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Amyloid inhibitor octapeptide forms amyloid type fibrous aggregate and affect in microtubule motility

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An amyloid inhibitor octapeptide simultaneously forms amyloid type fibrous aggregate on its own and interacts with microtubule lattice three times stronger than a Xenopus Microtubule Associated Protein (XMAP215).

The main cause of Alzheimer’s diseases (AD) is the accumulation of neuritc plaques, containing amyloid β (Aβ) fibrils which are generated from cross-β sheet like structures of Aβ42,2 There are many organic molecules, peptide and protein based drugs, which have potential to inhibit the amyloid fibrillation in vitro and raise hopes for treatment of neurodegenerative diseases such as AD.3 Among them, octapeptide ‘X’ (NAPVSPIPQ), derived from activity dependent neuroprotective protein (ADNP) exhibits in vitro and in vivo neuroprotective activity against cognitive dysfunction, anxiety, apolipoprotein E deficiency, cholinergic toxicity, closed head injury and stroke.3c,4 It is interesting to note that often small molecule amyloid inhibitors form aggregates itself.5 The probable mechanism of inhibition of fibrillation has been documented by electron microscopic studies for a few small molecules, which reveals that the colloidal like structure is localized to preform fibers and prevented new fiber formation.5 Now, the question remains, does all the molecules follow similar pathways and also form aggregates. It is well known that ‘X’ has huge potential in inhibition of tau fibrillation by inhibiting the hyper phosphorylation of tau as well as it binds with tubulin and microtubule in cell.5 However, it has never been studied before how ‘X’ alone behaves in solution, how ‘X’ interacts with tubulin, what are the interacting partners (amino acids) between peptide and tubulin and how strong it binds with tubulin. To understand these questions, we began our investigation for the first time in this work on the behaviour of ‘X’ alone in solution, its interaction with tubulin and microtubule lattice with the combination of computational and experimental studies. Herein, we report the self-assembly behaviour of ‘X’ by MD simulation, followed by characterization of self-assembled structure through various spectroscopic and microscopic techniques. Moreover, we have also studied (i) the inhibition of amyloid fibrillation in vitro by ‘X’, (ii) the interaction of ‘X’ with tubulin by docking on 2D micropattern surface and (iii) finally ‘X’ generated friction during microtubule motility on a dual functionalized surface.

Both ‘X’ and biotinylated-X (HX) (Figure 1a) has been synthesized through solid phase peptide synthetic method, purified through HPLC, passed through ion exchange Amberlite-IRA400 resin and characterized by MALDI Mass. We have performed MD simulation of the ‘X’ to gain atomistic detailed information about nature of self-assembly of ‘X’ alone in solution before performing detailed experimental analysis. Initially, two molecules of ‘X’ denoted as A and B in the simulation box was seperated by 2 nm and simulated for 200 ns. MD simulation studies revealed that ‘X’ form β-turn structure in the initial time period (Figure 1b). After 80 ns simulation, two ‘X’ start interacting with each other in parallel orientation and β-turn rich structure was observed (Movie S1). After 126 ns, they form antiparallel β-sheet structure with twist between Val, Ser of chain A with Ile, Pro of chain B. At 154 ns "reptation movement" between Ile, Pro of chain A and Val, Ser of chain B forces to reverse the antiparallel β-sheet structure. This interesting "reptation movement" once again takes place at 200 ns and inversion of β-sheet structure is observed (Figure 1b) . We found from "reptation movement" that four key amino acids Val, Ser, Ile and Pro in the ‘X’ backbone are responsible for the formation of β-sheet structure (Figure 1c, S1, Movie S1). From MD simulation movie, we envision that assemble process starts from β-turn
conformation and converts to β-sheet rich structure at the end, which is rich in fibrillar assembly. Therefore, this result further motivated us for exploration of self-assembling behaviour of this octapeptide by various experimental techniques.

We have performed FT-IR analysis of lyophilized samples of ‘X’ and ‘HX’ from 0, 7 and 30 days incubated solution and found the β-turn rich conformation for ‘X’ (Amide I is 1672 cm⁻¹) and ‘HX’ (Amide I is 1665 cm⁻¹) at 0 day incubation (Figure S4), β-turn rich conformation for ‘X’ (Amide I is 1671 cm⁻¹) and β-sheet rich conformation for ‘HX’ (Amide I is 1639 cm⁻¹) at 7 days incubation (Figure S2 and S3) and β-sheet (Amide I for ‘X’ is 1629 and for ‘HX’ 1640 cm⁻¹) rich conformation of both ‘X’ and ‘HX’ after 30 days incubation in physiological condition (Figure 2a, b). This result clearly indicates that both peptides have mixed conformation during assembly process, and it corroborates with previously described MD simulation studies (Movie S1). Recent reports also support our observation in MD simulation studies and FT-IR that during fibril nucleation, β-turn formation plays a significant structural role in the equilibrium leading to fibrils.

We have studied the morphologies of ‘X’ and ‘HX’ by transmission electron microscopy (TEM). Solutions of both ‘X’ and ‘HX’ were prepared in phosphate buffer at physiological conditions (37 °C and pH 7.4) for TEM studies and incubated for 0, 7 and 30 days. TEM images of freshly prepared sample of both ‘X’ and ‘HX’ reveal β-sheet structure (Figure S2a, f). Short peptide fibers with oligomeric structure after 7 days incubation (Figure 2c, f). Long fibrous network structure of 30 days incubated samples (c,h) of ‘X’ and ‘HX’ respectively. Inset shows twisted fibrous aggregate of ‘HX’.

Figure 2. FT-IR spectra of 30 days incubated samples of ‘X’ (a) and ‘HX’ (b) reveal β-sheet structure. TEM images of time dependent incubated solution of ‘X’ and ‘HX’. Oligomeric structure of freshly incubated samples (c,f). Short peptide fibers with oligomeric structure after 7 days incubation (d,g). Long fibrous network structure of 30 days incubated samples (e,h) of ‘X’ and ‘HX’ respectively. Inset shows twisted fibrous aggregate of ‘HX’.

We have further developed a novel in vitro assay for studying peptide-tubulin and peptide-microtubule lattice interaction using surface chemistry approach. Therefore, we used our recently developed biotin micropattern surface and immobilized freshly prepared ′HX′ onto the micropattern through neutravidin and followed by incubation of ‘HX’ immobilized micropattern surface with tubulin mix (80:20 unlabelled tubulin and Alexa568 labelled tubulin) in presence of GTP at 37 °C and observed under TIRF microscope. Initially, we observe weak tubulin binding on micropattern but on time progresses, we have observed localized binding of tubulin on ‘HX’ immobilized micropattern (Figure 3a). Control experiment was performed in absence of the peptide, following similar method as described results no tubulin binding on micropattern (data not shown). This result clearly indicates that binding mode of tubulin with ‘X’ is similar like microtubule associated protein (XMAP215) with tubulin on micropattern surface. This result motivated us to explore further about the interaction of ‘X’ with microtubule lattice and whether this interaction has direct effect on
microtubule motility, like recently we have observed that XMAP215 effects in microtubule motility and slowdown microtubule gliding speed in a concentration dependent manner.\textsuperscript{4b} For that purpose we prepared Tris-NTA and biotin functionalization as a surface.

Figure 3. Image reveals that the tubulin specifically binds with 'HX', immobilized on neutravidin pattern surfaces (a). Docking image reveals the specific interaction between the amino acid of 'X' and β-tubulin (b).

Effect of microtubule gliding speed on 'HX' and kinesin immobilized surface. Histogram reveals that the average gliding speed of GMP-CPP microtubule, in presence of 'HX' on kinesin immobilized surface is around 8.2 ± 0.48 µm/min (c) and in absence of 'HX' is 55.5 ± 14.2 µm/min (f). Example kymograph from a movie of microtubule gliding in presence of 'HX' (d). Gliding speed of GMP-CPP microtubules decreases around 7 times in presence of 'HX' (e). Example kymograph from a movie of microtubule gliding in absence of 'HX' (g). Cartoon represents the interaction of kinesin and 'HX' with the microtubule lattice (h). Scale bar corresponds to 10 µm.

- d glass surface, following previously described method\textsuperscript{4b} and immobilized kinesin (Kinesin612-His\textsubscript{10} and 'HX' on that surface. Next, we have observed motility of very short freshly prepared microtubules using GMP-CPP under TIRF microscope and gliding speed were calculated from various kymographs (Figure 3d,g), which are acquired from 15 min time lapse images and we observed the average speed of microtubules gliding, which is 8.2 ± 0.48 µm/min (Figure 3c, Movie S2). We have also performed control experiment following previously described method in the absence of 'HX'. Result from control experiment indicates that the average gliding speed is 55.5± 14.2 µm/min (Figure 3f, Movie S3). Above results clearly indicate, that the gliding speed of microtubules, in presence of peptide reduces close to 7 times (Figure 3e), which is three times stronger than XMAP215. Figure 3h represents the cartoon picture that peptide and Kinesin are interacting with microtubule lattice. Therefore, above results is the first report of a small octapeptide, which binds strongly with microtubule lattice, similar like XMAP215, and slowdown of gliding speed of microtubules on surface.\textsuperscript{4b}

In conclusion, we have shown that a short amyloid inhibitor octapeptide forms amyloid type fiber using various techniques such as FTIR spectroscopy, TEM and ThT assay. In addition, we have developed two novel assay one using 2D micropattern and another using Tris-NTA, biotin dual functionalized glass surface, which magnificently reveal how a short octapeptide binds with tubulin and generates very strong friction on microtubule lattice. To the best of our knowledge this is the first report, which will help for the screening of potential microtubule targeted anticancer and anti-Alzheimer’s peptides and small molecules tagged with biotin.

We thank Mr. D. Sarkar for microscopy, Dr. R. Natarajan and anonymous referees for their invaluable comments, CSIR (PK and BJ), UGC (AB), DST-Ramanujan (SG) for providing fellowships, CSIR-HICB (BSC0113) for funding (to SG).

Notes and references

Chemical Communications Accepted Manuscript


