6 Abstract

According to the protein-only hypothesis, the prion protein represents the sole infectious agent of so-called prion diseases, which are a group of transmissible neurodegenerative diseases in both humans and animals. The prion protein gains its disease-causing function by conversion of its apathogenic cellular form PrP^{C} into the pathogenic scrapie form PrP^{Sc}. This conversion causes a change of the predominantly α-helical secondary structure into a structure dominated by β-sheets ($\alpha \rightarrow \beta$ refolding) and thereby leads to an aggregation of the protein.

Within the scope of the work at hand, this $\alpha \rightarrow \beta$ refolding and aggregation were studied using several independent methods under a variety of conditions with recombinant Syrian hamster prion protein ($SHaPrP^{90–232}$) as a model. Methodical emphasize was placed on time-resolved Fourier transform infrared spectroscopy (FTIR spectroscopy).

Under appropriate conditions, the monomeric α-helical isoform of $SHaPrP^{90–232}$ was transformed into a β-sheet rich oligomeric isoform. The loss of α-helical parts of the secondary structure during $\alpha \rightarrow \beta$ refolding was represented by IR difference bands at $1\,653\,cm^{-1}$ (amide I region) and $1\,551\,cm^{-1}$ (amide II region), whereas the formation of β-sheets was indicated by difference bands at 1 691 and $1\,621\,cm^{-1}$ (amide I region) and at $1\,529\,cm^{-1}$ (amide II region). The position of the amide I β-sheet difference band is characteristic for intermolecular and antiparallel β-sheet structures with strong hydrogen bonds. The loss of α-helical secondary structure and the formation of β-sheets occurred concomitantly; an unfolded intermediate state has not been observed. Furthermore, it could be shown that no β-sheet was formed within the experimental dead time of 250 ms.

The aggregation process of $SHaPrP^{90–232}$, which paralleled the $\alpha \rightarrow \beta$ refolding, was investigated by time-resolved light scattering. Thereby, it was observed that monomeric protein aggregated to protofibrils via a transient stable oligomer: the so-called critical oligomer. Both the time required for formation of the critical

oligomer and its size were dependent on protein concentration. The minimal size of the critical oligomer was assigned to 8 monomer units.

By electron microscopy and atomic force microscopy the critical oligomer was morphologically characterised. Both compact and annular shaped oligomers were identified. Their diameter was 10–15 nm with their height being only a quarter of their width.

Pure critical oligomers of SHaPrP$^{90–232}$ were produced and stabilised by appropriate buffer conditions; their stability was checked by MALDI-TOF mass spectrometry.

Interactions between SHaPrP$^{90–232}$ in its monomeric and in its oligomeric form with model membranes were comparatively investigated by fluorescence resonance energy transfer spectroscopy and by experiments with reconstituted planar membranes. Significant interactions of both isoforms with membranes constituted of negatively charged phosphatidylserine could be observed. No interactions, however, were detected with membranes constituted of neutral phophatidylcholine. Compared to the monomer, the critical oligomer showed—depending on pH—equally strong or weaker interactions with the examined membranes. Neither the critical oligomer nor the monomeric SHaPrP$^{90–232}$ were able to form pores within the examined membranes. However, negatively charged membranes were significantly destabilised by both isoforms of the protein.

To distinguish between formation of critical oligomers and creation of temperature induced aggregates of SHaPrP$^{90–232}$, thermal denaturation of the protein was investigated by FTIR spectroscopy. The β-sheet structures formed by incubation above the denaturation temperature evoked absorption bands that could be clearly distinguished from those of the critical oligomers: Although also the β-sheet specific amide I difference bands at 1 690 and at 1 624 cm^{-1}, observed during thermal denaturation, were indicative for antiparallel intermolecular β-sheet structure, the hydrogen bonds of this structure were clearly weaker than those of the β-sheets of critical oligomers.

To gain high resolution information on the structure of critical oligomers, ^{13}C-^{15}N-SHaPrP$^{90–232}$ completely labelled with the carbon isotope ^{13}C and the nitrogen isotope ^{15}N was produced, transformed into critical oligomers and used for initial experiments by NMR spectroscopy. After primary evaluation of the data, mainly signals from the flexible N-terminal region of the protein could be observed in the NMR spectra of critical oligomers. Contrary, almost no signals from amino acids

lying in structured parts of the protein could be observed. After further evaluation of the data, more sequence specific structural information should be available, on the basis of which it should soon be possible to develop—in combination with the FTIR data of this work—a structural model of the critical oligomers of the prion protein.