The Plasma Cell Membrane Glycoprotein, PC-1, Is a Threonine-specific Protein Kinase Stimulated by Acidic Fibroblast Growth Factor*

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A §2P-labeled protein that co-purified with acidic fibroblast growth factor (aFGF) receptor from bovine liver proved to be a distinct membrane protein, which itself has kinase activity that is stimulated by aFGF. The protein was designated MAFP for major aFGF-stimulated phosphoprotein. MAFP was purified from bovine liver using immunoaffinity chromatography and was shown to be associated with fibroblast growth factor (aFGF) receptor from bovine liver using immunoaffinity chromatography. The purified MAFP showed molecular masses of 130 kDa and 260 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and nonreducing conditions, respectively. Purified MAFP elicited an aFGF-stimulated Thr-specific autophosphorylation activity and phosphorylation activity toward protein substrates (myelin basic protein and histone). Amino acid sequence analyses of 16 peptide fragments of MAFP, produced by endoproteinase Lys-C digestion followed by reduction and S-pyridylethylation, showed ~90-100% homology with the cDNA-deduced amino acid sequences of human and mouse plasma cell membrane glycoprotein, PC-1 (Buckley, M. F., Loveland, K. A., McKinstry, W. J., Garson, O. M., and Goding, J. W. (1990) J. Biol. Chem. 265, 17506-17511), suggesting that MAFP is the bovine version of PC-1. The amino acid sequences of bovine MAFP, human and mouse PC-1 reveal a putative ATP binding site in their extracellular domains. These results suggest that MAFP(PC-1) is an ectoprotein kinase. In addition to the kinase activity, MAFP(PC-1) was also found to possess alkaline nucleotide phosphodiesterase activity. It is now clear that several of the unique properties previously attributed to the aFGF receptor kinase are actually properties of this novel Thr-specific ectoprotein kinase, which co-purifies with the aFGF receptor and is responsive to stimulation by aFGF.

The plasma cell membrane glycoprotein PC-1 was discovered by Takahashi et al. (1) and has been used as a cell surface marker for plasma cells for two decades (1-3). PC-1 is absent from thymocytes, leukemia cells, and T-lymphocytes but present on cells of lymph nodes, hemolytic plaque-forming cells of spleen and myelomas, and cells of non-lymphoid tissues such as liver, kidney, and brains (1-3). PC-1 is a disulfide-linked homodimer with each monomer of M, ~ 120,000 (1-3). Recently, PC-1 cDNA from human and mouse were cloned and sequenced (2, 3). The deduced amino acid sequence of PC-1 cDNA reveals that PC-1 is a class II transmembrane protein that is oriented with its N terminus in the cytoplasm and an extracellular C terminus (2, 3). PC-1 consists of an extracellular domain of 826 amino acid residues, a transmembrane domain of 21 amino acid residues, and a cytoplasmic domain of 24 amino acid residues (2, 3). The physiological function of PC-1 is unknown. The specific cell surface expression on plasma cells but not T-lymphocytes suggests that it may play a role in the cellular physiology of plasma cells (1-3).

Our laboratory has been interested in the structure/function of acid phospholipid growth factor (aFGF) and the aFGF receptor (4, 5). We demonstrated that in Swiss mouse 3T3 cells the aFGF receptor is a 135-kDa protein tyrosine kinase (4). Recently, we characterized the protein tyrosine kinase activity of the aFGF receptor partially purified from bovine liver (6). During investigation of the kinase activity of the aFGF receptor from bovine liver, we were puzzled by the fact that a 135-kDa, aFGF-stimulated, §2P-labeled protein, which was believed to be the aFGF receptor, was only partially immunoprecipitated by anti-FGF receptor antiserum or anti-phosphotyrosine antibody (~10% of §2P labeling) (6). It seemed possible that the phosphoprotein(s) that was not immunoprecipitated by these antibodies represented one or more other kinases that were not tyrosine-specific and that appeared to co-migrate with the 135-kDa aFGF receptor on SDS-polyacrylamide gel electrophoresis under reducing conditions (6). To test this possibility, we raised monoclonal antibodies to the partially purified aFGF receptor fraction and found one monoclonal antibody, which allowed us to purify and characterize this major aFGF-stimulated phosphoprotein (MAFP) in bovine liver. In this communication, we show that amino acid sequence analyses of peptide fragments of the monoclonal antibody affinity gel-purified MAFP reveal ~90-100% homology with the cDNA-deduced sequences of human and mouse PC-1. The monoclonal antibody affinity gel-purified MAFP exhibits an aFGF-stimulated Thr-specific protein kinase activity. The structural and functional evidence of this work was supported by National Institutes of Health Grants CA 38806 and HL 41782. 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: aFGF, acidic fibroblast growth factor; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high performance liquid chromatography.

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dence suggest that the MAFP isolated from bovine liver is a Thr-specific ectoprotein kinase and is identical with PC-1.

**EXPERIMENTAL PROCEDURES**

**Materials**—$[γ-32P]ATP (4,500 Ci/mmol) was obtained from ICN Radiochemicals. Low molecular weight protein standard mixture, myelin basic protein, goat anti-mouse IgG-agarose, cyanogen bromide-activated Sepharose 4B, alkaline phosphatase-conjugated goat anti-mouse IgG, thymidine 5'-monophosphate-p-nitrophenyl ester, UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine, and histone were obtained from Sigma. Wheat germ lectin-Sepharose 4B was prepared as previously described (6). aFGF was isolated from bovine brain according to the procedure described previously (5).

**Endoproteinase Lys-C** was obtained from Boehringer Mannheim.

**Preparation of Partially Purified aFGF Receptor**—The aFGF receptor was partially purified from bovine liver by Triton X-100 extraction of plasma membranes, followed by sequential column chromatography, as described above, was used as antigen. About 200-300 μg of protein with complete avidat was injected intraperitoneally into BALB/c mice and was boosted with antigen in incomplete adjuvant at 3-4-week intervals. After 9 months, spleen cells from these mice were prepared and fused with myeloma cells (Ag), producing hybridomas,

**Identification of Monoclonal Antibodies to MAFP**—Since MAFP appears to co-purify with the aFGF receptor during purification of the latter (6), partially purified aFGF receptor from DEAE-cellulose column chromatography, as described above, was used as antigen. The ability to recognize a 130-135-kDa antigen on the conditioned media of hybridoma cells: Western blot analysis and immunoprecipitation. One of the monoclonal antibodies, designated D6.5, was specifically reactivated with MAFP.

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**Detection of Monoclonal Antibodies to MAFP**—Two procedures were employed for detection of monoclonal antibodies in the conditioned media of hybridoma cells: Western blot analysis and immunoprecipitation. Western blot analysis was carried out as previously described (7). The partially purified aFGF receptor was blotted onto nitrocellulose filters following 6.0% SDS-polyacrylamide gel electrophoresis. The ability to recognize a 130-135-kDa antigen on the nitrocellulose filter was used as one of the criteria for positive antibodies produced by hybridoma cells.

In the immunoprecipitation procedure, the partially purified aFGF receptor (3 μg) was incubated with 5 μg $[γ-32P]ATP (5 μCi)$ in the presence and absence of aFGF (600 pm) (6). After 20 min at 0 °C, the phosphorylation reaction was terminated by adding 0.2 ml of 1% Triton X-6 and 10 mM Tris buffer, pH 7.4 containing 5 mM EDTA, 100 μM sodium orthovanadate, 30 mM sodium pyrophosphate, 50 mM NaCl, and 50 mM NaF. The reaction mixture was then incubated with 1 ml of the hybridoma cell medium and 20 μl of goat anti-mouse IgG-agarose (4 °C overnight). The immunoprecipitates were analyzed by 6.0% SDS-polyacrylamide gel electrophoresis followed by autoradiography. The ability to immunoprecipitate a 130-kDa aFGF-stimulated, $32P$-labeled protein was used as identification of positive antibodies produced by hybridoma cells.

**Purification of MAFP Using Monoclonal Antibody D6.5-Sepharose 4B Affinity Chromatography**—Fifteen mg of monoclonal antibody D6.5 purified by protein G-agarose was coupled onto cyanogen bromide-activated Sepharose 4B (7 ml volume) to produce monoclonal antibody D6.5-Sepharose 4B (~1.7 mg/ml gel).

The aFGF receptor-containing fractions from wheat germ lectin-Sepharose 4B column chromatography, as described above, were pooled and applied onto a column of monoclonal antibody D6.5-Sepharose 4B. After sequential washing with 0.5 M NaCl, 0.2% Triton X-100, and 10% glycerol in 20 mM HEPES, pH 7.4 and 0.05% Triton X-100 in 20 mM HEPES, pH 7.4 (Triton X-100/HEPES buffer), MAFP was eluted with 3 M KSCN in Triton X-100/HEPES buffer, dialyzed against Triton X-100/HEPES buffer, and concentrated by ultrafiltration.

**Digestion of MAFP with Endoproteinase Lys-C Followed by S-Pyridylethylated Amino Acid Sequence Analysis**—The 130-kDa MAFP was electroeluted from 6.0% SDS-polyacrylamide gel electrophoresis, digested with endoproteinase Lys-C (~20-30:1, protein:enzyme, w/w), and S-pyridylethylated (8). The peptide fragments were then separated on reverse phase HPLC (C8 column) with a linear gradient of acetonitrile from 7 to 70%. The number of peptides are designated in the elution order in Table 1. The eluted peptides were determined on an Applied Biosystems model 477A gas/liquid phase protein sequenator with an on-line Applied Biosystems model 120A phenylthiohydantoin amino acid analyzer.

**Enzyme Assays and Phosphoamino Acid Analysis**—The kinase activity of MAFP was assayed as previously described (6). Briefly, MAFP (~3 μg) was incubated with 5 μM $[γ-32P]ATP (5 μCi), 0.5 mM MnCl₂, and 0.5 mM pyrophosphate in the presence of aFGF (37°C, 1-200 pm). The reaction mixture was then analyzed by 6.0% SDS-polyacrylamide gel electrophoresis and autoradiography. Alkaline nucleotide phosphodiesterase activity was assayed using thymidine 5'-monophosphate-p-nitrophenyl ester as substrate (9).

The phosphoamino acids of $32P$-labeled MAFP and histone were analyzed on thin-layer cellulose electrophoresis as previously described (4) following acid hydrolysis.

**RESULTS**

**Preparation of Monoclonal Antibodies to MAFP**—The aFGF receptor was partially purified from bovine liver by Triton X-100 extraction of plasma membranes followed by wheat germ lectin-Sepharose 4B affinity gel and DEAE-cellulose ion exchange chromatography, as previously described (6). Monoclonal antibodies raised to this preparation led to the discovery that MAFP is a distinct protein that co-chromatographed with the aFGF receptor during purification of the latter. To prepare monoclonal antibodies, the proteins from the aFGF receptor-containing fractions of DEAE-cellulose ion exchange chromatography were used as antigens. Immunoprecipitation and Western blot analysis were employed for screening monoclonal antibodies in the conditioned media of hybridoma cells. One of the monoclonal antibodies, designated D6.5, was used to specifically immunoprecipitate MAFP without precipitating the aFGF receptor. This monoclonal antibody D6.5 was coupled to cyanogen bromide-activated Sepharose 4B for purification of MAFP from bovine liver.

**Identification of MAFP as PC-1**—MAFP was purified by monoclonal antibody D6.5-Sepharose 4B affinity chromatography from bovine liver following Triton X-100 extraction of plasma membranes and wheat germ lectin-Sepharose 4B column chromatography. As shown in Fig. 1, purified MAFP exhibited molecular masses of 130 and 260 kDa on SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions, respectively (Fig. 1, lanes 1 and 2). A minor smaller molecular mass protein (~110 kDa) was also observed (Fig. 1, lane 1) and appeared to be a degradation product of the 130-kDa MAFP. The 130-kDa MAFP was electroeluted from the reducing SDS-polyacrylamide gel and subjected to N-terminal amino acid analysis and endoproteinase Lys-C digestion followed by reduction and S-pyridylethylated. No N-terminal acid residue was found in the 130-kDa MAFP molecule based on automated Edman degradation.

**FIG. 1.** SDS-polyacrylamide gel pattern of monoclonal antibody D6.5-Sepharose 4B-purified MAFP. MAFP (~3 μg), isolated from bovine liver by monoclonal antibody D6.5-Sepharose 4B column chromatography, was subjected to 6.0% SDS-polyacrylamide gel electrophoresis and autoradiography. The ability to immunoprecipitate a 130-kDa MAFP-stimulated, $32P$-labeled protein was used as identification of positive antibodies produced by hybridoma cells.

- 205 kDa
- 116 kDa
- 97 kDa
- 66 kDa
- 45 kDa
tion, suggesting that the N terminus of MAFP was blocked. The peptide fragments produced by digestion with endoprotease Lys-C followed by reduction and S-pyridylethylation were separated on reverse phase HPLC. The amino acid sequences of purified peptide fragments were analyzed by automated Edman degradation and are shown in Table I. A computer search of amino acid sequence homology of 16 peptide fragments with known proteins revealed ~80-100% homology of MAFP with the cDNA-deduced amino acid sequences of human and mouse PC-1 (2, 3). These findings, together with the similar dimeric structure of MAFP and PC-1 (2, 3), suggest that MAFP is the bovine version of PC-1. The amino acid sequences of MAFP and PC-1 also reveal an ATP-binding site (GXGXXG...A/GXK) in these molecules (Table I) (10, 11). It is of interest to note that this putative ATP-binding site locates in the extracellular domain of PC-1 (3). These results suggest that MAFP or PC-1 is a potential autokinase.

**MAFP(PC-1) Is a Thr-specific Ectoprotein Kinase—** Autophosphorylation is one of the distinct biochemical properties for most protein kinases (10-12). It seemed possible that the 3P labeling of MAFP might result from its autophosphorylation, which was stimulated by aFGF (6). To test for protein kinase activity of MAFP(PC-1), monoclonal antibody D6.5-Sepharose 4B-purified MAFP(PC-1) was incubated with [γ-32P]ATP in the presence and absence of 1.2 nM aFGF. The reaction mixture was then analyzed on 6.0% SDS-polyacrylamide gel electrophoresis. As shown in Fig. 2, aFGF stimulated the autophosphorylation of MAFP. The 130- and 260-kDa 32P-labeled MAFP were observed on SDS-polyacrylamide gel electrophoresis.

### Table I

**Comparison of the N-terminal amino acid sequences of the peptide fragments produced by digestion with endoprotease Lys-C of MAFP with the cDNA-deduced amino acid sequences of human and mouse PC-1**

| Protein | N-terminal amino acid sequences of peptide fragments Lys-C-1, Lys-C-16, Lys-C-3, Lys-C-21, Lys-C-12, Lys-C-18, Lys-C-15, Lys-C-25, Lys-C-8, Lys-C-13, and Lys-C-9 include amino acid residues 58-83, 204-224, 251-259, 265-276, 277-286, 284-303, 304-309, 357-380, 414-424, 452-476, 477-496, 505-536, 683-695, 696-720, 821-831, and 832-853, respectively. Lys-C-2/Lys-C-2' and Lys-C-3/Lys-C-3' co-chromatographed on reverse phase HPLC. Amino acid residues are numbered according to the cDNA-deduced amino acid sequences of human and mouse PC-1 (3). Asterisk above sequence indicates the identical amino acid residues in bovine MAFP, human and mouse PC-1. Dots above the sequence indicate the identical amino acid residues of bovine MAFP with human or mouse PC-1. Bars under the sequence indicate the putative ATP-binding site. X indicates the unidentified residues.

| Protein | N-terminal amino acid sequences | Human | Bovine | Mouse |
|---------|---------------------------------|-------|--------|-------|
| Lys-C-1 | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-16| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-3 | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-21| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-12| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-18| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-15| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-25| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-8 | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-13| G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-9  | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-2' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-3' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-21' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-12' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-18' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-15' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-25' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-8' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |
| Lys-C-13' | G-R-C-F-E-R-T-P-G-N-C-C-C-D-A-A-C-V-E-L-G-N-C-C-L-D | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- | T-F-P-H-Y-Y-S-I-V- |

**ATP Binding Site**

- Human: S-F-Y-Q-D-R-K Y-C-Q-G-S-F-G-H-S-G-S-N-F-Y-N-K-H-S-L-F-Y-G-Y-G-P-F-G-P-F-H-G
- Bovine: S-F-Y-Q-D-R-K Y-C-Q-G-S-F-G-H-S-G-S-N-F-Y-N-K-H-S-L-F-Y-G-Y-G-P-F-G-P-F-H-G
- Human: S-F-Y-Q-D-R-K Y-C-Q-G-S-F-G-H-S-G-S-N-F-Y-N-K-H-S-L-F-Y-G-Y-G-P-F-G-P-F-H-G
- Bovine: S-F-Y-Q-D-R-K Y-C-Q-G-S-F-G-H-S-G-S-N-F-Y-N-K-H-S-L-F-Y-G-Y-G-P-F-G-P-F-H-G
FIG. 2. aFGF-stimulated autophosphorylation of monoclonal antibody D6.5-Sepharose-purified MAFP. A, MAFP purified from monoclonal antibody D6.5-Sepharose 4B affinity chromatography (3 μg) was incubated with 5 μM [γ-32P]ATP, 0.5 mM MnCl₂, and 0.5 mM pyrophosphate with (+) or without (−) 1.2 nM aFGF. After 20 min at 0 °C, the reaction was terminated. The reaction mixture was then analyzed by 6.0% SDS-polyacrylamide gel electrophoresis under reducing (+ β-mercaptoethanol) and nonreducing (− β-mercaptoethanol) conditions followed by autoradiography. The arrowhead indicates the location of MAFP. B, MAFP purified from monoclonal antibody D6.5-Sepharose affinity chromatography (3 μg) was incubated with [γ-32P]ATP, 0.5 mM MnCl₂, and 0.5 mM pyrophosphate in the presence of various concentrations of aFGF as indicated. The reaction mixtures were then analyzed by 6.0% SDS-polyacrylamide gel electrophoresis under reducing conditions followed by autoradiography. The arrowhead indicates the location of MAFP.

gel electrophoresis under reducing (Fig. 2A, lanes 1 and 2) and nonreducing (Fig. 2A, lanes 3 and 4) conditions, respectively. This contrasts with the behavior of the aFGF receptor, which showed no change in mobility on SDS-polyacrylamide following treatment with reducing agents (4). The stimulation of MAFP autophosphorylation by aFGF appeared to be dose-dependent (Fig. 2B). At 600 pM of aFGF, maximum stimulation (−2-fold) was observed. Acid hydrolysis of 32P-labeled MAFP(PC-1) yielded phosphothreonine but not phosphotyrosine and phosphoserine (Fig. 3A), suggesting that MAFP(PC-1) is a Thr-specific protein kinase. For determination of substrate specificity of MAFP(PC-1), the activities of MAFP(PC-1) toward casein, bovine serum albumin, myelin basic protein, and histone were examined. In this experiment, the protein substrates (400 μg/ml) were incubated with monoclonal antibody D6.5-Sepharose 4B-purified MAFP(PC-1) (−5 μg/ml) in the presence of 5 μM [γ-32P]ATP and 1.2 nM aFGF. After 30 min at 0 °C, 32P-labeled proteins were then analyzed by 10% SDS-polyacrylamide gel electrophoresis and autoradiography. As shown in Fig. 3B, MAFP(PC-1) catalyzed the phosphorylation of histone and myelin basic proteins (lanes 5–8) but not casein and bovine serum albumin (lanes 1–4). This MAFP(PC-1)-catalyzed phosphorylation was augmented in the presence of aFGF (lanes 5 and 7). This result is essentially identical with our previous results when the partially purified aFGF receptor was used as the kinase (6). Thus, the activities toward these substrates in that preparation may have been because of the MAFP rather than the aFGF receptor. Together with the extracellular domain localization of the putative ATP-binding site of MAFP(PC-1) (2, 3), these results suggest that MAFP(PC-1) is a Thr-specific ectoprotein kinase.

MAFP(PC-1) is a Novel Enzyme with Multiple Functions—During investigation of potential inhibitors for the autophosphorylation activity of MAFP(PC-1), we found that nucleotides such as UDP, UTP, GDP, and GTP were potent inhibitors. Recently, Rebbe and co-workers (13) reported that mouse PC-1 was associated with alkaline nucleotide phosphodiesterase and pyrophosphatase activities. These observations prompted us to investigate these enzyme activities of monoclonal antibody D6.5-Sepharose 4B-purified MAFP(PC-1). Indeed purified MAFP(PC-1) showed a potent nucleotide phosphodiesterase activity with an apparent Kₘ = 0.2 mM and Vₘₐₓ = ~1,500 μmol/h/mg protein, using thymidine 5'-monophosphate-p-nitrophenyl ester as substrate (9). This enzyme activity of MAFP(PC-1) could be inhibited by nucleotide pyrophosphates such as UDP-glucose, UDP-galactose, and UDP-N-acetylgalactosamine (1 mM) (~80% inhibition). These results demonstrate that MAFP(PC-1) possesses nucleotide phosphodiesterase activity in addition to its kinase activity. It is of importance to note that the kinase activity and nucleotide phosphodiesterase activity of MAFP(PC-1) show different optimal pH of 7.4 and 9.8, respectively.

DISCUSSION

Two lines of evidence suggest that MAFP, isolated from bovine liver, is identical with PC-1. 1) Both MAFP and PC-1 possess the same homodimer subunit structure with each polypeptide subunit of 120-130-kDa molecular mass (2, 3). 2) The amino acid sequences of 16 peptide fragments of MAFP from bovine liver analyzed show ~80–100% homology with those deduced from human and mouse PC-1 cDNA (2, 3).

MAFP(PC-1) is a Thr-specific protein kinase that elicits typical autophosphorylation activity and phosphorylation activity toward specific substrates. Although like other protein kinases, MAFP(PC-1) possesses an ATP binding site (GXGXXG...A/GXK), the amino acid sequence of MAFP(PC-1) does not show close homology with those of...
well characterized Ser/Thr- and Tyr-specific protein kinases (10–12). It is very likely that MAFP(PC-1) is a new type of protein kinase that is distinct from other well characterized kinases with respect to structure and function. MAFP(PC-1) kinase appears to be unique in several aspects when compared with other known protein kinases. These include: 1) modulation of MAFP activity by divalent ions, Mn$^{2+}$, Mg$^{2+}$, and Ca$^{2+}$ (6); 2) stimulation of MAFP activity by an equimolar ratio of Mn$^{2+}$ and pyrophosphate (6); and 3) cell surface or lumen (the endoplasmic reticulum or Golgi complex) localization of the kinase domain of MAFP(PC-1). We previously recognized the first two unique properties in the kinase activity of the partially purified aFGF receptor preparation (6). It appears that these properties, which we attributed to the aFGF receptor, are really attributes of MAFP(PC-1) co-purified with the aFGF receptor (6).

Ectoprotein kinase activities have been demonstrated in several types of cells (14). However, none of these kinases has been isolated and characterized. MAFP(PC-1) may provide a good model to examine the physiological function(s) of an ectoprotein kinase. Many secretory proteins have been found to be phosphorylated at Ser/Thr residues (15). It is possible that ectoprotein kinases such as MAFP(PC-1) actually phosphorylate certain secretory proteins or plasma membrane proteins when functionally expressed in the lumen of the endoplasmic reticulum or Golgi complex, prior to transport of the kinase to the cell surface. As previously noted, MAFP(PC-1) is a class II transmembrane protein with a cytoplasmic domain of 24 amino acid residues. Under certain conditions (16), the kinase activity of MAFP(PC-1), by autophosphorylation or phosphorylation of neighboring plasma membrane proteins, may transduce a cellular signal(s). This could provide a rationale for the observation that the kinase activity of MAFP(PC-1) was responsive to aFGF.

In addition to the kinase activity, MAFP(PC-1) was also found to elicit an alkaline nucleotide phosphodiesterase activity. Several observations support the hypothesis that both kinase and nucleotide phosphodiesterase activities are derived from the same protein molecule MAFP(PC-1). These include the following. 1) Monoclonal antibody D6.5-Sepharose-purified MAFP(PC-1) showed both activities. 2) The kinase activity of MAFP(PC-1) could be inhibited by the substrates for nucleotide phosphodiesterase or nucleotide pyrophosphatase, such as thymidine 5'-monophosphate-p-nitrophenyl ester, UDP-galactose, UDP-glucose, and UDP-N-acetylglucosamine. 3) Both kinase and nucleotide phosphodiesterase activities were found to be sensitive to reducing agents. At 1 mM of dithiothreitol or β-mercaptoethanol, more than 90% of both kinase and phosphodiesterase activities of MAFP(PC-1) was abolished. 4) aFGF was found to stimulate both kinase and nucleotide-phosphodiesterase activities of MAFP. It is of interest to note that nucleotide phosphodiesterase or nucleotide pyrophosphatase isolated from rat liver was a disulfide-linked homodimer of 120-kDa subunits (17), which is similar to the subunit structure of MAFP(PC-1).

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