Ca^{2+}–Calmodulin Regulated Effectors of Microtubule Stability in Bovine Brain†

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ABSTRACT: Stable microtubules (as defined by resistance to Ca^{2+}, drug or cold temperature induced disassembly) form in abundance during tubulin assembly in brain crude extracts. We have previously shown that, in rat brain crude extracts, all microtubule stabilizing activity could be ascribed to a single Ca^{2+}–calmodulin binding and Ca^{2+}–calmodulin regulated protein, called "stable tubule only polypeptide", $STOP_{145}$ [Pirollet, F., Rauch, C. T., Job, D., & Margolis, R. L. (1989) *Biochemistry 28*, 835–842]. We have now performed an exhaustive study of STOP-like effectors in bovine brain high-speed supernatants. All activity binds to cation exchangers and to Ca^{2+}–calmodulin affinity columns. The activity can be resolved into two peaks on sizing columns. The first eluted peak contains a prominent 220-kDa protein. The second peak contains an apparently homogeneous 20-kDa polypeptide. A monoclonal antibody specific to rat brain $STOP_{145}$ recognizes the 220-kDa protein, but not the 20-kDa species. The 220-kDa protein can be purified on a STOP antibody column and accounts for the bulk of stabilizing activity in the first peak. The 20-kDa protein does not bind to STOP antibody affinity columns. Sequence analysis of oligopeptide fragments of the 20-kDa protein shows 100% homology with bovine myelin basic protein (MBP). Anti-MBP antibodies recognize the 20-kDa, but not the 220-kDa species. We conclude that the 220-kDa protein is the bovine equivalent to rat brain $STOP_{145}$ and that the 20-kDa species is MBP. Microtubule stabilization by MBP and $STOP_{220}$ is abolished in the presence of Ca^{2+}–calmodulin, and inhibition curves are similar for both proteins. Our findings, together with previously published evidence of microtubule–MBP functional relationships in vivo, offer the possibility that MBP and STOPs effect microtubule stability in similar ways although in different cell types.

In living organisms, microtubules can be either highly labile, disassembling in response to subtle variations of their physicochemical environment, or extremely stable and hard to dissociate into their component subunits (Dustin, 1984). In neuronal tissues, the existence and the functional importance of such highly stable microtubules has long been recognized (Heidemann et al., 1984; Baas & Heidemann, 1986).

Compared to labile microtubules, stable microtubules are enriched in acetylated and detyrosinated tubulin. However, it is now evident that these modifications of tubulin are the consequence, not the cause, of microtubule stability. It has been demonstrated that the microtubule stability state is not influenced by the tyrosination state of tubulin (Schultze et al., 1987; Paturle et al., 1989). Further, it has been shown that heavily acetylated tubulin derived from stable microtubules of the sea urchin sperm tail assembles into labile microtubules in vivo (Farrell, 1982).

It is almost certainly the case in mammalia that microtubule stability results from the action of specialized proteins. Unlike other MAPs,[1] these proteins are not purified by standard microtubule purification procedures which rely on cycles of warm temperature assembly of polymers, interspersed with cold temperature disassembly.

Their purification would therefore rely on biochemical procedures coupled with a sensitive assay of microtubule stabilization to cold temperature or dilution. The elucidation of these effectors has now been made possible by the development of such an assay ("STOP assay"), which allows the detection of microtubule stabilizing activity in complex extracts (Pirollet et al., 1989). Applied to rat brain crude extracts, this procedure has allowed us to demonstrate that all detectable activity can be ascribed to a single 145-kDa polypeptide ($STOP_{145}$) which could be purified by sequential elution from cation exchange and Ca^{2+}–calmodulin columns (Margolis et al., 1986; Pirollet et al., 1989).

In the present study, we have purified microtubule cold stabilizing activity from bovine brain. We find two distinct activities, one a protein of 220-kDa, and the other a protein of 20-kDa. Both activities are Ca^{2+}–calmodulin regulated. The 220-kDa protein reacts with a monoclonal antibody which is highly specific for $STOP_{145}$ in rat brain crude extracts. This protein can be purified to homogeneity by the use of a rat STOP antibody affinity column. We conclude that the 220-kDa protein is a beef brain homologue of rat brain STOP. The other protein does not react with the antibody. Sequence analysis shows it is apparently identical with myelin basic protein. As MBP is intimately associated with microtubules in cell bodies and processes of oligodendrocytes (Dyer & Benjamins, 1989a,b; Wilson & Brophy, 1989), our data suggest it may function as a microtubule stabilizing protein within these regions of oligodendrocytes.

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[1] Abbreviations: MBP, myelin basic protein; STOP, stable tubule only polypeptide; MAP, microtubule associated protein; MME, buffer composed of 100 mM Mes [2-(*N*-morpholino)ethanesulfonic acid], 1 mM $MgCl_2$, 1 mM EGTA [ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], pH 6.75; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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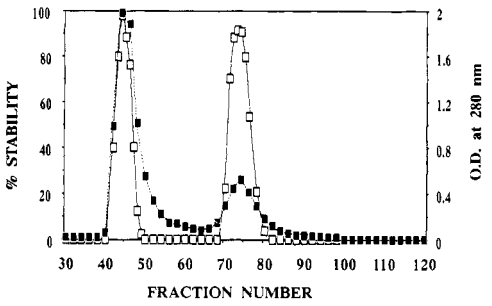

FIGURE 1: Separation of two peaks of bovine STOP activity. Bovine STOP activity was partially purified and loaded onto an Aca 54 column as described under Experimental Procedures. Protein concentration, as o.d., at 280 nm (closed squares) was recorded for each 300-μL column fraction. Aliquots (8 μL) of each fraction were then used for filter assay of cold stability (open squares), as described under Experimental Procedures.

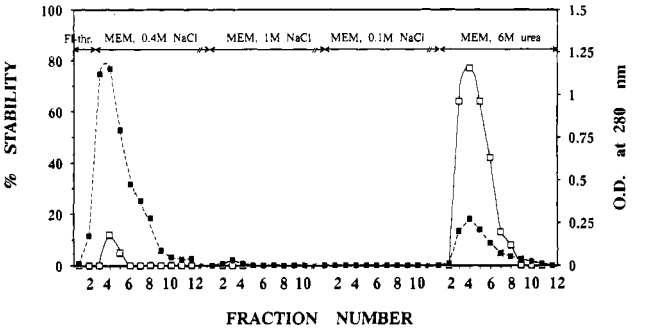

FIGURE 2: Immunoaffinity chromatography of STOP activity contained in the Aca 54 column peak 1. Active fractions from Aca 54 peak 1 were loaded onto an immunoaffinity column containing a covalently linked anti-rat STOP monoclonal antibody, as described under Experimental Procedures. Column fractions (300 μL) were assayed for protein content by $o.d._{280}$ measurement (closed squares). Aliquots (10 μL) of each fraction were then assayed for STOP activity (open squares), as described under Experimental Procedures. During the washes, assays were restricted to the fractions corresponding to the three first column volumes.

EXPERIMENTAL PROCEDURES

Purification of Beef Brain STOP Activities. Two beef brains (600 g) from a local slaughterhouse were processed at 0–4 °C within one hour after slaughter. All buffers contained 2 mM dithiothreitol, 10 μg/mL leupeptin, 10^4 units/mL aprotinin, 1 mM phenylmethanesulfonyl fluoride unless otherwise indicated. The brains were homogenized (1:1, g/mL) in MME buffer using a Waring Blendor (3 strokes, 15 s, low speed). The homogenate was centrifuged at 140000g for 30 min at 4 °C. The supernatant (=cytosol) was made 10% in glycerol (v/v) and loaded onto a 4 cm × 4 cm Sepharose-S Fast Flow column (Pharmacia) After extensive washing with 20 volumes of MME containing 0.1 M NaCl, STOP activity was eluted with MME containing 0.4 M NaCl. The eluate was adjusted to 2 mM calcium and passed through a 3 cm × 3 cm Calmodulin–agarose column (Sigma) equilibrated in 0.4 M NaCl, and 2 mM $CaCl_2$–MME buffer. All STOP activity bound to the column. The column was washed with the same buffer (10 volumes), and STOP activity was eluted with MME containing 0.4 M NaCl and 1 mM EGTA. After concentration with Ultraprep 30 (Millipore), the active pool was loaded onto a gel filtration column of Aca 54 (0.9 cm × 55 cm, IBF) in 0.4 M NaCl–MME buffer, containing no dithiothreitol. Two protein peaks were resolved, and each contained STOP activity. STOP activity from peak 1 was concentrated on Centricon 30 (Amicon) and further purified by an immunoaffinity column. This column was generated as previously described (Pirollet et al., 1989) with a monoclonal anti-rat-STOP antibody designated Mab-175. STOP activity was absorbed on the column (1 cm × 1.5 cm). The unabsorbed proteins were eluted with 10 mL of MME containing 0.4 M NaCl. The column was then washed with 10 mL of 1 M NaCl–MME buffer and further with 0.1 M NaCl–MME. STOP activity was eluted with MME containing 6 M urea as described for rat brain $STOP_{145}$ (Pirollet et al., 1989).

Purification of Rat Brain $STOP_{145}$. Pure $STOP_{145}$ was prepared from rat brain cytosol, according to published procedures (Pirollet et al., 1989).

Monoclonal and Polyclonal Antibodies. A series of monoclonal antibodies was raised against rat brain $STOP_{145}$, as previously described (Pirollet et al., 1989). They were designated Mab-296, Mab-175, and Mab-378. A polyclonal antibody was raised in a rabbit according to an injection schedule with 100 μg of rat $STOP_{145}$ for each injection. For antigen preparation, the affinity-purified $STOP_{145}$ was run into preparatice SDS–8% polyacrylamide gels, and the band of interest was excised and homogenized with PBS and Freund's adjuvant. Eight immunizations with the gel slice slurry were given subcutaneously. We have also produced a mouse monoclonal antibody, called Mab-5C3, against bovine $STOP_{220}$. BALB/c mice were injected with $STOP_{220}$ immunoaffinity-purified as described above, further run into preparative SDS–polyacrylamide gels, excised, and electroeluted. Spleen cell suspension from immunized mice was fused with SP2O myeloma cells, and hybridoma culture supernatants were screened for anti-bovine STOP reactivity in an ELISA assay. Hybridomas were selected, subcloned twice by limiting dilution, and produced in quantity in mouse ascitic fluid. The monoclonal antibody 5C3 is an IgM.

STOP Assay. The glass fiber filter assay of microtubule cold stability was performed as previously described (Pirollet et al., 1989). Briefly, cold-labile three-time recycled microtubule protein was assembled at 1.5 mg/mL in MME buffer in the presence of 0.05 mM [^{3}H]GTP, 10 mM acetyl phosphate, and 0.5 μg/mL acetate kinase. After a 30-min incubation at 30 °C, 40-μL aliquots were mixed with 2 mL of MME buffer equilibrated at 30 °C, containing 40% sucrose and 40 μM GTP. Samples were either immediately cross-linked by addition of 80 μL of 25% glutaraldehyde (total assembly level determination) or incubated an additional 5 min in the presence of an aliquot of a fraction to be assayed (up to 100 μL). The mixture was then maintained for 40 min on ice to disassemble all but cold-stable microtubules and then cross-linked as above (cold-stability level). Blanks correspond to cold-stability levels observed in the absence of added fraction. Samples were then applied to GF/C glass fiber filters (Whatman) on which microtubules are trapped and assayed for radioactivity. Results, expressed as percent of stability, are the ratio of the cold-stability levels minus blanks versus the total assembly levels minus blanks. In some experiments, pure tubulin microtubules were used in the assay. Pure tubulin was prepared and assembled into microtubules at 2 mg/mL as previously described (Job et al., 1985).

Microtubule Immunofluorescence Assay. Microtubules were cross-linked, centrifuged onto coverslips, and labeled with anti-β tubulin (Sigma) and FITC-anti-mouse (Biomakor) antibodies following published procedures (Pirollet et al., 1987).

Sequence Analysis. The peak 2 polypeptide (20 μg) from the Aca 54 column was digested with 0.5 μg of *Staphylo-*

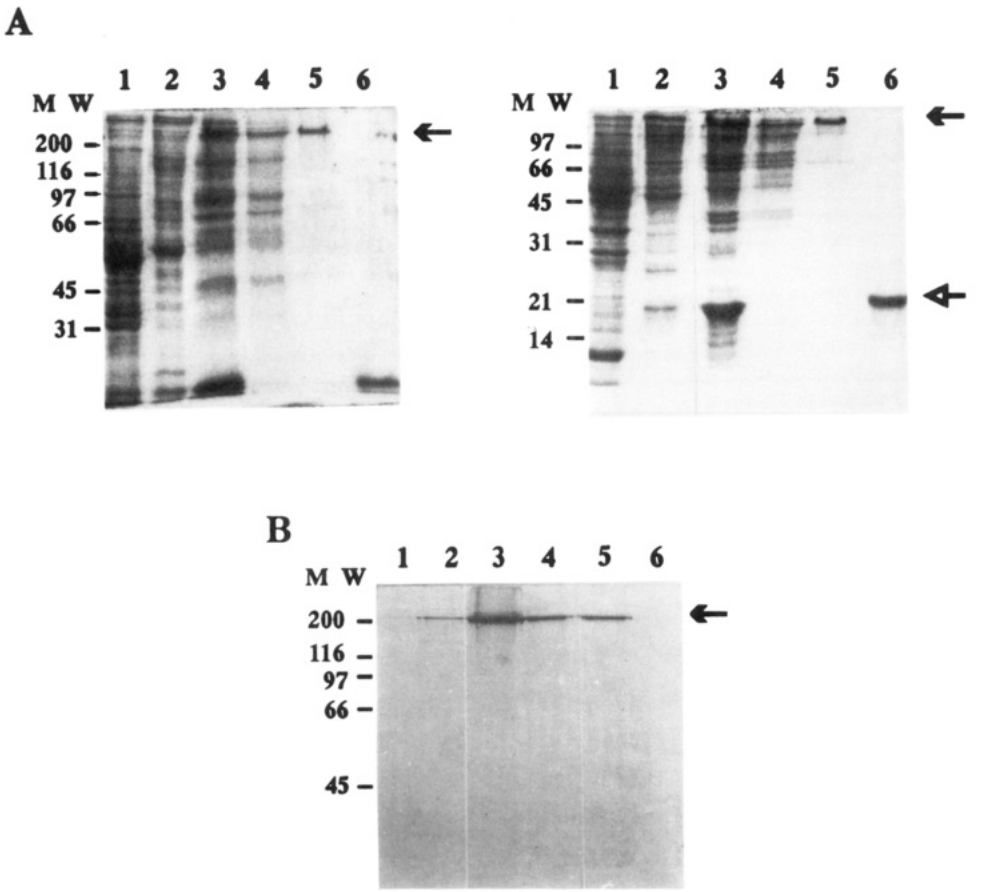

FIGURE 3: Electrophoresis and immunoblot analysis of fractions containing STOP activity, at each purification step. Active protein fractions were analyzed by 8% SDS–PAGE (panel A, left) or 12% SDS–PAGE (panel A, right): lane 1, cytosol; lane 2, Sepharose-S column eluate; lane 3, calmodulin-affinity column eluate; lane 4, Aca 54 column peak 1; lane 5, Mab-175 immunoaffinity column bound fraction (of peak 1); lane 6, Aca 54 column peak 2. The black arrow indicates the 220-kDa protein and the white arrow, the 20-kDa protein. Immunoblot analysis corresponding to the 8% SDS–polyacrylamide gel lanes is shown in panel B: Mab-175 is used as primary antibody.

coccus aureus V8-protease (Boehringer) overnight at 37 °C. Reversed-phase HPLC was carried out on a butyl Aquapore column (100 × 2.1 mm) with an acetonitrile gradient (0–60% in 0.1% trifluoroacetic acid) at a flow rate of 0.2 mL/min. Peptides, monitored by densitometry at 220 nm, were further purified with the same operating procedure on a C8 Aquapore column. Two peptides were selected for sequencing. Sequence determinations were performed using a 470.A gas-phase sequencer on-line coupled to a 120.A PTH analyzer (Applied Biosystems), both operated according to the manufacturer's specifications. Database searching and sequence management were done with the Bisance system from CITI2, Paris (Dessen et al., 1990).

Other Methods. SDS–polyacrylamide gel electrophoresis (using either 8% or 12% acrylamide gels) was performed as described (Laemmli & Favre, 1973). Proteins were stained with Coomassie-blue R or transferred to nitrocellulose sheets according to Towbin et al. (1979). Blots were exposed to various anti-STOP antibodies at 1:10 000 dilution or anti-MBP rabbit antiserum at 1:2 000 and reacted with alkaline phosphatase conjugated secondary antibodies (Promega). The protein concentrations were determined by Bradford assay (Bradford, 1976).

RESULTS

Bovine brain STOP activity shows chromatographic properties similar to the ones previously described for rat brain STOP activity (Pirollet et al., 1989). All the activity can be purified on Sepharose-S cation exchange and Ca^{2+}–calmodulin affinity columns (see Experimental Procedures and Figure 3). However, after these two steps of purification, the activity resolves into two peaks on Aca 54 sizing columns (Figure 1). The first peak (peak 1) contains a prominent 220-kDa polypeptide and other high molecular weight proteins (Figure 3A, left). The second peak (peak 2) contains a homogeneous 20-kDa protein (Figure 3A, right).

Chromatographic fractions were analyzed on immunoblots using monoclonal antibodies previously developed against rat $STOP_{145}$ (see Experimental Procedures). Three monoclonal antibodies, Mab-296, Mab-175, and Mab-378, were tested for cross-reactivity with bovine proteins. These antibodies react only with $STOP_{145}$ in rat brain crude extracts as was previously shown in the case of Mab-296 (Pirollet et al., 1989). However, Mab-296 and Mab-378 do not react with any protein in bovine crude extracts (data not shown). We find that Mab-175 reacts with a 220-kDa polypeptide in bovine crude extracts and that this antigen copurifies with peak 1 STOP activity (Figure 3B). In some preparations, Mab-175 also reacts with a polypeptide of apparent molecular mass 170-kDa (Figure 3B, lane 2). This protein does not bind to Ca^{2+}–calmodulin affinity columns. It might represent an inactive proteolytic product of the 220-kDa protein. Mab-175 does not show cross reactivity with the 20-kDa protein contained in peak 2.

Peak 1 was loaded onto an immunoaffinity column containing the covalently linked Mab-175, and absorbed proteins were eluted in urea as previously described (Pirollet et al., 1989) (Figure 2). Subsequent SDS–PAGE analysis showed a specific absorption of the major 220-kDa protein contained

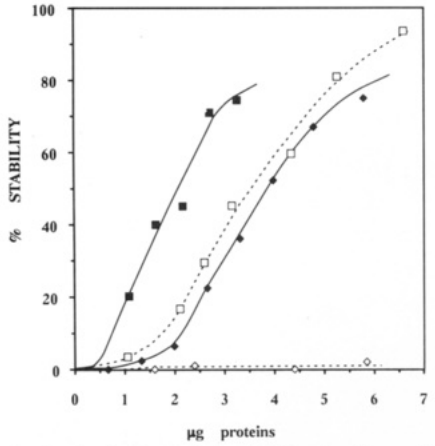

FIGURE 4: Analysis of microtubule stabilization by purified protein fractions. The panel shows the 3×-microtubule cold-stability assay described under Experimental Procedures upon addition of increasing titers of the protein contained in Aca 54 column peak 1 (open squares), peak 2 (closed diamonds), Mab-175 immunoaffinity unbound (open diamonds), and bound (closed squares) fractions.

Table I: Purification of STOP Activity from Bovine Brain Extract[a]

fraction	total act., STOP units	sp. act., STOP units/ mg of prot	yield, %	factor of purifn
cytosol	6981	1.6	100.00	1
Sepharose S-eluate	6495	54	93.04	34
calmodulin agar.-eluate	2341	140	36.04	88
Aca 54 peak 1	543	330	7.78	206
anti-STOP aff. col.-eluate	152	495	2.18	309
Aca 54 peak 2	522	250	7.48	156

[a] Conditions of cold-stability assays were as described under Experimental Procedures. Cytosol represents the protein fraction loaded onto a Sepharose-S column; Sepharose S-eluate represents the fraction eluted from a Sepharose-S column in 0.4 M NaCl. Calmodulin agar.-eluate represents the fraction eluted from a Calmodulin–agarose column in 0.4 M NaCl and 1 mM EGTA. Aca 54 peak 1 and Aca 54 peak 2 represent the first and the second pool of proteins separated onto the Aca 54 column; anti-STOP aff. col.-eluate represents the Aca 54 peak 1 that specifically bound to a Mab-175 affinity column and which was eluted with 6 M urea, as described under Experimental Procedures. Specific activity and total activity determinations: a dose–effect curve was constructed for STOP activity at each stage of purification. We define one STOP activity unit as the amount of STOP activity which yields a 50% cold-stability level under standard assay conditions. Total activity in STOP units is thus equal to the ratio of the total volume of the fraction under study to the volume of the aliquot which yields a 50% cold-stability level.

in peak 1 to the affinity column (Figure 3A). Fractions were also analyzed for STOP activity (Figure 2). The bulk of the peak 1 activity was absorbed onto the immunoaffinity column and could be recovered in the urea fractions containing the 220-kDa protein (Figure 2). A minor percentage of activity flowed through the column, but the corresponding specific activity was low as shown in Figure 2 and, in more quantitative terms, in Figure 4.

Taken together, these results show that, in bovine brain extracts, most STOP activity is accounted for by the presence of two proteins, of apparent molecular masses 220 and 20 kDa, respectively. A purification table of this activity is shown in Table I. Because of its reactivity with the highly specific Mab-175, and its similar chromatographic properties and activity, and because no antigen of 145 kDa is evident in bovine extracts, we conclude that the 220-kDa protein is the bovine equivalent of $STOP_{145}$ and designate it $STOP_{220}$. The immunological cross-reactivity of $STOP_{145}$ and $STOP_{220}$ was

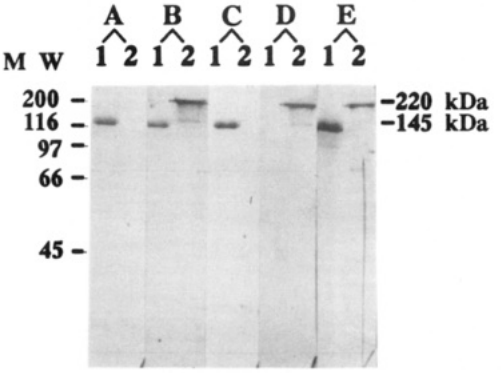

FIGURE 5: Western blot analysis of rat $STOP_{145}$ and bovine $STOP_{220}$ using various monoclonal or polyclonal antibodies as probes. 0.4 µg of purified rat $STOP_{145}$ (lanes 1) and bovine $STOP_{220}$ (lanes 2) were run into 8% SDS–polyacrylamide gels, transferred onto nitrocellulose sheets and immunostained. Various antibodies, described under Experimental Procedures, were used as primary probes: A, Mab-296; B, Mab-175; C, Mab-378; D, Mab-5C3; E, polyclonal anti-$STOP_{145}$ antibody.

further investigated by using a battery of monoclonal antibodies and one polyclonal antibody (Figure 5). Results confirm the homology of the two proteins since they react with equal intensity with the same polyclonal antibody and reveal the existence of interspecific differences since several monoclonal antibodies do not show cross-reactivity.

The protein with STOP activity present in peak 2 on the Aca 54 column did not bind to the Mab-175 affinity column. The question arose whether the 20-kDa peptide was a proteolytic fragment of $STOP_{220}$ or a different protein. The 20-kDa peak 2 protein was subjected to sequence analysis. After 20-kDa protein digestion with Staphylococcus V8 protease, two peptides, of 21 and 23 residues, when purified and sequenced, showed 100% homology with bovine MBP (Figure 6A). Further, specific anti-MBP antiserum recognized the isolated protein on Western blots but revealed no cross-reactivity between MBP and the 220-kDa protein (Figure 6B). We conclude that the 20-kDa peptide is MBP and that MBP and $STOP_{220}$ show no extensive structural homology. Comparison of $STOP_{220}$ and MBP microtubule stabilizing activity showed a higher specific activity, on a molar basis, for $STOP_{220}$ (Figure 4 and Table I).

Immunofluorescence images of pure tubulin microtubules stabilized by $STOP_{220}$ or MBP (Figure 7) show differences in their properties. Microtubules stabilized by $STOP_{220}$ appear as individual filaments. MBP, in contrast, appears to induce some microtubule cross-linking, although individual polymers are also visible. Interestingly, $STOP_{145}$ has been shown to generate cross-linked microtubule bundles similar to those observed with MBP (Margolis et al., 1990).

Both MBP and $STOP_{220}$ bind Ca^{2+}–calmodulin with high affinity (Figure 3). As $STOP_{145}$ stabilization of microtubules is regulated by Ca^{2+}–calmodulin (Margolis et al., 1986; Pirollet et al., 1989), we have examined the effect of Ca^{2+}–calmodulin on both MBP- and $STOP_{220}$-induced microtubule stability (Figure 8). In this assay, the level of free calcium ions is too low to induce the disassembly of stable microtubules in the cold by itself. The addition of calmodulin specifically inhibits microtubule stabilization by either MBP or $STOP_{220}$ (Figure 8). Therefore, MBP, like STOPs, appears to be a Ca^{2+}–calmodulin binding and Ca^{2+}–calmodulin regulated effector of microtubule stability.

DISCUSSION

From the early stages of development of the microtubule field, Ca^{2+} and Ca^{2+}–calmodulin regulated proteins have been

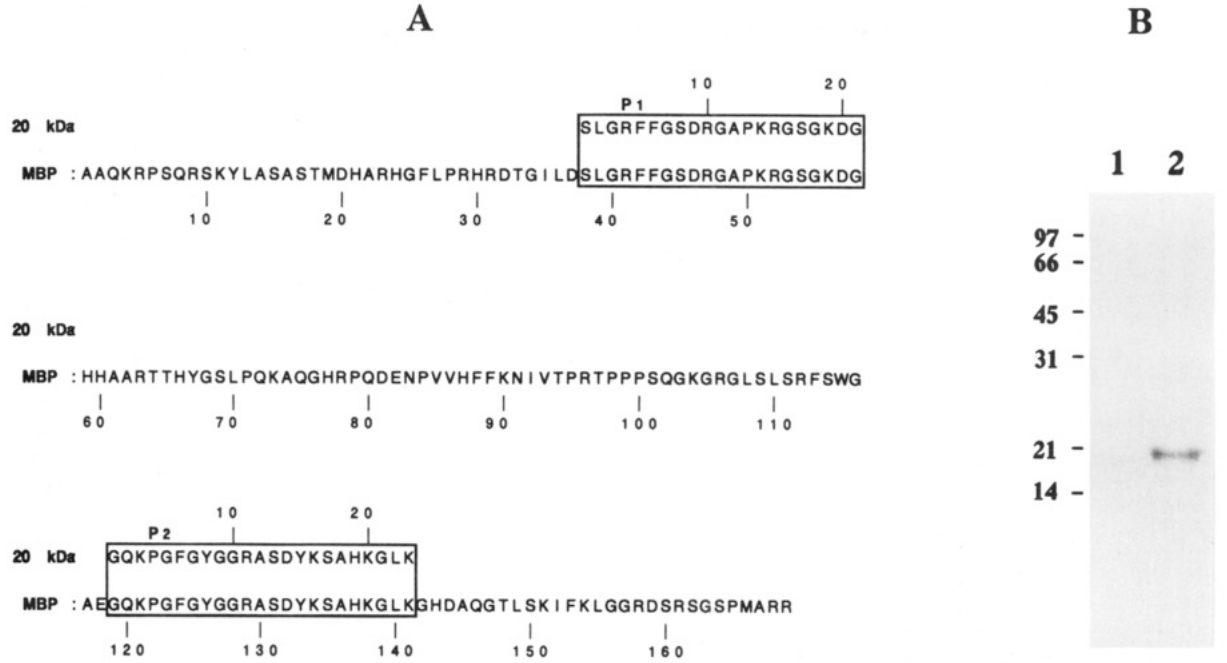

FIGURE 6: Sequence and immunological analysis of the 20-kDa peptide. The Aca 54 peak 2 was V8-protease digested, and two peptides P1 and P2 were sequenced after HPLC purification, as described under Experimental Procedures. In panel A, the peptide sequences were aligned with the published sequence of bovine MBP (Eylar et al., 1971; Brostoff et al., 1974) and identical residues were boxed. Panel B shows the immunoblot analysis corresponding to lanes 5 and 6 of the 12% SDS–polyacrylamide gel in Figure 3A, right, using a polyclonal MBP antibody as primary antibody.

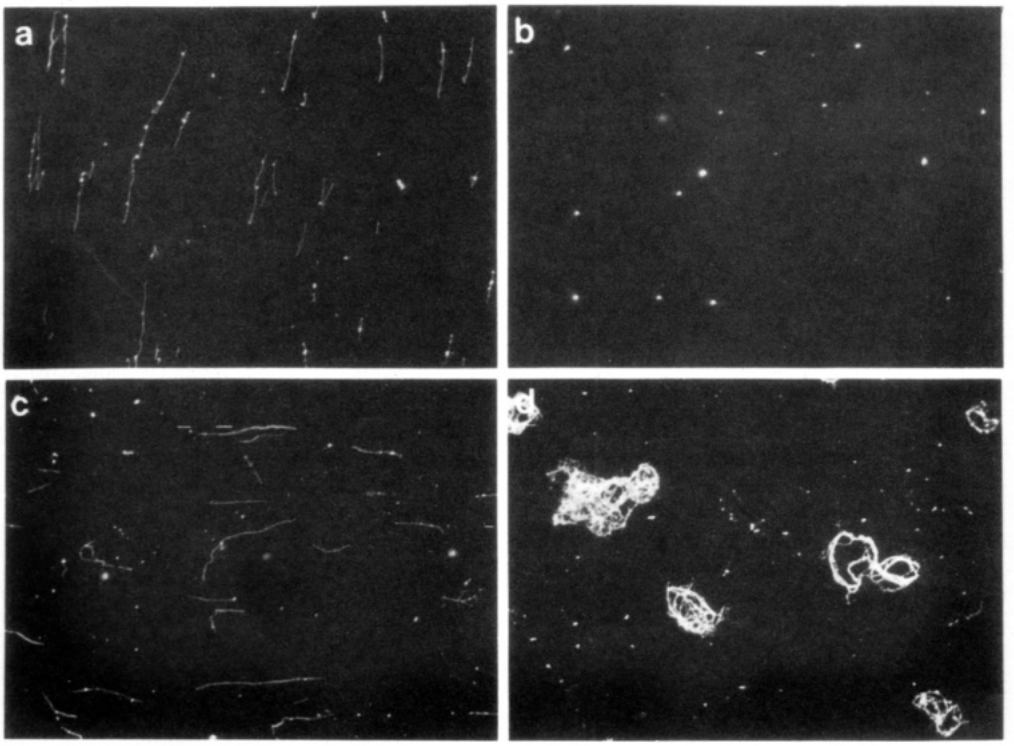

FIGURE 7: Immunofluorescence analysis of microtubule stabilization by $STOP_{220}$ or by MBP. Immunofluorescence analysis was performed in parallel with filter assays of microtubule cold stability. For description of the assays, see Experimental Procedures. Pure tubulin microtubules were used in this experiment. Immunofluorescence analysis was carried out as described under Experimental Procedures, on 2-μL aliquots withdrawn from the various microtubule solutions just prior to filtration. Images correspond to the following: a, total assembly levels; b, blanks; c, cold-stability level in the presence of 11.5 μg of Aca 54 column peak 1 proteins; d, cold-stability level in the presence of 11 μg of Aca 54 column peak 2 proteins.

thought to be important effectors of the microtubule assembly state and stability. Microtubules disassemble in the presence of Ca^{2+} (Weisenberg, 1972; Nishida & Sakai, 1977). This effect is enhanced in the presence of calmodulin (Berkowitz & Wolff, 1981). Classical microtubule-associated proteins, MAP_2 and τ have been proposed to be Ca^{2+}–calmodulin binding and Ca^{2+}–calmodulin regulated proteins (Lee & Wolff, 1984). However, their affinity for Ca^{2+}–calmodulin is probably low since they do not bind to immobilized calmodulin affinity columns in the presence of high ionic strength buffers.

We have developed assay procedures which have allowed an exhaustive characterization of microtubule effectors capable of inducing complete stability transition, i.e., which induce

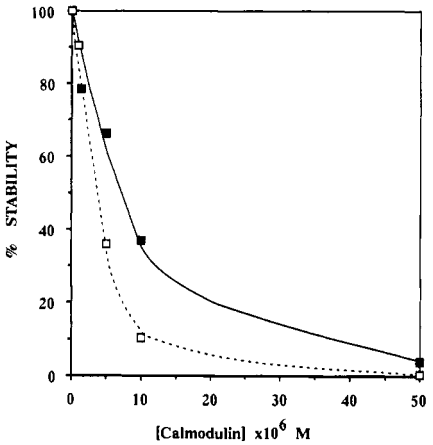

FIGURE 8: Effect of calmodulin on microtubule stabilization by $STOP_{220}$ or by MBP. Pure tubulin microtubules were assembled, diluted in sucrose MME, mixed with aliquots of Aca 54 column peak 1 (closed squares) or peak 2 (open squares), and subjected to filter assay of microtubule cold stability under conditions described under Experimental Procedures. The assay was performed in the presence of 0.7 mM $CaCl_2$ and variable amounts of VU-1 calmodulin (Roberts et al., 1985). In the absence of calmodulin, 30% of the assembled microtubules were stabilized to cold temperature by 17 μg of peak 1 protein or 19 μg of peak 2 protein. This value is represented as 100% in the graph.

microtubule resistance to cold temperature or millimolar Ca^{2+} concentration, in rat brain crude extracts (Pirollet et al., 1989). We have shown that a single protein, $STOP_{145}$, accounted for the whole microtubule-stabilizing activity. Rat $STOP_{145}$ behaves as a bona fide Ca^{2+}–calmodulin binding protein on calmodulin affinity columns, and its activity is abolished in the presence of Ca^{2+}–calmodulin.

We now report finding two proteins with similar microtubule cold-stabilizing activity in bovine brain tissue. One of the proteins, of 220 kDa, is almost certainly the beef brain homologue of rat brain $STOP_{145}$. A monoclonal antibody that is highly specific for rat brain STOP recognizes only this 220-kDa protein in beef brain extracts and is capable of selectively purifying it from a complex mixture of proteins. Like $STOP_{145}$, the 220-kDa protein has microtubule cold-stabilizing activity and is regulated by Ca^{2+}–calmodulin. Rat brain $STOP_{145}$ is uniquely neuronal and has been detected in this cell type in immunofluorescence studies (Margolis et al., 1990, and unpublished observations). This finding is expected to apply as well to bovine $STOP_{220}$. An intriguing observation is the difference in the apparent molecular weight of bovine and rat STOPs. It might have little functional significance. Large molecular weight differences have been previously observed among cytoskeletal components apparently having similar functions. This is the case, for example, of MAP_2, τ proteins (Goedert et al., 1991), and tektin proteins (Linck et al., 1985). What is special in the case of STOPs is that only one molecular form seems to be present in a given species while these proteins show interspecific variations. Knowledge of the size of the corresponding cDNAs will limit the number of assumptions which can be made regarding this observation.

The other protein is MBP. As is true of STOPs, MBP behaves as a bona fide calmodulin binding protein during purification, and its effect is inhibited by Ca^{2+}–calmodulin. However, the two proteins originate from different tissues. MBP, in contrast to neuronal STOPs, is uniquely present in oligodendroglial cells (Raine, 1984). The fact that we have not previously detected its microtubule-stabilizing activity in rat brain crude extracts is most likely due to differences in the homogenization procedures in preparation of rat and beef brain extracts. Using similarly harsh homogenization procedures, we have recently found MBP-dependent microtubule-stabilizing activity present in rat brain extracts (unpublished observations).

We have previously shown that nonneuronal tissues also contain Ca^{2+}–calmodulin effectors capable of triggering microtubule cold stability (Pirollet et al., 1989). However, these effectors appear to be somewhat different from brain STOPs since monoclonal antibodies specific for rat brain $STOP_{145}$ did not recognize antigens in microtubule-stabilizing fractions from other tissues (Pirollet et al., 1989). Taken together, these findings suggest that most tissues contain Ca^{2+}–calmodulin-dependent effectors capable of inducing complete microtubule stability transition from labile to stable states and that they have tissue specificity. Such diversity is common among cytoskeletal components, and examples include the different intermediate filament proteins (Steinart & Parry, 1985), distinctive actins (Schwob & Martin, 1992), tubulins (Pratt & Cleveland, 1988; Oakley & Oakley, 1989), and proteins with actin bundling or capping activity (Stossel et al., 1985).

The question arises as to whether MBP stabilization of microtubules has physiological meaning. In the early stages of research on microtubule biochemistry, the observation that several different proteins had similar effects on microtubule dynamics, and that these effects could be mimicked by other nonspecific basic proteins, raised questions about the specificity of these interactions (Dustin, 1984). However, subsequent work has, in all cases, shown that microtubule-associated proteins isolated in vitro were important physiological effectors of the microtubule system (Butner & Kirschner, 1991; Vallee, 1990).

MBP is a strongly basic protein (Lees & Brostoff, 1984), and this fact could revive old concerns. However, our exhaustive studies of STOP activity in brain extracts point to the extreme specificity of the assay procedure for microtubule cold stability, which has identified only two proteins with such activity. Furthermore, there is evidence for the association of MBP with microtubules in oligodendrocyte cell cultures (Dyer & Benjamins, 1989a,b; Wilson & Brophy, 1989). It is evident, from these studies, that there is a distinct spatial and functional relationship between MBP and the microtubule network in cell bodies, processes, and developing membrane sheets of cultured oligodendrocytes.

Due to its importance in pathology (Eylar et al., 1970; Itoyama et al., 1980; McKhann, 1982) and its abundance (Raine, 1984), MBP has been the subject of intense scrutiny. Among its properties, it has been reported to bind to Ca^{2+}–calmodulin and to interact with tubulin (Modesti & Barra, 1986; Chan et al., 1990). The present study suggests that these two properties are functionally related and indicates a well-defined biological activity for MBP that correlates with its physical association with the microtubule cytoskeleton (Dyer & Benjamins, 1989a,b). The relatively small size of MBP should facilitate the elucidation of the mechanisms of microtubule stabilization by MBP and of its regulation by Ca^{2+}–calmodulin.

Microtubules have been implicated as playing a major role in the morphogenesis of the shape of various cell types (Dustin, 1984). Oligodendroglial cells and MBP will present a unique model system for determining the role of the interaction between microtubules, microtubule-stabilizing proteins, and membranes in cell morphogenesis. Microtubules are believed to play a vital role in this process, and their ability to mold

oligodendrocyte cell shape is possibly controlled by proteins that link between microtubules and the plasma membrane (Dyer & Benjamins, 1989a,b; Fischer et al., 1990). Stabilization of microtubules within this framework can act to lock in changes in cell morphology in oligodendrocyte projections. The existence of MBP-defective mouse mutants (Tosic et al., 1990; Popko et al., 1987) should be of great help in order to establish the significance of MBP–microtubule interactions.

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