5 Abstract

The transmissible spongiform encephalopathies (TSEs) exhibit strain variations similar to genuine microorganisms. Strain diversity in TSEs has been suggested to be encoded by differences in the structure of the misfolded prion protein isoform (PrP^{Sc}). PrP^{Sc} is derived from the cellular prion protein (PrP^{C}) by posttranslational modifications and it is assumed to be the only component of the infectious unit called prion. The development of a reliable method for discrimination of different TSE strains causing scrapie, BSE, or Creutzfeldt-Jakob disease is a big challenge and remains an open issue. In the presented work, this problem was addressed using the Fourier transform-infrared (FT-IR) spectroscopy of PrP27-30, the proteinase K resistant core of PrP^{Sc}.

Various FT-IR techniques were used to explore the secondary structure, the structural stability, and hydrogen-deuterium exchange characteristics of PrP27-30 of four different TSE isolates (263K, ME7-H, 22A-H, and BSE-H) adapted to Syrian hamsters. Each of these variants showed characteristic incubation times and disease-specific symptoms. The strain differentiation capacity of the FT-IR approach was objectively proven for the first time by multivariate cluster analysis. The secondary structure of PrP27-30 was compared using samples suspended in H_2O or D_2O and samples dried for FT-IR microscopic measurements. Temperature induced secondary structure changes and H/D-exchange characteristics in PrP27-30 samples from 263K, ME7-H, and 22A-H were investigated in D_2O. Urea induced changes in PrP27-30 samples from strain 263K was investigated in the presence of 6M urea.

The results demonstrated that the second derivative FT-IR spectra obtained from dried protein films or samples hydrated in H_2O or D_2O consistently showed strain-specific infrared characteristics in the secondary structure sensitive amide I region. The secondary structure analysis of these spectra reveal strain dependent conformational diversities, assigned mainly to differences in the β-sheet structure but also to other structural components present in PrP^{Sc} such as turns and α-helices. These strain-specific hallmarks were complimented by strain-dependent spectral traits in the amide II and amide A absorption regions, and the different H/D-exchange behaviour of the various PrP27-30 samples. These variations were due to diversity in functional groups of the peptide backbone that are exposed to the solvent and/or to different structural flexibilities of sub-structures within the PrP27-30 aggregate. Such strain-specific spectra could serve as “fingerprints” for the determination and classification of the TSE-isolates. The multivariate cluster analysis suggested that the strain-specific structural

variations could be best investigated in D_2O. This approach was applied as well to differentiate between cases of sporadic CJD and its strains from a set of human brain samples including different forms of sporadic CJD and controls. This study showed that the discrimination of the CJD cases and the distinction between the sporadic CJD strains is hindered due to the heterogeneous structural composition of the purified samples. The latter was most probably due to the presence of protein contaminants, co-propagation of PrP^{Sc} with a different structure, or possibly both. However, before a final evaluation estimate of the potential of the FT-IR technique to discriminate human TSEs can be given, an analysis on PrP27-30 virtually free from contaminant proteins is required.

FT-IR spectra of PrP27-30 samples from 263K, ME7-H and 22A-H heated to 90°C showed that the specific resistance to heat treatment is associated with the thermal stability of different secondary structure components providing additional means for TSEs strain discrimination. Similar characteristics were observed in the time course of the urea-induced unfolding/denaturation of PrP27-30 aggregates from 263K. This strongly suggests that the spectral changes induced by the chaotropic agent should be sufficient to discriminate between TSEs strains. The temperature experiments suggested a possible protective role of the specific non-protein constituent of the prions. This was associated with the existence of tightly packed parts of the aggregates related with the structural stability of the prion rods exposed to 90°C and characterised by complete resistance to H/D exchange. The spectroscopic information extracted from temperature gradient and urea-induced unfolding/denaturation experiments suggested non-cooperative unfolding of the PrP27-30 aggregates. Prolonged treatment of PrP27-30 with 6 M urea caused the unfolding of most of the prion structural components. However, spectral features characteristic of aggregated protein indicated the presence of some PrP27-30 resistant to urea denaturation.

In conclusion, the FT-IR technique can be used for a reliable discrimination of the four TSE agents adapted to Syrian hamster, based on the secondary structure characteristics of purified strain specific PrP27-30 samples. Temperature gradient experiments and/or urea induced unfolding of PrP27-30, can also reveal differences between the TSE strains. Thus the presented results strongly suggest that FT-IR spectroscopy has a significant diagnostic potential for TSE strain differentiation.