"Interaction of recombinant and disease-associated prion proteins with organic and mineral soil colloids"

The normal prion protein is a cellular protein mainly located in the central nervous system. However the cellular protein, also indicated as PrP^{C}, can be converted in a misfolded pathogenic isoform, indicated as PrP^{Sc} from the Scrapie disease caused in sheep.

According to the "Prion-only" hypothesis, proposed by Prusiner in 1982, the PrP^{Sc} is the main, if not the sole, putative of prion diseases, also referred as Transmissible Spongiform Encephalopathies (TSEs). These neurodegenerative diseases affecting a variety of mammals are fatal, with pathogenesis in the central nervous system leading slowly but inexorably to death. The most known TSE in humans is the new variant of Creutzfeldt-Jakob disease (nvCJD), almost certainly occurred as a consequence of the consumption of meat cows affected by Bovine Spongiform Encephalopathy (BSE), commonly known as "mad-cow disease". Many others notably TSEs are known in humans, like Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, Fatal Familial Insomnia, while in animals, Scrapie affect sheep and Chronic Wasting Diseases (CWD) deer and elks.

The prion protein diseases cause an environmental issue because the environment and, in particular the soil compartment, can be contaminated and then become a potential reservoir and diffuser of TSEs infectivity as a consequence of (i) accidental dispersion from storage plants of meat and bone meal, (ii) incorporation of contaminated material in fertilizers, (iii) possible natural contamination of pasture soils by grazing herds and (v) burial of carcasses of contaminated animals.

In the environment the problem can be even more relevant because of the characteristic of the pathogenic prion protein. Very low environmental amounts of PrP^{Sc} are able to propagate the disease, PrP^{Sc} develops proteic amyloidal aggregates that exhibit resistance to protease attack in its pathogenic part, and many inactivating procedures are also ineffective against PrP^{Sc}. Moreover, for such of these diseases as in the case of Scrapie and CWD, environmental sources of infectivity can be spread by free ranging animals that in turn can sustain the disease diffusion. Therefore, these environmental sources of infection represent evident obstacles to control the spread of the disease.

In the past it was supposed that prion infectivity can persist in soil for years. This hypothesis was supported by an early study, in which in 1991 Brown and Gajdusek demonstrated that soil retained infectivity after 3 years of infectious material internment. More recent studies also confirmed this hypothesis; in 2006 Georgsson and colleagues reported that prion infectivity persisted in an old sheep-house for at least 16 years, and after this period healthy animals were infected, thereby strongly highlighting the prion capability to persist in soil in an infectious state.

In addition to intrinsic PrP^{Sc} properties, soil dynamics can affect the fate of a prion protein as well a generic protein in soil. Once introduced into the soil a protein may remain free in solution, and as such to be biologically or chemically degraded, or participate in the formation of organic and organo-mineral soil complexes. These complex phenomena could depend on properties of both the protein and soil. For instance, physicochemical properties of protein such the isoelectric point and flexibility of the polypeptide chain may affect the adsorption phenomenon on soil colloids such as clays and humic substances because of the presence of polar and non polar adsorption sites.

Therefore, it can be of crucial importance to identify the presence of soil components and colloidal particles able to minimize the migration of the prion protein in soil trough immobilization or to act as degrading agents and in turn reducing the environmental diffusion of the infectivity.

In the first part of the PhD thesis, the interaction of a recombinant ovine prion protein (PrP) with organo-mineral complexes obtained by birnessite-mediated oxidative polymerization of catechol, resembling humic-mineral complexes in soil was investigated. The first clear experimental result was that the investigated protein strongly interacted with birnessite-catechol complexes. The use of the C-terminal form of this protein showed that the part mainly involved in the binding with humic-like material was the N-terminal arm due to the presence in this region of several positively charged residues. As the prion protein conformation is linked with its pathogenic properties, the conformation of adsorbed/entrapped prion protein by FT-IR deconvolution of amide I signal was also investigated. Interaction with birnessite-catechol complexes caused a slight increase of beta sheet protein (PrP^{Sc} is beta sheet richer than the PrP^{C}), however the final conformation of the protein resulted still far from the pathogenic form. The high stability of the interactions of PrP was confirmed by performing several extraction tests, with a general ineffective result.

The second part of the study was devoted to the investigation of the interaction of PrP with organic complexes. Preformed natural humic acids extracted from an agricultural soil and humic-like substances were investigated. Interaction of PrP with soil organic matter as humic-like substances was performed by enzymatic polymerization of catechol. The decrease of UV-Vis absorbance, the disappearance of PrP from solution, the formation of insoluble aggregates clearly indicated that PrP adsorbed/entrapped in soil humic material. Also, the characterization of the insoluble aggregates by FT-IR analyses confirmed the presence of PrP in the precipitates. At the adopted sperimental conditions, the immobilization of the protein was complete in the humic-like system whereas resulted incomplete using as adsorbent soil humic acids.

The strength of the interaction between PrP and humic acids or humic-like substances also resulted different in the different systems. Notably, a stabilization effect of the interactions of PrP with humic acids was obtained by forming new humic-like material in solution. It is a reasonable conclusion that the contact between the protein and humic acid, as already formed soil organic substances, lead to superficial and weak adsorption processes, whereas ongoing transformation of organic material more strongly involved PrP molecules, mainly during the polymerization processes. Both the phenomena may normally occur in soil.

The third part of the thesis regarded the investigation of a possible degradation process acting in soil and leading to the inactivation of the PrP^{Sc}, the direct agent of the TSE. The degrading agent investigated was the birnessite, a high surface manganese soil mineral ranking among the strongest oxidants in soils. Incubations of PrP^{Sc} were performed in the presence of birnessite at different concentrations, at several soil pHs, and for different incubation periods. Birnessite caused a dose depending decline of residual PrP^{Sc}. The degradation of PrP^{Sc} occurred mainly at pH lower than 6. Two non-mutually exclusive factors possibly contributed to this behaviour. Firstly, MnO_2 redox potential and surface charge increase as pH declines. Secondly, solution pH may influence the degree of protein sorption to MnO_2 surfaces. As birnessite has a negative charge for all the pHs investigated, the maximal adsorption occurred at pHs closer to the apparent isoelectric point of PrP^{Sc} amyloidal aggregates (pI 4.6). The degradation of PrP^{Sc} was fast and maximal degradation was reached after a period of 24 hours. Protein misfolding cyclic amplification used to detect PrP^{Sc} in far dilutions was used to semi-quantify the entity of maximal degradation that resulted at least of a factor of 10^4 of the initial used amounts.

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Summarizing: soil organic matter as humic acids has a high capability of binding prion proteins; the interactions of these proteins can be even stronger when protein is involved in organic matter formation processes. The strength of binding between prion proteins and soil organic colloids was enhanced by new organic matter arriving in soil. As a consequence, through these mechanisms prion proteins can be bound to soil in the superficial organic rich layers, and its migration in soil is limited even if it can be more available to free ranging animals. Findings obtained by direct interaction of birnessite with the infectious protein, putative of TSE, suggested that birnessite may be employed as a reactive burial material in the disposal of prion-infected materials. Birnessite, being a natural occurring mineral, could be used for the decontamination of prion-contaminated soils and reducing the disease diffusion. While the majority of studies regarding the fate of prions in soil suggest that soil can only enhance the diffusion of TSE, the presence of such components could act reducing the environmental risk related to prions.

References

A full version of this thesis can be downloaded from:
http://www.fedoa.unina.it/1697/01/Russo_Agrobiologia_e_Agrochimica.pdf

Two papers about this thesis are already available as:

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