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MAP2c prevents arachidonic acid-induced fibril formation of tau: Role of chaperone activity and phosphorylation

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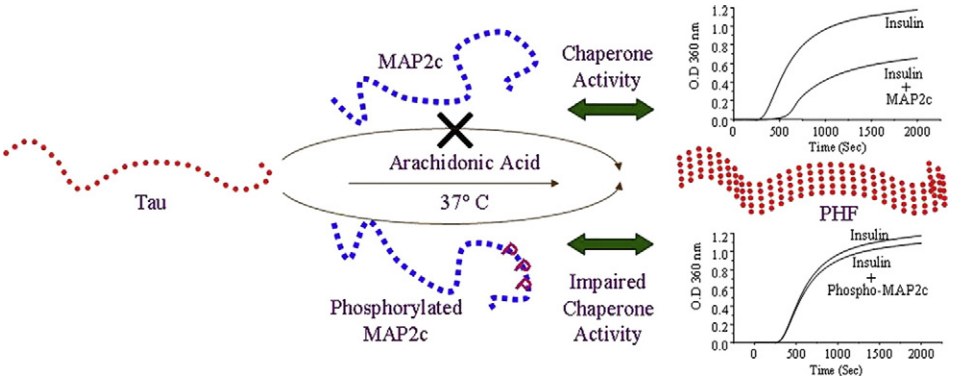
HIGHLIGHTS

- MAP2c prevents arachidonic acid-induced tau fibril formation in vitro.
- Phosphorylated MAP2c fails to prevent tau fibril formation in vitro.
- Phosphorylation impairs the chaperone activity of MAP2c.

GRAPHICAL ABSTRACT



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ABSTRACT

Tau has long been associated with Alzheimer's disease, where it forms neurofibrillary tangles. Here we show for the first time by electron microscopy that MAP2c prevents arachidonic acid-induced in vitro aggregation of tau. However, phosphorylated MAP2c failed to prevent the same. Previously we reported that MAP2c possesses chaperone-like activity while tau does not (Sarkar et al., 2004, *Eur J Biochem., 271(8)*, 1488-96). Here we demonstrate that phosphorylation severely impaired the chaperone activity of MAP2c, implying a crucial role of chaperone in preventing tau fibrillation. Additionally, the ability of MAP2c to induce microtubule polymerization was abolished completely upon phosphorylation. As tau and MAP2c possess highly homologous C-termini, we speculated that the N-terminus of MAP2c might account for its chaperone activity. Nevertheless, experiments showed that N-terminus of MAP2c alone is inactive as a chaperone. Our preliminary findings suggest that MAP2c/MAP2 could be one of the regulators maintaining tau homeostasis in the cell.

1. Introduction

Tau and MAP2 belong to the family of microtubule-associated proteins (MAPs). Members of this family bind to stabilize and regulate the microtubule network in neurons, thus maintaining several crucial cellular functions [1]. Structurally they constitute the class of intrinsically disordered proteins, which are devoid of well-defined secondary and

Abbreviations: HMW, high molecular weight; MAP2c, microtubule associated protein 2c; MTBD, microtubule binding domain; HSP, heat shock protein; AD, Alzheimer's disease; IPTG, isopropyl thio-β-D-galactoside; PMSF, phenylmethylsulfonyl fluoride; MDH, malic dehydrogenase.

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tertiary structures. The physiological functions including microtubule stabilizing activity of MAPs are critically regulated by phosphorylation/dephosphorylation, mediated through the intervention of several protein kinases and phosphatases [2–8]. While tau is majorly expressed in the axons, MAP2 is prevalent in the cell body and dendrites. Both the proteins have several isoforms generated due to the alternative splicing of exons, which are developmentally regulated [9–14]. Irrespective of the isoform, tau and MAP2 share a highly conserved carboxy-terminal domain consisting of the microtubule-binding repeats, and a variable amino-terminal projection domain which modulate the microtubule spacing.

The smallest isoform of MAP2, i.e., MAP2c, is the juvenile counterpart of the former, which is down regulated in the later stages of neuronal development. MAP2c contains the N and C-terminal domains of high-molecular-weight MAP2, lacking the middle intervening region [12]. The smaller isoform is believed to be involved in synaptogenesis, whereas the HMW MAPs participate in their stabilization [15]. In the current study, we have used MAP2c instead of MAP2 in all our experiments because of its smaller size and lesser complexity. It has been previously reported from our laboratory that MAP2 as well as MAP2c manifests chaperone-like activity, while tau is devoid of such activity [16].

Tau has been the focus of extensive research for several years, as its anomalous aggregation is the hallmark of various tauopathies including Alzheimer's disease (AD). AD is the most devastating dementia worldwide, characterized by the abundant extracellular plaques of β-amyloid peptide and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau. Hyperphosphorylation of tau results in its dissociation from microtubules. Consequently tau does not stabilize microtubule any more, thus collapsing axonal transport, ultimately leading to neuronal death. With 36 million cases documented in 2010 worldwide, which is predicted to reach 115 million by 2050 (http://www.alz.org/documents_custom/world_report_2012_final.pdf), AD constitutes an overwhelming threat to the nations. Currently the disease is irreversible and incurable. Tremendous efforts are being put forward to find better ways to treat the disease, and/or delay the progress. After the failure of innumerous compounds in clinical trial based on the amyloid β hypothesis, tau-mediated therapies have attracted the attention of researchers in recent years [17,18]. The tau-based therapies are based on several hypotheses — inhibition of tau aggregation by small molecules, intervention of chaperones in preventing tau aggregation, inhibition of protein kinases and/or activation of protein phosphatases (thus preventing tau hyperphosphorylation), etc. In recent years, a plethora of evidences have shown the efficacy of chaperones to prevent tau aggregation in vitro as well as in the mouse model.

In this report we show that MAP2c prevents the arachidonic acid-induced aggregation of tau in vitro. This unique property of MAP2c has been correlated with its chaperone-like activity, which on the other hand was modulated by phosphorylation of the protein. We have also explored the structural pre-requisite of MAP2c to function as a chaperone.

2. Materials and methods

2.1. Materials

IPTG, insulin, MDH, α-glucosidase, α-D glucopyranoside and oligonucleotides were from Sigma. The restriction enzymes were purchased from Bangalore Genei (India). All other reagents were of analytical grade.

2.2. Purification of MAP2c

Rat MAP2c (rMAP2c) consisting of 3 MTBDs was prepared following the procedure of Gamblin et al. [19], with a few modifications. In brief, pET 3a rMAP2c clone was transformed into *Escherichia coli* strain BL21(DE 3). Cells were lysed after IPTG induction and the supernatant was collected after separation of cell debris by centrifugation. The supernatant was boiled for 20 min in water bath with 500 mM NaCl. The protein was finally purified by SP ion-exchange chromatography using AKTA FPLC, followed by extensive dialysis in 50 mM phosphate buffer (pH 7.0). The purity of the protein was verified by SDS/PAGE and protein concentration was determined by the method of Lowry et al. [20].

2.3. Purification of tau

Human tau (htau40; consisting of 4 MTBDs) clone containing His-tag was transformed into *E. coli* strain BL21(DE 3). Cells were lysed after IPTG induction and the supernatant was collected after separation of cell debris by centrifugation. The supernatant was boiled for 20 min in water bath with 500 mM NaCl. Tau was finally purified by Ni-NTA column. Protein was eluted with 200 mM imidazole and was dialyzed extensively in 50 mM phosphate buffer (pH 7.0). The purity of the protein was checked by SDS/PAGE and protein concentration was determined by the method of Lowry et al. [20].

2.4. Cloning and expression of N-terminal of MAP2c

2.4.1. DNA isolation and manipulations

The N-terminal part of MAP2c corresponding to 1–366 bp coordinates was amplified by Proofstart polymerase (Qiagen GmbH, Germany) using the above plasmid DNA as template and the forward primer (5′-CGTAGAGGATCGAGATCTCGATCCCGCGA) and reverse primer (5′-AGCTGCGGATCCTCAAGCTGCAGGCTGATCCTTGTG). Using the former primer *Nde*I restriction endonuclease site was generated, while the latter primer harbors *Bam*HI site. The resulting PCR-made DNA fragment was digested together with *Nde*I and *Bam*HI and cloned into an *E. coli*-specific expression vector pET-3a at identical sites and subsequently sequenced by an automated DNA sequencer according to the manufacturer's protocol. The newly generated clone was designated as NMAP2c and the sequence was verified. NMAP2c was utilized to express the N-terminus of MAP2c and purified by Q-ion exchanger column using AKTA FPLC. The concentration of the protein was measured by the method of Lowry et al. and purity of the protein was checked by SDS-PAGE. The N-terminal band appeared at around 29 kD.

All other molecular biology techniques, digestion of DNA by restriction enzymes, ligation by T4 DNA ligase, transformation, etc. were carried out according to the standard procedures [21].

2.5. Assay of protein aggregation

Insulin aggregation assay in the presence of a chaperone was done according to Sarkar et al. [16].

2.6. Refolding assay

Refolding assay of α-glucosidase and MDH was performed as described by Sarkar et al. [16].

2.7. Phosphorylation of MAP2c by PKA

0.5 mg/ml of MAP2c was phosphorylated by 300 unit/ml of PKA catalytic subunit in a mixture of 0.1 M PIPES (pH 7.0), 1 mM ATP, 1 mM EGTA, 10 mM $MgCl_2$, 1 mM PMSF and 5 μg/ml aprotinin. The mixture was incubated at 37 °C for 60 min and stopped by boiling for 5 min. The incorporation of phosphate groups into MAP2c was confirmed by using radioactive ATP (γP^{32}) and running the samples on 11% SDS-PAGE. To visualize the phosphate incorporation, dried SDS gels were exposed to autoradiographic films.

For quantification of phosphorylation, aliquots were withdrawn at different time points during phosphorylation (using 1 mM cold ATP spiked with radiolabeled ATP γP^{32}). After boiling, the samples were filtered through a Millipore 0.45 μm filter paper and washed thoroughly by 50 mM phosphate buffer. The count was taken by liquid scintillation counter.

2.8. Polymerization assay

Glutamate-purified tubulin (1.5 mg/ml) in PIPES buffer was polymerized at 37 °C in the presence of 1 mM GTP. Polymerization was induced by 0.25 mg/ml of MAP2c. Reaction was monitored by measuring the absorbance at 360 nm with time using a Shimadzu UV-160 double-beam spectrophotometer, fitted with a temperature-controlled circulating water bath.

2.9. Electron microscopy with tau

To monitor in vitro fibril formation, tau was incubated with arachidonic acid. 4 μM of tau was incubated with 100 μM of arachidonic acid (dissolved in 100% ethanol) in the polymerization buffer containing 10 mM HEPES (pH 7.6), 100 mM NaCl, and 5 mM dithiothreitol at 37 °C [22]. For co-incubation of tau with MAP2c/phosphorylated MAP2c, the proteins were mixed in 1:1 ratio (mole/mole), prior to the addition of arachidonic acid and incubated for 10 min at room temperature. Only MAP2c or phosphorylated MAP2c was incubated under similar condition as control experiments. The final concentration of ethanol vehicle for arachidonic acid was 4.5%; hence, control sample of tau lacking arachidonic acid was also checked with 4.5% ethanol to rule out any effect of the latter. After 6.5 h of incubation at 37 °C, samples were removed and negatively stained with 1.5% uranyl acetate on formvar/carbon-coated 400-mesh grids. The fibril formation was viewed under a FEI Tecnai G2 Spirit Biotwin electron microscope at 80 kV.

3. Results

MAP2, MAP2c and tau share very high sequence homology among themselves, especially in the conserved MTBD of the C-terminal segment. Fig. 1 shows the schematic representation of the three proteins. They share a conserved 3 or 4-repeat region in the C-terminal end (R1-R3/R4), preceded by a proline-rich region (P), as depicted in Fig. 1A. The N-termini of MAP2 and MAP2c bear considerable sequence identity, while that of tau differ significantly. However, the projection domain of MAP2c is much shorter compared to its high molecular weight counterpart MAP2. Fig. 1B shows the schematic representation of sequence identity of MAP2/MAP2c/tau and MAP2c/tau respectively, as derived from multiple sequence alignment using NCBI Blastp. C-termini of all the three proteins and N-termini of MAP2 and MAP2c showed the highest scoring regions (black), while gray regions denoted short low scoring segments. While 4 repeat rat MAP2 and 3 repeat rat MAP2c showed 88% identity, rat MAP2c and human tau showed 54% sequence identity.

3.1. Phosphorylation of MAP2c by PKA

MAP2c was phosphorylated by PKA catalytic subunit, as described in Materials and methods. The extent of phosphorylation varied with concentration of PKA. Inset of Fig. 2 shows the autoradiogram of phosphorylation of MAP2c by labeled ATP (γP^{32}). The time of phosphorylation was 1 h in each case. From lanes 1 to 4, concentration of PKA varied from 25 unit/ml to 300 unit/ml and the extent of phosphorylation increased accordingly. Quantification of phosphorylation showed that it reached saturation at a maximal stoichiometry of ~3 mol phosphate/mol protein after 1 h with 300 unit/ml of PKA (Fig. 2). Our result correlated well with the previous report demonstrating Ser^{319}, Ser^{350} and Ser^{382} to be the major PKA-mediated phosphorylation sites on MAP2c, situated at the conserved KXGS motif in the microtubule-binding repeats [23].

3.2. Effect of native and phosphorylated MAP2c on arachidonic acid-induced fibril formation of tau

In native environment tau is an unusually soluble protein, even resistant to heat and acid denaturation. The sequence of the protein is highly governed by hydrophilic residues. Though tau exhibits little tendency towards aggregation under normal circumstances, it forms fibrils in the form of paired helical filaments and straight filaments in Alzheimer's disease [24]. In vitro, co-factors like arachidonic acid have long been used as an inducer of fibril formation of tau [25–27]. Arachidonic acid has been reported to induce tau fibrillization in micellar form with the intervention of a pre-fibrillar intermediate. The proposed pathway of anionic surface-induced tau aggregation has been documented in AD tissues and the intermediate has been suggested to be a potential therapeutic target [28]. These fibers reconstituted from recombinant tau by arachidonic acid are similar in morphology of straight filaments, as observed in Alzheimer's brain [23,28,29]. Thus in vitro fibrillation using arachidonic acid has served as a reliable model for studying tau aggregation and its prevention thereafter. Over the last decade, an increasing number of literatures have illustrated the involvement of chaperones in inhibiting the aggregation of tau, both in vitro and in vivo. Thus we were tempted to test the effect of the chaperone MAP2c on the arachidonic acid-induced fibril formation of tau by transmission electron microscopy. Samples for electron microscopy experiments were prepared by incubating tau with native or phosphorylated

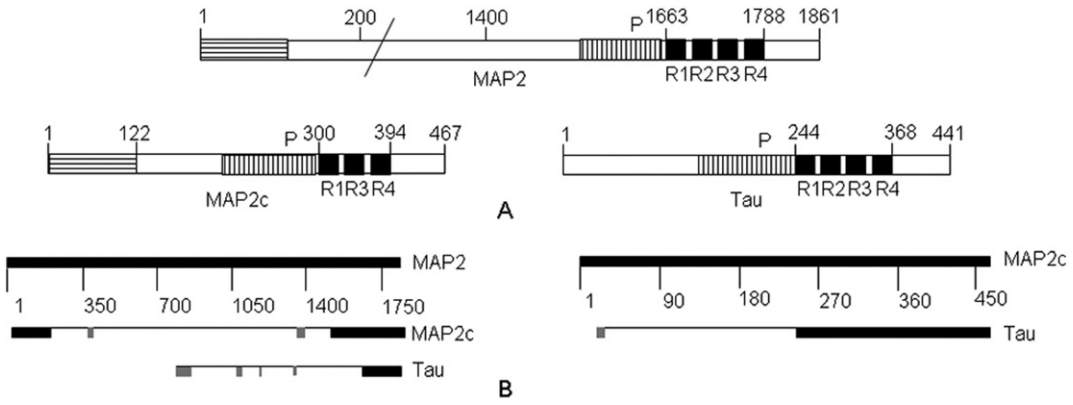

Fig. 1. (A). Schematic representation of MAP2, MAP2c and tau: ■ — microtubule-binding repeats (R1–R3/R4); ▥ — proline-rich region (P); ▭ — N-terminal cloned part of MAP2c which is highly homologous to MAP2. (B). Sequence alignment of MAP2/MAP2c/tau and MAP2c/tau respectively by NCBI Blastp. ■ — Highest scoring regions, ■ — short low scoring segments.

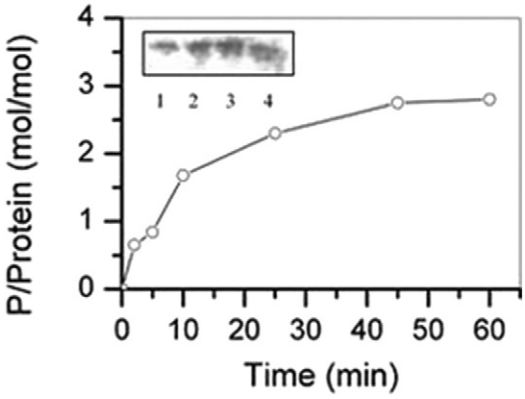

Fig. 2. In vitro phosphorylation of MAP2c by PKA: Phosphorylation of MAP2c reached saturation attaining a maximum stoichiometry of ~3 mol phosphate/mol MAP2c at 60 min with 300 unit/ml PKA. Inset shows autoradiogram of phosphorylation of MAP2c: Lanes 1 to 4 show phosphorylation for 1 h with varied amount of PKA unit. Lane 1, with 25 unit/ml PKA; Lane 2, with 50 unit/ml PKA; Lane 3, with 100 unit/ml PKA; Lane 4, with 300 unit/ml PKA.

MAP2c in 1:1 mole ratio respectively (tau:MAP2c). Fig. 3 shows the results of such experiments. Fig. 3A shows the formation of filaments after 6.5 h of incubation of native tau at 37 °C in the presence of 100 μM arachidonic acid. To our surprise, in the presence of MAP2c, no filament formation was observed with tau, as observed in Fig. 3B. Subsequently when MAP2c was phosphorylated followed by incubation with tau in the presence of arachidonic acid for the same period of time, fibrillar structure reappeared (Fig. 3C). MAP2c and phosphorylated MAP2c similarly incubated with arachidonic acid under identical condition as control did not show any such fibril (data not shown).

3.3. Effect of phosphorylation of MAP2c on its chaperone activity

Previously we had reported that MAP2c has excellent chaperone-like activity, which could attenuate the chemical and/or thermal aggregation of proteins and refold fully denatured substrate enzymes to a considerable extent [16]. Here we tested the effect of phosphorylation of MAP2c on its chaperone-like activity. Results of such experiments are shown in Fig. 4. Here, curve 1, Fig. 4A shows dithiothreitol-induced aggregation of insulin at 25 °C. Insulin concentration was 0.3 mg/ml. Using MAP2c to insulin ratio of 1:1 (w/w), the inhibition of insulin aggregation was found to be 40% (curve 2). However, phosphorylated MAP2c could no longer prevent the aggregation of insulin, as evident from curve 3. Assistance towards the recovery of lost biological activity during protein refolding from unfolded state is another characteristic of a chaperone. In our refolding experiment, MDH and α-glucosidase have been used as model substrates, which were initially unfolded in the presence of 8(M) urea. We have tested the role of both native and phosphorylated MAP2c in the context of protein refolding and the results are depicted in Fig. 4B and C. In Fig. 4B, curve 2 shows that MAP2c increased the enzymatic activity of MDH from its fully denatured state up to 38.5%, while phosphorylated MAP2c did the same to the extent of 28% (curve 3, Fig. 4B). In the absence of MAP2c, the self-refolding yield of MDH was 10% (curve 1). Similar scenario emerged when α-glucosidase was used as a model enzyme, as shown in Fig. 4C. The self-refolding yield of α-glucosidase from completely unfolded state was less than 2% (curve 1). In the presence of

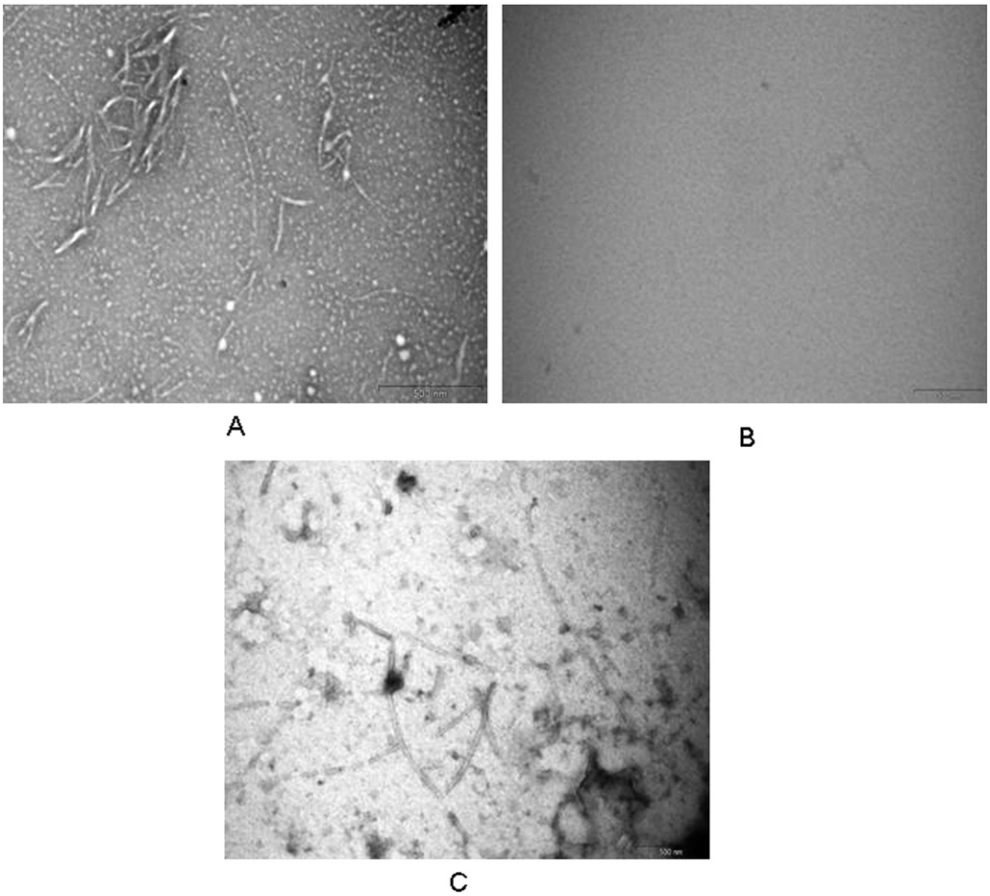

Fig. 3. Negative stain electron microscopy of tau and MAP2c with arachidonic acid: (A). Fibrils observed upon incubation of tau with arachidonic acid. (B). No fibril observed upon incubation of tau with arachidonic acid in the presence of MAP2c (1:1, mole/mole). (C). Fibrils observed upon incubation of tau with arachidonic acid in the presence of phosphorylated MAP2c (1:1, mole/mole).

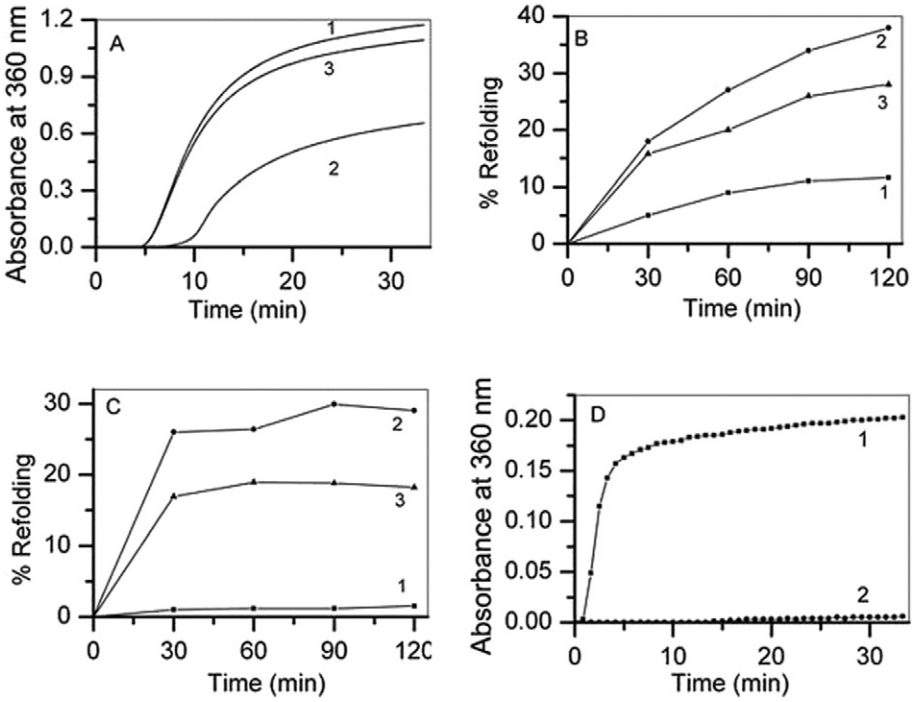

Fig. 4. Effect of phosphorylation of MAP2c on chaperone activity and tubulin polymerization: (A). Effect of phosphorylation of MAP2c on insulin aggregation: curve 1, insulin only; curve 2, insulin + native MAP2c (0.3 mg/ml); curve 3, insulin + phosphorylated MAP2c (0.3 mg /ml). Insulin concentration was 0.3 mg/ml in all cases. (B). Time course of reactivation of 0.03 μM MDH: curve 1, self-folding of MDH; curve 2, in the presence of 0.05 mg/ml native MAP2c; curve 3, in the presence of 0.05 mg/ml phosphorylated MAP2c. (C). Time course of reactivation of 0.06 μM α-glucosidase: curve 1, self-folding of α-glucosidase; curve 2, in the presence of 0.05 mg/ml native MAP2c; curve 3, in the presence of 0.05 mg/ml phosphorylated MAP2c. (D). Effect of phosphorylation of MAP2c on tubulin polymerization: curve 1, tubulin polymerization in the presence of native MAP2c; curve 2, tubulin polymerization in the presence of phosphorylated MAP2c; tubulin and MAP2c concentrations were 1.5 mg/ml and 0.25 mg/ml respectively.

native MAP2c, the increase in enzymatic activity of α-glucosidase was 29% (curve 2), while it was 18.2% in the presence of phosphorylated MAP2c (curve 3). The results clearly showed that phosphorylation of MAP2c significantly impaired its chaperone-like activity.

3.4. Phosphorylation of MAP2c and promotion of tubulin self-assembly

We have tested phosphorylated MAP2c for its ability to promote tubulin self-assembly, as PKA is known to phosphorylate MAP2c at the conserved microtubule-binding KXGS motif. We found that phosphorylation of MAP2c completely abolished its ability to induce tubulin polymerization (Fig. 4D), thus hinting towards the incorporation of phosphate moieties in the MTBD. Curve 1 depicts the self assembly of tubulin induced by native MAP2c, while curve 2 shows the same in the presence of phosphorylated MAP2c. Previous reports have shown that pseudophosphorylation at the above-mentioned serine residues strongly impaired the ability of MAP2c to bind and stabilize microtubules and failed to induce neurite initiation [23,30]. According to Ainsztein and Purich, phosphorylation of MAP2 at the microtubule-binding domain reduces its potency to induce tubulin polymerization, with distinct differences in the binding behavior depending on the extent of phosphorylation [31].

3.5. N-terminal of MAP2c and chaperone-like activity

Both tau and MAP2c, which co-purify with tubulin, have highly homologous C-termini and distinct N-termini. Although MAP2c possesses chaperone-like behavior, tau does not, as reported in our previous communication [16]. This had prompted us to speculate that the unique behavior of MAP2c might have originated from its N-terminal region, which differs significantly from tau. To demonstrate the role of N-terminus of MAP2c in chaperone activity directly, we have cloned, expressed and purified the N-terminal part of MAP2c, comprising of residues 1–122, as described in Materials and methods. The expressed segment of protein was tested for its chaperone-like activity using insulin aggregation assay (Fig. 5). Unlike full-length MAP2c (curve 2), the N-terminal truncated part was unable to prevent the DTT-induced aggregation of insulin (curve 3). This observation clearly suggested that the N-terminus of MAP2c alone cannot account for its chaperone-like property. Other segments of MAP2c are essential to make the protein active as a chaperone. As expected, the cloned N-terminal fragment also failed to induce tubulin polymerization, as it lacked the C-terminal microtubule-binding domain (data not shown).

4. Discussion

Alzheimer's disease is one of the most devastating protein misfolding diseases affecting a significant population worldwide. One

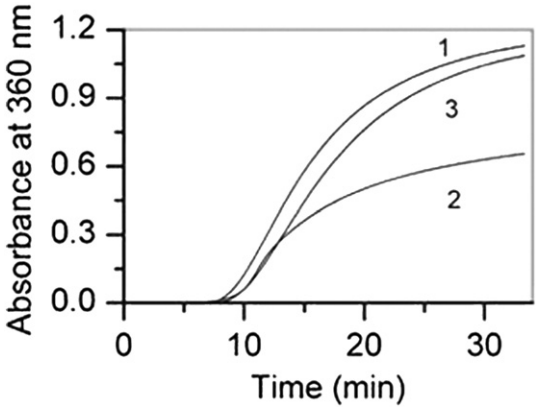

Fig. 5. Effect of N-terminal of MAP2c on chaperone activity: curve 1, insulin only; curve 2, insulin + whole MAP2c (0.3 mg/ml); curve 3, insulin + N-terminal of MAP2c (0.3 mg/ml). Insulin concentration was 0.3 mg/ml in all cases.

of the priority areas of Alzheimer's research is the prevention of aggregation of the pathological proteins i.e., tau and amyloid beta. In recent years, the crucial role of molecular chaperone like CHIP (Carboxyl-terminus of HSP70 Interacting Protein) in inhibiting tau aggregation has been established in vivo by several laboratories [32–34]. Also, HSP70 and HSP90 prevented amyloid formation of tau [35,36]. A correlation among the expression of molecular chaperones/HSPs, neurofibrillary tangle (NFT) and soluble tau protein levels in the brain of patients with Alzheimer's dementia has been reported [37–39]. A recent in vitro study showed that Protein Disulfide Isomerase could inhibit tau fibrillization [40] and S-nitrosylation of the former, which impairs its chaperone activity, has been implicated in Alzheimer's disease [41]. Several reports have also demonstrated up-regulation of chaperones in the proteome of Alzheimer's patients [42,43]. Probable benefit of curcumin has been suggested in AD-associated tauopathy, as it facilitates up-regulation of anti-tau co-chaperone BAG2 [44]. Thus tau-chaperone interaction is of immense importance in Alzheimer's pathogenesis and might provide a promise for the development of novel therapeutics [32,45]. Thus our current finding of tau aggregation inhibition by MAP2c could be well-attributable to the chaperone function of the latter.

Our subsequent experiments showed that phosphorylation of MAP2c no longer prevent the aggregation of tau in vitro. Simultaneously, it made the protein inactive as a chaperone, which reinforces the concept that the novel inhibition of tau aggregation by MAP2c is due to its chaperone-like behavior. Phosphorylation is the most prevalent post-translational modification of MAPs, which regulates productive MAPs–tubulin binding, mediated by C-terminal repeats and proline-rich region of MAPs [46–48]. Previous study has clearly established that S^{319}, S^{350} and S^{382} are the major PKA-mediated phosphorylation sites in the repeat region of MAP2c [23]. Also the study showed that in living cells phosphorylation within any single KXGS motif strongly reduced microtubule-binding function of MAP2 and eliminated microtubule bundling. Other in vitro studies reported as well that MAPs–microtubule binding is disrupted when phosphorylation occurs within repeats in the microtubule-binding domain [4,49]. Phosphorylation introduces negatively charged moieties in MAPs, which might then repel the C-termini of tubulin having similar polarity, posing an impasse to binding. In our experiment, the onset of tubulin polymerization was completely abolished by phosphorylated MAP2c. This puts forward the possibility that the three serines of KXGS motifs were majorly phosphorylated, which then disrupted the tubulin–MAPs interaction. Eventually the stoichiometry of phosphate incorporation was in good agreement with our assumption, as shown in Fig. 2. However, the possibility of some minor sites (viz, T^{220} at proline-rich region) also being phosphorylated, cannot be ruled out completely [50]. Here we report for the first time that along with other cellular functionalities, the unique chaperone property of MAPs is also modulated by phosphorylation. There is a plethora of literature information showing regulatory role of serine phosphorylation on chaperone function. Both positive and negative regulation of chaperone activity of α-crystalline and other HSPs has been reported by phosphorylation of serine residues [51–59]. A probable rationale behind the impairment of chaperone activity of MAP2c by phosphorylation could be that the site consisting of the specific serine residues recognizes the substrate during stress. A close scrutiny of the sequence revealed that in all the three microtubule binding domain of MAP2c, the consensus sequence motif containing the phosphorylated serine is 'hgsh', where 'h' is a hydrophobic residue. It is quite likely that MAP2c, normally unstructured, can transiently make the two hydrophobic residues, present at i and i + 3 positions, available for hydrophobic substrate binding site through incipient turn formation. Phosphorylation would then disrupt the interaction due to electrostatic and steric interference. A very recent NMR study has suggested that MAP2c might not be completely disordered in its unbound state and predicted the presence of several segments with a helical propensity [60]. Another report suggested that PKA-mediated phosphorylation might impart conformational change in MAP2c [50], which might alter its function as well. Also the fact that only N-terminal part of MAP2c is inactive as a chaperone, might indicate towards the involvement of C-terminal region of the protein in chaperone function.

In the Alzheimer's brain, hyperphosphorylated tau sequesters normal tau, as well as MAP2, thus inhibiting their ability to maintain the microtubule network [61]. Interestingly, this hyperphosphorylated tau forms tangles of filaments only with normal tau, but not with MAP2. However, the possibility that the hyperphosphorylated tau binds MAP2 and subsequently forms soluble complex with it, cannot be ruled out completely. Ludueña et al. reported that tau promoted vinblastine-induced spiral formation of tubulin, whereas MAP2 strongly inhibited the effect of tau even at very low concentration [62]. Therefore, in several instances, the opposite behaviors of tau and MAP2 have been documented, although no suitable explanation was put forward. We believe that these observations can be explained considering the chaperone-like activity of MAP2. It is noteworthy to mention here that the minimum template needed for tau fibril formation has been identified as ^{306}VQIVYK^{311}, a hexapeptide motif residing in the third microtubule-binding repeat of tau [63]. MAP2/MAP2c also possesses the motif with one altered residue, VQIVTK. The amyloidogenic motifs in protein are rich in amino acids possessing high beta sheet propensity [64]. Aromatic amino acid like tyrosine as well as beta branched amino acids like threonine, both possess high beta sheet propensity. Thus replacement of a tyrosine residue with a threonine might not alter the nature of the above-mentioned peptide segment. Despite that, MAP2c does not participate in amyloid formation in neurodegenerative diseases like tau. Rather MAP2c might prevent fibrillation, as suggested by our preliminary observation. We propose that the chaperone-like activity of MAP2c might be the underlying cause for the aforementioned event.

The in vivo relevance of our finding needs further investigation. However, while normal tau is enriched in the axons, hyperphosphorylated AD-tau aggregates and accumulates in the somatodendritic compartment [65], where HMW MAP2 proteins are primarily concentrated. Thus MAP2 might have a role in suppressing aberrant tau aggregation in vivo as well, which is further supported by the presence of MAP2 in AD brain PHF [66]. Again, significant reduction in the level and activity of major endogenous phosphatase PP2A has been manifested in Alzheimer's disease [67,68]. Soluble tau and MAP2 compete for binding to and dephosphorylation by PP2A/Bα for residues phosphorylated by PKA [69]. The normal PP2A–MAP2 interaction is influenced by the enrichment of cytosolic MAP2 and tau in neuronal cell bodies in AD, thereby disrupting several PP2A and MAP2-mediated events like microtubule stabilization. We hypothesize that diminished level and activity of PP2A in AD not only trigger tau hyperphosphorylation, but also fail to dephosphorylate MAP2 to the desired extent, thus perturbing its chaperone property and consequent tau homeostasis. It is tempting to speculate from our preliminary data that MAP2 might be a candidate of cellular defense arsenal against tau-mediated neurodegeneration. Further studies are needed to gain deeper insight into the event which might open a new avenue for chaperone-mediated therapies for the treatment of Alzheimer's disease.

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