Structure solution of misfolded conformations adopted by intrinsically disordered Alzheimer's tau protein

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ABSTRACT

Until now it was impossible to obtain atomic structure of intrinsically disordered protein (IDP) tau and/or its assembly in Alzheimer's paired helical filaments as neither of them could have been prepared in the form amenable to X-ray or NMR techniques. Using IDP tau property to attain regular tertiary structure after binding events during self-assembly or when complexed with its target we propose monoclonal antibodies as surrogate tau protein binding partners to form complexes and crystals for structure solution by X-ray technique.

Keywords: Alzheimer's disease; tau protein; intrinsically disordered protein; monoclonal antibody; crystal structure

List of abbreviations: IDP, intrinsically disordered protein; AD, Alzheimer's disease; PHF, paired helical filaments; MD, molecular dynamics; PBS, phosphate buffered saline; RMSD, root mean square deviation; mAb, monoclonal antibody

INTRODUCTION

It is well known that besides proteins that have clearly defined tertiary structure there exist a number of proteins, so called intrinsically disordered proteins (IDPs), which form regular tertiary structure only after self-assembling or binding to their targets [1]. Inherent physical properties of intrinsically disordered proteins have precluded so far in majority of cases their detailed structural analysis by X-ray crystallography or nuclear magnetic resonance. Since some IDPs play important role in neurodegenerative diseases, e.g. α-synuclein in Parkinson's disease, prion protein in transmissible spongiform encephalopathies and tau protein in Alzheimer's disease (AD) and related tauopathies [2], their structure determination is of the utmost importance. Neuronal damage in AD is accompanied by self-assembling of tau protein molecules into paired helical filaments (PHF), which consist mainly of six full-length isoforms of tau expressed in adult brain [3]. It was shown that the truncated form of tau may start the pathological cascade leading to neurodegeneration [4,5]. Removal of the PHF external fuzzy coat by pronase digestion leaves a PHF core composed of assembled microtubule binding repeats of tau protein [6].

The PHF core has defined tertiary structure, which can be mapped by monoclonal antibodies raised against the PHF core and determined by diffraction methods as has been shown in our previous work [7]. The principle of employed procedure exploits property of soluble tau, an intrinsically disordered protein, to adopt defined structure while interacting with its target. However, it is difficult to identify its natural binding partners as the *in vivo* specific interactions of IDPs are rather weak [8]. From this point of view

monoclonal antibodies raised against recombinant tau protein may serve as useful surrogate targets of tau facilitating an alternative way for its structure determination.

We have prepared monoclonal antibody MN423 recognizing core PHF tau and a panel of mAbs with high affinity to various segments of recombinant tau protein. High affinity facilitates formation of antibody-tau segment complexes, which are amenable to crystallization and structure determination by diffraction methods. We have shown that MN423 allows to determine the structure of a part of PHF core. Applying the procedure suggested in the present paper to a number of tau segments will facilitate structure determination of a large part of tau. The knowledge of structure will reveal novel information about the tau protein conformation and possibly opens the way for rational drug design targeting misfolded tau protein associated with neurodegeneration.

MATERIALS AND METHODS

Structure determination.

Preparation of crystals of the complex of MN423 with tau protein and data collection was described in [9]. Structure determination, refinement and analysis of results of the structure of the complex MN423 with C-terminal PHF core pentapeptide is in [7]. The PDB ID of the structure is 2v17.

Molecular dynamics simulation.

All molecular dynamics (MD) simulations of the PHF core tau peptide TDHGAE with acetylated N-terminus were carried out using explicit solvent in a 25x25x25 Å water box at 21 ºC for 1 ns in MOE 2007.0902 program (CCG Montreal, Canada). Calculations started with the energy-minimized peptide structure from the PDB entry 2v17 using forcefield CHARMM22 with non-bonded cutoff of 8 Å and reaction field solvation model. The simulation was carried out keeping the pressure constant; the energy of the system was calculated by NPA equation of motion with a time step of 0.5 fs. The lengths of peptide light bonds were constrained, whereas all other peptide and solvent bonds were left without constraints. Structure and energy outputs were taken from the simulation each 0.5 ps. After an initial 150 ps equilibration period the system was stabilized and energy minimization and analysis were done for structures output every 10 ps for subsequent 700 ps. The ensemble of structures was visualized and distribution of conformations was assessed by root mean square deviation (RMSD) of Cα atoms.

Enzyme-linked immunosorbent assay (ELISA).

Preparation of a panel of mAbs recognizing epitopes on tau297-391 will be described elsewhere. Deletion mutants of tau protein were expressed in *E. coli* and purified as described previously [10]. Immunoassay plates coated with 10 µg/ml of tau diluted in phosphate-buffered saline pH 7.4 (PBS) were incubated overnight at 37 °C. The plates were washed with PBS containing 0.05 % Tween 20 (PBS-Tween) and then blocked by PBS solution of 1 % non-fat dry milk for 1 hr at room temperature. Subsequently, after washing with PBS-Tween, the hybridoma culture supernatants were added, incubated for 1 hr at 37 °C and washed with PBS-Tween. Fifty microliters of peroxidase-conjugated secondary antibody (goat anti-mouse Ig, DakoCytomation, Glostrup, Denmark) diluted 1:2000 in PBS was applied to the plate for 1 hr at 37 °C. Immune complexes were visualized by o-phenylenediamine solution and quantified by absorbance measurement at 492 nm.

Affinity estimation.

BIACORE3000 instrument with CM5 sensorchip (Biacore AB, Uppsala) was used. All experiments were performed at 25 °C in PBS with 0.005 % of P20 as the running buffer. Typically, 5 000 RU (response units) of polyclonal anti-mouse antibody (No. Z 0420; DakoCytomation, Glostrup, Denmark) was coupled to the sensorchip at pH 5.0 via primary amines simultaneously in two flow cells, one of which was used as a reference in measurement. In each analysis cycle, diluted hybridoma supernatants were captured in the analytical flow cell to reach immobilization level of 200-400 RU. Duplicates of 40 nM solution of tau297-391 were injected at a flow rate of 50 ml/min over the sensorchip, 10 mM glycine-HCl buffer pH 1.5 was used for sensorchip regeneration. Kinetic binding data were double referenced [11] and fitted by BIA evaluation software

4.1 (Biacore AB) to two state reaction model. Kinetic rate constants were approximated globally, maximal responses were fitted locally and bulk response was set to zero.

RESULTS AND DISCUSSION

We have determined the structure of the complex of monoclonal antibody MN423 with the C-terminal hexapeptide of the core PHF tau at resolution 1.65 Å [7]. The complex MN423 - tau peptide was formed by mixing equivalent amounts of both partners including necessary additives and submitted to crystallization [9]. The structure of the complex was solved by molecular replacement and refined to a crystallographic R factor of 16 %. Clear electron density facilitated localization of the six C-terminal tau protein amino acids TDHGAE in the antibody recognition site formed by hypervariable loops of L and H chain.

MN423 recognizes the PHF core unit which has defined 3-D structure [12,13]. The unit is built by self-assembling of tau protein molecules in the process of Alzheimer's disease progression. Using hybridoma technology it is possible to prepare a number of mAbs directed against different parts of PHF core. In the process of maturation of monoclonal antibody raised against PHF core its paratope acquires a conformation which faithfully reflects the structure of tau protein in the PHF core. We hypothesize that the binding site of MN423 serves as a mould for recombinant tau core PHF segment and forces it to adopt the 3D structure of genuine, *in vivo* core PHF tau. This procedure is illustrated in Figure **1**.

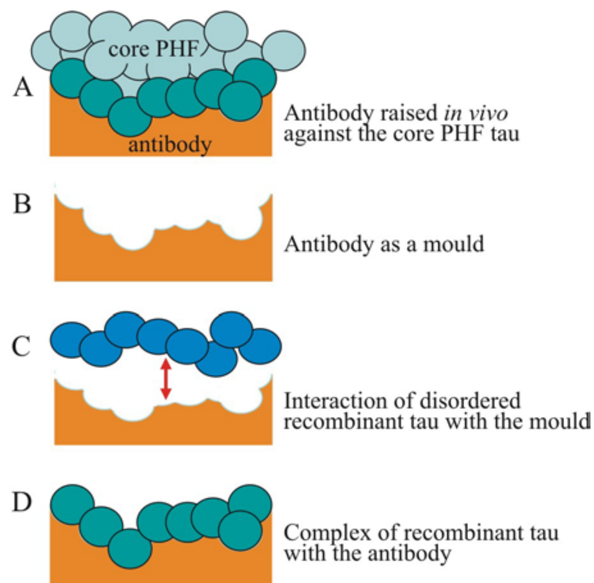

Figure 1. Formation of the *in vivo* tau protein fold on recombinant protein by monoclonal antibodies. (A) Monoclonal antibody specific for the core PHF tau segment (in dark color) raised *in vivo*. (B) Binding site of the antibody, which is the imprint of the core PHF tau structure, serves as a mould for structure formation of the disordered recombinant tau *in vitro*. (C) Interaction of antibody with disordered recombinant tau protein. (D) Formation of the tau protein-antibody complex, during which disordered recombinant tau protein attains the same fold as that of the *in vivo* tau PHF core segment against which the antibody was generated.

Interaction of the mould (MN423) with the core structure (PHF core tau peptide) as determined by X-ray analysis is shown in Figure **2**. As expected, MD simulation has shown that the peptide TDHGAE in solution adopts a large number of conformations (RMSD of Cα atoms in the range 2.1 to 5.8 Å) illustrating its disordered character (Fig.

2A). In the process of complex formation and binding to the mould the peptide obtains a unique conformation identical to that in the PHF core (Fig. **2B**, [7]).

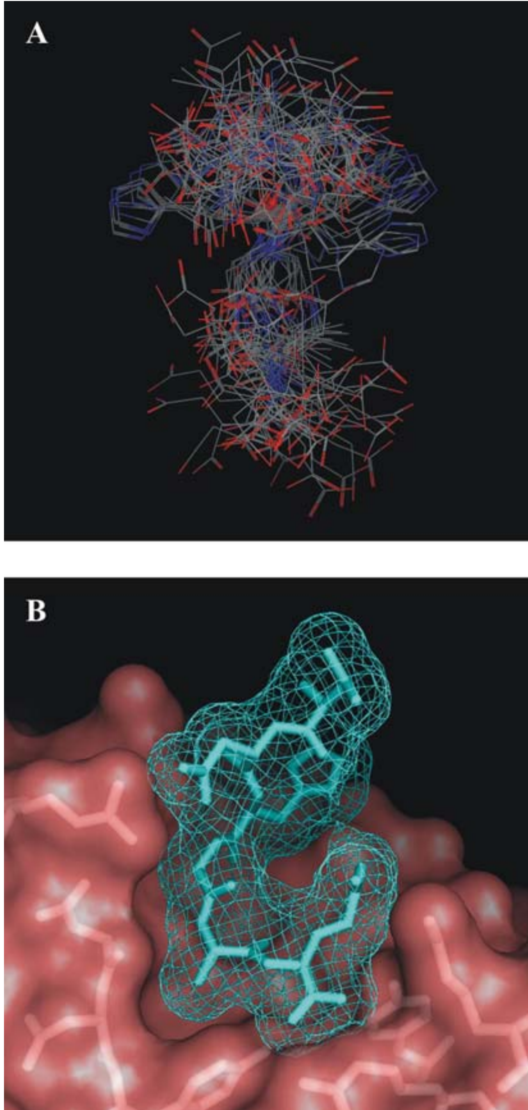

Figure 2. Interaction of monoclonal antibody MN423 with the PHF core tau peptide. (A) Conformations of the PHF core tau peptide ^{386}TDHGAE^{391} obtained by MD simulations (20 ps steps). (B) Molecular surfaces of the recombinant core PHF tau segment ^{386}TDHGAE^{391} (cyan) and the core PHF tau mould – the binding site of the antibody

(red) determined by X-ray analysis of the complex. The core PHF tau segment is disordered in solution, but upon complex formation it adopts conformation enforced by the mould (MN423 paratope). This ensures that the conformation is the same as that of the *in vivo* core PHF tau against which the antibody was raised. The figures **A** and **B** were drawn by *MOE* and *PYMOL* (http://pymol.sourceforge.net), respectively.

An alternative way of how to tackle the structure of IDP tau is to use complexes of recombinant tau peptides antigenic determinants (epitopes) with their targets, i.e. monoclonal antibodies, which substitute for their natural binding partners. We have prepared five monoclonal antibodies directed against the tau protein segment 297-391 (numbering according to the longest tau protein isoform tau40 [3]), which is contained in the PHF core [6]. The epitopes of these antibodies were mapped by ELISA (Fig. **3A**). They cover major part of the tau297-391 with binding constants in nanomolar range (Fig. **3B**), which gives a good chance to form stable complexes of mAbs with tau protein epitopes, thus facilitating crystallization and structure determination.

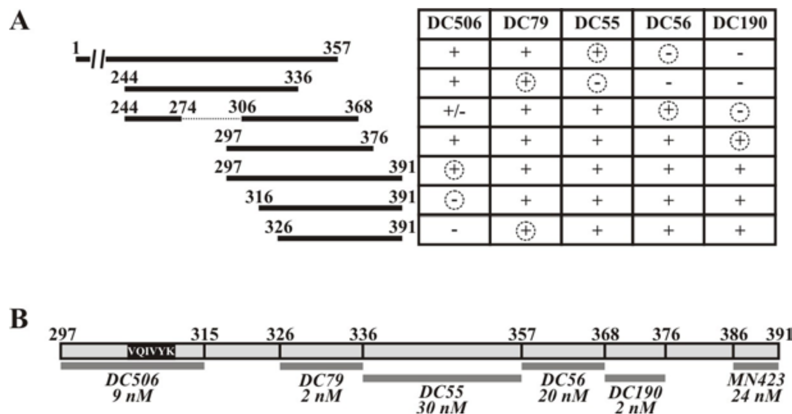

Figure 3. Monoclonal antibodies for structure determination of IDP tau. (A) Epitope mapping by ELISA, using the panel of tau protein deletion mutants (numbering according to the longest tau protein isoform [3]). The high, medium and low mAb reactivity are denoted by +, +/– and –, respectively. Highlighted (encircled) reactivities permitted to dissect the epitope boundaries. (B) Distribution of monoclonal antibody epitopes on the core PHF tau297-391 unit and their affinities determined by surface plasmon resonance (data for MN423 are from [7,14]). The segment VQIVYK forming the β-sheet structure important for the PHF core assembly [15,16] is present in all tau proteins sequenced from the native PHF core [6].

The two approaches to structure determination of intrinsically disordered protein tau described here open a new way to assess the structure of *in vivo* folded IDP tau by diffraction methods. Availability of a number of specific monoclonal antibodies will augment a chance to determine a large part of tau protein structure using antibody-tau complexes. We believe that this approach could reveal structural segments which may play an important role in the PHF core assembly, opening a possibility to study the mechanisms of the core PHF formation. Accumulation of sufficient structural data may facilitate the structure guided drug design for prevention and therapy of Alzheimer's disease. It is assumed that this procedure may be applicable also to other IDPs.

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