ELSEVIER

Soil Biology & Biochemistry 38 (2006) 1638–1644

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Interactions of prion proteins with soil

Liviana Leita[a,*], Flavio Fornasier[a], Maria De Nobili[b], Alessandro Bertoli[c], Sacha Genovesi[c], Paolo Sequi[d]

[a]*C.R.A. Consiglio per la Ricerca e Sperimentazione in Agricoltura—Istituto Sperimentale per la Nutrizione delle Piante, Sezione di Gorizia, via Trieste 23, I-34170 Gorizia, Italy*

[b]*Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, via delle Scienze 208, I-33100 Udine, Italy*

[c]*Dipartimento di Chimica Biologica, Università di Padova, viale G. Colombo 3, I-35121 Padova, Italy*

[d]*C.R.A. Consiglio per la Ricerca e Sperimentazione in Agricoltura—Istituto Sperimentale per la Nutrizione delle Piante, via della Navicella 2, I-00184 Roma, Italy*

Received 10 September 2004; received in revised form 3 November 2005; accepted 7 November 2005
Available online 6 February 2006

Abstract

Prions, are proteinaceous particles recognized as the agents of a class of neurodegenerative disorders, called transmissible spongiform encephalopathies (TSE), or prion diseases. Epidemiological data suggest that TSE-contaminated environments may serve as source of infectivity, but there is no information about adsorption of prions onto soil. We carried out experiments by mixing, healthy, or scrapie-infected hamster brains homogenates with three types of soil suspended in different buffers: (i) two saline buffers, i.e., phosphate buffer solution (PBS) and $CaCl_2$ solution; (ii) a mix of nondenaturing detergents, i.e., Triton X-100 and sodium deoxycholate (DOC) solution; (iii) a non-ionic detergent, i.e., lauryl maltoside; (iv) two anionic detergents, i.e., Sarkosyl or sodium dodecyl sulphate (SDS); and (v) a chaotropic agent, i.e., urea. The unbound prion proteins were detected in the supernatants (after centrifugation of soil suspension) by Western blotting. Results clearly demonstrate that both the no infectious (PrP^C) and infectious (PrP^{Sc}) forms are adsorbed by all soils. Only 1% sodium dodecylsulphate (SDS) partially impeded the association of PrP^C, but not that of PrP^{Sc} with the sandy loam soil. Agents with different interacting properties towards hydrophilic and/or hydrophobic domains failed to extract PrP^{Sc} from sediments of soil–brain homogenate mixtures. The strong interaction of PrP^{Sc} with soil favors the accumulation of prions in soils, especially if amended with prion-containing organic fertilizers and/or whenever TSE-affected animal carcasses, placenta, and excreta in general are buried or laid at the soil surface.

Keywords: Prion proteins; Scrapie; Prion diseases; Proteins adsorption

1. Introduction

Prion diseases (transmissible spongiform encephalopathies, TSEs) are a class of fatal neurodegenerative disorders that affect animals and human beings. TSEs include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob disease (CJD) in human beings. The ethiologic agent of TSEs is the prion, a proteinaceous particle composed mainly of PrP^{Sc}, a predominantly β sheet isoform of an α helix-rich glycosylphosphatidil-inositol linked cell surface glycoprotein, termed cellular prion protein (PrP^C), which is composed of about 250 aminoacids and is a normal constituent of mammalian cells (DeArmond and Bouramondo, 2002; Prusiner, 1998a).

The transition from the cellular form of the PrP^C protein to the pathogenic isoform (PrP^{Sc}), occurs spontaneously in infected cells, without any covalent modification of the protein, through a process by which, apparently PrP^{Sc} acts as a template for the conversion of a portion of the tertiary structure of PrP^C into an exceptionally stable β sheet conformation (Chiesa and Harris, 2001). This protein refolding confers novel physicochemical and biological properties to PrP^{Sc}, such as a propensity to aggregation, resistance to proteolysis, augmented hydrophobicity, and acquisition of neurotoxic and self-propagating potentials

*Corresponding author. Tel.: +39 0481 522041; fax: +39 0481 520208.
E-mail address: liviana.leita@entecra.it (L. Leita).

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.11.018

(Prusiner, 1998a). Scrapie is endemic in many geographical regions of the world except New Zealand and Australia (Detwiler and Baylis, 2003) and can be horizontally transmitted (Pálsson, 1979; Ryder et al., 2004). There is, however, little risk for human beings, as ovine prions do not appear to cross the sheep-primate species barrier contrary to BSE prions that generate a variant CJD form through the consumption of infected food (Collinge et al., 1996; Hill et al., 1997). Another TSE form, i.e., chronic wasting disease (CWD), previously endemic in captive herds of cervids, has recently spread among wild cervids in large areas of the United States (Belay et al., 2004; USDA, 2003).

The primary routes of infectivity of TSEs are unclear, but they may be transmitted via contaminated environments. Therefore, prions may spread through uncontrolled burying and dissemination of TSE-infected carcasses and excreta (Miller et al., 2004) or through use of contaminated bone meal fertilizers and sewage sludge (Gale and Stanfield, 2001). The unusual resistance of the PrP^{Sc} to proteases may cause the persistence and the adhesion of prions freed from easily decomposable organic materials such as tissues, fertilizers, etc. to soil components, thereby creating environmental sources of infectivity. A large number of laboratory experiments (Kimberlin, 1996) has shown that the intracerebral route is the most effective for transmission of the disease and the oral route the least effective, however, prions may behave differently in natural disease and may be spread more efficiently by peripheral routes (e.g., oral route) to genetically susceptible populations (Gosh, 2004). The oral infective dose (ID_{50}) of scrapie for a mouse is $4 \times 10{,}000$ infective units (IU), which represent between one hundredth and one hundred thousandth of a gram of infected brain tissue, and this however depends on the animal and eventually on the interspecies barrier: it is generally assumed that the oral ID_{50} for cattle is 0.1 g of infected bovine brain (DNV, 1997). Brains of an animals dying of TSE commonly contain between one million and ten thousand million IU per gram, but brain stems contain 100 times more (Kimberlin, 1996). Gale and Stanfield (2001) estimated a risk of BSE transmission of 7.1×10^{-5} per cow per year for cattle grazing on land to which sewage sludge has been applied, the risk of transmission through soil ingestion needs therefore to be seriously considered.

Epidemiology of scrapie in Iceland, where this disease has been known for about 100 years suggested, on one side, that contaminated pastures could transmit it to newly introduced lambs even after 3 years since they were left without sheep, but there was no conclusive evidence that soil could be a possible transmitting agent because the possibility that animals became infected during the long winter housing period could not be ruled out (Pálsson, 1979). The persistence and potential infectivity of PrP^{Sc} in different types of soils are still unknown, and prion–soil interactions have not been thoroughly examined. The first and, to our knowledge, only experiment on the prion persistence in soil was done by Brown and Gajdusek (1991) who, 3 years after burying in a garden soil perforated Petri dishes containing mixtures of soil and supernatant fluids derived from scrapie-infected brain homogenates, found that around 1% of the original infectivity remained in the soil, while little infectivity had leached into the underlying soil. Although this might seem at first sight a powerful and nearly complete deactivation, we have to keep in mind the large infectious potential of prions: the residual infectivity amounted in fact to between 2.2 and 3 log IU of the nearly 5 log IU buried in the soil. Unfortunately, we do not know whether prions were actually adsorbed on soil, and no information was given on the composition and pH of the soil employed for the experiment. It is therefore impossible at present to generalize these results. The adsorption of proteins onto soil, is in fact a complex phenomenon. It is strongly affected by the physicochemical characteristics of the proteins such as the isoelectric point (pI) and the flexibility of polypeptide chains (Quiquampoix et al., 1995; Norde and Giacomelli, 2000) and on the surface properties of soil colloidal particles, primarily clay minerals and humic substances, which are highly variable (Stotzky, 1986) and provide a wide range of polar and nonpolar adsorption sites (Stevenson, 1994; Senesi and Loffredo, 1999). Both clay and humic substances are surrounded by water films, which also affect protein adsorption via several mechanisms, involving enthalpic forces or entropic effects that promote conformational modifications of the adsorbed protein (Yong et al., 1992).

The influence of soil characteristics on the adsorption of proteins and on the retention of their activity has been the subject of several studies. Among these of interest in the present context, are those on toxins from *Bacillus thuringiensis* (Bt) which showed that the toxins adsorbed on clay minerals and humic acids maintained the insecticide property (Stotzky, 2000, 2002). Saxena et al. (2002) reported that Bt proteins exhibited stronger binding and persistence in soils with higher clay contents (especially of montmorillonite), and suggested that the adsorbed toxin in these soils could be transported to surface waters via runoff and erosion or colloid mediated transport (Kretzschmar et al., 1999), whereas in soils of lower clay content the protein may be more readily leached to groundwater.

Based on these premises, the results of investigations on the interactions of prion proteins, in both their normal and pathogenic forms with soils are reported. To this end a protocol was employed that consisted of mixing healthy or scrapie-infected brain homogenates with three types of soil with different contents of organic matter and clay, and in the immunodetection of unbound PrP molecules.

2. Materials and methods

2.1. Soils

Two arable and one woodland soils were sampled near S. Martino al Tagliamento (PN) in the North-East of Italy.