Relationship Between Magnetism and Prion Protein

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KEYWORDS: prion protein, magnetism, IR spectra.

Abstract

The mechanism of conversion of the normal prion protein (PrPC) into aggregates of its pathological conformer (PrPSc) remains unclear. The aim of this study was to evaluate the effects induced by exposure of biological samples containing PrPSC to a magnetic field. The results show that the magnetic field induced prominent molecular changes of samples indicated by the IR spectra located in the region that contains contribution primarily from absorption of amides. This finding suggests the existence of a strong correlation between magnetism and PrPsc and supports a new hypothesis that explains the conversion of normal PrPc to abnormal isoform PrPsc.

Introduction

Prion diseases represent a group of fatal transmissible spongiform encephalopathy (TSE) which includes Kreutzfeld–Jakob disease (CJD) in human beings, bovine spongiform encephalopathy (BSE) in cows and scrapie in sheep [1-3]. Such diseases are characterized by an accumulation of amyloid plaques in brain generated by the pathogenic prion protein form (PrPsc) of the normal protein (PrPc) [2, 4].
Protein PrPc functional role and the mechanisms leading to its conversion in the PrPsc form are still unknown. It has been shown that such transformation is associated to a strong increase of β-sheets in PrPsc probably due to the normal protein spatial rearrangement which is dominated by an α-helical structure [2, 5]. Here we show the effects induced by a magnetic field on biological samples containing the pathological form of prion protein.
Therefore, we assume that the PrPsc protein has such magnetic properties to interact with the induced electric field. This fact allows the PrPsc protein to transform the PrPc protein. Our hypothesis agrees with some protein chemical-physical characteristics: the elevated resistance to high temperatures [3, 6-9], the greater affinity for the Mn2+ regarding the Cu2+ [6, 10-12] and the increase of the b-sheets structures. In our opinion the magnetic field induced by the PrPsc is able to reorient the PrPc electrically promoting the highly polarized increase of the β-sheets structures, therefore more responsive to the field.

Materials and Methods

The brains of two sheep affected by scrapie (positive sheep 1 and 2) and the brains of two healthy sheep (negative sheep 1 and 2) were analyzed, homogenising them in Phosphate Buffered Saline (PBS, 5 mg/10 ml). Each sample was analyzed in triplicate. The PBS has been used as blank sample and each homogenate was divided in two aliquots of 5 ml. One of the aliquots was incubated with a small magnet for 15' at room temperature and then the magnet was removed. Both aliquots were then incubated with magnetic nanoparticles (NPM) at room temperature with gently stirring for two hours [4, 7, 8]. Then the NPM were removed and dried at 60°C overnight. The dried NPM were mixed with KBr (1% p/p), pressed to form pill and then analyzed through infrared Fourier – transform spectroscopy (FT-IR). The IR spectra were acquired between 400 and 4000 cm^{-1} with FT-IR spectrometer and analyzed by OPUS 6.0 and Origin PRO 8 software.
Infrared Fourier-transform (FT-IR) spectroscopy is a technique used to analyze biological materials, to obtain specific biochemical information about the distribution of the proteins in different tissues [4].
The figure 1 shows a typical FT-IR spectrum obtained through an analysis on positive and negative samples treated with (NPM), as reported by Kneipp [4]. The adsorption bands correspond to different functional groups vibrations contained in the proteins, lipids, carbohydrates and nucleic acids present in the sample. In particular, the most interesting bands, in gray in the figure, are found to be between 3000 e 2838 cm^{-1}, closely related to the vibrational stretching of the groups CH_2 and CH_3, and between 1750 and 1480 cm^{-1}, related to the adsorption of the amide I and II of the proteins [4]. Statistical analysis of the data was performed using SPSS software, analyzing samples differences with the ANOVA procedure. Differences were considered to be significant when p value was $< 0,05$.

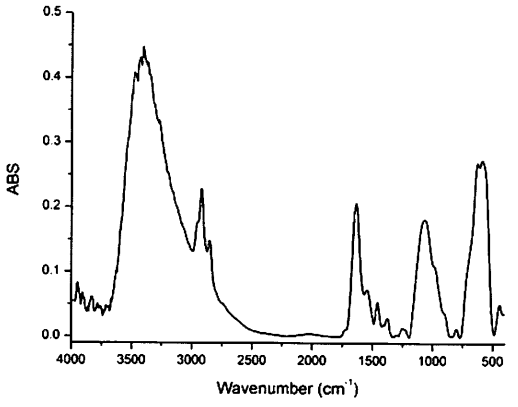

Fig. 1 - Typical FT-IR spectrum obtained from the analysis of healthy ovine brain treated with NPM. ABS: Absorbance

Results

Our results show significant differences between the samples treated with magnet and those untreated, particularly in the region of the amides (1750-1480 cm^{-1}).
As illustrated in figure 2, the IR spectra of the positive sample 1, unexposed to the magnet (orange lines), show an amide-I adsorption band at 1640 cm^{-1} and an amide II low and enlarged band at 1537 cm^{-1}. The magnet effect on the positive samples induces adsorption spectrum modifications (violet lines) with a shift of the amide I band at 1636 cm^{-1} and a peak of the amide II at 1540cm^{-1}. Thus, the spectrum profiles of the pathogenic samples treated with magnet result to be equal to the negative samples deriving one (green line). In order to simplify the diagram reading a single negative tracing has been used.
The spectra of the sample positive 2 is shown in figure 3. In this case also the positive treated sample spectrum is comparable with the negatives ones.
As illustrated in figure 4, in the spectrum region of 3600 - 2800 cm^{-1}, corresponding to the CH_2 and CH_3 groups vibrational stretching, we clearly see a shift of approximately 20 cm^{-1} in the positive treated samples.
The variance analysis has shown that the differences between the analyzed samples are statistically significative ($p<0.01$).

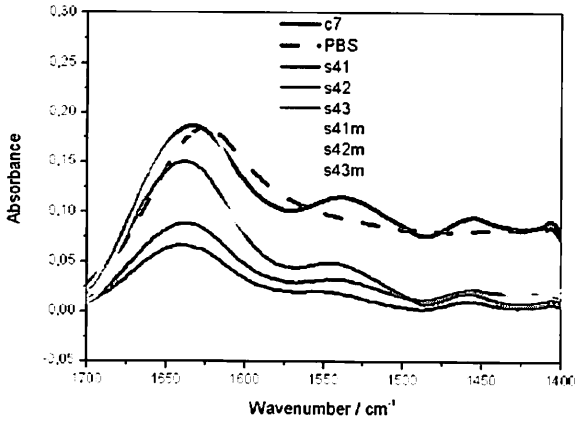

Fig. 2 - IR spectra of the positive sample 1 treated with magnet (light gray line) et untreated (gray line) respect to the negative sample (black line). The samples were analyzed in triplicate. In black PBS.

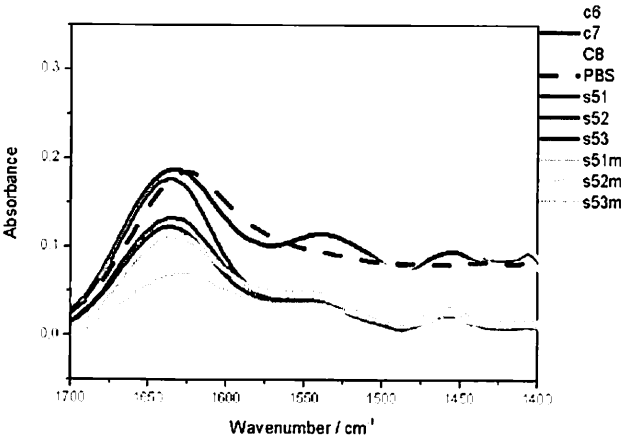

Fig. 3 - IR spectra of the positive sample 2. The sample untreated in gray colour, the sample treated in light gray, the negative sample in black. The samples were analyzed in triplicate. In dashed black PBS

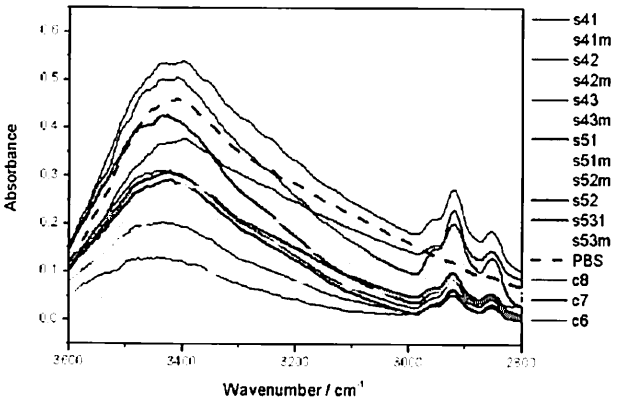

Fig. 4 - IR spectra of all the samples analyzed. In black the negative sample treated and untreated, in dashed black PBS, in gray the positive sample 1 and 2 treated, in light gray positive sample 1 and 2 untreated. The samples were analyzed in triplicate.

Discussion

The strong analogy encountered between pathological samples treated with magnet and the negative ones suggests that PrPsc could have been attracted by the magnet, and therefore removed. By means of this evidence, we can speculate that the conversion of normal PrPc to abnormal isoform PrPsc is associated with a change in its physical properties, due to the acquisition of magnetic properties in the globular domain 27-30. This suggestive hypothesis could explain the rearrangements of the chemical bonds which take place in the molecule at the level of the secondary and ternary structures. The following chemical-physical and structural characteristics differentiate the two isoforms. PrPc is sensitive to the degradation with low concentrations of Proteinase K that do not digest PrPsc. The PrPc has in the N-terminal region 4 copies of octapeptides, which are able to bind the Cu^{2+}, together with the amino acid His96 and His111. Other sites for binding the Cu^{2+} are located in the C-terminal region of the protein. Therefore, the PrPc has high affinity for Cu^{2+}, while, on the contrary, the PrPsc has greater affinity for Mn^{2+} than for Cu^{2+}. Our hypothesis agrees with the

increase of the paramagnetic characteristics of Mn^{2+} about Cu^{2+}. Actually calculating the magnetic moment of both ions with the spin formula a difference of 3.4 MB is evidenced.
This hypothesis agrees also with the resistance to high temperatures of the PrPsc, whose inactivation temperature seems to coincide with the Curie temperatures of the magnet demagnetizations.
These preliminary results, if confirmed, could support the previously formulated idea and find interesting applications in biological fluids treatment (milk and blood) contaminated by prionic proteins. Moreover, it opens future perspectives in the diagnostic field, making easier the method, avoiding the use of proteinase K.

Acknowledgments

The authors tank Professor P. Innocenzi, Professor A. Pusino, Dr. M.V. Pinna, Dr. L. Ligios, Dr. C. Santucciu, Dr. M.G. Tilocca, Dr. S. Fresu and Professor S. Stoccoro.

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