Identification and Characterization of an ATP-Mg-dependent Protein Phosphatase from Pig Brain*

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Substantial amounts of ATP-Mg-dependent phosphorylase phosphatase (Fc*·M) and its activator (kinase Fα) were identified and extensively purified from pig brain, in spite of the fact that glycogen metabolism in the brain is of little importance. The brain Fc·M was completely inactive and could only be activated by ATP-Mg and Fα, isolated either from rabbit muscle or pig brain. Kinetical analysis of the dephosphorylation of endogenous brain protein indicates that Fc·M could dephosphorylate 32P-labeled myelin basic protein (MBP) and [32P]phosphorylase a at a comparable rate and moreover, this associated MBP phosphatase activity was also strictly kinase Fα/ATP-Mg-dependent, demonstrating that MBP is a potential substrate for Fc·M in the brain. By manipulating MBP and inhibitor-2 as specific potent phosphorylase phosphatase inhibitors, we further demonstrate that 1) Fc·M contains two distinct catalytic sites to dephosphorylate different substrates, and 2) brain MBP may be a physiological trigger involved in the regulation of protein phosphatase substrate specificity in mammalian nervous tissues.

Protein phosphorylation is now recognized as an important control mechanism in the regulation of nervous functions and may represent the only approach available to study the molecular basis for a wide variety of neurophysiological phenomena. A diversity of brain proteins for the kinases have been found and the functional role of these substrate proteins in the brain have been gradually established (1). In contrast to the extensive study of the kinases, very little attempt has been made to identify and characterize the nature of phosphoprotein phosphatases in the nervous tissues (1-4). To our knowledge, calcineurin, a Ca2+/calmodulin-dependent protein phosphatase (5, 6) is the only well-characterized brain phosphatase that is capable of dephosphorylating many substrates including phosphorylase kinase and inhibitor-1 (5), phospho-casein and phosphohistone (6), phosphoesters and free phosphotyrosine (7, 8), and 4 endogenous brain substrates (9).

An ATP-Mg-dependent protein phosphatase has been identified in liver, heart, and skeletal muscle from rat and rabbit (10). The enzyme exists in an inactive form consisting of a catalytic protein Fc and a modulator subunit M, a unique protein previously characterized as a phosphatase inhibitor-2 (11). The inactive Fc·M complex can be activated in the presence of ATP-Mg and a protein factor termed Fα (12), which has also been identified as a glycogen synthase kinase (13, 14). The activated phosphatase becomes able of modifying activities of several enzymes involved in the regulation of glycogen metabolism (12, 15, 16). The ATP-Mg-dependent protein phosphatase is likely a prime target for regulation (17, 18). In the present study, we extend the observation and find out that tremendous amount of Fc·M and its activator Fα also exist in the brain, in spite of the fact that glycogen metabolism is of little importance in the nervous tissues. We further characterize brain Fc·M and find out that the concentration of the inactive Fc·M is relatively high in sharp contrast to the awfully low content of the active phosphatase-1 (2) in the pig brain, when comparing with the rabbit skeletal muscle or liver system (2, 12). By a combination of anion-exchange chromatography, gel filtration, hydrophobic and affinity columns, brain Fc·M and Fα were purified. In an attempt to further identify the possible physiological role that Fc·M might play in the nervous tissues, we have tested the endogenous pure brain protein as substrate and demonstrate that brain Fc·M is a very potential myelin basic protein phosphatase, using 32P-MPB1 and [32P]phosphorylase a as substrates for competitive study and by manipulating MBP and inhibitor-2 as specific phosphorylase phosphatase inhibitors, we further demonstrate that Fc·M contains two distinct catalytic sites to dephosphorylate different substrates. The possible physiological role of the brain MBP involved in controlling the substrate specificities of the ATP-Mg-dependent protein phosphatase in mammalian nervous tissues is proposed.

EXPERIMENTAL PROCEDURES
Materials—[γ-32P]ATP was from ICN; ATP, polylysine, cAMP, H2A histones, dihydrotestosterone from Sigma; DEAE-Sephadex A-50, CM-Sephadex C-50, Sephacryl S-200, Blue Sepharose CL-6B, and CNBr-activated Sepharose 4B from Pharmacia; ACA 34 Ultragel from LKB, and DEAE-cellulose and CM-cellulose were from Whatman.

Protein Purification—Phosphorylase b (19), phosphorylase kinase (20), inhibitor-2 (21), ATP-Mg-dependent protein phosphatase (Fc·M) (12) and its activator (Fα) (13) were purified from rabbit skeletal muscle. The catalytic subunit of CAMP-dependent protein kinase was isolated from pig heart following the procedures essentially as described in Ref. 22. The eucaryophilic MBP was purified to homogeneity from pig heart basically according to Ref. 20 and kindly provided by Dr. W.-C. Chang, Institute of Biological Chemistry, Academia Sinica and Institute of Biochemical Sciences, National Taiwan University. In addition to its ability to induce experimental encephalomyelitis in guinea pigs, this protein exhibited the characteristic properties of mammalian MBPs; high content of glycine and basic amino acids, presence of methylated arginine residues, and absence of cystine or cystein and high isoelectric point (pI > 10). The molecular weight as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was 20,000.

*This work was supported in part by the National Science Council and the National Tsing Hua University in Taiwan, Republic of China. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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The abbreviations used are: MBP, myelin basic protein; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
Preparation of 32P-labeled Protein Substrates—[32P]Phosphorylase a was prepared from phosphorylase b using 0.15 mM [γ-32P]ATP (200 cpm/pmol), 15 mM Mg acetate, 0.1 mM CaCl2, and pure phosphorylase b kinase. 32P-labeled MBP was prepared by incubating 1 mg of pure MBP with 0.2 mM [γ-32P]ATP, 20 mM Mg acetate, and catalytic amount of cAMP-dependent protein kinase at 30 °C for 60 min. The reaction was terminated by 20% trichloroacetic acid and resuspended in buffer containing 20 mM Tris, pH 7.0.

Enzyme Assay—Spontaneously active phosphorylase phosphatase, ATP-Mg-dependent phosphatase and its activator Fa activities were determined essentially as described in Refs. 12 and 13. The activity of ATP-Mg-dependent phosphorylase phosphatase was commonly measured after a 10-min preincubation at 30 °C with 0.1 mM ATP, 0.5 mM Mg acetate, and saturating amounts of Fa required for full activation under these conditions. One unit of phosphatase was defined as the amount of enzyme which releases 1 nmol of [32P]phosphate/min at 30 °C from 32P-phosphorylated protein substrates. Fa, as an activating factor for the ATP-Mg-dependent phosphatase was measured by the formation of activated Fa-M in a 10-min preincubation at 30 °C. The assay mixture contained appropriate dilutions of Fa, 0.1 mM ATP, 0.5 mM Mg acetate, and saturating amounts of purified Fa-M. The assay time is 5 min. One unit of Fa was defined as the amount of protein which produces 1 unit of activated ATP-Mg-dependent phosphatase activity after a 10-min preincubation. MBP phosphatase activity was assayed at 30 °C in buffer containing 20 mM Tris, pH 7.0, 1 mg/ml of bovine serum albumin, 0.16 mg/ml of 32P-labeled MBP and different dilutions of enzyme by measuring the release of 32P as described in Ref. 12. One unit of MBP phosphatase was defined as the amount of enzyme which releases 1 nmol of [32P]phosphate/min at 30 °C from 32P-MBP.

Analytical Methods—Protein determinations were carried out by the method of Bradford (24) or estimated from the absorbance at 280 nm. Native polyacrylamide gel electrophoresis was performed according to Davis (25). A pre-run was done without sample in order to remove substance used in the preparation of the gels, which could interfere with the subsequent determination of the enzyme activities.

RESULTS

Identification of the ATP-Mg-dependent Phosphorylase Phosphatase (Fa-M) in the Pig Brain—200 grams of fresh pig brain were homogenized in 2.5 volumes of 50 mM Tris-HCl, pH 7.8, 30 mM 2-mercaptoethanol, 4 mM EDTA, and 1 mM EGTA. The homogenate was centrifuged and the resulting supernatant was directly absorbed onto a DEAE-cellulose column (2.6 × 40 cm). The column was first extensively washed with buffer A (20 mM Tris, pH 7.0, 15 mM 2-mercaptoethanol). The breakthrough proteins were saved for assay later (13) and the DEAE column was eluted with a 1200-ml linear gradient of 0.05-0.35 M NaCl in buffer A and 104 fractions were collected. Optical density (O.D.) at 280 nm was measured at each fraction. Inactive type-2 phosphatase activity was measured after a 10-min preincubation with Fa and ATP-Mg. The inactive Fa-M activity was calculated by subtracting the active phosphatase-1 activity from the total phosphatase activity.

<table>
<thead>
<tr>
<th>Tissue species</th>
<th>Active phosphatase-1</th>
<th>Inactive Fa-M</th>
<th>Total type-1 phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig brain</td>
<td>6,500</td>
<td>5,500</td>
<td>12,000</td>
</tr>
</tbody>
</table>

Fig. 1. Chromatography of pig brain extracts on DEAE-cellulose. Pig brain extracts were filtered through glass wool and applied to a DEAE-cellulose column (2.5 × 40 cm). The column was eluted with a 1200-ml linear gradient of 0.05-0.35 M NaCl in buffer A and 10-ml fractions were collected. Optical density (O.D.) was measured at each fraction. Inactive type-1 phosphatase activity was measured after a 10-min preincubation with Fa and ATP-Mg. The inactive Fa-M activity was calculated by subtracting the active phosphatase-1 activity from the total phosphatase activity.
To simplify the purification schemes, only the major peak fractions were collected. Optical density were applied to a column (2.5 x 34 cm) and eluted with buffer B containing 0.2 M NaCl and eluted with a 100-ml breakthrough proteins which contained virtually all the Fc-M enzyme was successively applied to polylysine-Sepharose 4B (1 x 10 cm). The resin was first washed extensively with buffer B containing 0.2 M NaCl and eluted with a 100-ml linear gradient going from 0.2 to 0.6 M NaCl. As seen in Fig. 3A, brain Fc-M was eluted in a sharp peak but with a rather broad shoulder, which may be the degraded products. In order to simplify the purification schemes, only the major peak fractions were pooled, dialyzed, and rechromatographed on the second polylysine-Sepharose column in an identical way. We found that brain Fc-M somehow was much easier than rabbit muscle enzyme (12) to be purified to near homogeneity, because brain Fc-M activity was successively applied to polylysine-Sepharose 4B column were applied to a second one and processed in the same way. Symbols are as in Fig. 2.

**TABLE II**

Purification of the pig brain ATP-Mg-dependent phosphatase

Starting material was 200 g of fresh pig brain. The ATP-Mg-dependent phosphatase activity was measured at maximum dilution and assayed as indicated under "Experimental Procedures." As shown in Fig. 3B where we can see that the brain Fc-M activity was perfectly overlapped with the protein profile. The enzyme was thus pooled and concentrated by dialysis against 20% polyethylene glycol to 1 ml and then against 60% glycerol in buffer B. The enzyme was stored as such at -20 °C and used as brain Fc-M for all the further experiments throughout the whole text. The extent of purification and yields at each separation step in the preparation of this enzyme are presented in Table II. The reason why the amount of enzyme at DEAE-cellulose stage was taken as 100% activity was simply because Fc-M activity could not be easily detected in the extracts. It is interesting to note that on 10% polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate, the purified brain Fc-M still contained three major protein bands with molecular weights corresponding to the rabbit muscle enzyme reported in Refs. 11 and 34 (not illustrated), although on nondenaturing 6% native gel electrophoresis, only one major protein band could be revealed (see below). Therefore, it is really difficult at this moment to rule...
Identification and Purification of ATP-Mg-dependent Phosphatase Activator Fₐ from Pig Brain—The DEAE-cellulose breakthrough solution as previously described was used as starting material for identification and purification of brain Fₐ, and directly applied to a CM-cellulose column (2.6 × 40 cm). The resin was first washed with buffer A and then eluted with a 1200-ml linear gradient from 0 to 0.2 M NaCl. [³²P]Phosphorylase a and purified rabbit skeletal muscle Fₐ-M (12) were used to assay the Fₐ activity as the ATP-Mg-dependent phosphorylase phosphatase activator (13) in the collected fractions. As shown in Fig. 4A, a rather sharp symmetrical Fₐ peak was obtained, demonstrating that the ATP-Mg-dependent phosphatase activator Fₐ-M, which has also been identified as a specific glycogen synthase kinase (13, 14) indeed exists in the brain, despite that glycogen metabolism is of little importance. This brain Fₐ enzyme was therefore also decided to be purified and thus pooled as indicated in Fig. 4A and concentrated by 30–60% ammonium sulfate fractionations. After extensive dialysis, the Fₐ fractions were next applied to a column (1 × 10 cm) of Blue Sepharose CL-6B and eluted. As can be seen in Fig. 4B, most of the impurities were separated out and Fₐ activities were eluted at a place where almost no protein could be detected. The peak fractions were pooled as indicated in Fig. 4B and concentrated by dialysis against 20% polyethylene glycol down to 1 ml and then against 40% glycerol. This enzyme preparation (specific activity around 40,000 units/mg as the Fₐ-M activator) was stored as such at -20 °C and used as brain Fₐ throughout the whole text.

Identification of Brain Fₐ-M as a Potent Myelin Basic Protein Phosphatase—As shown in Table III, the purified brain Fₐ-M was found to be completely inactive towards [³²P]phosphorylase a and could only be activated very specifically in the presence of 0.1 mM ATP, 0.5 mM Mg acetate, and saturating amounts of kinase Fₐ-M, isolated either from pig brain or rabbit skeletal muscle, indicating that both Fₐ-M and Fₐ enzymes purified from nervous and non-nervous tissues could be used interchangeably. However, since Fₐ-M is the major phosphorysine, phosphorylase kinase and glycogen synthase phosphatase are responsible for the coordinate control of glycogenolysis and glycogenesis in liver, heart and skeletal muscles (10, 12, 15–18), and since glycogen metabolism is of little importance in the nervous tissues, it is believed that this kinase Fₐ-M/ATP-Mg-dependent protein phosphatase may play another important role other than glycogen metabolism in the brain. In an attempt to identify the possible physiological substrate for brain Fₐ-M, the MBP purified from pig brain and ³²P-labeled by cAMP-dependent protein kinase was used as a testing substrate. As can be seen in Table III, the brain Fₐ-M was found to be able to catalyze the dephosphorylation of ³²P-labeled MBP and [³²P]phosphorylase a at a comparable rate and moreover, this MBP phosphatase activity like phosphorylase phosphatase activity was also strictly kinase Fₐ-M/ATP-Mg dependent (see Table III). In addition, the apparent Kₘ value of ³²P-MBP for brain Fₐ-M was found to be only 4 μM which was more than 3 times lower than that of [³²P]phosphorylase a (12.5 μM). Further evidence that brain Fₐ-M represents the only entity responsible for the dephosphorylation of both phosphorylase a and phosphorylated MBP was obtained by showing that both phosphatase activities were co-purified throughout all the possible separation steps including DEAE-cellulose ion exchange chromatography, ACA 34 Ultrogel filtration, ammonium sulfate fractionation, Blue-Sepharose CL-6B and polysine-Sepharose 4B columns and even on native polyacrylamide gel electrophoresis, both enzyme activities were found to co-migrate perfectly and correspond to the major staining protein band (Fig. 5). All the results taken together demonstrate that brain Fₐ-M is a very potent myelin basic protein phosphatase. It is interesting to note that even when the myelin basic protein was ³²P-labeled by rabbit skeletal muscle phosphorylase kinase on the serine residues totally unrelated to those sites phosphorylated by cAMP-dependent protein kinase (27), we found that the activated Fₐ-M was also capable of removing all the ³²P labels from the phosphorylated...
TABLE III

<table>
<thead>
<tr>
<th>Kinase F_2/ATP-Mg-mediated activation of brain F_2, M on phosphorylase phosphatase and MBP phosphatase activities</th>
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<tr>
<td>Assay conditions were as described under “Experimental Procedures.”</td>
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<thead>
<tr>
<th></th>
<th>Phosphorylase phosphatase</th>
<th>MBP phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain F_2, M</td>
<td>units/mg</td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ATP, Mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+Brain F_2</td>
<td>11,700</td>
<td>9,240</td>
</tr>
<tr>
<td>+Muscle F_2, ATP, Mg</td>
<td>11,700</td>
<td>9,240</td>
</tr>
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FIG. 5. Co-migration of the kinase F_2/ATP-Mg-dependent phosphorylase and MBP phosphatase activities on the 6%
native polyacrylamide gel electrophoresis. 10 μg of brain F_2, M were applied to two identical 6% native polyacrylamide gels. After completion of the electrophoresis, one gel was stained for proteins, the other was cut in 2-mm slices and extracted overnight in 0.3 ml of buffer B. The kinase F_2/ATP-Mg-dependent phosphorylase (O) and MBP (●) phosphatase activities were determined on the proteins extracted from each slice. Assay conditions were as described under “Experimental Procedures.”

MBP at a comparable rate (data not shown). The results further implicate that F_2, M may be the major MBP phosphatase in the nervous tissues controlling all the dephosphorylation reactions involved in mammalian myelin basic proteins.

The Catalytic Mechanism of Brain F_2, M on the Dephosphorylation of [32P]Phosphorylase a and 32P-MBP—In a substrate competitive study, we found that nonradioactive phosphorylase a (concentrations up to 0.1 mM) could not slightly inhibit the F_2, M-associated MBP phosphatase activity, even when the experiment was performed in the presence of 0.001 mM 32P-MBP (Fig. 6). Conversely, the dephosphorylation of 32P-labeled phosphorylase a (0.03 mM in the assay) catalyzed by the activated F_2, M was completely blocked by the nonradioactive phosphorylated MBP even in the concentration of 0.004 mM (not shown). However, this strange phenomena became explainable later on when we further identified that the dephosphoform of MBP itself, which obviously was produced during the phosphatase assay experiments, was a very potent factor capable of specifically inhibiting the endogenous phosphorylase phosphatase activity in F_2, M but had no effect on the associated MBP phosphatase activity (Fig. 7). The possibility that this MBP inhibition on the F_2, M-associated phosphorylase phosphatase activity was simply due to the
substrate-directed effect has been ruled out, since the $K_i$ value of MBP was only ~0.1 μM (Fig. 7), which was 120 times lower than the $K_m$ value of $[^{32}P] $phosphorylase α (~12 μM). For instance, as can be seen in Fig. 7, we need only 7.5 pmol of MBP to completely block all the phosphorylase phosphatase activity in an assay mixture where about 1000 pmol of $[^{32}P]$-labeled phosphorylase α was used as substrate. All the results taken together strongly implicate that 1) the MBP inhibitory activity is due to a direct effect on the phosphatase itself and 2) Fc-M may contain two distinct catalytic sites to dephosphorylate different phosphorylated substrates. In addition, the results further indicate that during the dephosphorylation of $[^{32}P]$-MBP, Fc-M is producing its own inhibitor to block the other endogenous phosphatase activities. The nonphosphorylated MBP turned out to be a potent effector capable of controlling the substrate specificity of the kinase Fα/ATP-Mg-dependent protein phosphatase in mammalian nervous tissues. Further evidence that brain Fc-M does contain two active sites was obtained as shown in Fig. 8 that in contrast to the myelin basic protein, even the phosphatase inhibitor-2, a very acidic protein, could also very specifically block the phosphorylase phosphatase activity in Fc-M but caused no effect at all on the associated MBP phosphatase activity, demonstrating that this is not simply due to a conformational modification of the Fc-M enzyme to a form which only acts on $[^{32}P]$-MBP but instead the enzyme itself indeed possesses two distinct catalytic sites to catalyze the dephosphorylation of different phosphorylated substrates.

**DISCUSSION**

This is the first report to demonstrate the existence of the multisubstrate ATP-Mg-dependent protein phosphatase and its unique activator Fα, a CAMP/Cs⁺, calmodulin-independent synthase kinase (12-14) in the mammalian nervous tissues. Although ATP-Mg-dependent phosphatase (Fc-M) is generally believed (2, 10, 12, 15-18, 28) to be the major phosphorylase, phosphorylase kinase, and glycogen synthase phosphatase responsible for the termination of glycogenolysis and initiation of glycogenesis in most mammalian tissues including liver and heart and skeletal muscles, in the present report, substantial amounts of this enzyme were detected and isolated from the pig brain (see Table 1), in spite of the fact that glycogen metabolism in the brain is of very minor importance. The results strongly suggest that brain Fc-M should play an important additional role in the regulation of brain functions other than in glycogen metabolism. The discovery that MBP was a very potential physiological endogenous brain substrate for Fc-M may be just one of the many cases which are as yet undefined. It is believed that the multiple brain phosphoproteins can possibly be identified as the endogenous substrates for this phosphatase in the coming future. In sharp contrast to the present study, Ingebritsen et al. (35) has reported that the brain tissue extract contains very little protein phosphatase-1 when compared with rabbit skeletal muscle. One good explanation for this discrepancy may be that they have greatly underestimated the high content of the Fc-M enzyme in the brain as shown in Table 1 and Fig. 1, since this type of phosphatase-1 has been reported to be not easily detected in the tissue extracts (10, 12).

The inactive Fc-M and the active protein phosphatase-1 (2) have recently been identified as the two interconvertible forms (15, 25, 29) and the distribution of these two enzyme forms in the tissues is even proposed under hormonal and neural control (17, 18, 36). In the present study, it is interesting to note that when the pig brain tissue extracts were chromatographed on DEAE-cellulose, almost no active phosphatase-1 could be detected in the salt-eluted fractions, however, when rabbit skeletal muscle extracts were treated in an identical way, a substantial amount of type-1 phosphatase (2) was found to stay in an active form (Table 1). Although at this moment, it is still premature to assess which component is the key factor to cause this discrepancy, the results strongly implicate that the control mechanism for the activation and inactivation of this phosphatase system in the nervous and non-nervous tissues may be distinctly different. For instance, the unique phosphatase activator Fα and the phosphatase modulator (11) (previously characterized as a specific type-1 phosphatase inhibitor-2) (26), which has been reported as a key factor controlling the interconversion process between the active phosphatase-1 and inactive Fc-M may be distinctly regulated between pig brain and rabbit skeletal muscle. Whether this is true or not deserves further investigation.

Although the precise functions of the brain myelin basic proteins are as yet undefined, it is believed that they are metabolic in nature, involving phosphorylation and dephosphorylation (30). The results that activated Fc-M was capable of exhaustively dephosphorylating $[^{32}P]$-MBP prepared either by CAMP-dependent protein kinase or phosphorylase kinase, support the notion that Fc-M is the major MBP phosphatase in the brain. In sharp contrast to the reported data in Refs. 32 and 33 that one substrate acts as an inhibitor of phosphatase action on another substrate, we did find that the dephosphorylation of $[^{32}P]$-MBP by the activated Fc-M was not slightly inhibited by 100 times higher concentration of nonradioactive phosphorylase α (Fig. 6). Together with the results that MBP and acidic inhibitor-2 protein are two potential inhibitors which can specifically block the Fc-M-associated endogenous phosphorylase phosphatase activity, we further demonstrate that Fc-M may contain two distinct catalytic sites to dephosphorylate different phosphorylated substrates. It is noted that in agreement with the data reported in rabbit muscle system (11, 12, 29, 31), the phosphatase modulator (inhibitor-2) was also found to be complexed with the brain.
ATP. Mg-dependent phosphatase throughout all the chromatographic steps and when the purified brain Fc-M was boiled, the modulator protein (M) could be extracted out of the catalytic entity (Fc) and be used to inactivate and convert the active phosphatase to the inactive ATP-Mg-dependent form and simultaneously to stimulate the free Fc activity isolated from rabbit muscle in a dose-dependent manner (data not shown). Since all the phosphatase preparations used in Refs. 32 and 33 were all free of any modulator content, it is highly possible that this Fc-complexed modulator protein may be the key factor responsible for these two distinct catalytic site mechanisms. In addition, the results in Fig. 7 further indicate that during the dephosphorylation of 32P-MBP, Fc-M is actually producing its own inhibitor to block the other associated phosphatase activities. Whether this is another new type of regulatory mechanism to control the substrate specificity of phosphoprotein phosphatases in mammalian nervous tissues presents an intriguing problem which needs further investigation. On the other hand, the existence of a substantial amount of phosphatase activator Fc/glycogen synthase kinase-3 (13, 14) in the brain where almost no glycogen synthase is available also implicated that this bifunctional protein Fc may as well function as a different protein kinase totally unrelated to glycogen synthase kinase in the nervous tissues (Fig. 4). While not stressing the physiological events, it is noteworthy that by manipulating the acidic inhibitor-2 protein and myelin basic protein as specific inhibitors and by exploiting brain tissue as more suitable material for the better isolation of homogeneous preparations of ATP-Mg-dependent phosphatase (see Figs. 1–3 and Table II), we might be able to work out the more detailed precise molecular catalytic and kinase Fc/ATP-Mg-mediated activation mechanisms of this unique multisubstrate protein phosphatase (34, 37–39). These are under current investigation in this laboratory.

Acknowledgments—We thank Drs. W. Merlevede and J. R. Vandenhede for their encouragement and helpful suggestions.

REFERENCES