presence of dark brown color produced after the suspension of the produced PrPres precipitates induced by these two iron salts. The intensity of the produced color was proportional to the iron salt concentration used. The chemical reduction of the iron III salts to iron II was produced in presence of the Alzheimer disease A beta peptide and was recently observed using synchrotron based techniques [1].

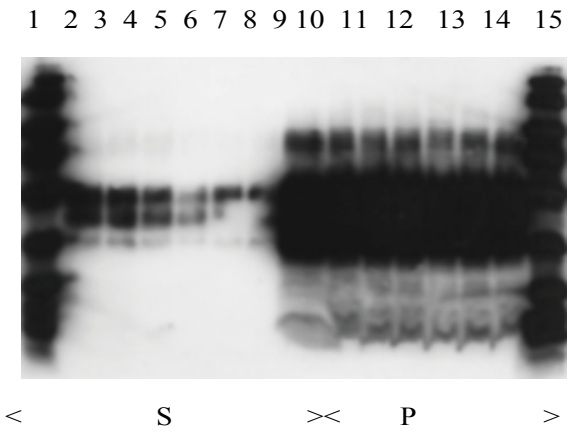

Figure 1: Immune blot.

Figure 2a: Immune blot.

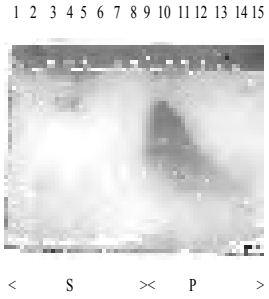

Figure 2b: Photography of the gel after the transfer

Figure 3a: Immune blot.

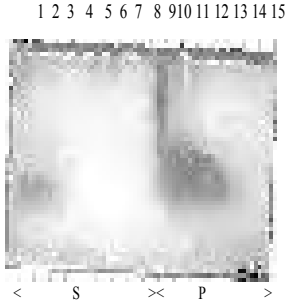

Figure 3b: Photography of the gel after the transfer

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