The Difference in the Binding of Phosphatidylinositol Distinguishes MAP2 from MAP2C and Tau†

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ABSTRACT: The interactions of bovine brain MAP2 and tau, recombinant murine MAP2C, and recombinant human tau with phosphatidylinositol vesicles yield apparent K_d values of 51 ± 6 nM, 2.4 ± 0.6 μM, 1.4 ± 0.1 μM, and 1.6 ± 0.2 μM, respectively. Examination of the binding of MAP2 and/or MAP2C to phosphatidylcholine vesicles doped with phosphatidylinositol or to phosphatidylserine vesicles and of thrombin-digested MAP2C to phosphatidylinositol vesicles demonstrates that the observed high affinity of the MAP2: phosphatidylinositol binding is due to the contributions of two separate interactions. A low-affinity site (K_d = 1.5–2.5 μM) is located within the C-terminal domain and affects the nonspecific interaction of MAP2, MAP2C, and tau with anionic phospholipids. The second site, with an apparent K_d of 221 ± 25 nM, is located within the MAP2-specific peptide, which is eliminated from MAP2C by differential gene splicing. It is proposed that the high affinity of MAP2 for phosphatidylinositol contributes to the spatial and temporal regulation of the dendritic cytoskeleton.

MAP2 is a dendrite-specific microtubule associated protein (MAP)[1] which promotes microtubule assembly and cross-links actin filaments. Modulation of these activities is therefore important to the control of the dendritic cytoskeleton. Indeed, the phosphorylation of the tubulin- and actin-binding domain of MAP2 by a cAMP-activated protein kinase, which copurifies with microtubules, increases the dynamic instability and the steady-state critical concentration for microtubule assembly (Burns et al., 1984; Raffaelli et al., 1992) and weakens the MAP2:microtubule and MAP2:actin interactions (Nishida et al., 1981; Selden & Pollard, 1983; Burns et al., 1984). In addition, both activities are inhibited by phosphatidylinositol (PI; Yamauchi & Purich, 1987, 1993; Surridge & Burns, 1992). Analysis of this inhibition of microtubule assembly using phosphatidylcholine (PC) vesicles doped with increasing amounts of PI demonstrated that MAP2 has a single, high-affinity PI-binding site (Surridge & Burns, 1992). PI has also been reported to inhibit the cross-linking of actin filaments by MAP2 but not that by tau, another neuronal MAP (Yamauchi & Purich, 1993). We have now determined the dissociation constant (K_d) of the MAP2:PI interaction, and we demonstrate that both tau and MAP2C, a differential splice product of the MAP2 gene, lack the MAP2 site which confers the high affinity for PI binding.

MATERIALS AND METHODS

Microtubule protein was prepared from bovine brain by two cycles of assembly and disassembly in MEM (100 mM Mes, 2.5 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, pH 6.4 with KOH; Burns & Islam, 1981) and was stored (15 mg·mL^{-1}) under liquid nitrogen. MAP2 was purified immediately prior to experimentation by fractionating the microtubule protein on phosphocellulose (Whatman P11) and sequentially eluting with MEM and 0.2 M NaCl in MEM. Whole brain tau was purified from bovine brain by heat treatment followed by fractionation on DEAE–cellulose (Kotani et al., 1985). Protein concentrations were determined by the method of Hartree (1972) or the Coomassie plus protein assay (Pierce), using bovine serum albumin as a standard.

Clones expressing murine MAP2C (pET NCO/MAP2c, a gift of Dr. Andrew Matus) and the human tau isoform (4RT/383; Goedert et al., 1989) were grown in *Escherichia coli*, and the overexpressed proteins were purified by carboxymethylcellulose chromatography (Goedert & Jakes, 1990). The *E. coli* cells (500 mL) were grown in SOB (20 mg·mL^{-1} bactopeptone, 5 mg·mL^{-1} bacto-yeast extract, 0.5 mg·mL^{-1} NaCl, 2.5 mM KCl, pH 7.0 with NaOH) at 37 °C with continuous shaking. When the A_{600} was OD 0.6–1.0, expression was induced by the addition of IPTG (0.4 mM, 2 h, 37 °C). The cells were harvested, resuspended in MEM buffer (10 mL), and ultrasonically disrupted (MSE Soniprep, 3 × 15 s, 4 °C, 20 μm). The cell homogenate was centrifuged (11 500*g*, 1 min, Eppendorf 5413), and the resulting supernatant was applied to a carboxymethyl-Sepharose column (Pharmacia CM-Sepharose Fast Flow: 8 cm × 0.9 cm diameter), preequilibrated with MEM buffer. The recombinant proteins were eluted at between 0.1 and 0.3 M NaCl in MEM, dialyzed against MEM buffer, and stored at −70 °C.

MAP2C (≈14 μM) was incubated with bovine plasma thrombin (10 units·mL^{-1}, 37 °C, 1 h). Tubulin was prepared by phosphocellulose chromatography of the microtubule protein and assembled into microtubules by incubation (20 min, 37 °C) with 40 μM taxol, 200 μM GTP, 1 mM phosphoenolpyruvate, and 1 unit·mL^{-1} pyruvate kinase. The microtubules were harvested by sedimentation through a 30% sucrose cushion (w/v in MEM, 100 000*g*, 20 min) and resuspended (≈20 μM tubulin) in MEM buffer containing 40 μM taxol.

The binding of MAPs to lipid vesicles was assessed by means of a quantitative lipid sedimentation assay. Lipid suspensions were prepared from solutions of the lipids (10 mg·mL^{-1}) in chloroform. Small volumes of these lipid solutions (typically 2 μL) were evaporated to dryness in a vacuum desiccator (15

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[1] Abbreviations: PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; MAP, microtubule associated protein.

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h), allowed to slowly form a suspension in 1 mL of MEM, and then vortexed immediately before use. The protein solutions were clarified by centrifugation (100 000*g*, 30 min) immediately before the assays were prepared. The assay mixture (typically 200 μL) contained lipid (5 μg·mL^{-1}), 67 mM NaCl, and protein (5–5000 nM) in MEM buffer. The protein binding was permitted to reach equilibrium (10 min, 20 °C) before the lipid vesicles were pelleted (100 000*g*, 30 min). The pellets were then resuspended in MEM buffer, and the protein content was determined by dot blotting onto nitrocellulose (Schleicher and Schull, pore size 0.45 μm) and staining with colloidal gold (Hunter & Hunter, 1987; Bio-Rad Laboratories Inc). The filters were well dried, soaked in mineral oil (Nakamura et al., 1985), and read at A_{405} using an Elisa microplate reader (Labsystems Uniskan II). The staining was calibrated with reference to the concentration of the stock protein. The quoted standard deviations of the slopes of the Scatchard plots were calculated by weighted least-squares regression.

All reagents were obtained from Sigma Chemical Co. Ltd., except where otherwise stated. The PI, PC, and PS were the ammonium salts of the respective bovine liver L-α-phospholipids and consisted primarily of the stearic and arachidonic acids.

RESULTS

While we showed earlier that bovine MAP2 sediments with pure PI vesicles (Surridge & Burns, 1992), we were unable to report the MAP2:PI dissociation constant (K_d). We have since established (data not shown) that an accurate determination of this K_d requires an assay using low concentrations of both the lipid and the protein, which in turn necessitates using an extremely sensitive protein assay. While one approach would be to use [γ-^{32}P]ATP to phosphorylate the MAP2, such a posttranslational modification might alter the MAP2:PI interaction. Furthermore, preliminary experiments established that the direct measurement of the protein concentration using the colloidal gold assay yielded more reliable results than a variety of more indirect methods, such as separating the bound and free MAP2 and then measuring the MAP2 concentrations by labeling the protein with [^{3}H]-iodoacetamide.

Increasing concentrations of MAP2 (50 nM to 1 μM) were incubated with a constant concentration of pure PI vesicles (≈5 μg·mL^{-1}). The vesicles were then pelleted (100 000*g*, 30 min, room temperature), and the MAP2 contents of the pellets and supernatants were determined by staining with colloidal gold. Applying a Scatchard analysis to the bound and unbound MAP2 concentrations yields an apparent K_d of 51 ± 6 nM MAP2 (Figure 1a). This value is consistent with the effects of PI on MAP2-dependent microtubule assembly, which indicated a MAP2:PI K_d of <1 μM MAP2 (Surridge & Burns, 1992).

The abscissa intercept of a Scatchard plot conventionally yields an estimate of the binding stoichiometry. This requires an accurate estimate of the available PI concentration, yet the method for preparing the lipid vesicles most probably yields a mixture of single and multilamellar vesicles. The concentration of PI available to bind MAP2 is consequently unknown, and therefore we have not sought to estimate the binding stoichiometries. Studies on the PI-induced inhibition of MAP2-dependent microtubule assembly did, however, indicate that MAP2 has a single, high-affinity PI-binding site (Surridge & Burns, 1992).

The inhibition by PI of MAP2-dependent microtubule assembly (Surridge & Burns, 1992) suggests that it binds to

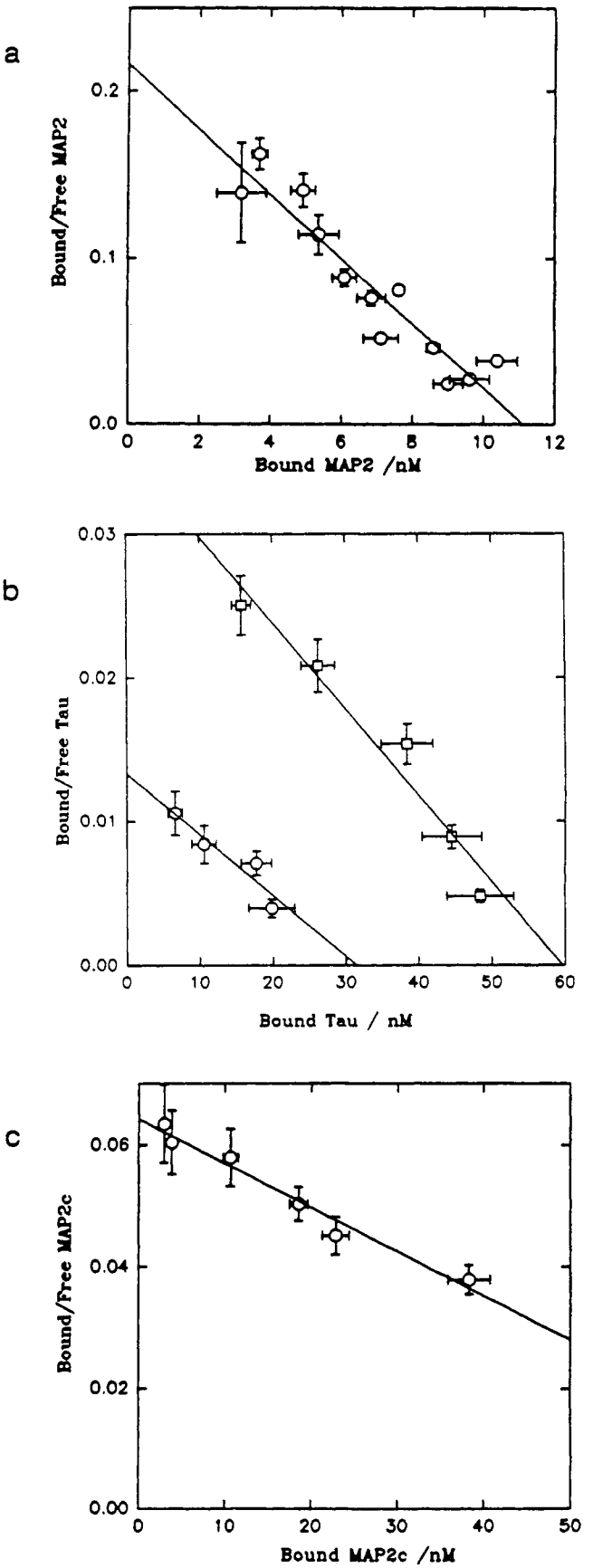

FIGURE 1: Binding of MAP2, tau, and MAP2C to pure PI vesicles. Scatchard plots of the binding of (a) native bovine MAP2, (b) native bovine brain tau (○) or recombinant human tau383 (□), and (c) recombinant mouse MAP2C to pure PI vesicles. The slopes yield the apparent K_d values: (a) 51 ± 6 nM for MAP2; (b) 2.4 ± 0.6 μM for native bovine brain tau and 1.6 ± 0.2 μM for the recombinant human tau383 construct; and (c) 1.4 ± 0.1 μM for recombinant mouse MAP2C.

the tubulin-binding site specified by peptides close to the C terminus of MAP2. We have sought to test this by comparing the binding of PI to MAP2 with that to MAP2C and tau, as each of these proteins has C-terminal peptides which are either identical or highly homologous to each other. In particular, MAP2C is a differential gene-splicing product of the MAP2 gene and differs from MAP2 only in lacking an insert of almost 1400 residues separating the common N- and C-terminal domains (Papandrikopoulou et al., 1989; Kindler et al., 1990). By contrast, MAP2 and tau share significant homology only within the C-terminal peptide. Both proteins, as well as MAP2C and the ubiquitous MAP4 (Aizawa et al., 1990), which also binds to PI vesicles (data not shown), have a series of 18-residue quasirepeats which are separated by more divergent "spacer" peptides. MAP2 (and MAP2C) have three such tandem repeats, while the differing tau isoforms have, as a result of differential gene splicing, either three or four (Himmler, 1989; Goedert et al., 1989). In addition to this high homology sequence, MAP2, MAP2C, MAP4, and tau each contain a less highly conserved proline-rich peptide which lies immediately N-terminal to these tandem repeats.

Studies involving the microtubule-binding and/or assembly properties of either expressed truncated tau isoforms (Butner & Kirschner, 1991; Kanai et al., 1992; Brandt & Lee, 1993) or synthetic peptides of certain of the individual MAP2 or tau repeats (Ennulat et al., 1989; Joly et al., 1989; Joly & Purich, 1990; Novella et al., 1992; Yamauchi et al., 1993) have clearly demonstrated that the tandem repeats contribute directly to the tubulin-binding site and to the actin-binding site (Correas et al., 1990). Similar studies with MAP4 peptides (Aizawa et al., 1990) and tau constructs (Kanai et al., 1992) have, however, indicated that there may be an additional contribution from the flanking proline-rich peptide. The inhibition by PI of both microtubule assembly and actin gelation might therefore suggest that the PI-binding site lies within the tandem repeats or this flanking peptide and that the K_d for PI binding to MAP2C and tau would be identical to the observed value for MAP2 (51 ± 6 nM).

The Scatchard analysis of the binding of unfractionated bovine brain tau to pure PI vesicles yields an apparent K_d of 2.4 ± 0.6 μM (Figure 1b), that is, the binding is nearly 50-fold weaker than predicted. As this bovine tau consists of a mixture of the 3- and 4-repeat isoforms, we have also examined the binding of an expressed human 4-, repeat isoform: the apparent K_d is 1.6 ± 0.2 μM (Figure 1b). The marked difference between these values and that observed for MAP2 suggests that the PI-binding site is specified by the subtle sequence differences within either (a) the tandem repeats of MAP2 and tau, (b) the more divergent intervening "spacer" peptides, (c) the less highly conserved proline-rich peptide, or (d) by a peptide lying outside the sequence normally considered to define the tubulin-binding (and actin-binding) domain. We have examined these alternatives by considering the interaction between PI and MAP2C.

Scatchard analysis of expressed murine MAP2C binding to pure PI vesicles yields an apparent K_d of 1.4 ± 0.1 μM (Figure 1c), i.e., a value which is comparable to that observed for the bovine brain and expressed human tau proteins but approximately 30-fold weaker than that for the native bovine MAP2. We have examined this striking difference by considering the binding of MAP2 to PI-doped PC vesicles, of proteolytic fragments of MAP2C to pure PI vesicles, and of both MAP2 and MAP2C to pure PS vesicles.

We have previously shown that PI differs from other anionic phospholipids in that microtubule assembly can be inhibited

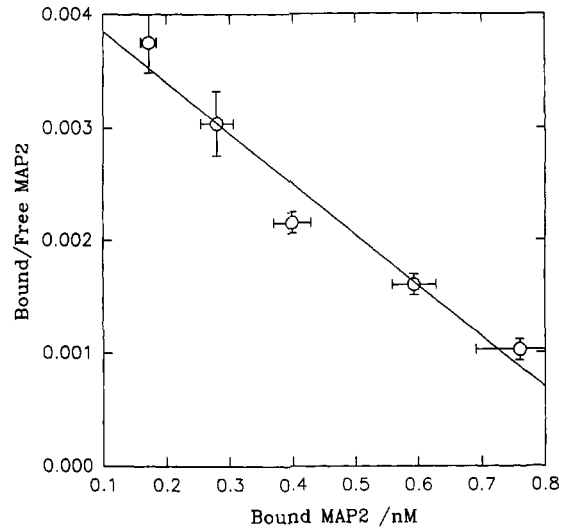

FIGURE 2: Binding of MAP2 to PI-doped PC vesicles. Scatchard plot of the binding of native bovine brain MAP2 to PC vesicles doped with small amounts of PI (1:500 PI:PC, ≈5 μg·mL^{-1} total lipid). The slope yields an apparent K_d of 221 ± 25 nM MAP2.

by PC vesicles which are doped with only trace amounts of PI (<1:1000 PI:PC; Surridge & Burns, 1992). We have therefore examined the binding of bovine MAP2 to PI-doped PC vesicles (1:500 PI:PC). The apparent K_d is 221 ± 25 nM (Figure 2); the apparent curvature of the plot is not reproducible. The affinity of this interaction is therefore much weaker than that with pure PI vesicles, in contrast with the earlier effects of PI doping on the inhibition of microtubule assembly. Lower levels of doping yielded intermediate values for the apparent K_d, while a further reduction in the PI:PC ratio did not result in any additional increase in the K_d (data not shown). Significantly, there was no detectable binding of either MAP2C or the expressed tau to PC vesicles doped with PI, even at PI:PC ratios as high as 1:9 (data not shown). Consequently, dispersion of PI molecules within a PC environment weakened the binding of MAP2 relative to the pure PI vesicles and resulted in the loss of any detectable MAP2C or tau binding.

Two approaches have been used to examine this unexpected result. First, we have examined the binding of MAP2 and MAP2C to PS vesicles. The head groups of PI and PS are of similar size and are uncharged at neutral pH, so any nonspecific anionic phospholipid effects should be common to the two lipids. Indeed, pure PS vesicles inhibit MAP2-dependent microtubule assembly (Yamauchi & Purich, 1987; Surridge & Burns, 1992). Both MAP2 and MAP2C bind to pure PS vesicles with similar apparent K_d values (1.3 ± 0.3 and 1.8 ± 0.2 μM, respectively, Figure 3). This strongly suggests that K_d values in the 1–2 μM range reflect a nonspecific interaction between the MAP molecules and charged phospholipids.

We have also examined the binding of MAP2C proteolytic fragments to pure PI vesicles. MAP2 can be cleaved by either α-chymotrypsin (Gottlieb & Murphy, 1985) or thrombin (Joly et al., 1989) to yield a C-terminal fragment which includes the tandem repeats and part of the proline-rich peptide and a second, N-terminal fragment. We have, for the first time, used thrombin to digest MAP2C (Figure 4); there is minimal secondary cleavage of MAP2C, unlike the observed secondary degradation of the full-length MAP2. From the known site of thrombin digestion (Joly et al., 1989), the calculated molecular weights of the two products are 22 543 and 26 775 Da. The two peptides are, however, readily separated by SDS-

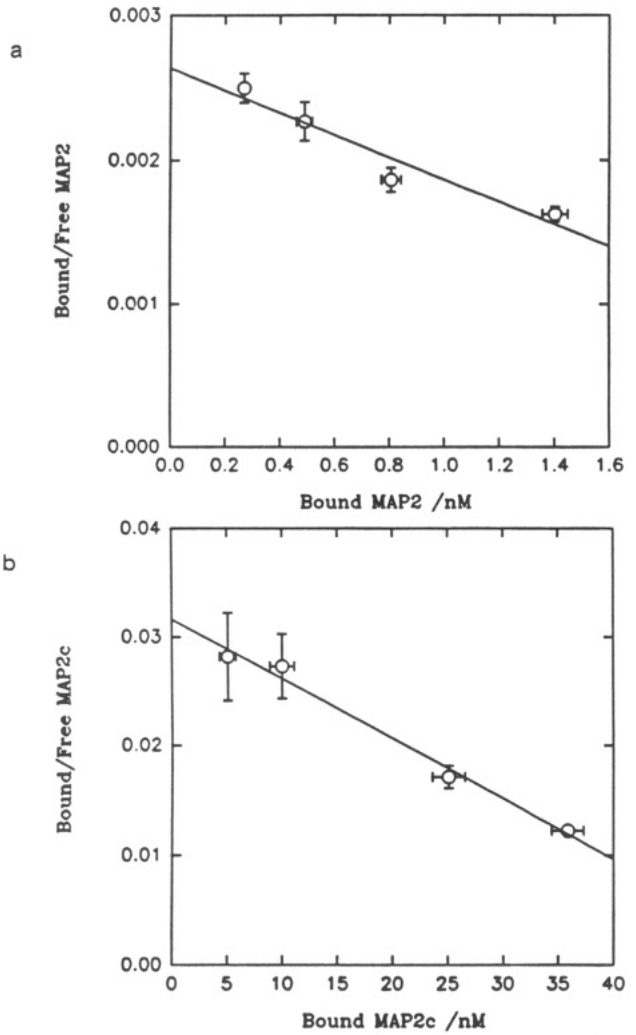

FIGURE 3: Binding of MAP2 and MAP2C to pure PS vesicles. Scatchard plots of the binding of (a) native bovine brain MAP2, apparent K_d = 1.3 ± 0.3 μM MAP2, and (b) recombinant mouse MAP2C, apparent K_d = 1.8 ± 0.2 μM MAP2C.

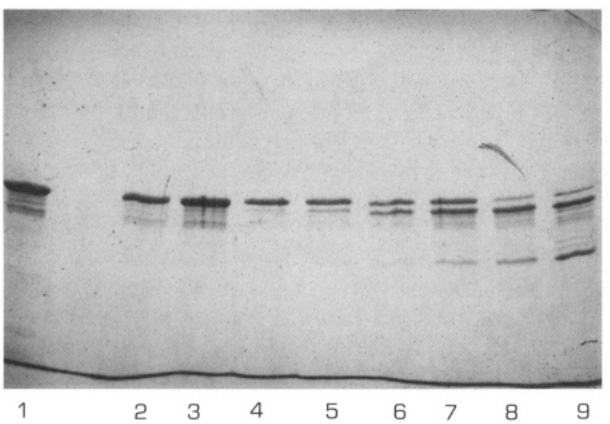

FIGURE 4: Thrombin digestion of MAP2C. Recombinant mouse MAP2C (lane 1) was digested with thrombin (1 unit·mL^{-1}, 37 °C) for 0 (lane 2), 1 (lane 3), 2 (lane 4), 5 (lane 5), 10 (lane 6), 15 (lane 7), 30 (lane 8), and 60 (lane 9) min. There are two principle proteolytic fragments, one running immediately ahead of the undigested MAP2C and the other with a much faster mobility, even though the calculated molecular weights are 49 301 Da for MAP2C and 22 543 and 26 775 Da for the two fragments.

PAGE (Figure 4): one has a mobility which is about twice that of the intact MAP2C, while the other migrates only slightly ahead of the undigested protein. In order to identify which of these two peptides contains the C-terminal tubulin-

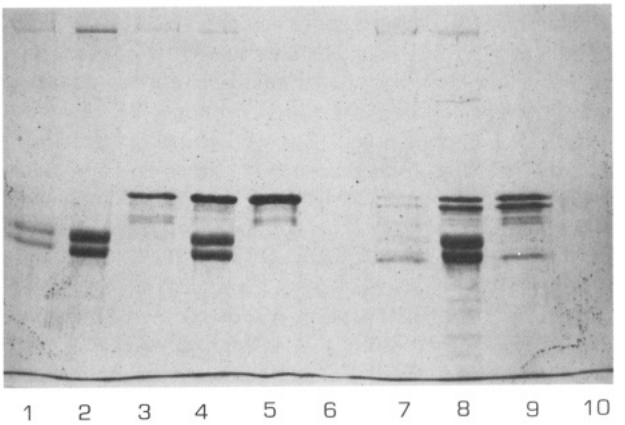

FIGURE 5: Characterization of the mcirotubule-binding fragment of MAP2C. Recombinant mouse MAP2C (≈14 μM) and thrombin-digested MAP2C (≈14 μM, see Figure 4 for digestion conditions) were incubated with or without taxol-stabilized microtubules (≈20 μM assembled tubulin) and then pelleted (100 000*g*, 30 min, room temperature). The 10% SDS–PAGE shows the supernatants (lanes 1, 3, 5, 7, 9) and the pellets following resuspension to an equivalent volume (lanes 2, 4, 6, 8, 10). Lanes 1 and 2: the supernatant and pellet of the control taxol-stabilized microtubules which contain a trace amount of contaminating MAP2. The supernatants and pellets following the co-incubation of undigested MAP2C with (lanes 3 and 4) and without (lanes 5 and 6) taxol-stabilized microtubules. The supernatants and pellets following the co-incubation of thrombin-digested MAP2C with (lanes 7 and 8) and without (lanes 9 and 10) taxol-stabilized microtubules. Note the selective recovery of the less mobile thrombin fragment with the assembled microtubules (lane 8), while both it, the faster migrating thrombin fragment, and the residual undigested MAP2C are primarily recovered in the supernatant if the assembled microtubules are omitted (lane 9).

binding domain, the digested protein was incubated with taxol-stabilized microtubules. Fractionation of the pelleted protein and the supernatant by SDS–PAGE shows that the slower migrating fragment preferentially binds to the pelleted microtubules (Figure 5) and therefore is the C-terminal peptide. The anomalous migration of this peptide, which contains the tandem repeats, may relate to its highly cationic nature. A fraction of the N-terminal peptide may also bind weakly (compare Figure 5, lanes 7 and 9), but any quantitation will require a N-terminal-specific antibody as this peptide comigrates with β-tubulin.

This characterization of the MAP2C thrombin fragments has permitted us to map the low-affinity interaction of MAP2C with pure PI vesicles. The thrombin-digested protein was incubated with the pure PI vesicles, which were then pelleted (100 000*g*, 30 min, room temperature). Analysis by SDS–PAGE of the pelleted and supernatant fractions shows that only the more slowly migrating, C-terminal peptide binds to the vesicles (Figure 6).

DISCUSSION

MAP2, the differential splice product MAP2C, and tau each bind to pure PI vesicles (Figure 1a–c): MAP2C and tau have very similar dissociation constants (1.4–2.4 μM), while MAP2 binds with a markedly lower apparent K_d (51 nM). In summary, the high-affinity PI binding (apparent K_d = 51 nM) is observed with native MAP2 but not with recombinant MAP2C or with either native or recombinant tau. This indicates that the 1364-residue MAP2-specific peptide, which is eliminated from MAP2C by differential gene splicing, contributes to the PI-binding site and/or that the native MAP2 has a posttranslational modification which specifically *enhances* PI binding. Phosphorylation of MAP2, however, *weakens* the MAP2:tubulin and MAP2:actin interactions [e.g.,

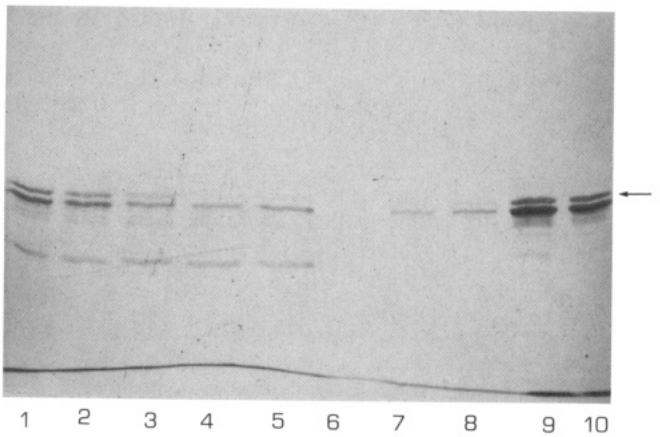

FIGURE 6: Characterization of the PI-binding fragment of MAP2C. Thrombin-digested recombinant mouse MAP2C (see Figure 4 for details) was incubated with increasing amounts of pure PI vesicles and then pelleted (100 000*g*, 30 min, room temperature). The resulting supernatants (lanes 1–5) and pellets (lanes 6–10) are shown following incubation with 0 (lanes 1 and 6), 0.1 (lanes 2 and 7), 0.2 (lanes 3 and 8), 0.5 (lanes 4 and 9), and 1.0 (lanes 5 and 10) mg·mL^{-1} PI vesicles. Note the increasing recovery in the pellet of the undigested MAP2C (arrowed) and the slowly migrating fragment, which corresponds to the tubulin-binding domain (Figure 5), and minimal recovery of the faster migrating N-terminal domain.

Nishida et al. (1981); Selden and Pollard (1983), Burns et al. (1984), Raffaelli et al. (1992)] and is unlikely to enhance the binding of an anionic phospholipid. The possible role of a posttranslational modification in enhancing PI binding is further diminished by the similarity between the binding constants of native MAP2 and recombinant MAP2C to PS vesicles (apparent K_d values of 1.3 and 1.8 μM respectively, Figure 3). It is therefore concluded that the MAP2-specific peptide contributes to the high-affinity interaction between MAP2 and PI.

The apparent binding constants of MAP2 and MAP2C to PS vesicles (apparent K_d values of 1.3 and 1.8 μM, respectively, Figure 3) are similar to those for MAP2C and tau to PI vesicles (1.4 and 1.6–2.4 μM, respectively, Figure 1b and c). This strongly suggests that a nonspecific, low-affinity interaction is defined by the highly homologous C-terminal peptides of MAP2, MAP2C, and tau. The importance of this C-terminal peptide, with its tandem repeats of the tubulin-binding domain, to the low-affinity interaction is further demonstrated by its specific binding to PI vesicles following thrombin digestion of MAP2C (Figure 6). Apparent K_d values in the range of 1.5–2.5 μM are therefore due to the nonspecific interaction between anionic phospholipids and the highly cationic C-terminal peptides common to MAP2, MAP2C, and the tau isoforms, while the high-affinity MAP2:PI interaction (K_d = 51 nM) appears to require the MAP2-specific peptide.

There are a variety of possible reasons for the small difference in the binding of native bovine brain tau and the recombinant human tau to pure PI vesicles (2.4 ± 0.6 μM *vs* 1.6 ± 0.2 μM, Figure 1b). In addition to the differing sources and potential levels of phosphorylation, the bovine tau was isolated by boiling under denaturing conditions (Kotani et al., 1985), while the recombinant tau was not heat-treated. The difference may, however, relate to the differing isoform composition, since brain tau consists of a mixture of up to six isoforms (Himmler, 1989; Himmler et al., 1989; Goedert & Jakes, 1990) containing three or four of the tandem repeats, while the recombinant protein (tau383) specifically contains the four repeats. The tighter binding of the recombinant protein may therefore reflect the enhanced charge of the more extensive and highly cationic tubulin-binding domain.

The inhibition of MAP-dependent microtubule assembly by vesicles composed of various anionic phospholipids is retained even if they are doped with small amounts of inert phospholipid such as PC. This inhibition is lost if the PC content of these vesicles rises above a critical value, typically about 50% (Reaven & Azhar, 1981; Surridge & Burns, 1992). This does not apply to PI, in that PC vesicles which are doped with only trace quantities of PI (PI:PC < 1:1000) retain their ability to inhibit microtubule assembly (Surridge & Burns, 1992). Surprisingly, this doping reduces the MAP2:PI interaction, with the apparent K_d increasing from 51 ± 6 nM for the pure PI vesicles to 221 ± 25 nM for the 1:500 PI:PC doped veiscles (Figures 1a and 2).

There are a number of possible reasons for this apparent difference. First, there is a problem in interpreting data relating to the binding of large ligands to a homogenous lattice in that a ligand bound to a single site may obstruct the binding to a number of surrounding sites. This results in a nonlinear (convex downwards) Scatchard plot (McGhee & von Hippel, 1974), although the plots may appear linear at low ligand concentrations. The slopes of such plots give an underestimate of the dissociation constant, such that:

$$K_d^{app} = K_d(n/2n - 1)$$

where K_d and K_d^{app} are the actual and apparent dissociation constants and n is the number of lattice points covered by a single ligand molecule. This analysis, achieved by considering the binding of a ligand to a one-dimensional lattice, has been applied to the binding of protein ligands to DNA (Kowalczykowski et al., 1986), tropomyosin to actin (Wegner, 1979), and MAP2 to taxol-stabilized microtubules (Wallis et al., 1993). The binding of MAP2, MAP2C, and tau to phospholipid vesicles differs from these examples in that it involves the interaction of a large ligand with a two-dimensional lattice. This complicates the analysis considerably since a complete theoretical description would need to include the orientations of the ligands on the lattice and the consequential loss of entropy as the ligand binding approaches saturation. Experimental evidence shows that the binding of MAP2 masks about 200 PI molecules (Surridge & Burns, 1992), and so the McGhee and von Hippel analysis might predict a 2-fold underestimate of the dissociation constant. Additional discrepancies may result from the two-dimensional nature of the phospholipid lattice. The difference between the observed 51 nM K_d for pure PI vesicles and the observed 221 nM K_d for the PI:PC doped vesicles may therefore reflect this "footprinting" effect.

There is, however, an alternative explanation which is consistent with the doping data, the low-affinity (apparent K_d = 1.5–2.5 μM) binding of MAP2C and tau to pure PI vesicles, the similar K_d for MAP2 and MAP2C to PS vesicles, and the weaker binding of the bovine brain tau to pure PI vesicles than that to the four-repeat recombinant protein. This alternative proposes that MAP2 differs from MAP2C and tau in having an additional high-affinity PI-binding site, which is located within the MAP2-specific peptide and which is detected when the PI molecules are dispersed in a neutral PC environment. The PI:PC doping experiments indicate that the K_d of this specific PI-binding site is 221 ± 25 nM (Figure 2). It is proposed that the tighter binding of MAP2 to pure PI vesicles results from the interaction at this site coupled with a second, low-affinity site corresponding to the nonspecific interaction of the highly cationic C-terminal tubulin-binding domain with the anionic phospholipids. By contrast, only this second interaction can be detected when assaying the binding

of MAP2C or tau to pure PI vesicles or the binding of MAP2 or MAP2C to PS vesicles.

This generation of a high-affinity interaction from the additive effects at two weaker binding sites resembles the high-affinity binding to tau to the microtubule lattice resulting from the tandem arrangement of the low-affinity repeats within the tubulin-binding domain (Butner & Kirschner, 1991). The apparent K_d of the MAP2-specific site (221 nM) would be equivalent to an apparent binding free energy of -37.9 kJ·mol^{-1}, while the free energy for the MAP2 binding to pure PI vesicles would equate to -41.6 kJ·mol^{-1}. This difference is less than that potentialy available from the nonspecific interaction (ΔG $-14.8/-16.1$ kJ·mol^{-1}) and may relate to the discrepancies resulting from an analysis of a two-dimensional lattice. Alternatively, the nonadditive contributions may imply that binding at the high-affinity site does not inevitably result in binding at the nonspecific site. This would contrast with the additive contributions and consequently cooperative binding by the individual repeats (and "spacers") to the total free energy of tau binding to microtubules (Butner & Kirschner, 1991).

The striking difference between the binding of MAP2 and MAP2C to pure PI vesicles, coupled with the undetectable binding of MAP2C to PI-doped PC vesicles, strongly suggests that the high-affinity PI-binding site lies within the 1364-residue, MAP2-specific insert. While the tertiary structure of MAP2 is unknown, this insert presumably contributes to the 90-nm side arm observed by electron microscopy (Gottlieb & Murphy, 1985). The high-affinity PI-binding site may therefore be located some distance from the C-terminal tubulin-binding domain. Indeed, spatial separation of the high- and low-affinity binding sites is consistent with the nonadditive contributions to the total free energy of binding.

The reported difference in the effects of PI on MAP2- and tau-induced actin gelation (Yamauchi & Purich, 1993) could be due to tau lacking the MAP2-specific high-affinity PI-binding site. This, however, seems unlikely as the reported concentration of PI (50–100 μM) would be sufficient to saturate both the MAP2-specific high-affinity site and the low-affinity site of the common C-terminal peptides: a specific effect would be expected only when using PI doping of an inert lipid. Significantly, phosphatidylethanolamine, PS, and PC showed no effect on the MAP2-induced gelation, yet PS vesicles would be predicted to bind to the highly cationic C-terminal peptide containing the actin-binding (and tubulin-binding) site. Finally, it may be relevant that the molar concentration of tau required to effect an equivalent gelation of the F-actin was 5-fold higher than that for MAP2. The MAP-dependent promotion of microtubule assembly may therefore involve a different mechanism to the MAP-dependent actin gelation.

In addition to the deletion of the MAP2-specific insert, MAP2C differs from MAP2 in both the timing of its expression during development and its spatial localization. MAP2C is preferentially synthesized during early embryogenesis, while MAP2 accumulates at a latter stage (Tucker et al., 1988; Matus, 1991). Furthermore, MAP2C is distributed throughout the neuron, while MAP2 is specifically localized to dendrites and dendritic spines (Caceres et al., 1984; Bernhardt & Matus, 1984; De Camilli et al., 1984; Tucker et al., 1988). By contrast, tau is synthesized throughout development, although there is a developmentally related difference in the expression of specific isoforms, and is predominantly found in axonal compared with dendritic processes. The MAP2:PI interaction may contribute to these differing temporal and spatial distributions of MAP2 compared with MAP2C and tau. In particular, the MAP2:PI interaction may modulate the availability of MAP2 in the dendritic process. This could be analogous to the PIP2:profilin and PIP2:gelsolin interactions of macrophages and other cells, particularly if a receptor-mediated event were to activate a phospholipase and result in the release of membrane-associated MAP2. *In vitro* studies show that MAP2 both promotes microtubule assembly by lowering the steady-state critical concentration and cross-links F-actin. Consequently, a local or temporal increase in the free MAP2 concentration with the dendritic process might be expected to enhance microtubule assembly and/or actin gelation. Significantly, we have found that phospholipase C-γ_1 releases MAP2 from PI vesicles and abolishes the PI-induced inhibition of MAP2-dependent microtubule assembly and that both processes have similar time courses (data not shown).

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