SYSTEMATIC REVIEW

Intrinsically disordered tau protein in Alzheimer's tangles: a coincidence or a rule?

Skrabana R[1,3], Skrabanova M[1,3], Csokova N[1,3], Sevcik J[2], Novak M[1,3]

Institute of Neuroimmunology AD Centre, Slovak Academy of Sciences, Bratislava, Slovakia.
Michal.Novak@savba.sk

Abstract

Tau protein, the major constituent of neurofibrillary tangles in Alzheimer's disease (AD) and related tauopathies, is classified as intrinsically disordered protein (IDP). IDPs in contrast to globular proteins contain high proportion of polar and charged amino acids in their sequence, which results in the absence of a well-defined three-dimensional structure of the free protein. Structural flexibility of IDPs is required to perform their important role in many cellular processes. In the course of tauopathies, highly soluble disordered tau protein acquires rigid fold and forms highly insoluble filaments. Beneficial intrinsic disorder transforms into a fatal order: is it a coincidence, or is there an underlying reason for preferential IDPs assembly? In this review we present the structural characteristics of tau protein filamentous lesions in AD and discuss the tendency of IDPs to assembly and to form amyloid deposits *(Ref. 65)*.
Key words: Alzheimer's disease, intrinsically disordered proteins, tau protein, paired helical filaments, amyloidogenicity.

At the beginning of the 20th century, Alois Alzheimer described a pathological process visualised by silver staining of neurofibrils in brain tissue sections from a demented patient aged 52 years (Alzheimer, 1907). In 1963 and 1964 Kidd published electron microscopy images of the major structural constituents of these neurofibrils: paired helical filaments (PHF; Kidd, 1963; Kidd, 1964). It took another 25 years of research to uncover molecular nature of PHF.

Paired helical filaments composition, morphology and structure

Early immunological studies showed that tau protein was associated with PHF (Grundke-Iqbal et al, 1986a; Grundke-Iqbal et al, 1986b; Kosik et al, 1986; Delacourte and Defossez, 1986; Wood et al, 1986), but only with generation of a monoclonal antibody (mAb) MN423, raised against highly purified protease-resistant PHF core (Novak et al, 1989) it was possible to identify unequivocally the tau protein as a constitutive element of neurofibrillary pathology (Wischik et al, 1988 a; Wischik et al, 1988 b). Consequently, the gene coding for human tau protein was cloned and sequenced (Goedert et al, 1988). It was shown that in the central nervous system tau protein has six isoforms resulting from alternative splicing of mRNA transcribed from a single gene (Goedert et al, 1989). The tau isoforms differ each from the other by the presence or absence of one or two short inserts in the aminoterminal part and have either 3 or 4 microtubule-binding repeat motifs in the carboxyterminal part. Further work with mAb MN423 revealed that protease-resistant PHF core, composed of truncated tau protein, is 93–95 residues long (Novak et al, 1993). It consists of six N-terminally distinct peptides from which is 70 % derived from a three-repeat tau isoform (Jakes et al, 1991). Initial observations suggested a conformational epitope of MN423 (Novak et al, 1991; Harrington et al, 1991).

Ultrastructural description of PHF morphology by electron microscopy supplemented their biochemical characterisation. Three-dimensional image reconstruction of electron micrographs of highly purified PHF suggested their structural organisation into double helical stack of transversely oriented subunits, each

[1]Institute of Neuroimmunology AD Centre, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia, and [3]Centre of Excellence for Alzheimer's Disease and Related Disorders, Slovakia

Address of correspondence: M. Novak, Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.
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of which with three domains (Crowther and Wischik, 1985; Wischik et al, 1985). Remarkably, later high-resolution transmission electron microscopy studies using platinum-carbon replicas of Pronase-treated and untreated PHF did not support a double-helical filament arrangement but proposed a single-helical morphology (Ruben et al, 1995; Ruben et al, 2005). Morphological comparison based on computer maps obtained from electron micrographs of PHF and those of straight filaments, a minor class of fibrillar deposits found in AD pathology, revealed that both filament types shared similar morphological units, though differing in their relative arrangement in the two types of filaments (Crowther, 1991).

On the level of molecular structure, a dense PHF core is composed of the tau microtubule-binding region in the cross-beta arrangement (Novak et al, 1993; Berriman et al, 2003), whereas more relaxed folding of an external protease-sensitive fuzzy region could contribute to the alpha helix structure occasionally observed in PHF preparations (Goux, 2002; Sadqi et al, 2002; Barghorn et al, 2004). The mass per unit length of the PHF core were determined as 65 kDa/nm (Wischik et al, 1988a) with approximately 80 nm^2 cross-sectional area assuming 0.00122 nm^3/Da protein-packing density (Crowther, 1991). Considering the 0.47 nm cross-beta structure spacing, there would be three 95-amino-acid core PHF peptides per one layer of PHF cross-beta structure (Berriman et al, 2003).

Substantial insolubility of PHF precludes any direct determination of their atomic structure by nuclear magnetic resonance or X-ray crystallography. Using spin-labelled tau protein and electron paramagnetic resonance spectroscopy, evidence was provided about beta-helical structure in PHF prepared *in vitro* from full-length tau (Margittai and Langen, 2004). However, observed spin exchange between paramagnetic labels inserted into the PHF core fits to other models of PHF structure as well. Other group used electron microscopy together with limited proteolysis experiments in study of the core PHF assembly and collected important constraints for the core PHF conformation (von Bergen et al, 2006). As an alternative approach we recently proposed conformation-dependent anti-tau monoclonal antibodies as a specific tool for disentangling the conformation of PHF (Skrabana et al, 2006). Biochemical characterization of mAb MN423 recognizing conformational epitope on tau protein assembled into core PHF led to important conclusions about PHF core conformation: C-terminus of protease-resistant PHF core subunit formed by pentapeptide 387DHGAE391 is exposed to the solution and protrudes from the compact core assembly (Khuebachova et al, 2002) and the spatial proximity of the three segments 306VQIVYK311, 321KCGSL325 and 387DHGAE391 determines the intramolecular folding of tau protein in the PHF core (Skrabana et al, 2004). For survey about *in vitro* studies of PHF formation and about determination of PHF structure see paper Skrabana et al. (2006).

Disordered character of tau protein

Not all members of the proteome of living organisms exhibit a three-dimensional molecular structure. The number of proteins and protein domains that have been shown to adopt disordered conformation under physiological conditions is rapidly increasing (Uversky et al, 2005). These so-called natively unfolded or intrinsically disordered proteins have little or no ordered structure in their molecules (Uversky, 2002 a; Tompa, 2003 b; Uversky et al, 2005). Over the last two decades there has been a continuous increase in the number of studies explicitly dealing with IDPs, starting from approximately one PubMed hit per year in 1989–1999 and ending with more than 100 hits in 2005. The list of experimentally verified IDPs includes more than 300 proteins; its non-redundant subset with maximum of 40% sequence similarity consists of 183 non-homologous sequences (Linding et al, 2004). This collection comprises full-length proteins; there are other disordered domains with chain length of more than 30 amino acid residues in proteins with prevalent globular folded scaffold (Uversky, 2002 b). It was estimated that over 50 % of proteins in eukaryotes may carry unstructured regions of more than 40 residues in length (Vucetic et al, 2003). Protein disorder, thus, is a general phenomenon. IDPs perform essential functions (Wright and Dyson, 1999; Dunker et al, 2001; Tompa, 2003 a; Dyson and Wright, 2005; Uversky et al, 2005), which are most of the time intimately linked with their disordered structural state. In terms of their modes of action, they can be classified into several broad functional classes, serving as entropic chains, display sites, chaperons, effectors, assemblers or scavengers (Gunasekaran et al, 2003; Tompa, 2005).

Intrinsic disorder is encoded by the specific amino acid composition of IDPs, which include considerable high content of charged and polar amino acids, low overall hydrophobicity and presence of repeat regions. All these features are present in the tau protein. DisEMBL IDPs predictor (Linding et al, 2003) assigns nearly 90 % of tau protein sequence to the disordered state. Accordingly, recent survey using different secondary structure predictors showed that only about 12 % of tau sequence has propensity to form regular secondary structure (Gamblin, 2005). Experimental data from UVCD measurements consistently revealed very low content of regular secondary structure in tau (Cleveland et al, 1977; Ruben et al, 1991; Schweers et al, 1994; Barghorn et al, 2004). Summarising, tau protein fulfils all the conditions to be counted among the IDPs (Uversky, 2002 a; Tompa, 2003 b; Uversky et al, 2005).

IDPs and amyloidoses: implication for tau protein

Nowadays we know that more than twenty diseases are characterised by deposition of misfolded proteins. These so called amyloidoses afflict either the central nervous system or a variety of peripheral tissues (Stefani and Dobson, 2003). Amyloid diseases imply pathologic formation of insoluble fibrillar protein aggregates packed into cross-beta structure (Tycko, 2004). Since the majority of available experimental data witnessed prevalent cross-beta structure for the tau protein assembled into PHF, diverse tauopathies including AD could be considered as a form of brain amyloidosis (Goedert and Jakes, 2005). It was found that certain combinations of primarily hydrophobic amino acids in

the protein sequence could form "hot spots" of amyloid formation in polypeptide chain (Ventura et al, 2004; Sanchez de Groot et al, 2005; Rousseau et al, 2006). Experimental data served to develop high accuracy predictors of such motifs from the protein primary amino acid sequence (Fernandez-Escamilla et al, 2004; Pawar et al, 2005). Tau protein and other IDPs adopt an open conformation, which would imply high accessibility of potential "hot spots" of fibrillization. Moreover, open and extended conformation itself could be prone to form beta sheets in the terms of easy dehydration and forming intramolecular main-chain hydrogen bonds. Is there an elevated propensity to amyloid formation for IDPs? Two striking complementary evidences emerged from the latest studies: (1) protein fibrillogenesis indeed requires partly unfolded conformation, yet not the fully extended, prevalent in IDPs (Uversky and Fink, 2004); (2) fibrillization-prone "hot spots" of primary amino acid structure are much less frequent in IDPs (which account for their good solubility) than in globular proteins (Linding et al, 2004). It seems that IDPs are evolutionary protected from amyloid formation, to which they would be otherwise well predisposed due to their extended conformation. On the other hand, beta-structure promoting hot spots contained in the globular proteins are equally well protected against aggregation by burial in the hydrophobic core. Amyloidogenesis of globular proteins requires destabilization of their native structure and introduction of certain degree of disorder (Uversky and Fink, 2004).

In *in vitro* studies of PHF formation, two short polypeptide sequences in tau protein molecule, 275VQIINK280 and 306VQIVYK311, were experimentally proven to represent tau-tau interaction motifs (von Bergen et al, 2000). Additional three "hot spots" of tau assembly were computer-predicted in the microtubule-binding region of tau (Pawar et al, 2005). Notwithstanding this, there is no elevated tendency to assembly for tau protein, neither when it performs its physiological functions in the cell nor when it is prepared by heterologous expression *in vitro*. As discussed above, intrinsic disorder of tau molecule does not necessary imply its amyloidogenicity (Linding et al, 2004). More probably there have to be present some external factors, which promote pathologic folding of tau by exposing its fibrillizating "hot spots". Among the various factors, there are three dominating: phosphorylation (Cruz and Tsai, 2004; Drewes, 2004; Gong et al, 2005), template assisted misfolding and truncation (Novak, 1994; Binder et al, 2005). It is truncation, which could cause severe destabilisation of residual secondary structure elements found in tau (Jeganathan et al, 2006), exposing previously partially buried regions with high beta-forming potential.

Concluding remarks

Notwithstanding the evolutionary protection of IDPs against assembly, there are many examples of amyloid lesions composed of these proteins in various neurodegenerative diseases (tau protein in AD and frontotemporal demetia, synuclein in Parkinson's disease, prion protein in spongiform encephalopathies, huntingtin in Huntington chorea (Ross and Poirier, 2004; Skrabana et al, 2006)). Under physiological condition of a healthy organism, there is no way for IDPs to form pathologic deposits from a disordered state. Malignant IDPs transformation needs an intervention of a "rule of coincidence" of pathology triggering factors, like truncation and phosphorylation of tau protein. These factors make the previously harmless IDP vulnerable to fibrillization and disqualify it from its physiological function with fatal consequences for the whole organism.

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