Purification of a Fatty Acid-stimulated Protein-serine/threonine Phosphatase from Bovine Brain and Its Identification as a Homolog of Protein Phosphatase 5*

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An arachidonic acid-stimulated Ser/Thr phosphatase activity was detected in soluble extracts prepared from rat pituitary clonal GHGH4 cells, rat or bovine brain, and bovine heart. The enzyme activity was purified to homogeneity from bovine brain as a monomer with a Mr of 63,000 and a specific activity of 32 nmol of Pi released per min/mg of protein when assayed in the presence of 10 μM phosphocasein in the absence of lipid. Arachidonic acid stimulated activity 4–14-fold, with half-maximal stimulation at 50–100 μM, when assayed in the presence of a variety of phosphosubstrates including casein, reduced carboxamidomethylated and maleylated lysozyme, myelin basic protein, and histone. Oleic acid, linoleic acid, and palmitoleic acid also stimulated activity; however, saturated fatty acids and alcohol or methyl ester derivatives of fatty acids did not significantly affect activity. The lipid-stimulated phosphatase was identified as the bovine equivalent of protein phosphatase 5 or a closely related homolog by sequence analysis of proteolytic fragments generated from the purified enzyme. When recombinant rat protein phosphatase 5 was expressed as a cleavable glutathione S-transferase fusion protein, the affinity-purified thrombin-cleaved enzyme exhibited a specific activity and sensitivity to arachidonic acid similar to those of the purified bovine brain enzyme. These results suggest that protein phosphatase 5 may be regulated in vivo by a lipid second messenger or another endogenous activator.

Four major types of protein-Ser/Thr phosphatases have been described: PP1,1 PP2A, calcineurin, and PP2C. The catalytic subunits of PP1, PP2A, and calcineurin constitute a large family of structurally related enzymes (1–3). Cloning studies have also revealed several novel protein-Ser/Thr phosphatases related to PP1 and PP2A, the properties and functions of which are not yet understood (2). In addition to a catalytic subunit, PP1, PP2A, and calcineurin each contain one or more regulatory subunits. Mechanisms regulating the activity of these enzymes include the control of localization and substrate specificity by regulatory subunits; direct phosphorylation; the binding of a second messenger; and inhibition by regulatory domains, subunits, and interacting proteins (2). Several members of the PP1/PP2A family have been shown to be sensitive to putative lipid second messengers (4–8).

Arachidonic acid and several of its lipoxigenase or cytochrome P-450 metabolites have been reported to modulate the activity of a variety of ion channels (9–13). Although ion channel responses may be directly regulated by lipids (9, 10), in several cases, lipid-dependent responses can be blocked by Ser/Thr phosphatase inhibitors such as microcystin, okadaic acid, and calyculin (11–13). This suggests that a Ser/Thr phosphatase related to PP1 or PP2A may mediate the effect of some lipids on ion channel activity (14). The possibility that a lipid-activated Ser/Thr phosphatase may in turn regulate ion channel function prompted us to look for such an enzyme. In this report, we describe the purification of a fatty acid-stimulated Ser/Thr phosphatase from bovine brain. The purified enzyme contains a single Mr 63,000 polypeptide and is identical or closely related to the recently cloned Ser/Thr phosphatase PP5 (15–17).2 We also demonstrate that recombinant rat PP5 is similarly activated by the unsaturated fatty acid arachidonic acid (AA). Protein phosphatase 5 and its yeast homolog (PPT1) contain a C-terminal catalytic domain that is structurally related to PP2A and PP1 and an N-terminal domain consisting of several tetratricopeptide repeats (TPRs) that is not shared with other members of the PP1/PP2A family (15–17). The stimulation of PP5 in vitro by unsaturated fatty acids suggests that this enzyme may be regulated in vivo and raises the possibility that it is a target for activation by a lipid second messenger or some other endogenous effector.

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1 The abbreviations used are: PP, protein phosphatase; AA, arachidonic acid; TPR, tetratricopeptide repeat; CMC, critical micellar concentration; HPLC, high performance liquid chromatography; GST, glutathione S-transferase; pNPP, para-nitrophenyl phosphate; SM-PP1M, smooth muscle myosin light chain phosphatase 1.

2 Protein phosphatase 5 cloned from a human teratocarcinoma cell cDNA library (16), PPT cloned from a rat fat cell cDNA library (15), and PPK cloned from a mouse lung cDNA library (17) are >90% identical in amino acid sequence, but PP5 is only 40% identical to PPT1 cloned from S. cerevisiae (16). We will therefore refer to mammalian forms of this enzyme as PP5 and reserve the term PPT for the yeast form of the enzyme. This nomenclature is consistent with the majority of other reports on this enzyme (18–23) following the initial cloning studies.
phosphorylation for these substrates (expressed as mol of phosphate/mol of protein) was 0.27 for casein, 0.21 for RCML, 0.15 for RBBP, 0.33 for Kemptide, and 0.4 for phosphorylase a. During purification, casein was phosphorylated with 100 μM [γ-32P]ATP (3.5 × 10^11 cpm/mmol ATP) to a stoichiometry of 0.05 mol of phosphate/mol of casein and then subjected to assay column flow fractions and pools.

**Phosphatase Assays—**Phosphatase reactions conducted during purification were carried out for 15 min at 30 °C with 0.6 μM 32P-casein in the presence and absence of 50 μM AA (Calbiochem) in 60 mM Tris, pH 7.6 (25 °C), 1 mM EDTA, 1 mM EGTA, and 0.1% β-mercaptoethanol (buffer B). Reactions were initiated by adding 20 μl of substrate containing 0.1 or 0.5 μl of an enzyme sample. Where the phosphatase was purified, the assay conditions were modified slightly. All solutions were pre-equilibrated to room temperature just before assay. Ten microliters of lipid or ethanol vehicle solution were mixed with 10 μl of phosphatase, and then the reaction was immediately initiated by adding 10 μl of substrate and incubated for 10 min at 30 °C in buffer B. Reactions were terminated with 100 μl of ice-cold 10% (w/v) trichloroacetic acid and 20 μl of 7.5 mg/ml bovine serum albumin and then microcentrifuged at 12,000 g for 5 min. Reactions with 32P-RBMP were terminated instead with 100 μl of ice-cold 20% (w/v) trichloroacetic acid. Assays using 32P-Kemptide, 32P-histone, and 32P-BMP were quenched with 450 μl of 4% (w/v) activated charcoal in 0.6 M HCl, 90 mM Na2PO4, and 2 mM NaH2PO4 and then centrifuged at 12,000 g for 5 min. Acid-soluble radioactivity was quantified by liquid scintillation counting. The phosphatase released from each substrate was <20% of the total present. The final ethanol concentration in lipid-containing reactions was 0.8–1.7% and had no significant effect on phosphatase activity.

**Fluorometric Determination of the Lipid Critical Micellar Concentration—**The CMCs of lipid solutions in 60 mM Tris, pH 7.6 (25 °C), 1 mM EDTA, 1 mM EGTA, 0.1% β-mercaptoethanol, 1.3% ethanol, and a 2 μM concentration of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene were determined by fluorescence spectroscopy (31). Lipids were dispersed by sonication or vigorous vortexing, equilibrated to room temperature, and then incubated with fluorescent probe in the dark for at least 30 min. Fluorescence intensity was measured at excitation and emission wavelengths of 358 and 430 nm, respectively, on a Hitachi F-2000 fluorescence spectrophotometer.

**Peptide Sequencing and Analysis—** Fifty micrograms of purified phosphatase were concentrated by trichloroacetic acid precipitation, resuspended in 8 μl urea and 0.4 mM NH4HCO3, reduced and carboxymethylated (32), and then digested with 1.8 μg of endoproteinase Lys-C (Waco Chemicals) at 37 °C overnight. Peptides were purified by reverse-phase chromatography on a Vydac C18 column (2.1 mm) at 37 °C overnight. The major 1.5-kilobase pair cDNA containing the entire PP5 coding region was then amplified by polymerase chain reaction together with PP5-specific primers using an ALFexpress automatic DNA sequencer (Pharmacia Biotech Inc.) as a template in polymerase chain reaction using the pCI-PP5 clone as a template. The 5'-sense primer (5'-GAC- GATCCATTGGGGATGGCGAGGCGGAGG) and the 3'-antisense primer (5'-GACTGATATTCTACATTCCAGTCG) utilized in this polymerase chain reaction contained initiation and stop codons, respectively, as well as BamH I and EcoRI restriction linkers. The major product containing the full-length PP5 coding region was then amplified by polymerase chain reaction using the pCI-PP5 clone as a template. The 5'-sense primer (5'-GACTGATCCATTGGGGATGGCGAGGCGGAGG) and the 3'-antisense primer (5'-GACTGATATTCTACATTCCAGTCG) utilized in this polymerase chain reaction contained initiation and stop codons, respectively, as well as BamH I and EcoRI restriction linkers. The major product containing the full-length PP5 coding region was then cloned into the BamH I-EcoRI sites of pBluescript II (pBlII KS‘) (Stratagene) and sequenced using an ALFexpress automatic DNA sequencer (Pharmacia Biotech Inc.) to verify its authenticity. To perform bacterial expression, the entire PP5 cDNA was excised from the pBlII-PP5 construct using BamH I and EcoRI restriction enzymes and cloned immediately downstream of the GST coding region into the BamH I-EcoRI sites of pGEX-2T (Stratagene). The newly inserted glycine-termin- 3 A. Law, J. Skinner, and S. Rossie, manuscript in preparation. 4 Constructed as described by Taylor et al. (G. Taylor, Y. Liu, C. Baskerville, and H. Charbonneau, submitted for publication).
sion was induced with 50 μM isopropylthiogalactoside. Cells were lysed in 50 mM Tris, pH 7.6, 0.1% β-mercaptoethanol, 2 mM EDTA, 4 mM MnCl₂, 1 mM phenylmethylsulfonyl fluoride, and 1 mM each aproti-

nin, leupeptin, and pepstatin. The GST-PP5 fusion protein was affinity-purified with glutathione-agarose and then treated with thrombin (34, 35). Proteolysis was terminated with 1 mM phenylmethylsulfonyl fluo-

ride, and recombinant PP5 was dialyzed in 50% glycerol, 1 mM EGTA, 0.1% β-mercaptoethanol, 20 mM Tris, pH 7.6 (4°C), and 4 mM MnCl₂ overnight and then stored at −20°C. The amino acid sequence of recombinant PP5 is identical to that reported by Becker et al. (15) except for four residues (GSGS) remaining at the N terminus after thrombin cleavage.

**RESULTS**

**Detection and Purification of a Fatty Acid-stimulated Ser/Thr Phosphatase from Soluble Brain Extract**—When soluble extracts prepared from rat brain were subjected to Mono Q anion-exchange chromatography and fractions were assayed for phosphatase activity using 32P-casein as substrate in the presence of 50 mM AA, a peak of activity eluting at −110 to −125 mM NaCl was observed (data not shown). Its activity ranged from 615 g of bovine brain (Table I). The pure enzyme exhibited a specific activity of 6 nmol of Pi released per min/mg of enzyme when assayed in the presence of 50 mM AA and 0.6 mM 32P-casein for 15 min at 30°C (Table I).

**Identification of the Fatty Acid-stimulated Phosphatase as a Homolog of PP5**—The purified phosphatase was inhibited by okadaic acid (IC₅₀ = 6 nM) and microcystin (IC₅₀ = 4 nM). The sensitivity of the lipid-stimulated enzyme to these inhibitors (36), together with its apparent molecular weight, suggested that it may represent a recently cloned protein-Ser/Thr phosphatase, PP5 (15–17). Protein phosphatase 5 has a predicted molecular mass of 58 kDa, and recombinant human PP5 is inhibited by okadaic acid (IC₅₀ = 1.5 nM) and microcystin (IC₅₀ = 1 nM) (16) when assayed at an enzyme concentration similar to that used in the present study.

To determine its identity, the amino acid sequences of peptides derived from the lipid-stimulated phosphatase were ob-

**TABLE I**

| Purification step | Total protein | Total activity | Specific activity | Yield | Purification |
|-------------------|---------------|---------------|------------------|-------|--------------|
| Soluble extract   | 4100          | 3281.0        | 0.80             |       |              |
| DEAE-cellulose    | 1300          | 453.1         | 0.35             |       |              |
| CM-Sepharose-CL-6B| 144           | 21.0          | 0.15             | 100   |              |
| Sephadex G-100 supernatant | 3.2  | 9.5          | 3.0              | 45    | 20           |
| α-casein-agarose | 2.5           | 8.3           | 3.3              | 40    | 22           |
| Mono S            | 0.4           | 2.4           | 6.0              | 11    | 40           |

**a** Protein was estimated by the Bradford method (51).

**b** Phosphatase activity was assayed toward 0.6 μM 32P-casein in the presence of 50 mM arachidonic acid.

**c** Yield and purification were compared to the DEAE pool since this was the first step at which the lipid-activated activity was separated from other phosphatases and could be measured accurately.

**d** Assays represent pooled fractions dialyzed against buffer A containing 50% glycerol.
Assays performed in triplicate. Values at each substrate concentration are from three independent.

Stimulation of purified phosphatase activity by AA substrates—32P-casein, half-maximal stimulation occurred at

The activity of the PP5 homolog toward other substrates was also stimulated by AA. In the absence of lipid, phosphatase activity toward histone, MBP, and RCML was comparable to that toward casein, ranging from 24 to 62 nmol of Pi released per min/mg of phosphatase ± S.E. Values at each substrate concentration are from three independent assays performed in triplicate.

homologous to the catalytic subunits of PP1, PP2A, and calcineurin (15–17). These peptides are 96% (B), 89% (C), 100% (D), and 84% (E) identical to the corresponding residues of rat PP5. The remaining peptide (peptide A in Fig. 2) is similar to a peptide corresponding to peptides A–E. We will refer to the enzyme purified from bovine brain as a PP5 homolog in this report.

Effect of AA on Dephosphorylation of Casein and Other Substrates—Stimulation of purified phosphatase activity by AA was concentration-dependent. When assayed with 1 or 2.5 μM 32P-casein, half-maximal stimulation occurred at ~50 μM AA, with 6-fold maximal stimulation (Fig. 3). At these low substrate concentrations, the response of the enzyme to lipid was biphasic, with stimulation declining above 250 μM lipid. At higher levels ofstrate (5–10 μM), the concentration of lipid required for half-maximal stimulation was increased 2-fold, greater maximal activation (9–14-fold) was observed, and the biphasic nature of the stimulatory effect was diminished. The loss of this biphasic effect and the increase in lipid concentration required for half-maximal activation may be due to the binding of lipid by casein.

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The activity of the PP5 homolog toward other substrates was also stimulated by AA. In the absence of lipid, phosphatase activity toward histone, MBP, and RCML was comparable to that toward casein, ranging from 24 to 62 nmol of Pi released per min/mg of phosphatase when assayed with 10 μM phospho-substrate (Table II). Arachidonic acid stimulated phosphatase activity toward all of these substrates in a concentration-dependent manner, although its effect was less pronounced than on activity for casein. Half-maximal stimulation by lipid occurred between 75 and 100 μM lipid, and maximal stimulation ranged from 4 to 12-fold, with dephosphorylation of histone being the least sensitive and dephosphorylation of MBP the most sensitive. As with casein, a biphasic response to lipid was observed for MBP; however, stimulation of histone or RCML dephosphorylation reached a plateau at high lipid concentrations. Since AA can increase the activity of the bovine brain PP5 homolog for a variety of substrates, the stimulatory effect of lipid is likely to be enzyme-directed. Little or no activity was seen with phosphorylase a or Kemptide.

Structural Requirements for Lipid Stimulation—To determine whether the activating lipid demonstrates specific structural requirements, the activity of various fatty acids or fatty acid analogs to stimulate the activity of the purified phosphatase toward 32P-casein or 32P-RCML was assessed. At 200 μM, the unsaturated fatty acids AA, oleic acid, linoleic acid, and palmitoleic acid each stimulated phosphatase activity 4–9-fold toward 32P-casein and 4–6-fold toward 32P-RCML (Table III); AA and oleic acid were the most potent. Methyl ester or alcohol derivatives of AA and oleic acid had little or no effect on phosphatase activity. The saturated fatty acids arachidic acid, stearic acid, and caproic acid did not significantly affect activity at concentrations of 200 μM, whereas myristic acid and lauric acid slightly stimulated activity toward 32P-RCML. These results suggest that stimulation by fatty acids is not simply a hydrophobic effect and that the carboxyl moiety and at least...
Activation of Ser/Thr Phosphatase 5 by Unsaturated Fatty Acids

Table II
Effect of arachidonic acid on phosphatase activity with various \(^{32}\)P-labeled substrates

| Substrate     | Specific activity | Relative activity |
|---------------|-------------------|-------------------|
|               | Vehicle + 250 \(\mu\)M AA | nmol P', released/min/mg | fold |
| Casein        | 32 ± 9            | 443 ± 17          | 11 ± 1  |
| MBP           | 40 ± 6            | 474 ± 56          | 12 ± 1  |
| RCML          | 62 ± 6            | 353 ± 46          | 6 ± 1   |
| Histone       | 24 ± 2            | 89 ± 14           | 4 ± 1   |

Table III
Effects of fatty acids and analogs on purified phosphatase activity

| Lipid additive | Carbon chain | Relative activity |
|----------------|-------------|-------------------|
|                | RCML        | Casein            |
| Arachidonic acid | 20:4       | 4.6 ± 0.2         | 8.5 ± 1.7 |
| Arachidonic acid | 20:4       | 1.3 ± 0.1         | 1.1 ± 0.1 |
| Arachidonic acid methyl ester | 20:4 | 0.6 ± 0.0 | 0.9 ± 0.5 |
| Arachidonic acid | 20:0       | 0.9 ± 0.1         | 1.0 ± 0.3 |
| Oleic acid     | 18:1       | 6.2 ± 0.3         | 6.9 ± 0.3 |
| Oleic acid     | 18:1       | 0.7 ± 0.1         | 0.9 ± 0.2 |
| Oleic acid methyl ester | 18:1 | 0.6 ± 0.1 | 0.8 ± 0.2 |
| Stearic acid   | 18:0       | 0.9 ± 0.1         | 1.2 ± 0.5 |
| Linoleic acid  | 18:2       | 4.4 ± 0.2         | 4.3 ± 0.7 |
| Palmitoleic acid | 16:1      | 4.0 ± 0.1         | 4.7 ± 0.7 |
| Myristic acid  | 14:0       | 2.4 ± 0.1         | 1.5 ± 0.2 |
| Lauric acid    | 12:0       | 3.2 ± 0.2         | 1.7 ± 0.3 |
| Caprylic acid  | 6:0        | 1.3 ± 0.0         | 1.5 ± 0.4 |

* Prepared in distilled water.

one double bond are required for stimulation.

The concentration of AA at which half-maximal activation occurred corresponds to its CMC, 50 \(\mu\)M, measured under conditions similar to those used in the phosphatase assay. Other activating fatty acids exhibited similar CMCs under these conditions: oleic acid, 43 \(\mu\)M; linoleic acid, 92 \(\mu\)M; and palmitoleic acid, 70 \(\mu\)M. In contrast, arachidonic acid and the methyl ester of oleic acid did not significantly stimulate activity at 200 \(\mu\)M (Table III), which is above their CMCs measured under the assay conditions used in this study. This suggests that unsaturated fatty acids of a shorter chain length are not the lipids of the phosphatase, but that micelle formation alone is not sufficient.

Recombinant PP5 Is Activated by AA—To determine whether PP5 is activated by AA, a cDNA encoding rat PP5 was expressed in bacteria as a cleavable GST fusion protein, which was then affinity-purified, cleaved from carrier GST, and tested for sensitivity to lipid. The nucleotide sequence of the rat PP5 cDNA generated from GH4C1 cells is identical to that of the PP5 cDNA isolated from a rat fat cell cDNA library (15) that encodes a protein of 58 kDa. After affinity purification with glutathione-agarose and subsequent thrombin cleavage, the recombinant enzyme migrated as a single polypeptide of 63,000 on Coomassie Blue-stained SDS-polyacrylamide gels (data not shown). On SDS gels, the purified bovine brain PP5 homolog and recombinant rat PP5 have nearly identical mobilities. Recombinant rat PP5 was also similar to the bovine brain PP5 homolog in its sensitivity to inhibition by okadaic acid or microcystin (data not shown). While the concentration of AA required for half-maximal stimulation was the same for recombinant PP5 and the purified bovine brain PP5 homolog, maximal stimulation of recombinant PP5 ranged from 20 to 25-fold, compared with a 14-fold maximal stimulation seen with the purified enzyme (Fig. 4A). This difference may occur due to species variation in structure, or a decreased responsiveness may result from the more complicated and prolonged purification required in the case of the bovine brain PP5 homolog. As with the purified bovine brain PP5 homolog, stimulation of recombinant PP5 by AA was observed with both \(^{32}\)P-casein and...
Activation of Ser/Thr Phosphatase 5 by Unsaturated Fatty Acids

Fig. 5. Stimulation of the bovine brain PP5 homolog or PP2A by AA. The phosphatase activity of the purified PP5 homolog (○) or heterotrimeric PP2A containing a Mr 55,000 B' subunit (●) was assayed for 10 min at 30 °C with 10 μM 32P-casein and increasing concentrations of AA as described under “Experimental Procedures.” Data are presented as the activity relative to control in the absence of added lipid. Values at each lipid concentration are the average ± S.E. from three independent assays performed in triplicate. Control activity was 48 nmol of Pi released per min/mg of enzyme for the PP5 homolog and 627 nmol of Pi released per min/mg of enzyme for PP2A.

32P-MBP (Fig. 4B). Thus, recombinant PP5 is similar in its properties to the PP5 homolog purified from bovine brain. In contrast, under conditions in which AA activated PP5 14-fold with 32P-casein, PP2A containing a Mr 55,000 B' subunit was only modestly stimulated (Fig. 5).

Stimulation of recombinant PP5 by AA was also observed in assays with the substrate pNPP. As with phosphoprotein substrates, stimulation of PP5 activity for pNPP by AA was biphasic, although some differences were noted. Half-maximal stimulation by AA occurred at ~50 μM, a 5-fold maximal stimulation was seen at 100 μM, and higher lipid concentrations led to a sharper decline in stimulation (Fig. 6A). These results obtained with an aryl phosphomonoester are consistent with an effect of lipid that is enzyme-directed. Since pNPP could be assayed at a high molar ratio of substrate to enzyme and contained a single hydrolyzable phosphate group, this substrate was used to examine the effect of lipid on catalysis by PP5. Control PP5 activity for pNPP showed Michaelis-Menten kinetics, yielding a $K_m$ of 27 mM and a $V_{max}$ of 660 pmol of Pi released per min (Fig. 6B). Arachidonic acid caused a modest decrease in $K_m$ and an increase in $V_{max}$ at lipid concentrations below the observed CMC. At 40 μM AA, we observed a $K_m$ of 10 mM and a $V_{max}$ of 1400 pmol of Pi released per min, leading to a 6-fold increase in catalytic efficiency ($k_{cat}/K_m$) from 47 to 270 $M^{-1}s^{-1}$.

Effect of Salt on PP5 Activity—Control and lipid-stimulated PP5 activities were each affected by NaCl and KCl in a concentration-dependent manner. When the recombinant enzyme was assayed in the presence and absence of 150 mM KCl, control activity was inhibited ~50% by KCl, and the concentration of AA required for half-maximal stimulation was increased 1.7-fold in the presence of salt (data not shown). The threshold for stimulation by AA and the maximal activity with AA were not altered by salt, and the CMC for AA (50 μM) was unaffected by salt (data not shown). Thus, at an ionic strength similar to intracellular conditions, AA stimulates PP5 as much as 40-fold over control values.

Fig. 6. Stimulation of recombinant PP5 activity for pNPP by AA. A, rat PP5 phosphatase activity was assayed for 30 min at 30 °C with 250 ng of enzyme, 50 mM pNPP, and 0–500 μM AA as described under “Experimental Procedures.” Results are shown as the average μmol of Pi released per min/mg of enzyme ± S.E. from a representative assay performed in triplicate. The experiment was performed twice with similar results. Control PP5 activity released 920 ± 6 nmol of Pi/min/mg of enzyme. B, phosphatase activity was assayed for 15 min at 30 °C with 500 ng of rat PP5, 10–150 mM pNPP, and 0–40 μM AA as described under “Experimental Procedures.” The Lineweaver-Burk double-reciprocal plot shows the average values for control (●) and 10 (□), 20 (■), 30 (○), or 40 (●) μM AA-treated samples from three independent assays. The percentage of error for velocity averaged <3% and was independent of lipid and substrate concentration. Weighted (fourth power) least-squares fitting was used to estimate $K_m$ and $V_{max}$.

DISCUSSION

We have purified a Mr 63,000 monomeric fatty acid-activated Ser/Thr phosphatase from soluble extracts of bovine brain and have identified this enzyme as a homolog of PP5. The bovine brain PP5 homolog is activated by AA over a 10-fold range in concentration, with half-maximal stimulation occurring at 50–100 μM. Stimulation by AA and other unsaturated fatty acids is observed with a variety of substrates, indicating that lipid activation represents a direct effect on the enzyme itself. The response of recombinant rat PP5 to AA is similar to that of the purified phosphatase from bovine brain, consistent with our conclusion that the bovine brain enzyme is similar or identical to PP5. Others have also shown that recombinant human PP5 and native PP5 partially purified from rabbit liver can be stimulated by polyunsaturated fatty acids (37). Little is known concerning how the activity of PP5 is controlled. The activation of PP5 by unsaturated fatty acids reveals this enzyme's potential for regulation and suggests that PP5 may be controlled in vivo by a bioactive lipid or by some other endogenous activator.

Activation by AA is not a general property of the PP1/PP2A family since PP2A which contains a Mr 55,000 B' subunit is only modestly stimulated by AA. However, at least two other
phosphatases in the PP1/PP2A family are sensitive to AA: smooth muscle myosin light chain phosphatase (SM-PP1M), which is a form of PP1 (4), and an unidentified form of PP2A. SM-PP1M, which responds to a similar range of AA concentrations in vitro as that required for PP5 activation, is a potential physiologic target for AA in mediating the sensitizing effect of calcium on smooth muscle contraction (38). Treatment of SM-PP1M with AA decreases dephosphorylation of heavy meromyosin, but increases activity for another substrate, phosphorylase A (4). Physiologic targets for PP5 have not yet been defined. Although we have observed stimulation with a number of artificial substrates, it remains possible that AA or some other effector may inhibit, rather than stimulate, dephosphorylation of a physiologically relevant substrate by PP5. In the case of SM-PP1M, AA dissociates two regulatory subunits from the catalytic subunit, which itself is insensitive to AA (4). A region of the 130-kDa M subunit of SM-PP1M that may mediate the response to AA has recently been identified (39). A region corresponding to this stretch of sequence is not obviously present in PP5.

Protein phosphatase 5 and PTP1, its homolog from the yeast Saccharomyces cerevisiae, are distinguished from other members of the PP1/PP2A family in containing a unique N-terminal domain consisting of several TPRs (15–17). Tetratricopeptide repeats are hypothesized to form amphipathic helices (40) and have been shown to mediate protein-protein interactions (41). Although the locus of fatty acid binding is not known, it is tempting to speculate that the putative amphipathic helices of the N-terminal TPR domain may confer lipid sensitivity to PP5 since amphipathic helices have the potential to interact with lipid bilayers (42). Unsaturated fatty acids may disrupt interaction between the TPR domain and the catalytic domain of PP5 in a manner analogous to the dissociation of SM-PP1M subunits by AA (4). Alternatively, lipids may promote an activating interaction between these domains or may mimic the binding of an activating protein. Protein phosphatase 5 has been shown to bind an atrial natriuretic factor receptor in vitro (17) and to interact with glucocorticoid receptors and hsp90 in vitro (23); however, no effect of these interacting proteins on PP5 activity was reported.

It remains to be established whether AA, other bioactive lipids, or some other biomolecule regulates PP5 in vivo. The levels of lipid required in this study are higher than those required to activate protein kinase C, a potential intracellular target for AA activation (43), but are similar to the levels of fatty acid shown to affect the activity of SM-PP1M (44), Gα (44), Ras GTPase-activating protein (45), and Rac (46). These high levels may be required to mimic the action of a more potent lipid or a protein activator. Alternatively, in the context of a membrane or in combination with some other signal, the sensitivity of PP5 to AA may be enhanced. It will be important to evaluate the effects of other bioactive lipids on PP5 and to analyze the structural basis of lipid activation.

Arachidonic acid and its metabolites participate in cellular functions such as secretion (47, 48), apoptosis (49), and mitogenesis (50). In addition, as mentioned earlier, a Ser/Thr phosphatase has been implicated in ion channel regulation by various AA metabolites. Protein phosphatase 5 may be a candidate for mediating the effects of AA or one of its metabolites in one or more of these lipid-sensitive processes. Since protein kinase C is a potential intracellular target for AA (43), AA, like calcium, may control two opposing processes, the phosphorylation and dephosphorylation of Ser/Thr residues.

Regulation of ion channels or hormone receptors would require that PP5 be present in the cytoplasm or associated with the plasma membrane. Chen et al. (16) observed that PP5 is predominantly nuclear in normal and transformed fibroblasts. However, the association of PP5 with atrial natriuretic factor receptors (17) and to interact with glucocorticoid receptors and hsp90 (23) suggests that, under some circumstances, PP5 may be localized to the cytoplasm or the plasma membrane. To understand the biological function of PP5, it will be important to determine whether PP5 is regulated by a lipid second messenger or some other effector under physiologic circumstances, to establish whether PP5 is present in other subcellular compartments in addition to the nucleus, and to define its intracellular targets.
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*Activation of Ser/Thr Phosphatase 5 by Unsaturated Fatty Acids*