Human prostatic acid phosphatase has phosphotyrosyl protein phosphatase activity

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The major secreted isoenzyme of human prostatic acid phosphatase (PACp) (EC 3.1.3.2), which catalyses *p*-nitrophenyl phosphate (PNPP) hydrolysis at acid pH values, was found to have phosphotyrosyl protein phosphatase activity since it dephosphorylated three different phosphotyrosine-containing protein substrates. Several lines of evidence are presented to show that the phosphotyrosyl phosphatase and PACp are the same enzyme. (1) A highly purified PACp enzyme preparation which contains a single *N*-terminal peptide sequence was used to test for the phosphotyrosyl phosphatase activity. (2) Both activities comigrated during gel filtration by high performance liquid chromatography. (3) Phosphotyrosyl phosphatase activity and PNPP acid phosphatase activity exhibited similar sensitivities to different effectors. (4) Both phosphatase activities showed the same thermal stability. (5) Specific anti-PACp antibody reacted to the same extent with both phosphatase activities. (6) PNPP acid phosphatase activity was competitively inhibited by the phosphotyrosyl phosphatase substrate. To characterize further the phosphotyrosyl phosphatase activity, the K_m values using different phosphoprotein substrates were determined. The apparent K_m values for phosphorylated angiotensin II, anti-pp60src immunoglobulin G and casein were in the nM range for phosphotyrosine residues, which was about 50-fold lower than the K_m for phosphoserine residues in casein.

INTRODUCTION

Phosphorylation and dephosphorylation is a major mechanism for enzyme regulation in eucaryotic systems (Greengard, 1978; Krebs & Beavo, 1979; Cohen, 1982). Several oncogene proteins and growth factor receptors have been found to possess protein phosphorylation activity specific for tyrosine residues. Thus tyrosyl protein kinases are believed to be important in normal and malignant growth of cells (Bishop, 1983; Heldin & Westermark, 1984).

Since the level of tyrosine phosphorylation is apparently important in neoplastic transformation and in normal cell growth control, the relative rate of tyrosine phosphorylation compared with phosphotyrosine dephosphorylation may be an important regulatory mechanism in cell growth control. Although much effort has been directed at characterizing tyrosyl kinases, relatively little is known regarding phosphotyrosine phosphatases and their physiological significance. The observation that dephosphorylation of phosphotyrosine residues occurs directly following the loss of the transformed phenotype in cells transformed by temperature-sensitive mutants of Rous sarcoma virus and Fujinami sarcoma virus (Bishop, 1983; Foulkes, 1983), following the initial increase in phosphotyrosine residues upon the addition to cells of platelet-derived growth factor, epidermal growth factor (Heldin & Westermark, 1984) and diverse mitogenic agents (Nakamura *et al.*, 1983; Kohno, 1985), suggest an important physiological role for phosphotyrosine phosphatase in regulation of the growth of normal and transformed cells.

Human prostatic acid phosphatase (PACp) is composed

of a group of acid phosphomonoesterases. The major PACp isoenzyme (M_r 100000) is a glycoprotein which has previously been shown to hydrolyse phosphomonoesters with optimal activity at pH 4–6 (Yam, 1974; Lin *et al.*, 1983a). The major isoenzyme has been found to be expressed at a high level in the normal prostate gland and to have repressed expression in prostate tumour tissue (Yam, 1974; Loo *et al.*, 1981). To determine the physiological function, we purified the major PACp isoenzyme and tested the enzyme for its phosphotyrosyl protein phosphatase activity. The results support the hypothesis that PACp may play a role in determining the phosphorylation state of phosphotyrosine-containing proteins.

MATERIALS AND METHODS

Materials

$[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (sp. radioactivity 2900 Ci/mmol) was purchased from New England Nuclear, Boston, MA, U.S.A. Angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), *p*-nitrophenyl phosphate, *p*-nitrophenol standard solution, *O*-phosphotyrosine, L(+)-tartaric acid, hydrolysed, partially dephosphorylated casein, protein A-Sepharose CL-4B, and NaF were from Sigma Chemical Co., St. Louis, MO, U.S.A. Bio-Rad dye was from Bio-Rad Laboratories, Richmond, CA, U.S.A. Rabbit anti-PACp antibody was a gift from Dr. C. L. Lee. The catalytic subunit of the cyclic AMP-dependent protein kinase was a gift from Dr. R. Roskoski. All other materials were obtained as previously described (Clinton *et al.*, 1982; Lin *et al.*, 1983a,b).

Abbreviations used: PACp, prostatic acid phosphatase; pp60src, the phosphoprotein encoded by *src*, the transforming gene of Rous sarcoma virus; PNPP, *p*-nitrophenyl phosphate.

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Purification of prostatic acid phosphatase

The major PAcP isoenzyme with M_r 100 000 was purified from human seminal plasma as we have previously described by using a purification scheme which included $(\text{NH}_4)_2\text{SO}_4$ fractionation, concanavalin A-Sepharose 4B affinity chromatography, DEAE-cellulose ion exchange chromatography, and Sephadex G-100 and G-150 gel filtrations (Lee *et al.*, 1978; Lin *et al.*, 1983a,b). The purified PAcP was found to have a single *N*-terminal protein sequence (Lin *et al.*, 1983b; Taga *et al.*, 1983). The purified enzyme was stored at -20°C in 0.1 M-citrate, pH 6.0, where it was stable for several months. The protein concentration was determined by the Bio-Rad dye protein assay with bovine serum albumin as the standard. Purified PAcP was also subjected to gel filtration by h.p.l.c. on a Spherogel-TSK SW 4000 column (Beckman Instruments, Berkeley, CA, U.S.A.) equilibrated with 5 mM-citrate, pH 5.8, containing 0.2 M-NaCl. A 200 μl sample of purified PAcP (0.4 mg/ml) was chromatographed on the column at 24°C and fractions were stored at 0°C . A 3 μl aliquot of each fraction was removed for the phosphotyrosyl phosphatase assay with phosphoangiotensin as the substrate and for the acid phosphatase determination.

Acid phosphatase determinations

PNPP at 3 mM in 50 mM-citrate, pH 6.0 (final volume 0.4 ml), was used to determine esterase activity. Incubation was performed at 34°C for the time indicated in the Figure legends. The reaction was terminated by the addition of 2.0 ml of 0.1 M-NaOH. The released *p*-nitrophenol was measured spectrophotometrically at 410 nm and *p*-nitrophenol standard solution was used to determine the amount of phosphate released (Lin *et al.*, 1983a).

Preparation of ^{32}P -labelled phosphoproteins

^{32}P -labelled angiotensin II was prepared by using protein kinase obtained from human serum (Lin *et al.*, 1985). The phosphorylated angiotensin II was purified by high voltage paper electrophoresis, eluted from the paper with water, lyophilized and stored at -20°C .

^{32}P -labelled anti-pp60src IgG and casein were phosphorylated on tyrosine residues by partially purified pp60src protein in immunocomplexes as previously described (Clinton *et al.*, 1982). After the kinase reaction the ^{32}P -labelled proteins were precipitated with trichloroacetic acid and washed with acetone. The washed pellet was dialysed against 50 mM-citrate, pH 6.0, and stored at -20°C .

^{32}P -labelled casein, phosphorylated on serine residues, was prepared by using the catalytic subunit of the cyclic AMP-dependent protein kinase (Roskoski, 1983). The phosphorylation reaction was carried out at 34°C for 30 min. The reaction was terminated and unincorporated radioisotope was removed by precipitating the protein with trichloroacetic acid and washing the pelleted protein with acetone as described above.

To characterize the above products, SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) followed by autoradiography was conducted to show that the ^{32}P migrated as a single band corresponding to the substrate protein. The phosphoamino acid content of the substrates was analysed as described (Clinton *et al.*, 1982). This involved partial acid hydrolysis, and separation of the

phosphoamino acids by high voltage paper electrophoresis in two dimensions and detection by ninhydrin staining and autoradiography. ^{32}P -labelled angiotensin, anti-pp60src IgG, and casein phosphorylated by partially purified pp60src were found to contain ^{32}P -labelled phosphotyrosine. No radioactive phosphoserine or phosphothreonine could be detected in any of these substrates. Only ^{32}P -labelled phosphoserine could be detected in the casein phosphorylated by the catalytic subunit of the cyclic AMP-dependent protein kinase. The specific radioactivity of each substrate was calculated from the incorporation of ^{32}P into trichloroacetic acid-precipitable protein and the specific radioactivity of the $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Preparations of phosphorylated substrates routinely contained 1 mol of phosphate/mol of angiotensin, 0.3–1.0 mol of phosphate/mol of IgG, and 0.1–0.9 mol of phosphate/mol of casein containing phosphotyrosine and casein containing phosphoserine.

Phosphoprotein phosphatase determinations

The assay was carried out in a reaction volume of 30 μl containing 50 mM-citrate, pH 6.0, and 0.024 μg of enzyme unless otherwise specified. The reaction was terminated by boiling for 5 min. The incubation time as well as incubation temperature in each assay was adjusted so that less than 25% of the $^{32}\text{P}_i$ was released from the phosphoproteins. The released $^{32}\text{P}_i$ was separated from other reaction components by electrophoresis at pH 4.4 for 20 min at 1500 V. The product that comigrated with the $^{32}\text{P}_i$ marker was localized by autoradiography, cut from the paper, and quantified by Čerenkov counting.

Immunoprecipitation of phosphatase activities

Antiserum made in rabbits against purified PAcP was determined to be specific for PAcP by the solid-phase immunofluorescence assay (Lee *et al.*, 1978; Chu *et al.*, 1982). The titre of specific IgG prepared from antiserum as previously described (Lee *et al.*, 1978) was determined by titrating different dilutions of IgG with 250 ng of purified enzyme. A 50% binding of IgG to PAcP was chosen for comparing inhibition of the two phosphatase activities. For the immunoprecipitations, the purified enzyme was incubated at 4°C for 1 h in 50 mM-citrate, pH 6.0, with the IgG fraction. Protein A-Sepharose 4B at an equivalent concentration to the IgG was added for a 30 min incubation. After centrifugation at 4°C , the supernatant was removed. PNPP acid phosphatase and phosphotyrosyl phosphatase activities in the supernatant were determined.

RESULTS

Prostatic acid phosphatase functions as a phosphotyrosyl protein phosphatase

The purpose of our study was to test whether the major isoenzyme of PAcP, an enzyme previously characterized to hydrolyse PNPP at acid pH, can function to hydrolyse phosphomonoesters to tyrosine in proteins. The PAcP used in these studies was purified to homogeneity from seminal plasma according to a procedure we have used previously (Lin *et al.*, 1983a,b). The homogeneity of PAcP was confirmed by polyacrylamide-gel electrophoreses under denaturing and non-denaturing conditions (results not shown; Lin *et al.*, 1983b). The purified PAcP was found to have a single *N*-terminal amino acid (lysine) and

Fig. 1. Kinetics of dephosphorylation of ^{32}P -labelled angiotensin II by PACP

For the phosphoprotein phosphatase assay, the reaction was carried out at 24 °C in a volume of 150 μl containing 50 mM-citrate, pH 6.0, 0.5 nM-phosphoangiotensin II and 24 ng of enzyme protein. At the indicated time intervals, aliquots (25 μl) were removed for the determination of $^{32}\text{P}_i$ released. For the acid phosphatase assay, 3 mM-PNPP was used as substrate and the activity was measured at 34 °C as described in the Materials and methods section.

Fig. 2. Effect of PACP concentration on dephosphorylation of ^{32}P -labelled angiotensin II

Phosphoprotein phosphatase assay at 24 °C and acid phosphatase assay at 34 °C were performed as described in the Materials and methods section with 1 min incubation. A concentration of 0.6 nM-phosphoangiotensin II was used.

a single *N*-terminal peptide (of about 24 amino acids) by this purification procedure (Lin *et al.*, 1983b).

One phosphotyrosine-containing substrate we tested for dephosphorylation by PACP was phosphorylated angiotensin II. Angiotensin II has been found to be phosphorylated by several tyrosyl kinases (Wong & Goldberg, 1983; Lin *et al.*, 1985) and since it contains tyrosine, but not serine or threonine which are the other major amino acids phosphorylated by the known protein kinases, angiotensin II provides a substrate which can be exclusively phosphorylated in tyrosine. Fig. 1 shows that

Fig. 3. Gel filtration of PACP by h.p.l.c.

Purified PACP was loaded onto a TSK SW4000 gel filtration column (0.75 cm $\times$ 30 cm) equilibrated with 5 mM-citrate, pH 5.8, containing 0.2 M-NaCl, and then eluted with the same buffer. The flow rate was 0.5 ml/min and 250 μl fractions were collected.

Table 1. Inhibition of prostatic acid phosphatase activity

The phosphatase assays were performed as described in the Materials and methods section with 0.2 nM-phosphoangiotensin II or 3 mM-PNPP as substrates.

Effector	Relative activity (%)	
	Phosphoangiotensin II	PNPP
None	100	100
Vanadate (50 μM)	11	22
		3*
ZnCl ₂ (50 μM)	99	99
(200 μM)	95	98
L(+)-Tartrate (10 mM)	8	7
PNPP (10 mM)	7	N.D.†
NaF (10 mM)	2	13
		3*

* PNPP was used at 30 μM .

† Not determined.

dephosphorylation of both the phosphopeptide, ^{32}P -labelled angiotensin II, and the organic phosphate, PNPP, was catalysed by purified PACP. The release of $^{32}\text{P}_i$ from phosphorylated angiotensin II exhibited linear kinetics with an incubation time of up to 3 min at 0.5 nM substrate concentration. When the reaction mixture was incubated for 1 min, the release of $^{32}\text{P}_i$ from angiotensin II showed first-order kinetics with respect to enzyme concentration up to 50 ng in the 30 μl reaction (Fig. 2). Although the concentration of phosphorylated angiotensin II was in the nanomolar range (0.5 nM), the dephosphorylation reaction was time-dependent (Fig. 1) and enzyme-concentration-dependent (Fig. 2). These results obtained using highly purified enzyme and substrate indicate that PACP has phosphotyrosyl phosphatase activity.

To demonstrate the copurification of PACP and the phosphotyrosyl phosphatase, the enzyme activities were measured after h.p.l.c. gel filtration of purified PACP. As shown in Fig. 3, a single protein peak contained both the phosphotyrosyl phosphatase and the PNPP acid phosphatase activities.

Fig. 4. Competitive inhibition of PNPP dephosphorylation by phosphoangiotensin II

PNPP was used as the substrate with different concentrations of phosphoangiotensin II as indicated in the inset. The assay was performed at 34 °C under conditions where the rate of PNPP hydrolysis was linear at the PNPP concentrations used. The inset is the slope of the Lineweaver-Burk plot versus inhibitor concentration. The concentrations of phosphoangiotensin used were 1.6 nM (Δ), 1.1 nM ($\times$), 0.7 nM ($\bullet$) or 0 ($\circ$).

Effects of inhibitors on the phosphatase activities

To characterize further and compare both phosphatase activities, the effects of different inhibitors on PNPP hydrolysis and on phosphoangiotensin II dephosphorylation by PACP were investigated (Table 1). PNPP, a substrate for PACP, inhibited the dephosphorylation of ^{32}P -labelled angiotensin II by 93%. Tartrate, a relatively specific inhibitor of PACP but not of alkaline phosphatases or of several other acid phosphatases (Fishman & Lerner, 1953; Yam, 1974), exhibited the same inhibitory effect on phosphotyrosyl protein phosphatase (92%) and PNPP acid phosphatase (93%) activities. Moreover, both enzymic activities responded similarly to Zn^{2+} . While Zn^{2+} has been found to inhibit several phosphotyrosyl phosphatases, neither dephosphorylation of PNPP or of ^{32}P -labelled angiotensin II were significantly affected by ZnCl_2 . Both vanadate and fluoride have been shown to be inhibitory toward PACP activity (Sawada *et al.*, 1981; Lin *et al.*, 1983a). As shown in Table 1, both phosphatase activities were inhibited by these compounds. Phosphotyrosyl phosphatase activity, however, appeared somewhat more sensitive to the inhibition by these two inhibitors. When a lower concentration of PNPP (30 μM) was used for the assay, the enzyme was more effectively inhibited, with about 97% loss of the PNPP phosphatase activity in the presence of vanadate and of NaF (Table 1). These data show that both PNPP and phosphoangiotensin II phosphatase activities associated with PACP were affected similarly by different effectors.

If the same enzyme molecule is catalysing the hydrolysis of PNPP and of phosphoangiotensin II, then inhibition of PNPP dephosphorylation by phosphoangiotensin II should be competitive. To test this, PNPP dephosphorylation by PACP was measured under conditions of increasing substrate concentration and under conditions of different concentrations of phosphoangiotensin II. Fig. 4 shows a double-reciprocal plot of the initial rate of the reaction plotted against substrate (PNPP) concentration. Inspection of this plot reveals that

Fig. 5. Thermostability of PACP

The purified PACP was preincubated at 56 °C in 50 mM-citrate, pH 6.0. At the indicated time intervals, aliquots were removed and measured for acid phosphatase activity ($\bullet$) with PNPP or for phosphotyrosyl phosphatase activity ($\circ$) with phosphoangiotensin II as substrate.

at increasing inhibitor (phosphoangiotensin II) concentration the K_m of the enzyme for PNPP is increased whereas the $V_{\max}$ remains unchanged. Furthermore, the apparent K_i of about 0.9×10^{-9} M is nearly equal to the apparent K_m (1.1×10^{-9} M) for phosphoangiotensin II (see Table 3). Therefore, phosphoangiotensin II is a competitive inhibitor of the PNPP acid phosphatase.

Temperature effects on phosphatase activities

To explore further the phosphotyrosyl phosphatase activity and the PNPP acid phosphatase activity associated with PACP, their thermostabilities were compared. As shown in Fig. 5, the two enzymic activities exhibited the same thermal sensitivities at 56 °C. After incubation at 56 °C for 30 min, about 30% of the activities remained for both acid phosphatase and for phosphotyrosyl phosphatase.

Effects of anti-PACP antibody on phosphatase activities

Immunoassays have been developed to distinguish prostate-specific acid phosphatase (PACP) from other acid phosphatases (Henderson & Neelson, 1981; Chu *et al.*, 1982). An antibody made to the purified PACP and a control, preimmune, serum were tested for their reactivities toward the phosphotyrosyl phosphatase and the PNPP acid phosphatase activities. The antibody was used at a concentration previously determined to cause about 50% loss of PNPP phosphatase activity. After absorption with the specific antibody and precipitation of the immune complex with protein A-Sepharose, the enzyme remaining in the supernatant was assayed for

Table 2. Precipitation of phosphotyrosyl phosphatase activity by anti-PAcP antibody

The substrate concentrations of PNPP and phosphoangiotensin II were 3 mM and 7.4 nM respectively. The dephosphorylation was performed at 34 °C as described in the Materials and methods section. The values in parentheses represent the percentage of the phosphatase activities remaining after incubation with anti-PAcP antibody in comparison with the control which was incubated with preimmune IgG.

Antibody	Phosphatase activity remaining in the supernatant	
	PNPP (nmol/min)	Phosphoangiotensin II (fmol/min)
Rabbit preimmune IgG	32.2 (100)	61.9 (100)
Rabbit anti-PAcP IgG	13.4 (41.6)	24.0 (38.8)

Fig. 6. Lineweaver-Burk plot

³²P-labelled angiotensin II was used as the substrate with 0.3 nM-PAcP. The assay was for 1 min at 24 °C under conditions where the rate of dephosphorylation was linear with all substrate concentrations. The K_m was calculated to be 1.1×10^{-9} M by the method of Lineweaver & Burk (1934).

PNPP acid phosphatase as well as for phosphotyrosyl phosphatase activity. As shown in Table 2, about 60% of both the phosphotyrosyl phosphatase activity and the PNPP acid phosphatase activity was lost following the reaction with antibodies to PAcP.

Kinetic properties of prostatic acid phosphatase

PAcP is capable of hydrolysing a wide variety of organic phosphomonoesters (Lin *et al.*, 1983a), but little is known regarding its activity toward phosphoproteins. In order to study the specificity of this enzyme for phosphoproteins, angiotensin II, IgG, and casein, each

Table 3. Activity of prostatic acid phosphatase with different substrates

Each value is the average of two different determinations. The K_m and V_{max} for phosphoangiotensin II and *O*-phosphotyrosine were obtained from reactions at 24 °C; IgG containing phosphotyrosine, casein containing phosphotyrosine, casein containing phosphoserine and PNPP were incubated at 34 °C. To measure dephosphorylation of phosphotyrosine, the released tyrosine was separated from phosphotyrosine by paper electrophoresis at pH 3.5 at 1000 V and was quantified by reaction with ninhydrin.

Substrate	Apparent K_m (M)	V_{max} (mol/min per mg)
Phosphoangiotensin II	1.1×10^{-9}	1.5×10^{-10}
Phosphotyrosine-IgG	1.7×10^{-9}	8.2×10^{-11}
Phosphotyrosine-casein	9.6×10^{-10}	5.6×10^{-11}
Phosphoserine-casein	6.3×10^{-8}	1.0×10^{-10}
<i>O</i> -Phosphotyrosine	5.0×10^{-3}	8.0×10^{-4}
PNPP	2.8×10^{-4}	2.7×10^{-4}

containing phosphotyrosine, and casein containing phosphoserine, were compared as substrates. Each substrate was assayed under conditions where the reaction rate was linear (results not shown). A K_m value of 1.1×10^{-9} M for phosphoangiotensin II was obtained from a Lineweaver-Burk plot (Fig. 6). Using ³²P-labelled anti-pp60src IgG as the substrate, a K_m of 1.7×10^{-9} M, which was similar to that for phosphoangiotensin II, was obtained (Table 3). To compare the affinity of PAcP for phosphotyrosine with that for the major phosphoamino acid in cells, phosphoserine, the apparent K_m values for the two were determined. As shown in Table 3, the apparent K_m value for casein containing phosphotyrosine was about 50-fold less than that for casein which contained phosphoserine.

The kinetic data obtained for several substrates revealed other properties of the enzyme. The enzyme was found to have a lower K_m for protein substrates than for small phosphate-containing compounds. The K_m values determined for PNPP and for free *O*-phosphotyrosine were found to be more than five orders of magnitude higher than for phosphotyrosine proteins (Table 3). Although PAcP showed a higher affinity for protein substrates, the V_{max} values for PNPP and for phosphotyrosine were greater than for the phosphoproteins (Table 3).

DISCUSSION

The results of this study demonstrate that the major acid phosphatase from seminal plasma, PAcP, is a phosphotyrosyl protein phosphatase. Several lines of evidence indicate that phosphotyrosyl phosphatase and PNPP phosphatase activities are mediated by the same enzyme. First, the two activities copurified. Second, tartrate, an inhibitor which distinguishes PAcP from several other alkaline and acid phosphatases, and PNPP, a substrate for PAcP, were both efficient inhibitors of the phosphotyrosyl phosphatase activity. Third, both phosphatase activities showed the same sensitivity to inactivation by heat and similar reactivity with antibody to PAcP. Most importantly, phosphoangiotensin II

showed competitive inhibition of PNPP dephosphorylation by PAcP. Therefore, we conclude that both enzymes have the same active site.

While this work was in progress, Li *et al.* (1984) reported that phosphotyrosyl phosphatase activity copurified with the major acid phosphatase of 100 000 Da from prostate tissue. The acid phosphatase used in our studies was purified from human semen and may be the secreted form of the enzyme investigated by Li *et al.* (1984). In addition to confirming the finding that phosphotyrosyl phosphatase activity copurifies with acid phosphatase from prostate, our study provides evidence that both PNPP and phosphotyrosine dephosphorylation occur at the same active site. We also provide additional kinetic analyses of the phosphotyrosyl phosphatase activity, including K_m determinations for phosphotyrosine- and phosphoserine-containing proteins.

PAcP showed substrate selectivity that may be of physiological importance. The enzyme showed a very high affinity (K_m in the nanomolar range) for phosphotyrosine in different substrates. In fact PAcP has the highest affinity for phosphotyrosine residues in proteins of any phosphotyrosyl phosphatase yet investigated (Foulkes, 1983; Lau *et al.*, 1985). Phosphotyrosine in proteins is present at very low levels in most cells and is the least abundant of any of the acid-stable phosphoamino acids (Hunter & Sefton, 1980). Therefore, it is expected that a phosphotyrosyl phosphatase which functions in the regulation of phosphotyrosine levels in cells would have an exceptionally high affinity for phosphotyrosine. Moreover, PAcP was also found to have a greater affinity for phosphotyrosine than for phosphoserine in proteins. The K_m for proteins which contained exclusively phosphotyrosine, including angiotensin II, IgG, and casein, was about 50-fold lower than for casein which contained only phosphoserine. The finding that the V_{max} was about the same for casein which contained either phosphoserine or phosphotyrosine suggests that, once associated with the enzyme, the rate of phosphate hydrolysis was the same when the phosphomonoester was adjacent to a polypeptide. The V_{max} was relatively low when phosphoprotein substrates were used. Kinetic analysis of PAcP with authentic cellular phosphotyrosine-containing substrates in their native state may result in a higher V_{max} . This can be tested only when the endogenous substrates have been identified. Although the V_{max} is low, the concentration of PAcP is very high in prostate tissue and appears to be the major phosphotyrosyl phosphatase in prostate tissue (Li *et al.*, 1984).

There are some puzzling aspects of the kinetics of phosphotyrosine dephosphorylation by PAcP. When the substrate phosphoangiotensin was at lower concentrations than the enzyme (0.5 nM compared with 1.6 nM of enzyme protein) and less than the K_m of 1.1 nM, the reaction rate was linear for up to 3.0 min (Fig. 1). Moreover, the rate was proportional to the enzyme concentration up to 16.7 nM (Fig. 2) even though the substrate was only at 0.6 nM. One possible explanation for linear kinetics under these conditions is because of the slow rate of release of the enzyme-product complex. Li *et al.* (1984) also observed a slow phosphoprotein substrate turnover rate using a tartrate-sensitive acid phosphatase purified from prostate tissue. They observed only about 40% dephosphorylation of the substrate (0.4 nM-substrate and 1.24 nM-enzyme) in a 1 h reaction at 37 °C. Another possible explanation which we cannot rule out is that a

subpopulation of PAcP, perhaps a modified form, is active as a phosphotyrosyl phosphatase. It is unlikely that there is a minor contaminant of the PAcP which is responsible for the phosphotyrosyl phosphatase activity, since the enzyme was extensively purified and since the phosphotyrosyl phosphatase substrate, phosphoangiotensin, was a competitive inhibitor of PAcP activity.

The very high affinity (K_m in nM range) of the substrate we used, phosphoangiotensin II, for PAcP can lead to non-linear double reciprocal plots (see Dixon & Webb, 1979). However, a linear relationship was obtained in several independent experiments (see Fig. 6). Apparently the phosphoangiotensin II concentrations we used were in the linear range of double-reciprocal plotting. The expected kinetics were observed, however, when higher concentrations (3–16 nM) of phosphoangiotensin were used. In this case a curved double-reciprocal plot was obtained (results not shown).

Our finding that PAcP is capable of dephosphorylating phosphoserine groups in proteins is in contrast with the results of Li *et al.* (1984) which showed the lack of activity of an acid phosphatase purified from prostate toward phosphoserine. It is possible that the differences observed in activities toward phosphoserine may have been caused by the different substrates used. The casein used in our study was first partially dephosphorylated and then phosphorylated by a serine-specific protein kinase and [γ - 32 P]ATP. However, the α -casein used in the studies of Li *et al.* (1984) was not apparently first dephosphorylated and may have contained higher amounts of unlabelled phosphoserine. If the level of unlabelled phosphoserine was high enough, measurements of dephosphorylation by the 32 P released may have been obscured. It is also possible that the secreted form of PAcP used in our study has slightly different biochemical properties than the cellular form of PAcP.

The identification and partial characterization of PAcP as a phosphotyrosyl phosphatase provides a new biochemical tool with which to probe the role of phosphotyrosine residues in cell transformation. There is suggestive evidence that this phosphatase may play a role in the malignant growth of prostate tumours. The major PAcP isoenzyme is reduced by at least 75% in tumour tissue from prostate (Yam, 1974; Loo *et al.*, 1981). Furthermore, the growth rate of prostate tumour cell lines is inversely correlated with the level of PAcP produced by the cells. If PAcP functions as a phosphotyrosyl phosphatase, then a reduction of its activity should result in an increased level of phosphotyrosine in proteins. Recent studies in our laboratory show that the phosphotyrosine, but not the phosphoserine or phosphothreonine, residues are specifically amplified in prostate tumour cells; moreover, this is associated with repressed levels of PAcP (M.-F. Lin & G. M. Clinton, unpublished work). The combined data suggest that expression of prostatic acid phosphotyrosyl phosphatase is at least partially responsible for determining the net amount of phosphotyrosine residues in proteins, and possibly the malignant growth of the cells.

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REFERENCES

- Bishop, J. M. (1983) *Annu. Rev. Biochem.* **52**, 301–345
- Chu, T. M., Wang, M. C., Lee, C. L., Killian, C. S. & Murphy, G. P. (1982) in *Biochemical Markers for Cancer* (Chu, T. M., ed.), pp. 117–136, Marcel Dekker, New York
- Clinton, G. M., Guerina, N. G., Guo, H. Y. & Huang, A. S. (1982) *J. Biol. Chem.* **257**, 3313–3319
- Cohen, P. (1982) *Nature (London)* **296**, 613–619
- Dixon, M. & Webb, E. C. (1979) *Enzymes*, pp. 464–465, Academic Press, New York
- Fishman, W. H. & Lerner, F. (1953) *J. Biol. Chem.* **200**, 89–97
- Foulkes, J. G. (1983) *Curr. Top. Microbiol. Immunol.* **107**, 163–180
- Greengard, P. (1978) *Science* **199**, 146–152
- Heldin, C.-H. & Westermark, B. (1984) *Cell* **37**, 9–20
- Henderson, A. R. & Neelson, D. A. (1981) *Clin. Chim. Acta* **115**, 9–32
- Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1311–1315
- Kohno, M. (1985) *J. Biol. Chem.* **260**, 1771–1779
- Krebs, E. G. & Beavo, J. A. (1979) *Annu. Rev. Biochem.* **43**, 923–959
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lau, K.-H. W., Farley, J. R. & Baylink, D. J. (1985) *J. Biol. Chem.* **260**, 4653–4660
- Lee, C. L., Wang, M. C., Murphy, G. P. & Chu, T. M. (1978) *Cancer Res.* **38**, 2871–2878
- Li, H.-C., Chernoff, J., Chen, L. B. & Kirschenbaum, A. (1984) *Eur. J. Biochem.* **138**, 45–51
- Lin, M. F., Lee, C. L., Li, S. S. L. & Chu, T. M. (1983a) *Biochemistry* **22**, 1055–1062
- Lin, M. F., Lee, C. L., Sharief, F. S., Li, S. S. L. & Chu, T. M. (1983b) *Cancer Res.* **43**, 3841–3846
- Lin, M. F., Lee, P. & Clinton, G. M. (1985) *J. Biol. Chem.* **260**, 1582–1587
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Loor, R., Wang, M. C., Valenzuela, L. & Chu, T. M. (1981) *Cancer Lett.* **14**, 63–69
- Nakamura, K. D., Martinez, R. & Weber, M. J. (1983) *Mol. Cell. Biol.* **3**, 380–390
- Roskoski, R., Jr. (1983) *Methods Enzymol.* **99**, 1–6
- Sawada, H., Shinoda, M., Maruyama, H. & Nakayama, T. (1981) *Chem. Pharm. Bull.* **29**, 3624–3629
- Taga, E. M., Moore, D. L. & Van Etten, R. L. (1983) *Prostate* **4**, 141–150
- Wong, T. W. & Goldberg, A. R. (1983) *J. Biol. Chem.* **258**, 1022–1025
- Yam, L. T. (1974) *Am. J. Med.* **56**, 604–616

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