Autotaxin (ATX) is an extracellular enzyme and an autocrine motility factor that stimulates pertussis toxin-sensitive chemotaxis in human melanoma cells at picomolar to nanomolar concentrations. This 125-kDa glycoprotein contains a peptide sequence identified as the catalytic site in type I alkaline phosphodiesterases (PDEs), and it possesses 5′-nucleotide PDE (EC 3.1.4.1) activity (Stracke, M. L., Krutzsch, H. C., Unsworth, E. J., Årestad, A., Cicco, V., Schiffmann, E., and Liotta, L. (1992) J. Biol. Chem. 267, 2524–2529; Murata, J., Lee, H. Y., Clair, T., Krutzsch, H. C., Årestad, A. A., Sobel, M. E., Liotta, L. A., and Stracke, M. L. (1994) J. Biol. Chem. 269, 30479–30484). ATX binds ATP and is phosphorylated only on threonine. Thr210 at the PDE active site of ATX is required for phosphorylation, 5′-nucleotide PDE, and motility-stimulating activities (Lee, H. Y., Clair, T., Mulvaney, P. T., Woodhouse, E. C., Aznavoorian, S., Liotta, L. A., and Stracke, M. L. (1996) J. Biol. Chem. 271, 24408–24412). In this article we report that the phosphorylation of ATX is a transient event, being stable at 0 °C but unstable at 37 °C, and that ATX has adenosine-5′-triphosphatase activity toward histone, myelin basic protein, or casein. These results lead to the proposal that ATX is capable of at least two alternative reaction mechanisms, threonine (T-type) ATPase and 5′-nucleotide PDE/ATP pyrophosphatase, with a common site (Thr210) for the formation of covalently bound reaction intermediates threonine phosphate and threonine adenylate, respectively.

Autotaxin (ATX) is an extracellular enzyme and an autocrine motility factor that stimulates pertussis toxin-sensitive chemotaxis in human melanoma cells at picomolar to nanomolar concentrations. This 125-kDa glycoprotein contains a peptide sequence identified as the catalytic site in type I alkaline phosphodiesterases (PDEs), and it possesses 5′-nucleotide PDE (EC 3.1.4.1) activity (Stracke, M. L., Krutzsch, H. C., Unsworth, E. J., Årestad, A., Cicco, V., Schiffmann, E., and Liotta, L. (1992) J. Biol. Chem. 267, 2524–2529; Murata, J., Lee, H. Y., Clair, T., Krutzsch, H. C., Årestad, A. A., Sobel, M. E., Liotta, L. A., and Stracke, M. L. (1994) J. Biol. Chem. 269, 30479–30484). ATX binds ATP and is phosphorylated only on threonine. Thr210 at the PDE active site of ATX is required for phosphorylation, 5′-nucleotide PDE, and motility-stimulating activities (Lee, H. Y., Clair, T., Mulvaney, P. T., Woodhouse, E. C., Aznavoorian, S., Liotta, L. A., and Stracke, M. L. (1996) J. Biol. Chem. 271, 24408–24412). In this article we report that the phosphorylation of ATX is a transient event, being stable at 0 °C but unstable at 37 °C, and that ATX has adenosine-5′-triphosphatase (ATPase; EC 3.6.1.3) and ATP pyrophosphatase (EC 3.6.1.8) activities. Thus ATX catalyzes the hydrolysis of the phosphodiester bond on either side of the β-phosphate of ATP. ATX also catalyzes the hydrolysis of GTP to GDP and GMP, of either AMP or PPi to Pi, and the hydrolysis of NAD to AMP, and each of these substrates can serve as a phosphate donor in the phosphodiester bond, and the hydrolysis of NAD to AMP, and each of these substrates can serve as a phosphate donor in the phosphodiester bond.

Materials—Histone II A, myelin basic protein, casein, 2-mercaptoethanol, magnesium chloride, sodium chloride, dibasic potassium phosphate/ATP Pyrophosphatase and ATPase Activities*

(Received for publication, September 5, 1996)

Timothy Clair‡, Hoi Young Lee, Lance A. Liotta, and Mary L. Stracke

From the Laboratory of Pathology, Division of Clinical Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892

EXPERIMENTAL PROCEDURES

Materials—Histone II A, myelin basic protein, casein, 2-mercaptoethanol, magnesium chloride, sodium chloride, dibasic potassium phosphate

‡To whom correspondence should be addressed: Laboratory of Pathology, Division of Clinical Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892

The abbreviations used are: ATX, autotaxin; rATX, recombinant ATX; PDE, phosphodiesterase; 9-TMP, 5′-nitrophenyl-thymidine monophosphate; AMP-CP, adenosine 5′-(β,γ-methylenetriphosphate); AMP-PCP, adenosine 5′-(β,γ-methylenetriphosphate); PAGE, polyacrylamide gel electrophoresis.

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.

This paper is available on line at http://www-jbc.stanford.edu/jbc/jbc
phosphate, p-nitrophenyl thymidine-5'-monophosphate and other nucleotides, and Tris-HCl were from Sigma. HEPES buffer was from Life Technologies, Inc. Electrophoresis buffer was from Bio-Rad. Ethylene glycol was from Fisher. Radioactive materials were from ICN (Costa Mesa, CA) (ATP, 8-azido-ATP, and GTP), DuPont NEN (ATP, P, P, and Na, Amersham Corp. (AMP), and American Radiolabeled Chemicals (St. Louis, MO) (ATP).

**Purification of ATX**—The purification of ATX from A2058 cells was performed as described previously (1) through the weak anion exchange step. rATX was purified to homogeneity as follows. ATX cDNA, which included the full-length open reading frame, was subcloned into the plasmid vector pMIG01 (14) and then transfected into vaccinia virus (15). BS-C-1 cells were infected with recombinant virus, and the culture lysate was collected and filtered with an Easy Flow filter, molecular mass cutoff, 300 kDa (Sartorius), to remove virus particles. The lysate was concentrated on an Amicon ultrafiltration device, a Diaflo YM30 membrane, and then was sequentially fractionated through agarose-bound concanavalin A (Vector Laboratories, Inc., Burlingame, CA) as described (1) and either anion exchange on ZORBAX BioSeries-WAX (MAC-MOD, Chadds Ford, PA) as described (3) or ATP-agarose. For the ATP-agarose step active fractions from concanavalin A chromatography were concentrated and dialyzed into T/EG buffer (50 mM Tris-HCl, pH 7.5, 0.75 ml sample of this concentrate was applied to a 2-ml bed of ATP-agarose resin (C-8 linked through an amine-carbon spacer) in a 10-ml Econocolumn (Bio-Rad), which had been equilibrated with 10 volume of T/EG buffer. An additional 6 ml of T/EG buffer was added, and the column was stopped and gently rocked at 4°C. After 2 h the column was drained, and the resin was washed with an additional 12 ml of T/EG buffer. At this point rATX was eluted from the ATP-agarose resin by the addition of 6 ml of T/EG buffer containing 1 M NaCl, stopping the column, and rocking it gently at room temperature for an additional 2 h. The column was drained at room temperature and washed with 12 ml of T/EG buffer containing 1 M NaCl. Fractions were tested for motility in chemotaxis assays and for purity by silver stain of an SDS-PAGE gel. The pooled fractions were dialyzed in T/EG buffer and stored at 5°C.

**Analytical Gel Electrophoresis**—Protein samples were analyzed by SDS/PAGE in a Tris glycine buffer system using precast 8–16% gradient minigels (Novex, San Diego, CA). Gels were stained using a Daiichi Silver Stain II kit (Integrated Separation Systems, Natick, MA) and investigated on a 300-kDa (Sartorius) membrane, and then was fractionated as described above for 32P-labeled protein bands in gels. The reaction mixture was quantified by reading the absorbance at 410 nm (Amax = 64 nmol of p-nitrophenol).

**Protein Phosphorylation and Hydrolysis of Phosphoester Bonds**—Various 32P-labeled substrates ([α-32P]ATP or [γ-32P]ATP (25 Ci/mmol), [α-32P]GTP or [γ-32P]GTP (25 Ci/mmol), [α-32P]AMP (3000 Ci/mmol), [22P] (1 Ci/mmol), or [32P] (adenylate) NAD (800 Ci/mmol), each at a concentration of 10 μM, were incubated with and without ATX, at the indicated times and temperatures, in 1.8-ml microtubes in a final volume of 20 μl containing 100 μM MgCl2, 50 mM HEPES, pH 7.3. For analysis of protein, 20-μl aliquots were incubated in 1.8-ml microtubes in a final volume of 200 μl containing 100 μM MgCl2, 50 mM HEPES, pH 7.3. For analysis of protein, 20-μl reactions were terminated by addition of 10 μl of 2 × sample buffer (Novex), and products were resolved by SDS-PAGE, silver staining, and autoradiography (XAR film, Eastman Kodak Co.). Where indicated, using the autoradiogram and silver stain to localize the band, radioactivity in individual protein bands in dried gels was quantified directly using a BioScan SpotCount apparatus (BioScan Inc., Washington, DC), inventor’s prototype, kindly provided by Richard Braverman, NCI). Nucleotide and phosphate products (2-μl aliquots) from ATX-catalyzed reactions (10 μl) were resolved by ascending TLC on polyethyleneimine-coated sheets (J. T. Baker, Phillipsburg, NJ) in 0.85 M dibasic potassium phosphate, pH 3.4. Radioactive spots were localized and quantified as described above for 32P-labeled protein bands in gels. The reaction product concentration in a radioactive spot was calculated as a fraction of total radioactivity in all spots in the chromatogram. ATX-dependent hydrolysis was calculated as the difference in product concentration in corresponding areas of chromatograms from reactions performed in the presence and absence of ATX.

**Phosphoaffinity Labeling**—The binding of ATP to ATX was detected by phosphoaffinity labeling (16). Samples (10 μl) were incubated in 1.8-ml microtubes in a final volume of 20 μl containing 100 μM MgCl2, 50 mM HEPES, pH 7.3, and 10 μl [α-32P]ATP (10 Ci/mmol, ICN). After 90 min at 0°C this ligand was photoactivated by irradiation at 253 nm using a hand-held UV lamp (UVG-54; UVP, Inc., San Gabriel, CA) placed directly over the uncapped tubes for 30 s. Immediately following photolysis, reactions were terminated, and protein products were analyzed as described above for 32P-labeled protein bands. No radioactive bands were detected in samples that had not been irradiated.

**RESULTS**

**ATX Has 5'-Nucleotide PDE Activity at Physiological pH**—Type I PDE enzymes have characteristic alkaline pH optima, whereas the cellular motility-stimulating activity of ATX, which depends on the presence of an intact PDE active site, is expressed at neutral pH. We therefore sought to determine whether ATX displays 5'-nucleotide PDE activity under conditions of physiological pH and low substrate concentrations. The 5'-nucleotide PDE activity of rATX was assayed at pH 7.3 and 8.9 (Fig. 1). This activity, measured at high concentrations (5 mM) of substrate (ATP-TMP), is 3-fold greater at alkaline than at physiological pH. Measurement of the PDE activity of ATX at substrate concentrations below 1 mM, however, reveals that the reaction velocity measured at pH 7.3 is not significantly different from that detected at pH 8.9. Thus, at physiological temperature and pH and at low substrate concentrations, ATX has readily detectable 5'-nucleotide PDE activity.

**Autoptatin Catalyzes a Phosphorylation-Dephosphorylation Cycle**—The observation (3) that phosphorylation-deficient (K209L) ATX stimulates cellular motility suggested that dephospho-ATX is biologically active and led us to investigate the stability of phospho-ATX. The incorporation of the γ-phosphate of ATP into A2058 ATX and the dephosphorylation of phospho-ATX at physiological temperature are depicted in (Fig. 2). Samples of A2058 ATX were incubated at 0°C with 10 μM [γ-32P]ATP for the indicated times, and reaction products were analyzed by SDS-PAGE and autoradiography (Fig. 2, A and C). To detect the dephosphorylation of phospho-ATX, the phospho- catalyzed product of the reaction was resolved at 4°C, divided into aliquots, and further incubated either at 0 or 37°C for the indicated times (Fig. 2, B and C). Silver staining (data not shown) showed equal protein loading, and only one band of labeled protein from each reaction was detected by autoradiography. Phospho-ATX accumulates for about 90 min and re-
for the on and off reactions, respectively. Radioactivity in phospho-ATX immediately began to a co-purifying protein kinase and/or phosphoprotein phosphatase activity in ATX, we have used histone, casein, and myelin basic protein as possible phosphoacceptors. Incubation with 32PPi (data not shown). In an attempt to detect protein phosphorylation, we incubated A2058 ATX with 32P-labeled ATP in 20-μl reactions, as described under “Experimental Procedures,” at 0°C for various times from 15 to 120 min and resolved by SDS-PAGE. A, resulting silver stain and autoradiogram of the sole labeled band. A separate reaction (100 μl) containing A2058 ATX (50 μl) was incubated for 120 min, dialyzed, divided into 20-μl aliquots, further incubated for various times from 0 to 30 min at either 0 or 37°C, and resolved by SDS-PAGE. B, resulting autoradiogram of the sole labeled band. Lane 0 contains sample after dialysis without further incubation. The radioactivity incorporated into ATX was quantified as described under “Experimental Procedures,” and the combined data are represented in the C as percentage of maximum. Radioactivity in phospho-ATX immediately before (120 min) and immediately after dialysis are used as 100% values for the on and off reactions, respectively.

ATX displayed similar properties of phosphorylation (Fig. 3A) and dephosphorylation (Fig. 3B). The stability of phospho-ATX at 37°C in the presence of SDS (autoradiogram; Fig. 3B, last lane) indicates that the dephosphorylation of phospho-ATX is an enzymatic activity, requiring the native conformation of ATX. The ATX protein itself is stable under dephosphorylation conditions (silver stain; Fig. 3B, third lane). These results with homogeneously pure ATX demonstrate that the on-off cycle of phosphorylation is an intrinsic property of ATX and is not due to a co-purifying protein kinase and/or phosphoprotein phosphatase.

In addition to this activity toward ATP, ATX can be phosphorylated using [γ-32P]GTP, [γ-32P]AMP, [γ-32P] (adenylate) NAD or 32PPi, (data not shown). In an attempt to detect protein kinase activity in ATX, we have used histone, casein, and myelin basic protein as possible phosphoacceptors. Incubation of each of these proteins with [γ-32P]ATP resulted in the incorporation of label into histone or myelin basic protein but not into casein in the absence of any exogenous catalyst, whereas the inclusion of A2058 ATX in these reactions resulted in the phosphorylation of ATX as well but had no effect on the endogenous incorporation of label into these proteins (data not shown). These experiments fail to demonstrate protein kinase activity in ATX but leave open the possibility that such activity exists given appropriate cofactors and/or specific substrates.

ATX Has ATPase and ATP Pyrophosphatase Activities—The production of nucleotides and phosphates from ATP by ATX was detected using ATP that had been labeled with 32P in either the α- or the γ-phosphate position. Incubation of ATX with [α-32P]ATP results in the production of [32P]ADP and [32P]AMP (Fig. 4, A and B). Identical incubations were performed with [γ-32P]ATP and resulted in the production of 32Pi and 32PPi (Fig. 4, C and D). ADP and Pi are produced at more than twice the rate as AMP and PPi, respectively. Under the assay conditions used, each of these reaction products accumulates at a constant rate for 60 min (data not shown); the chromatograms shown are from 40-min incubations. These results demonstrate that ATX is able to hydrolyze the phosphodiester bonds in ATP on either side of the β-phosphate, which is then contained in either of the reaction products, ADP or PPi, resulting from either ATPase (EC 3.6.1.3) or ATP pyrophosphatase (EC 3.6.1.8) activities, respectively.

The substrates other than ATP that serve as phosphate donors in the phosphorylation of ATX were tested for their susceptibility to hydrolysis by ATX. Incubation with rATX results in the production of [32P]GDP and [32P]GMP from [α-32P]GTP, 32Pi from [32P]AMP or 32PPi, and [32P]AMP from [32P] (adenylate) NAD (data not shown).

There Is Competition between Substrates for the 5′-Nucleotide PDE, ATP Binding, and Phosphorylation Activities of ATX—ATX binds noncovalently to ATX (3), and ATX uses nucleotides as substrates, but the nature and number of nucleotide binding sites in ATX are not known. The data on enzyme catalysis by ATX presented here can be explained by the existence of a single nucleotide binding site, and this interpretation is supported by experiments showing competition between substrates for the various activities of ATX, as depicted (Fig. 5). Either 5′-TMP or ATP can compete with [α-32P]-azido-ATP in the ATP binding assay (Fig. 5A) or with [γ-32P]ATP in the phosphorylation of ATX (Fig. 5B). 5′-TMP and ATP are comparable in their ability to inhibit ATP binding, whereas 5′-TMP competes markedly less well than ATP in the phosphorylation assay.

A comparison of the ability of ATP and various ATP derivatives to inhibit the 5′-nucleotide PDE reaction is shown in Fig. 5C. The nucleotide analogs, which lack a hydrolyzable phosphate at the β-γ position (AMP-CP and AMP-PCP), are relatively less efficient as inhibitors than those that contain this phosphate (AMP-CPP and ATP), showing that the ability of...
ATP to inhibit the reaction depends at least partially on the presence of a hydrolyzable phosphate in the γ position.

**DISCUSSION**

In this study we have shown that homogeneously pure rATX catalyzes 5'-nucleotide PDE activity (Fig. 1) under physiological conditions and is indistinguishable from A2058 ATX (purified from a human melanoma cell line), based on the kinetics of threonine phosphorylation and dephosphorylation (Figs. 2 and 3). In addition we have shown that, with ATP as a substrate, ATX has ATPase (producing ADP and Pi) and ATP pyrophosphatase (producing AMP and PPi) activities (Fig. 4).

Since both the 5'-nucleotide PDE and ATP pyrophosphatase activities of ATX hydrolyze the α–β phosphodiester bond in their respective nucleotide substrates, it is probable that these two activities result from the same reaction mechanism. ATX is labeled by either [32P]adenylate NAD or [32P]AMP (this report) but not by [32P] ATP (3; data not shown). It is possible that ATX preferentially hydrolyzes, and incorporates phosphate from, the highest energy phosphoester bond in the substrate, which, in the case of ATP, is the β–γ phosphodiester bond. Such a preference would also explain the observations that ATP and Φ-TMP are comparable in their ability to compete for the noncovalent binding of [α-32P]8-N3-ATP to ATX (Fig. 5A), but that ATP is effective at much lower concentrations than Φ-TMP in inhibiting phosphorylation of ATX by [γ-32P]ATP (Fig. 5B). Consistent with this possibility is the observation (Fig. 5C) that ATP derivatives that lack a hydrolyzable bond at the β–γ position (AMP-CP and AMP-PCP) are less effective as inhibitors of the ATX-catalyzed 5'-nucleotide PDE reaction than derivatives that contain a hydrolyzable bond at this position (AMP-CP and ATP). The interesting suggestion (17) that there may be competition between the phosphorylation and phosphodiesterase activities of PC-1 may be relevant to these unresolved questions regarding ATX. The simplest interpretation of the competition between substrates for ATP binding, 5'-nucleotide PDE, and phosphorylation (Fig. 5) is that a single nucleotide binding site is used by ATX for each of these enzymatic functions, but definitive resolution of this question awaits more extensive enzyme inhibition and nucleotide binding studies.

GTP, NAD, AMP, and PPi are susceptible to hydrolysis by ATX and serve as phosphate donors in its phosphorylation. The hydrolysis of PPi to Pi occurs in a number of intracellular energy-conserving reactions (18), but the relationship between these reactions and the inorganic pyrophosphatase activity of ATX is not clear. The predominant products of ATP hydrolysis by ATX in vitro are ADP and P3, but the substrates and products of in vivo catalysis by ATX in the stimulation of tumor cell motility are not known. With the ability to hydrolyze nucleoside polyphosphates at a variety of positions, ATX may catalyze nucleotidase cascades (19, 20). ATX hydrolyzes substrates other than ATP, and the facility with which these substrates phosphorylate ATX suggests that in catalyzing each of the various hydrolytic reactions, ATX uses a covalently bound, phosphate-containing reaction intermediate. The data presented in this article strongly suggest that this is indeed the case for the ATPase reaction catalyzed by ATX. Fig. 6 depicts a

![FIG. 4. ATPase and ATP pyrophosphatase activity of rATX.](image)

Samples of homogeneously pure rATX were incubated, as described under “Experimental Procedures,” for 40 min at 37°C with either [α-32P]ATP (A and B) or [γ-32P]ATP (C and D). Reaction products were analyzed as described under “Experimental Procedures.” A and C, autoradiograms of TLC (one chromatogram of a duplicate pair is depicted). B and D, quantification of ATX-dependent activity in A and C, respectively. Bars, ±1 S.D. of duplicate chromatograms.

![FIG. 5. Competition between substrates for ATX-catalyzed reactions.](image)

Homogeneously pure rATX was assayed for the noncovalent binding of [α-32P]8-azido-ATP (A) and for phosphorylation by [γ-32P]ATP (B), as described under “Experimental Procedures,” in the presence of the indicated concentrations of Φ-TMP or ATP. This same material was assayed for 5'-nucleotide PDE activity, as described under “Experimental Procedures,” at pH 7.3 with a substrate (Φ-TMP) concentration of 1 mM in the presence or absence of 0.1 mM ATP or ATP analogs (C). Bars, ±1 S.D.
The proposed model for the formation of covalently bound reaction intermediates in the catalytic action of ATX toward ATP. ATX is proposed to be capable of at least two alternative mechanisms, ATPase and 5'-nucleotide PDE/ATP pyrophosphatase, each of which uses Thr210 as the site for the formation of the covalently bound reaction intermediate. The phosphothreonine intermediate in the ATPase reaction mechanism (Fig. 6, reaction 1) contains only the γ-phosphate from ATP and is stable at 0 °C and unstable at 37 °C (Figs. 2 and 3). The depiction formed of the adenyllyl threonine intermediate (Fig. 6, reaction 2) is based on the reported mechanism for 5'-nucleotide PDE (4). According to this proposal the phosphorylation-dephosphorylation cycle of ATX is a integral part of the ATPase reaction mechanism, and ATX is atypical among known ATPases (21, 22) in that it uses a phosphorylated threonine as a covalently bound reaction intermediate. Uniquivalent demonstration of the identity of the phosphorylation-dephosphorylation cycle of ATX with its ATPase activity awaits analysis in progress designed to show that a single point mutation simultaneously abolishes both of these activities. This mutational analysis is also being used to investigate the possibility that the same relationship holds between the other phosphorylation substrates and their hydrolysis by ATX.

Among the proteins with sequence homology to ATX the most well characterized is the ectoprotein PC-1. ATX and PC-1 each contain two tandem somatomedin B regions, the loop region of an EF-hand, and a type I PDE catalytic site and possess 5'-nucleotide PDE activity (2, 23). Studies on the effect of pH on the PDE activity of PC-1 (12), assayed at a substrate (β-32P]ATP) concentration of 0.5 mm, show optimum activity at alkaline pH, a characteristic that is typical of type I PDE enzymes. The 5'-nucleotide PDE activity of ATX at submillimolar substrate concentrations (Fig. 1) does not show this preference for alkaline pH. These data suggest that ATX and PC-1 may differ in this respect, and that catalysis of the 5'-nucleotide PDE reaction by ATX is physiologically relevant. [α-32P]ATP has been reported to label purified PC-1 (threonine at the PDE active site) (12) as well as immunoprecipitated or cell surface PC-1 (24). Attempts to label ATX with [α-32P]ATP have been unsuccessful (3; data not shown). It is possible that adenyllyl ATX, formed during incubation of ATP with ATX, exists only as a short-lived 5'-nucleotide PDE/ATP pyrophosphatase reaction intermediate and that its extremely transient nature precludes detection under the conditions and quantities of ATX used. Such a characteristic would also explain the efficiency of this ATX-catalyzed reaction at physiological pH, a property previously unreported among the type I PDE enzymes.

The dephosphorylation of phospho-ATX also differs from that of PC-1 in that it occurs after dialysis to remove exogenous nucleotides, which are reported to be stimulatory and necessary for the dephosphorylation of phospho-PC-1 (17).

This and other distinctions in the enzymatic characteristics between PC-1 and ATX may arise, at least in part, from a difference in the sequence of the nucleotide binding site. PC-1 (5) contains the glycine-rich GXGXXG sequence found in nucleotide-binding proteins (25) along with the downstream lysine invariably found in protein kinases (26), and this region may serve as an ATP binding site. Although ATX (2) has extensive homology to PC-1, it does not contain this sequence, nor does ATX contain a perfect match to any of the other P-loop type sequences found in adenine and guanine nucleotide-binding proteins (27). Although the nature of the ATP binding site(s) in PDE enzymes is not yet defined, ATP clearly binds to ATX (3; this report), and both PC-1 (28) and ATX (3; this report) have been purified to homogeneity using ATP-agarose chromatography. The failure to detect protein kinase activity in ATX is not unexpected considering the lack of sequence similarity to known protein kinases.
reuptake of nucleosides (46). Cell adhesion molecule 105 has been identified as an ecto-ATPase with implications for cell-cell interaction (47), and a rat liver ecto-ATPase has been identified as a canalicular bile acid transport protein (48). Since phosphorylation-deficient ATX (K209L) is biologically active (3), the ATPase activity of ATX may be dispensable for the stimulation of cellular motility. On the other hand, the stability of phosphorylated forms of ATX is apparently an active state, the possibility of a regulatory role for the phosphorylation of ATX is not excluded.

Continuing investigations on autotaxin are designed to test the hypothesis that the phosphorylated forms of ATX are enzyme-bound reaction intermediates in the hydrolysis of phosphoester bonds and to study the relationship between the 5'-nucleotide PDE/ATP pyrophosphatase activity of ATX and its stimulation of cellular motility, as well as the influence of the phosphorylation state and ATPase activities on these properties of ATX.

Acknowledgment—We thank Dr. Elliott Schifffmann for invaluable discussions throughout the course of this work.

REFERENCES
1. Stracke, M. L., Krutzsch, H. C., Unsworth, E. J., Årestad, A., Cioce, V., Schifffmann, E., and Liotta, L. (1992) J. Biol. Chem. 267, 2524–2529
2. Murata, J., Lee, H. Y., Clair, T., Krutzsch, H. C., Årestad, A. A., Sobel, M. E., Liotta, L. A., and Stracke, M. L. (1994) J. Biol. Chem. 269, 30479–30484
3. Lee, H. Y., Clair, T., Mulvaney, P. T., Woodhouse, E. C., Aznavoorian, S., Liotta, L. A., and Stracke, M. L. (1996) J. Biol. Chem. 271, 24408–24412
4. Culp, J. S., Blytt, H. J., Hermodson, M., and Butler, L. G. (1985) J. Biol. Chem. 260, 8320–8324
5. Buckley, M. F., Loveland, K. A., McKinniry, W. J., Garson, O. M., and Goding, J. W. (1990) J. Biol. Chem. 265, 17506–17511
6. Kawagoe, H., Soma, O., Goji, J., Nishimura, J., Nakamura, H., and Sano, K. (1995) Genomics 30, 380–384
7. Deissler, H., Lottspeich, F., and Rajewsky, M. F. (1995) J. Biol. Chem. 270, 9849–9855
8. Razell, W. E., and Khorana, H. G. (1989) J. Biol. Chem. 234, 2105–2113
9. Decker, K., and Bischoff, E. (1971) FEBS Lett. 1, 96–98
10. Evans, W. H., Hood, D. O., and Gurd, J. W. (1973) Biochem. J. 135, 819–826
11. Morley, D. J., Hawley, D. M., Ulbright, T. M., Butler, L. G., Culp, J. S., and Hodes, M. E. (1987) J. Histochem. Cytochem. 35, 75–82
12. Oda, Y., Kuo, M.-D., Huang, S. S., and Huang, J. S. (1995) J. Biol. Chem. 268, 27318–27326
13. Lee, H. Y., Murata, J., Clair, T., Polymeropoulos, M. H., Torres, R., Manrow, E., Liotta, L. A., and Stracke, M. L. (1996) Biochem. Biophys. Res. Commun. 218, 714–719
14. Davison, A. J., and Moss, B. (1990) Nucleic Acids Res. 18, 4285–4286
15. Fuerst, T. R., Earl, P. L., and Moss, B. (1987) Mol. Cell. Biol. 7, 2538–2544
16. Haley, B. E., and Hoffman, J. F. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3367–3371
17. Uriarte, M., Stalmans, W., Hickman, S., and Bollen, M. (1995) Biochem. J. 306, 271–277
18. Reeves, R. E. (1976) Trends Biochem. Sci. 1, 53–55
19. Pearson, J. D., Carleton, J. S., and Gordon, J. L. (1980) Biochem. J. 190, 421–429
20. Trams, E. G. (1980) J. Theor. Biol. 87, 609–621
21. Pedersen, P. L., and Carafoli, E. (1987) Trends Biochem. Sci. 12, 146–150
22. Fiers, W. (1995) Int. Rev. Cytol. 158, 141–214
23. Rebbe, N. F., Tong, B. D., Finlay, E. M., and Hickman, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5192–5196
24. Belli, S. I., Mercuri, F. A., Sali, A., and Godin, J. W. (1995) Eur. J. Biochem. 229, 669–676
25. Schulz, G. E. (1992) Curr. Opin. Struct. Biol. 2, 61–67
26. Bossemeyer, D. (1994) Trends Biochem. Sci. 19, 201–205
27. Saraste, M., Siibald, P. R., and Wittinghofer, A. (1990) Trends Biochem. Sci. 15, 430–434
28. Madixus, B. A., Shrawcd, P., Kamakura, S., Sasson, S., Youngren, J., Fisher, A., Spencer, S., Grupe, A., Henzel, W., Stewart, T. A., Reaven, G. M., and Goldfine, I. D. (1985) Nature 317, 448–451
29. Gilman, A. (1995) Biosci. Rep. 15, 65–97
30. Shapiro, R. (1986) Biochemistry 25, 3527–3532
31. Haraguchi, M., Miyadera, K., Uemura, K., Sumizawa, T., Furukawa, T., Tamada, K., and Akiyama, S. (1994) Nature 368, 198
32. Drawbridge, J., Scherson, T., Ersm, J. F., Basaviah, P., and Steinberg, M. S. (1991) J. Cell Biol. 115, 145a (abstr.)
33. Stiles, G. (1992) J. Biol. Chem. 267, 6451–6454
34. Chen, Z.-F., Levy, A., and Lightman, S. L. (1995) J. of Neuroendocrinol. 7, 83–96
35. vanDriel, J. R., Wilks, A. F., Pietersz, G. A., and Goding, J. W. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8619–8623
36. Lin, S.-H. (1989) J. Biol. Chem. 264, 14403–14407
37. Ehrlich, Y. H., Hogan, M. V., Pawlewksa, Z., Naik, U., and Kornecki, E. (1990) Ann. N. Y. Acad. Sci. 603, 401–416
38. Najjar, S. M., Acioli, D., Phillippe, N., Jerneberg, J., Margolis, R., and Taylor, S. J. (1993) J. Biol. Chem. 268, 1201–1216
39. Wang, T.-F., and Guidotti, G. (1996) J. Biol. Chem. 271, 9898–9901
40. Strobel, R. S., Nagy, A. K., Knowles, A. F., Buegel, J., and Rosenberg, M. D. (1996) J. Biol. Chem. 271, 16323–16331
41. Duseau, J. W., and Hutchins, P. M. (1988) Respir. Physiol. 71, 33–44
42. Meiningen, C. J., Schelling, M. E., and Granger, H. J. (1988) Am. J. Physiol. 255, H554–H562
43. Rose, F. R., Hirchhorn, R., Weissman, G., and Cronstein, B. N. (1988) J. Exp. Med. 167, 1186–1194
44. Garcia-Castro, I., Mate, J., Vasanthakumar, G., Weissman, W., Schifffmann, E., and Chiang, P. (1983) J. Biol. Chem. 258, 4345–4349
45. Suprenant, A., Rassendren, F., Kawashima, E., North, R. A., and Buell, G. (1996) Science 272, 725–728
46. Che, M., Nishida, T., Gatmaitan, Z., and Arias, I. M. (1992) J. Biol. Chem. 267, 9681–9688
47. Aurivillius, M., Hansen, O. C., Lazrek, M. B. S., Bock, E., and Öbrink, B. (1990) FEBS Lett. 264, 267–269
48. Sippel, C. J., Suchy, F. J., Ananthanaryanan, M., and Perlmutter, D. H. (1993) J. Biol. Chem. 268, 2083–2091