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Prion protein structure and pathology of transmissible spongiform encephalopathies (TSE)

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Introduction

Transmissible spongiform encephalopathies (TSE) are neurodegenerative diseases for which it has been proposed that they are related to a novel infectious agent, the prion [1, 2]. TSEs have been reported to occur as infectious, inherited, and spontaneous diseases. Following the 'protein-only hypothesis' [2, 3] the causative agent is a pathogenic conformation of the prion protein (PrP). PrP is ubiquitous in mammalian cells in a benign, cellular conformation (PrP^{C}). In rare cases it may be transformed into the infectious scrapie conformation (PrP^{Sc}), which has been observed in the form of insoluble, protease-resistant aggregates in the brain of affected individuals [4, 5]. The most widely discussed TSEs are Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE). Other human prion diseases include kuru, the Gerstmann–Sträussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI). The 'mad cow crisis' in Europe has greatly added to public concern about TSEs and raised keen interest in the biological foundations of TSE pathology and TSE transmission. Thus, questions relating to the 'species barrier' [4, 5], *i.e.*, the relative ease of infection between individuals of different species relative to that between individuals of the same species, have attracted intensive interest, in particular regarding possible transmission of BSE from cows to humans through the food chain [6, 7]. Another focus are the point-mutations in human PrP that have been linked with inherited prion diseases, *i.e.*, GSS, FFI and certain forms of CJD [1]. To provide a foundation for discussions of these phenomena on the molecular level, we undertook to solve the three-dimensional structure of the cellular form of the prion protein in solution, using nuclear magnetic resonance (NMR) spectroscopy.

Results

For the NMR structure determination we started out using the standard protocol [8], with modern techniques that rely on isotope labeling of the protein with ^{15}N and ^{13}C [9]. The general strategy followed is illustrated with

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Figure 1: The decisive breakthrough toward a structure determination came about when it was realized that the fragment of residues 121–231 of the mouse prion protein, mPrP(121–231), forms a self-folding domain with sufficient long-time stability to enable data collection for a NMR structure determination [10, 11]. Subsequently, once we succeeded in expressing the full-length polypeptide chain, mPrP(23–231) [12], it turned out that knowledge of the resonance assignments and the structure of mPrP(121–231) greatly aided in the data analysis of the intact protein, since much of the work could be done by difference spectroscopy [13].

The NMR structure of mPrP(121–231) (Figure 2) contains three α-helices and a two-stranded antiparallel β-sheet. The helices extend from residues 144 to 154, 175 to 193, and 200 to 219, and the β-strands contain the residues 128 to 131 and 161 to 164. The first turn of the second helix and the last turn of the third helix are linked by the single disulphide bond in the protein. The

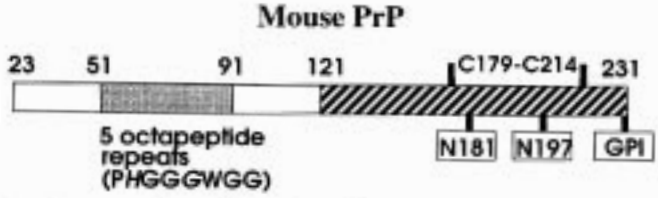

Figure 1. Schematic presentation of the amino acid sequence of mammalian prion proteins, with residues 23–231. The *C*-terminal fragment of residues 121–231 is shaded, and the posttranslational modifications are indicated. In the *N*-terminal part of the molecule the octapeptide repeats are indicated, where the residues written in italics are variable.

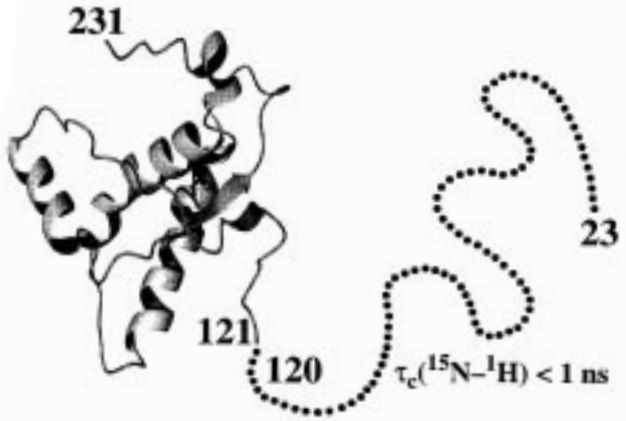

Figure 2. Structure of mPrP(23–231). A *C*-terminal domain of residues 126–231 forms a globular structure with three α-helices and a short β-sheet, which was also observed in mPrP(121–231). The backbone $^{15}N–^1H$ moieties of the residues 126–230 manifest rotational correlation times of several ns, which is typical for a folded protein of this size. The residues 121–125 and the *N*-terminal segment 23–120 (represented by black dots) show features of a flexible, 'random coil-like' polypeptide, with rotational correlation times for the $^{15}N–^1H$ groups of $\tau_c < 1$ ns (adapted from ref. [13]).

twisted V-shaped arrangement of the helices 2 and 3 forms the scaffold onto which the β-sheet and the first helix are anchored. The regular secondary structure elements and the connecting loops are well defined, with the sole exceptions of residues 167 to 171 and the chain-terminal segments 121–125 and 220–231. The polypeptide fold is stabilized by hydrophobic interactions in a core that contains primarily side chains of the second and third helices and the β-sheet, one side chain of the first, mostly hydrophilic helix, and the side chains of the residues 134, 137, 139, 141, 158 and 198, which are located in loop regions. With the exceptions of Ile 139, Ile 184 and Val 203, the residues of the hydrophobic core are invariant in the known mammalian prion protein sequences [14]. Hydrophobic surface patches in mPrP(121–231) are located near the β-sheet and the loop preceding the first helix. The surface of mPrP(121–231) is otherwise characterized by a markedly uneven distribution of positively and negatively charged residues [10]. Overall, although it has a polypeptide fold that is not so far represented by the protein structures in the Brookhaven Protein Data Bank [15], the polypeptide mPrP(121–231) displays typical features of a globular protein (see also [11]).

The NMR structure determination of mPrP(23–231) [13] showed that the *C*-terminal domain of residues 126–231 has the same fold as in mPrP(121–231) and that the *N*-terminal polypeptide segment 23–120 is flexibly disordered. From a technical point of view it should be emphasized that the information on the segment 23–120 of mPrP shown in Figure 2 could not have been obtained with any other technique but NMR: for each residue except the prolines the time scale for intramolecular mobility of the polypeptide backbone could be determined to be shorter than 1 nanosecond (10^{-9} s). In addition, for the indole rings of all tryptophan residues in the segment 23–120 similar mobility could be demonstrated, which is of particular interest for understanding possible roles of the octapeptide repeats (see Figure 1). Overall, the NMR approach shows that the structure of the *C*-terminal domain 126–231 is the same as in mPrP(121–231), that the *N*-terminal domain 23–120 forms an extended, highly flexible structure, and that the linker peptide 121–125 is also in an extended conformation, with mobility that is intermediate between that of the tail and that of the globular domain [13] (Figure 2).

The results obtained for mPrP(23–231) have recently been confirmed by studies of two fragments of the prion protein from Syrian hamster, shPrP(90–231) [16] and shPrP(29–231) [17], which show coinciding NMR structures except for local differences in the least well defined molecular region at the end of helix 3.

Discussion

Prior to the structure determination of mPrP(121–231) [10] discussions on structure-function correlations of the prion protein were based on structure

predictions [18, 19] that are, at least in the case of PrP^C, different from the real structure. The NMR structure for PrP^C now presents, for the first time, a rational basis for such discussions. By analogy with other areas of biomedical research it can be expected that structure-based rational thinking will support future development of diagnosis and possibly treatment of TSEs.

Space only permits to indicate some lines of thought based on the NMR structure of mPrP, which are elaborated in detail elsewhere. For example, the presence of an extensive polypeptide segment with the properties of a flexible extended coil in PrP^C could enable structural transitions to PrP^{Sc} aggregates that might display sizeable β-sheet content [20] without major conformational rearrangements in the *C*-terminal domain 126–231. In particular, the combination of the result presented in Figure 2 with earlier findings that the entire polypeptide segment 90–231 is protected against protease K digestion in PrP^{Sc} [2] emphasizes that there is a major change in the structural arrangement of the residues 90–125 between PrP^C and PrP^{Sc}. Furthermore, the NMR structure determination has already resulted in identification of the location in the three-dimensional structure of a likely contact site for a hypothetical host protein, 'protein X' [21, 22], which may be required for the formation of PrP^{Sc} and might play an important role for the species barrier, and of the epitopes for a PrP^{Sc}-specific monoclonal antibody [23]. Finally, the availability of the three-dimensional structure enables detailed studies of possible effects of the amino acid replacements in human PrP that segregate with inherited human prion diseases [24].

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