

# Structure of DC11 Fab fragment specific for the pre-aggregation conformation of intrinsically disordered protein tau

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A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and the mechanism of the transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of AD patient. The antibody recognizes also the recombinant truncated tau proteins up to the shortest fragment tau321-391 [1].

It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation [2]. This may imply possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds up the tau aggregation. The information about conformational epitopes of these antibodies are therefore of high significance.

To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using <sup>13</sup>C, <sup>15</sup>N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e. its C-terminal region.

We have solved the X-ray structure of DC11 Fab fragment to a resolution of 1.33 Å crystallized in a space group P 1 21 1 and deposited it into the PDB database with PDB ID 9H8H. We have also obtained crystals of DC11 Fab and crystals of tentative complexes between DC11 and either tau321-391 or tau peptide tau371-387 in different space groups. We have further measured the synchrotron SAXS data to characterize the conformational ensembles of tau321-391 and tau297-391 in both batch and SEC-SAXS modes. We have also attempted to characterize the complexes between tau proteins and DC11 Fab fragment.

The results highlight the importance of the R' region of tau, that was recently shown to be important also for tau interaction with microtubules [3]. This sequence forms the interface of rigid filament core and flanking fuzzy C terminal segment in solved tauopathy filaments.

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