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In Vitro Glycation of the Pathogenic Prion Protein

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The prion protein (PrP) possesses two glycosylation sites at the position 181 and 197. After electrophoresis the PrP is detected as a 3 isoforms; bi-glycosylated, mono-glycosylated and non-glycosylated. The interaction of ion, chemical or protein ligands with the prion protein leads to an increase of the molecular weight of the 3 isoforms either as aggregation or oligomerization [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].

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). In addition, the AGEs also contributes to the generation of elevated release of intracellular reactive oxygen species (ROS) and nitrogen oxygen species, including the radical's superoxide and peroxynitrite leading to development of oxidative stress, inflammation, and increased glycation of key intracellular as well extracellular proteins leading to the development of neurodegenerative disorders and progressively ending by death [4].

Here was investigated the effect of adding individual sugars to proteinase K treated pathogenic prion strains (PrPres) and heating at 100°C for variable time.

In vitro and at high temperature; after adding reducing sugars to PrPres it became attached to the amine group of the amino acid Lysine copies present on the prion protein through a non-enzymatic Maillard reaction. The quantity of the attached saccharide molecules will physically increase the molecular weight of the PrP peptide bands. Also the protein configuration can be changed due to the presence of sugar molecules leading to better exposure of certain epitopes and resulting in a better immune detection by their specific antibodies.

The results in Figure 1 showed the effect of adding 8 µl of 1M lactose to 4 µl of dilutions (P, 1:2, 1:4 and 1:8) of a proteinase K treated bovine brain suspension (PrPres), then adding 4 µl Laemmli buffer to each of the 15 tubes, votexing and heating during variable periods (0, 5, 10 and 20 min respectively). The tubes were then centrifuged for two min. at 10000 RPM. The supernatants were collected and a constant volume from each was deposited and run on 15% SDS acrylamide gel, transfer on nitrocellose membrane and immunoblotted using an anti-PrP monoclonal antibody and peroxidase conjugated anti-mouse polyclonal serum [5].

The results in Figure 2 were obtained after adding to 4 µl of proteinase K treated PrPsc of previously confirmed cases from either ovine, bovine or mice adapted ovine strains 8 µl of a 1 M solution of either galactose (Ga), glucose (G), lactose (La) or mannose (Ma) and finally to each tube 4 µl of Laemmli buffer were added. The control tubes (C) without any sugar were prepared by adding 4 µl from the same prion strains to 12 µl of Laemmli buffer. The mixtures were vortexed, heated for 15 min. at 100°C and centrifuged for two min. at 10000 RPM. The supernatants were collected and a constant volume from each was deposited and run on 15% SDS acrylamide gel, transfer on nitrocellose membrane and immunoblotted using an anti-PrP monoclonal antibody and peroxidase conjugated anti-mouse polyclonal serum [5].

In Figure 3 was tested the capasity of 5 different prion strains to interact with mannose at 100°C during variable duration. To three sets

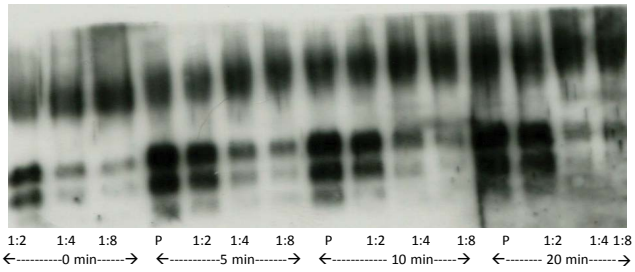

Figure 1: The molecular masse of the prion protein bands as well as the immune detection intensity of bands were substentially increased after heating 5 min. in presense of lactose. This effect was continuly amplified after heating during 10 min. but less after 20 min.

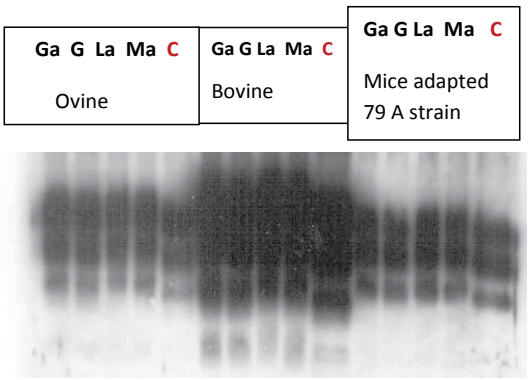

Figure 2: The position of 3 prion bands of mixures with the disacchrides (La and Ma) were slightly higher than those with the added monosaccharides (Ga and G). Where the controles without sugers did'nt showed any increase of its molecular weight compared to those with sugars.

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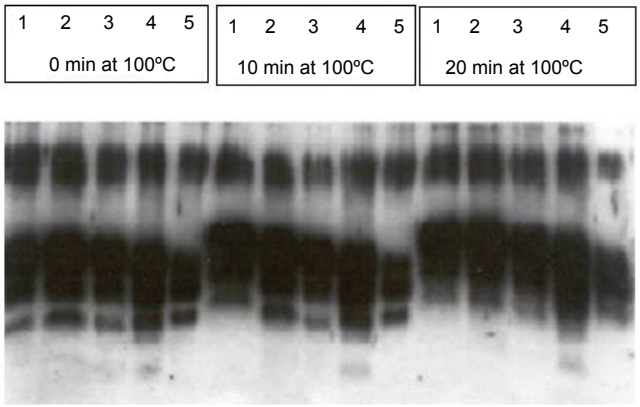

Figure 3: The molecular masse of the three prion protein bands of all the five prion strains were increased after heating for 10 mins in presence of mannose and were further slightly increased when heated for 20 minutes at 100°C compared to those of non heated ones. Another observation concerning strian difference was noted after heation 10 min. concerning the ovine strain in position 1 which showed a higher increase in its molecular weight than the other ovine in tube 2 of the same set. This result can be explained by the presence of higher copy numbers of lysine in the prion protein of the first strain compared to the second one.

of 5 tubes each was added 8 µl mannose and 4 µl Laemmli buffer in each of the 15 tubes. to the tubes 1 of three set was added 4 µl of an ovine field isolated strain, to the tubes 2 of three set was added 4 µl of another ovine field isolate, to the tubes 3 of three set was added 4 µl of a bovine field prion strain, to the tubes 4 of three set was added 4 µl of another bovine field isolate and to the tubes 5 of three set was added 4 µl of a mouse brain suspension of a mouse adapted ovine strain. After vortexing the 5 tubes of the first set were not heated, those of the second sete were heated for 10 min. at 100°C and those of the third set were heated for 20 min. at 100°C. The tubes were then centrifuged for two min. at 10000 RPM. The supernatants were collected and a constant volume from each was deposited and run on 15% SDS acrylamide gel, transfer on nitrocellose membrane and immune blotted using an anti-PrP monoclonal antibody and peroxidase conjugated anti-mouse polyclonal serum.

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