

## Techniques to elucidate the conformation of prions

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### Abstract

Proteinaceous infectious particles (prions) are unique pathogens as they are devoid of any coding nucleic acid. Whilst it is assumed that prion disease is transmitted by a misfolded isoform of the cellular prion protein, the structural insight of prions is still vague and research

for high resolution structural information of prions is still ongoing. In this review, techniques that may contribute to the clarification of the conformation of prions are presented and discussed.

**Key words:** Prion; Amyloid; Neurodegenerative disease; Protein structure; Fourier-transform infrared spectroscopy

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**Core tip:** Prions (proteinaceous infectious particles) are misfolded isoforms of cellular proteins that cause neurodegenerative diseases in mammals and humans. Several structural models are available for prions but a 3D-structure does still not exist. More structural information is demanded for the understanding of the conversion process and finally for the design of efficient therapeutic approaches. In this review, techniques that may contribute to the clarification of the conformation of prions are presented.

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### INTRODUCTION

Prions are infectious proteins that cause fatal neurodegenerative diseases<sup>[1-5]</sup>. Rearrangements of the structure of the cellular prion protein (PrP<sup>C</sup>) are the key of infection. PrP<sup>C</sup> is characterized by a high  $\alpha$ -helical content and conversion in a  $\beta$ -sheet rich conformer (PrP<sup>SC</sup>) transforms it into an infectious protein<sup>[6,7]</sup>. According to the "prion hypothesis" the protein itself is the causative agent of disease even though several cofactors that may influence the conversion process are discussed in the literature<sup>[2,8-12]</sup>. A 3D-structure of PrP<sup>SC</sup> does still not exist, albeit several structural models for

PrP<sup>Sc</sup> are described<sup>[13]</sup>. Definite structural information about prions is indispensable for the understanding of the conversion process and eventually for the design of new therapeutic strategies.

In the present review, several techniques to assess the conformation of prions are presented and discussed with a main focus on the application of Fourier-transform infrared spectroscopy. The intention of this mini-review article is to give an overview of current techniques used in prion research to elucidate the structure of prions. For details readers are referred to the literature cited in the text.

## FOURIER-TRANSFORM INFRARED SPECTROSCOPY

Fourier-transform infrared (FTIR) spectroscopy was one of the fundamental techniques that had demonstrated the high  $\beta$ -sheet content of PrP<sup>Sc</sup> in contrast to PrP<sup>C</sup><sup>[14-16]</sup>. This technique has several advantages compared to other analytical approaches. With FTIR spectroscopy protein samples can be analyzed (1) without labeling; (2) under native conditions; (3) with only minute amounts of material (in particular in case of micro-FTIR); and (4) in a dynamic process. Moreover results can be obtained within a very short time<sup>[16,17]</sup>. FTIR spectroscopy elucidates primarily the secondary structure of proteins and originates essentially from C=O stretching vibrations of the amide groups of the protein backbone. For the structural characterization of PrP<sup>Sc</sup> the amide I band is the most useful infrared absorption band<sup>[18-20]</sup>.

FTIR of proteins typically is performed in H<sub>2</sub>O, D<sub>2</sub>O or under dried conditions. For spectroscopic analyses the purity of sample material is of utmost importance<sup>[16,21]</sup>. This is particularly challenging when sample material has to be extracted from infected tissue and when the protein of interest is present in only small amounts. Improved purification protocols for the extraction of prions from tissue have been published and allowed us to analyze tiny amounts of sample material by micro-FTIR<sup>[16]</sup>. Micro-FTIR can be applied on dried protein samples using a FTIR spectrometer linked to an IR-microscope. This enables the performance of measurements on pure protein samples in the range of only a few nanograms.

Micro-FTIR has been shown to be an appropriate tool for the screening of different prion strains and *in vitro* generated prion protein (PrP)<sup>[16,17]</sup>. Particularly in case of *in vitro* generated PrP only very small amounts of prions are expected to be extractable. This could impede an analysis by other structure-sensitive techniques [e.g., circular dichroism (CD) spectroscopy]. We recently recorded spectra from PrP<sup>Sc</sup> molecules generated by protein misfolding cyclic amplification (PMCA) using micro-FTIR<sup>[16]</sup>. PMCA mimics the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> *in vitro*<sup>[22,23]</sup>. Comparing spectra from native- and PMCA-derived PrP<sup>Sc</sup> revealed structural differences

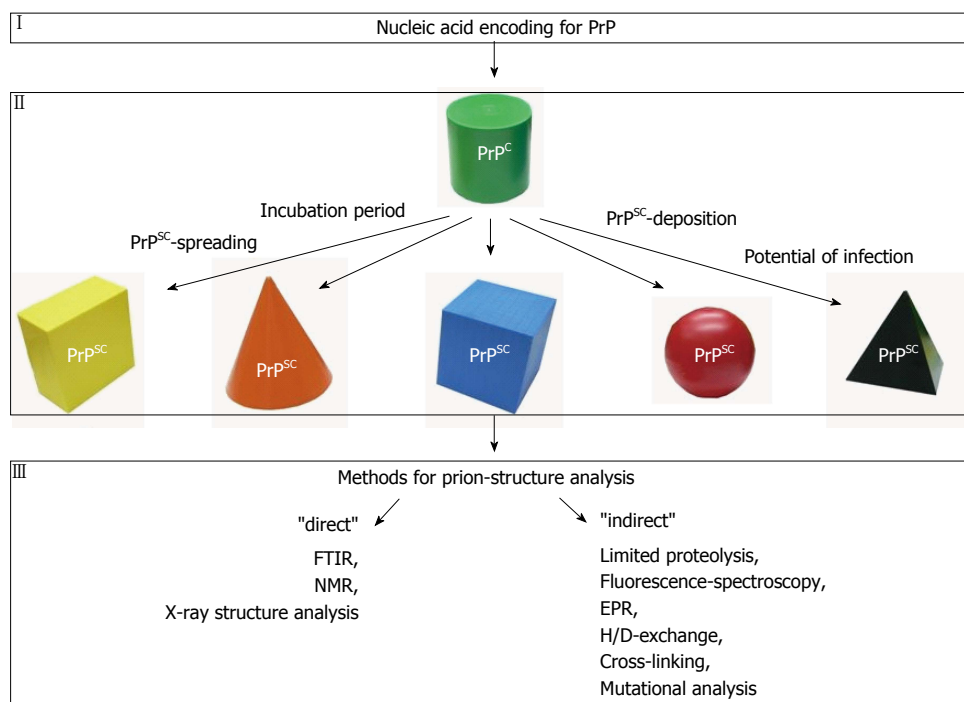
of molecules before and after PMCA. This technical progress provides the possibility to analyze the influence of cofactors on the conversion activity and putative correlating structural rearrangements in future experiments.

As mentioned above, micro-FTIR is also suitable to discriminate different prion strains<sup>[14,24,25]</sup>. In prion research, strains are defined as prion-isolates that, after inoculation into distinct hosts, cause disease with consistent characteristics, such as incubation period, distinct patterns of PrP<sup>Sc</sup> distribution and spongiosis in the brain (Figure 1). A concrete example of use would be strain typing for chronic wasting disease (CWD)<sup>[17]</sup>. CWD is a prion disease occurring in cervids in North America and rapidly spreads among free ranging deer and elk<sup>[26,27]</sup>. To test whether and to which extent there are different strains for CWD in the environment or whether new strains emerge in the future, micro-FTIR (with only 100 mg of infected tissue needed to obtain a sufficient amount of protein extract for FTIR analysis) can be applied. The use of small amounts of sample material allows the preparation of multiple samples within a short time. Spectra from CWD field samples collected in the past, present or future could be compared. As different strains can correlate with different zoonotic potentials a rapid structural scan by FTIR may be suitable for a proper risk assessment.

## OTHER TECHNIQUES TO DETERMINE THE STRUCTURE OF PRIONS

Electron microscopy analyses directly reveal the morphological difference between PrP<sup>C</sup> and PrP<sup>Sc</sup> as the latter appears as amyloid fibrils<sup>[28,29]</sup>. Moreover, it is possible to distinguish between some prion strains due to their distinct morphological shape. Electron crystallography had a limited scope to predict general conformational features because prions tend to aggregate<sup>[30,31]</sup>. Atomic force microscopy (AFM) is used as an alternative technique that needs no staining of samples<sup>[16,32]</sup>. AFM can be combined with infrared- and Raman-spectroscopy suitable for site-directed sample characterization. Tip enhanced infrared- or Raman-spectroscopy principally allow measurements on defined positions within a prion fibril<sup>[33,34]</sup>. These techniques could be useful for the analyses of mixtures of prion fibrils ("quasi species") and would allow a precise biochemical characterization of prions<sup>[17,35]</sup>.

The impact of specific amino acids on the prion conversion process can be determined by mutational analyses when subsequently tested in animal-, cell culture- or *in vitro* conversion assays<sup>[36-39]</sup>. By this means it could be demonstrated that specific amino acids accelerate or decelerate the conversion process and that the N-terminus is not essential for prion infectivity<sup>[40]</sup>. Limited proteolysis with Proteinase K is used to discriminate PrP<sup>C</sup> from PrP<sup>Sc</sup> as the conversion in a  $\beta$ -sheet rich conformation (PrP<sup>Sc</sup>) prevents the



**Figure 1 Different conformations of the prion protein.** In mammals and humans both isoforms of prion protein (PrP) [the cellular isoform (PrP<sup>C</sup>) and the misfolded isoform (PrP<sup>SC</sup>)] are encoded by the same gene ( I ). PrP<sup>C</sup> is shown in green ( II ). Under specific conditions PrP<sup>C</sup> can be transferred into a misfolded and then putatively infectious conformation (PrP<sup>SC</sup>). Prion strains can adopt different conformations (indicated by different symbols and colors). When a misfolded isoform of the prion protein becomes infectious it is characterized by specific features as incubation period, PrP<sup>SC</sup>-spreading, PrP<sup>SC</sup>-deposition and a distinct potential of infection. For structural analyses of misfolded prion proteins several methods are available. While FTIR, NMR and X-ray structure analyses provide information about the secondary structure as well as 3D-structural information ("direct"), limited proteolysis, fluorescence spectroscopy, EPR, H/D-exchange, cross-linking or mutational analyses reveal more "indirect" structural information that is focused on specific residues within the protein. FTIR: Fourier-transform infrared.

C-terminus from degradation while the less structured N-terminus is cleaved off<sup>[41-46]</sup>. Limited proteolysis at different pH values, with rising amounts of protease or in the presence of chaotropic salts can give further structural information and may help to discriminate prion strains with different conformations.

Recombinant PrP can (after site-directed mutagenesis) site-specifically be labelled with fluorophores or spin labels and then analyzed by fluorescence- or EPR spectroscopy, respectively. Such studies propose a parallel in-register  $\beta$ -sheet structure of PrP amyloids and enable the calculation of distances between specific residues within an amyloid fibril<sup>[11,47-49]</sup>. It has to be mentioned that misfolded recombinant PrP without the addition of cofactors usually lacks infectivity and may therefore not exactly represent the *in vivo* structure of PrP<sup>SC</sup><sup>[11]</sup>.

The exchange of hydrogen for deuterium ions and subsequent analysis by mass spectrometry allows the detection of unstructured and highly structured regions within PrP<sup>SC</sup><sup>[50-52]</sup>. The C-terminal part of prions shows low exchange rates indicating a highly structured conformation<sup>[53]</sup>.

While the above mentioned techniques reveal basic and to some extent indirect structural insights, high resolution structure information is needed to understand the conversion process of PrP. High resolution 3D-structural data from X-ray fiber diffraction and NMR are available for PrP<sup>C</sup> but not for the complete PrP<sup>SC</sup>

molecule. Because of the insolubility and the propensity to aggregate only parts of PrP<sup>SC</sup> are structurally resolved until now<sup>[54-57]</sup>.

## CONCLUSION

Misfolding of a cellular protein into an infectious isoform sharing the same primary structure is unique for prions. This phenomenon is currently discussed to be a general principle for other neurodegenerative diseases<sup>[58-60]</sup>. Prions lack a coding nucleic acid thus diagnosis and the detection of misfolded isoforms cannot be done by genetics but *e.g.*, by structural characterization.

A lot of structural models for PrP<sup>SC</sup> exist but as discussed recently by Requena and Wille "the results and/or their interpretation remain controversial"<sup>[61]</sup>. For a better understanding of the conversion of PrP<sup>C</sup> to PrP<sup>SC</sup>, more structural data from biochemical and biophysical experiments are required for PrP<sup>SC</sup> (Figure 1). Therapeutic strategies that aim at the prevention of this misfolding process would benefit from such a progress.

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