

Two Functional Guanidine Groups Are Responsible For the Biological Activity of Streptomycin and Functionally Equivalent Molecules

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Here is presented some results obtained with a group of molecules composed of two antibiotics and 4 chemicals having in common the presence of two guanidine groups each possess an imine group and aminoacetal functional group. These molecules when added in low concentrations interact rapidly with the infectious prion proteins (PrP^{sc}) via hydrogen bond transfer between each of the guanidine groups and amino acids on one or several peptides and so increases the apparent molecular weight of the protein. With increased quantities and incubation with the PrP^{sc} for one hour at 37°C; these molecules form multimolecular protein aggregates and allowed its recovery via a low centrifugation step. Also other consequences of these interactions are either a drop or even a complete reduction of the prion PrP^{sc} infectivity. These molecules though their interaction with microbes provoked an anti-bacterial activity.

The guanidine-containing compounds constitute a very important class of therapeutic agents suitable for the treatment of a wide spectrum of diseases as well as disinfecting agents. Several molecules possesses two guanidine groups as streptomycin, dihydrostreptomycin, triethylenetetramine, bis-3-aminoproylamine, guanidine hydrochloride, and spermine tetra-hydrochloride. The presence of these 2 functional guanidine groups within a non-polymeric hydrophilic molecular system was suspected to be the chemical structure of streptomycin implicated in the interaction with proteins [1]. This interaction takes place through hydrogen bond transfer among the 2 guanidine groups on streptomycin and the amino-acids of one or several prion peptides. The addition of low concentration of streptomycin to a constant amount of non-soluble fraction of the infectious prion protein (PrP^{sc}) followed by electrophoresis on polyacrylamide gel and immuno detection revealed an increase in the apparent molecular mass of each of the three bands and this increase of the protein molecular mass was proportional to the added streptomycin quantity. When higher streptomycin concentration were added and incubated with PrP^{sc} for one hour at 37°C aggregation and flocculation of the prion protein occurred due to cross-linking by such a proportionally small streptomycin molecule and the PrP^{sc} isoforms or fragments [1].

Streptomycin induced infectivity reduction after inoculating into mice of a mixture of the PrP^{sc} and streptomycin. The drop in infectivity can be attributed to changes of the surface electrostatic charges due to hydrogen bond transfer between the two guanidine

groups present on streptomycin and the different amino acids on the PrP^{sc} peptides [2] but didn't interfere with proteinase K resistance. Similarly guanidine hydrochloride markedly reduced and, at higher concentrations, even destroyed the infectivity of PrP^{sc} [3] but can reduce also the PrP^{sc} resistance to proteinase K. Finally as streptomycin and dihydrostreptomycin possess antibacterial activity also guanidine containing molecules possessed antibacterial activity.

To prove that chemicals as triethylenetetramine, bis-3-aminoproylamine, guanidine hydrochloride, and spermine tetra-hydrochloride similarly interact with proteins as streptomycin the following experiments were under taken.

Adding 0, 30, 60, 120, 180 and 240 mM triethylenetetramine (**tri**) or 0, 1, 2, 4, 6 and 8 µl of 0.5 M bis-3-aminoproylamine (**Bis**) to a constant quantity of the PrP^{sc}, vortexing, heating 5 min. at 100°C, centrifugation at 12,000 g for 5 minutes, recovering supernatants, loading, on SDS-PAGE, electrophoresis, transfer and monoclonal antibodies immuno-detection showed an increase of the molecular weight of the prion proteins proportional to the concentration of the added molecule. The PrP^{sc} three bands tend to migrate altogether in a smeared distribution of aggregated proteins Figure 1.

Guanidine called also carbamidine and has biotechnological application in protein purification and as a protein denaturant and is used as fungicides or disinfectant. Also guanidine hydrochloride increased the apparent molecular mass of the 3 prion protein bands proportional to its concentration added when tested as in the previous experiment Figure 2.

Spermine is a polyamine and its intervention occurs during replication of eukaryote and prokaryote cells. Here we present 2 different experiments, in (A) directly after mixing spermine and PrP^{sc}, heating, centrifugation, then collection of the supernatants and loading onto SDS-PAGE. In (B) the spermine and PrP^{sc} mixtures were first incubated at 37°C for one hour before collecting the supernatants and suspending the pellets in 50% SDS-Laemmli buffer as below.

In experiment (A) spermine tetra-hydrochloride was added as 0, 1, 2, 2.5, 3, 3.5, 4 and 5 mg to a constant volume of 6 µl of PrP^{sc} extracted from 3000 µg equivalent BSE infected cattle brain. After vortexing, heating at 100°C for 5 minutes, centrifugation, collection of the supernatants and loading onto SDS-PAGE followed by transfer

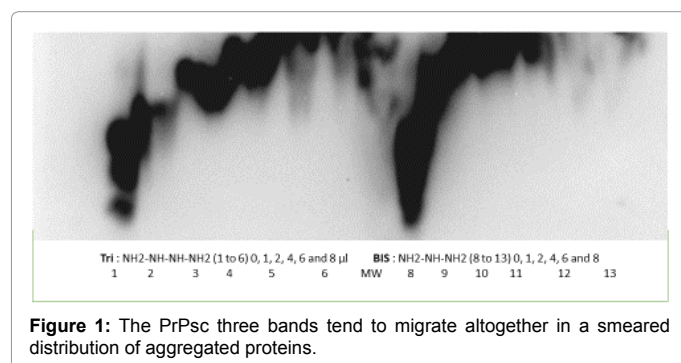


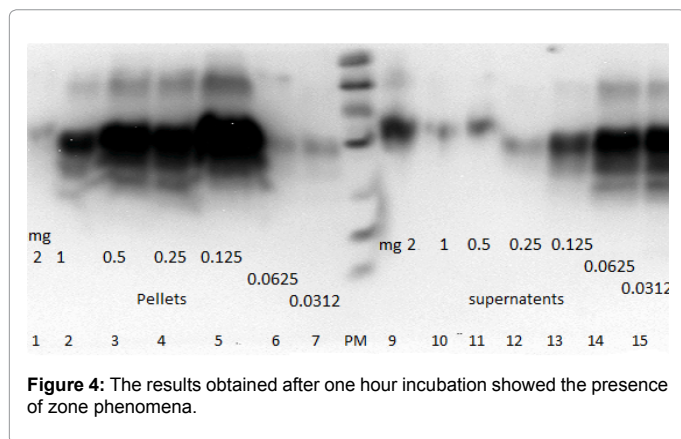
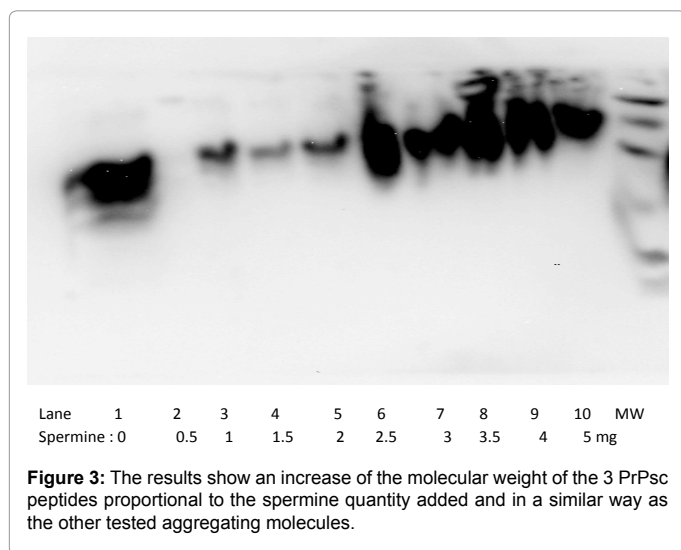
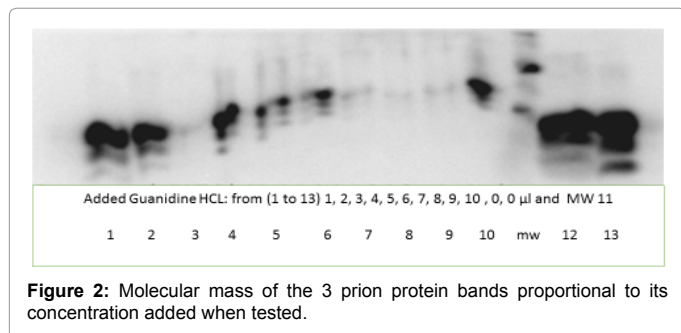
Figure 1: The PrP^{sc} three bands tend to migrate altogether in a smeared distribution of aggregated proteins.

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and immuno-blotting. The results (Figure 3) show an increase of the molecular weight of the 3 PrP^{Sc} peptides proportional to the spermine quantity added and in a similar way as the other tested aggregating molecules.

In experiment (B), 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.0312 mg concentrations of spermine in 8 μl volume were added to 8 μl volume PrP^{Sc} extracted from 4 mg equivalent positive BSE cattle brain followed by vortex stirring, incubation at 37°C for one hour, centrifuged at 12,000 g, collection of the supernatants separately and the pellets were suspended in 8 μl 50% v/v 8M urea and Laemmli denaturing buffer, after vigorous vortexing, 5 minutes heating at 100°C, 2 minutes centrifugation at 12,000 g and collection of the second supernatants.

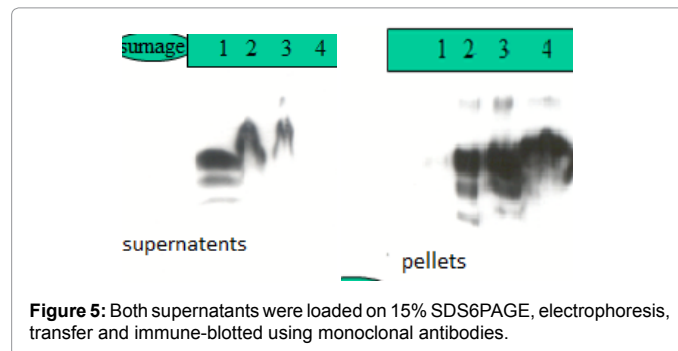
Both supernatants were deposited on SDS-PAGE, electrophoresis, transfer and immunodetection.

The results obtained after one hour incubation (Figure 4) showed the presence of zone phenomena. The lower spermine concentrations 0.0625 and 0.0312mg showed very low interaction with the PrP^{Sc} as the prion protein were mostly present in the supernatant (lane 14 and 15) and has a MW of 29 Kd. From lane 2 to 5 (corresponding to decrease in spermine concentration from 1 mg to 0.125 mg) most of the PrP^{Sc} were more progressively accumulated and precipitated in the pellets successively. In presence of 0.125 spermine was observed the highest PrP^{Sc} accumulation. Few PrP^{Sc} amount were still present in the supernatants (lane 10 to 13) with higher molecular mass. No precipitation was revealed in presence of 2 mg spermine and most of the PrP^{Sc} was present in the supernatant with increased molecular mass. This result is in accord avec that present in Figure 3 where higher doses of spermine resulted in increase of the molecular weight of the protein bands. Also these observations can be explained by the necessity for a longer incubation after the interaction to bring to aggregates formation. The presence of an equilibrium zone between the amount of PrP^{Sc} and the quantity of spermine can explain the zone phenomena.

In order to show that the interaction with streptomycin did not affect the Proteinase K resistance of the prion protein the following experiment was done. After homogenization of a BSE positive brain at 10% (weight/volume) in glucose 5%, 100 μl were distributed in each of 4 Eppendorf tubes then 0, 5, 10, 20 μl of 0.7M streptomycin, and finally 10 μl Proteinase K (at 200 μg/ml) were added successively. The tubes were incubated at 37°C for one hour, heat to 100°C for 5 minutes, centrifugation at 12,000g for 5 min. and collection of the supernatants. The pellets were suspended in 100μl of 50% volume:volume 8 M urea and Laemmli denaturing buffer per tube, after vigorous vortexing, heat at 100°C for 5 minutes then centrifugation for 5 minutes at 12,000g and collection of the second supernatant (representing the pellet) Both supernatants were loaded on 15% SDS6PAGE, electrophoresis, transfer and immune-blotting using monoclonal antibodies (Figure 5).

The results obtained showed that with or without streptomycin the PrP^{Sc} has a MW of 29KD (lane1 in supernatants without streptomycin and lane 2 with streptomycin in pellet). In absence of streptomycin the PrP^{Sc} was mainly present in the supernatant where after addition of more of streptomycin small quantities were still in the supernatant with a higher molecular mass and progressively the rest of the protein was aggregated and recuperated in the pellet only. This result gave a proof that streptomycin didn't affect the PrP^{Sc} resistance to Proteinase K.

The prion protein PrP^{Sc} is rich in beta-sheets, aggregates into prion rods and show infectivity and proteinase K (PK) resistance. Dissociation of prion rods and breakdown of beta-sheets by denaturation results in loss of both infectivity and PK-sensitivity. The effects of guanidine



(Gdn), which solubilizes and denatures proteins by breaking down their higher structure on the infectivity of PrP^{sc} of scrapie strain 263K was assayed by intracerebral inoculation into hamsters. Gdn markedly reduced or, at higher concentrations, even destroyed the infectivity of PrP^{sc}. These results confirmed that all the alternations in the PK-resistance and the infectivity of PrP^{sc} caused by Gdn resulted from changes in its higher structure and emphasized that a complete loss of PK-resistance of PrP^{sc} may not necessarily mean its full non-infectivity [3]. In conclusion, infectivity and PK resistance are not associated on the same region on the PrP^{sc} peptide.

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