FEBS Letters 406 (1997) 42–48 FEBS 18380

Identification of a protein-tyrosine phosphatase (SHP1) different from that associated with acid phosphatase in rat prostate

Ana-Montserrat Valencia[a], Jose L. Oliva[a], Guillermo Bodega[b], Antonio Chiloeches[a], Pilar López-Ruiz[a], Juan C. Prieto[a], Christiane Susini[c], Begoña Colás[a,*]

[a]*Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain*
[b]*Departamento de Biología Celular y Genética, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain*
[c]*INSERM U151, CHU Rangueil L3 31054 Toulouse, France*

Received 2 February 1997

Abstract Using [^{32}P]poly(Glu,Tyr) as substrate, we have identified, for the first time, in the rat prostatic gland a protein-tyrosine phosphatase activity different from that associated with prostatic acid phosphatase. Concanavalin A-Sepharose 4B was used to separate the two protein-tyrosyl phosphatases activities. The activity retained by the lectin had characteristics of the prostatic acid phosphatase. It was sensitive to inhibition by PNPP and the optimum pH shifted towards physiological values when [^{32}P]poly(Glu,Tyr) was used as substrate. However, the major protein-tyrosine phosphatase activity was not retained by the lectin, and corresponded, at least in part, to SHP1 as probed by the presence of the protein, its mRNA and the loss of PTPase activity after immunodepletion of SHP1. This enzyme is localized within the epithelial cells. Thus, the coexistence of two protein-tyrosine phosphatase activities in rat prostate, one associated with the acid phosphatase and the other related to SHP1, makes it necessary to analyze the importance of both activities in vivo and their possible function regarding prostatic cell growth and its regulation.

Key words: Prostate; PTPase; SHP1; Prostatic acid phosphatase

1. Introduction

Important cellular activities such as cell proliferation, oncogenesis and signal transduction are regulated, in part, by the balance between the activities of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPases) [1]. PTKs are notable in that they are the cytoplasmic catalytic portion for many growth factor receptors and the genes that encode them are potential oncogenes. PTPases, by contrast, have recently been identified. These enzymes represent a structurally diverse family of transmembrane receptor-like and non-receptor enzymes which play a crucial role in cellular processes involving phosphorylation on tyrosine residues, such as growth and differentiation [2,3]. Typical non-receptor PTPases have accessory domains of varied structural motives, at either the N- or C-terminal end of the catalytic domain. Some accessory domains are related to well-characterized protein families [4]. Recently, several laboratories have identified a subfamily of non-receptor PTPases, including SHP1 [5–8], *Drosophila* corkscrew gene product [9] and SHPTP2 [10,11]. These three PTPases contain two Src homology 2 (SH2) domains N-terminal to their PTPase domain. SH2 domains are found in a variety of signal-transducing proteins and mediate its association with tyrosine-phosphorylated proteins [12]. Thus, these PTPases may associate with activated receptor PTKs or substrates of these kinases. One of these PTPases, SHP1 is predominantly expressed in hematopoietic cells, where it negatively regulates cell growth [13]. It is also expressed in malignant epithelial cell lines and in tissues replenished from stem cells, suggesting that this enzyme may play a role in cellular signal transduction of non-hematopoietic cells [5,6,14].

The human prostate gland, unlike all other male sex accessory tissues, has an extraordinary tendency towards growth disorders. In this gland, which is normally under androgen regulation, progression from normal epithelium to malignancy and metastasis may be associated with changes in the expression of specific growth factors and oncogenes that replace the need for androgens [15].

Over the past several years, a PTPase activity associated with prostatic acid phosphatase (PAcP) (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) has been identified in human prostate. This enzyme is a glycoprotein that contains two 50 kDa subunits [16] and possesses phosphatase activity towards Tyr-phosphorylated proteins [17–19]. Recently different groups have described the cloning and cDNA sequencing of human PAcP [20,21] showing that there are no significant sequence similarities between this phosphatase and the members of the PTPase family. They also have a different reaction mechanism. The importance of the normal and pathological growth-related processes in the prostate, together with the involvement of PTPases in these process, supports the notion that species of PTPases different from PAcP may be present in the prostate. However, so far, there is apparently little information about the spectrum of PTPases in the prostate. In the present study, we examine PTPase activity in the rat prostate gland. The results reveal the presence of a PTPase activity distinct from that associated with PAcP. The non-receptor PTPase, SHP1, which is present in very large amounts in the rat prostatic gland, is responsible, at least in part, for this activity.

2. Materials and methods

2.1. Materials

Triton X-100, soybean trypsin inhibitor (STI), bovine serum albumin (fraction V), bacitracin, phenylmethylsulfonyl fluoride (PMSF), poly(Glu, Tyr) (4:1, by mass), ammonium molybdate (1% in 1.32 M HCl) and acid phosphatase kit were from Sigma (St. Louis, MO, USA).

[^{32}P]ATP and Western blotting detection system were from Dupont NEN (Boston, MA). Sephacryl S-300 HR was from Pharmacia (Uppsala, Sweden). Anti-human SHP1 polyclonal antibody was from Up-

*Corresponding author. Fax: (34) (1) 8854585.

PII S0014-5793(97)00235-4

state Biotechnology Inc. (Lake Placid, NY, USA) and anti-human PTP1B monoclonal was from Transduction Laboratories (Kentucky, USA). Protein standards for calibration of SDS electrophoretic gels, were obtained from Bio-Rad (Munich, Germany). RNAzol B was from Bioprobe (Montreuil/Bois, France). First strand cDNA synthesis kit was from Boehringer Mannheim (Germany). Taq polymerase was from Promega (Madison, WI, USA). Sense and antisense oligonucleotides were synthesized and purified by Eurogentec (Seraing, Belgium).

2.2. Preparation of the cytosolic and particulate fractions

Wistar rats were killed by decapitation. Pooled ventral prostates were homogenized in an Omni-Mixer with buffer (50 mM Tris-HCl, 0.25 M sucrose, and 0.03% STI at pH 7.5). The homogenate was centrifuged at $150\times g$ for 5 min. The supernatant was centrifuged at $100\,000\times g$ for 1 h. The supernatant corresponded to the cytosolic fraction, and the pellet (particulate fraction) was resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 0.01% STI, 0.1 mM PMSF and 5% glycerol (buffer A). The particulate fraction was then solubilized using the same buffer with 1% Triton X-100. After stirring for 60 min at 4°C, detergent-insoluble protein was removed by centrifugation at $400\,000\times g$ for 10 min.

2.3. Concanavalin A-affinity chromatography

The solubilized particulate fraction was incubated with concanavalin A-Sepharose 4B, previously equilibrated with buffer A containing 0.02% Triton X-100. The slurry was stirred slowly for 150 min at 4°C and then packed into a 10×1 cm chromatographic column. The unbound material was collected and assayed for PTPase and acid phosphatase activity. The column was washed exhaustively until no protein was detected eluting from the column. The retained material was eluted with the same buffer containing 0.6 M methyl-D-mannopyranoside (MM). Fractions were collected and separately assayed for PTPases and acid phosphatase activities.

2.4. Assays for PTPase activity

Phosphorylated poly(Glu,Tyr) was prepared by incubating 7.2 mg polymer with 1–2 mg of A-431 cell membranes, in 50 mM Tris-HCl buffer containing 0.1 μM EGF, 250 μM [^{32}P]ATP (700 dpm/pmol), 100 μM orthovanadate, 5 mM $MnCl_2$, and 10% NP-40 at pH 7.5, as previously described [22]. PTPase activity was measured by the release of [^{32}P]phosphate from ^{32}P-labelled poly(Glu,Tyr) in a 100 μl reaction mixture containing 30 000 cpm ^{32}P-labelled poly(Glu,Tyr) (final concentration 0.3 μM) and 8–15 μg sample in buffer containing 50 mM Tris-HCl, 0.1% albumin, 5 mM DTT, 0.01% STI, and 0.1 mM PMSF at pH 7 (PTPase buffer). The reaction was allowed to proceed for 5 min at 30°C, then stopped by the addition of 100 μl 30% trichloroacetic acid. The liberated inorganic [^{32}P]phosphate was extracted using the molybdate extraction procedures and radioactivity was counted by liquid scintillation [22].

2.5. Western immunoblotting and immunoprecipitation

Samples (25 μg) of different preparations were fractionated by SDS-PAGE and electrotransferred to nitrocellulose membrane Hybond C. The blots were incubated either with anti-SHP1 or with anti-PTP1B and the immunoreactive bands were detected by use of a horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence system from Dupont NEN.

For immunoprecipitation, solubilized samples (500 μg) were incubated with 4 μg of antibody prebound to Sepharose protein A for 3 h at 4°C. The immunoprecipitate was collected by centrifugation, washed three times in buffer containing 50 mM Tris-HCl (pH 7), 150 mM NaCl, 0.01% STI, 2 mM EDTA and 0.1 mM PMSF, and collected in Laemmli's sample buffer [23] for SDS-PAGE. The immunoprecipitation supernatant was also collected and immunoblotted using anti-SHP1 antibody.

2.6. Reverse transcription PCR

RNA was extracted with the RNAzol B method. Total RNA (5 μg) was denatured at 4°C for 10 min. cDNA was synthesized using a first strand cDNA synthesis kit. The reverse transcription mixture was diluted twofold with sterile water. PCR for SHP1, PTP1B and actin was then performed in a final volume of 50 μl containing 5 μl of reverse transcribed total RNA, 2.5 units of Taq polymerase, specific sense and antisense primers (1 μM), 250 μM of dNTPs in 10 mM Tris-HCl buffer (pH 9) containing 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Triton X-100. Sense and antisense oligonucleotides derived from the cDNA sequence of SHP1, PTP1B and actin. The resulting cDNA amplification products for SHP1, PTP1B and actin were predicted to be 469, 398 and 517 bp in length, respectively. Thermal cycling parameters were 94°C for 1 min, 54°C for 1 min 30 s, 72°C for 3 min for 26 or 35 cycles with a final extension of 72°C for 10 min, in a Perkin Elmer thermal cycler. PCR products were separated on 7.5% polyacrylamide gels and visualized with ethidium bromide.

2.7. SHP1 immunohistochemistry

Rat ventral prostate was fixed in 1.5% paraformaldehyde in 0.1 M phosphate buffer for 1 h 30 min. After fixation, prostatic fragments were dehydrated and embedded in paraffin and sections of 9 μm thick were obtained. Then, sections were deparaffinized and treated with rabbit non-immune serum diluted 1:30 in Tris-HCl (0.05 M, pH 7.6) for 30 min to reduce non-specific background staining. Sections were incubated overnight at 4°C with the same polyclonal antibody anti-SHP1 used previously diluted 1:500. Following three 10 min washes, the sections were incubated for 1 h at 20°C in peroxidase-conjugated goat anti-rabbit immunoglobulin (Sigma) at 1:100 dilution and rinsed twice. Peroxidase activity was revealed with 0.025% DAB and 0.005% H_2O_2 for 15 min, and then sections were washed with distilled water, dehydrated in graded concentrations of ethanol and mounted in Depex. Control sections were performed replacing the primary antibody by normal serum; these control sections showed no immunoreactive product. Some sections were also counterstained with 0.1% toluidine blue to facilitate the visualization of epithelial cells.

2.8. Other procedures

SDS-PAGE was performed according to Laemmli [23]. The protein concentration was estimated by the method of Bradford [24] using bovine serum albumin as a standard. The acid phosphatase activity was determined colorimetrically using an acid phosphatase kit, with PNPP as substrate, as the manufacturer described.

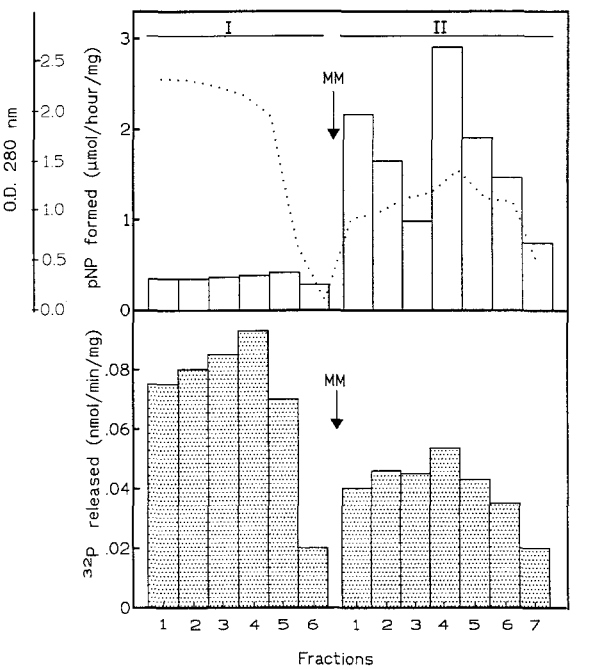

Fig. 1. Concanavalin A affinity chromatography of solubilized particulate fraction. The procedures for this chromatography are described in Section 2. The dotted line indicates the absorbance at 280 nm. The column was washed until no protein could be detected in the eluate, and the glycoprotein was eluted with MM (arrow). Aliquots of each fraction were assayed for PNPase activity (upper panel) and PTPase activity (lower panel), using PNPP and [^{32}P]poly(Glu,Tyr) as substrates. The figure is representative of three experiments. The peaks of PTPase activity are designated I and II as indicated.

3. Results

Using [^{32}P]poly(Glu,Tyr) as substrate, we identified PTPase activity in the crude homogenate as well as in the 100 000 × *g* supernatant and pellet derived from rat ventral prostate. Membrane and cytosolic fractions showed similar total PTPase activities, the specific activity being nearly twofold higher in the particulate fraction than in the cytosolic fraction, 0.43 μmol/mg protein/min and 0.26 μmol/mg protein/min, respectively.

To further identify the prostatic PTPase activity, we attempted to separate PTPase activity from that of acid phosphatase by concanavalin A-affinity chromatography. This lectin has been successfully used previously for the purification of the PAcP [17,18]. When the solubilized particulate fraction was chromatographed on a concanavalin A-Sepharose 4B column, two peaks of PTPase activity were obtained (Fig. 1, lower panel). The fractions with higher PTPase activity, which was not bound to the lectin column, corresponded to peak I. The other peak, termed peak II, was retained by the resin and was eluted with methyl-D-mannopyranoside (MM). Both peaks exhibited PNPPase activity, but the PNPPase activity of peak II was at least 6-fold higher than that of peak I. On the other hand, the PTPase activity of peak I was at least 3-fold higher than that of peak II.

To further characterize the PTPase activity in peaks I and II, we tested a variety of potential modulatory agents of PTPase activity. The results are presented in Table 1. Firstly, peak I PTPase activity was examined for its sensitivity to the widely known PTPase inhibitors, sodium vanadate and acidic compounds such as non-phosphorylated poly(Glu,Tyr). Vanadate and poly(Glu,Tyr), at 100 μM, inhibited this activity by 81% and 69% respectively. Tartrate and fluoride, both inhibitory agents of PAcP activity, had no effect. The PTPase activity was not inhibited by PNPP at 10 mM. We tested the effect of the same modulatory agents on PTPase and PNPPase activity of peak II. Both phosphatase activities were inhibited by vanadate and poly(Glu,Tyr). PTPase activity, however, appeared to be more sensitive to the inhibition induced by these two agents. The PNPPase activity was effectively inhibited by NaF and slightly by tartrate whereas these agents had no effect on PTPase activity. If the dephosphorylation of [^{32}P]poly(Glu,Tyr) observed in peak II was catalyzed by acid phosphatase, one would expect PNPP to inhibit this reaction. As shown in Table 1, 10 mM PNPP inhibited the PTPase activity by 40%. Furthermore, we found that when [^{32}P]poly(Glu,Tyr) was used as the substrate, peak II exhibited a higher specific activity at neutral pH (0.009 nmol/mg/min) than at acidic pH (0.0012 nmol/mg/min) (data not shown). In marked contrast, PNPP hydrolysis was higher at acidic pH (data not shown). These findings suggest that peak II is, probably, due to PAcP. The peak I exhibiting high PTPase activity could represent a PTPase activity different from that associated with PAcP.

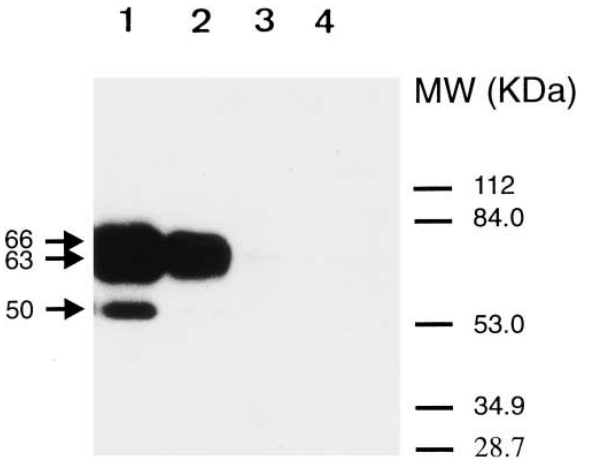

Fig. 2. Detection of PTPase by Western blotting. Preparations obtained from concanavalin A chromatography were subjected to SDS-PAGE, transferred to nitrocellulose membrane and Western-blotted using anti-SHP1 antibody. Revelation by a chemiluminescence-Western immunoblotting detection system is shown and corresponds to the analysis of the solubilized particulate fraction (lane 1), peak I (lane 2), peak II (lane 3) and bovine prostatic acid phosphatase (Sigma) (lane 4). In all cases, there are equal amounts of extract (25 μg of protein). The figure is representative of three experiments. The positions of the molecular mass markers are shown.

Among the recently cloned PTPases, the 66 kDa phosphotyrosine phosphatase (SHP1) is expressed primarily in hematopoietic cells, but also in epithelial cells [5,6]. To test whether, at least in part, the PTPase activity detected in peak I is immunologically related to SHP1 we used a rabbit polyclonal antibody against this enzyme. Immunoblotting of the solubilized particulate fraction showed that the antibody specifically recognized a 66 kDa protein (Fig. 2, lane 1). The pooled fractions of peaks I and II were also subjected to immunoblotting using the same antibody; the 66 kDa protein was again observed to be present in peak I (Fig. 2, lane 2) but not in peak II (Fig. 2, lane 3). The antibody did not recognize the partially purified bovine PAcP (Fig. 2, lane 4).

To test the relative contribution of SHP1 to this PTPase

Table 1
Influence of various agents on PTPase activity of peak I and on PTPase and PNPPase of peak II

Agent	Concentration	% of control		
		Peak I	Peak II	
		[^{32}P]poly(Glu,Tyr)	pNPP	[^{32}P]poly(Glu,Tyr)
Vanadate	1 μM	98	98	97
	100 μM	19.3	62.2	54
Poly(Glu,Tyr)	100 μM	31	84.8	34
Tartrate	1 mM	104	98	114
	10 mM	111	70.5	91
NaF	10 mM	83.9	37.3	119
pNPP	10 mM	96.2	–	58

The activities were measured as described in Section 2 in the presence of each of the agents at the indicated concentrations. The enzyme activity is expressed as a percentage of the control (in the absence of the agents).

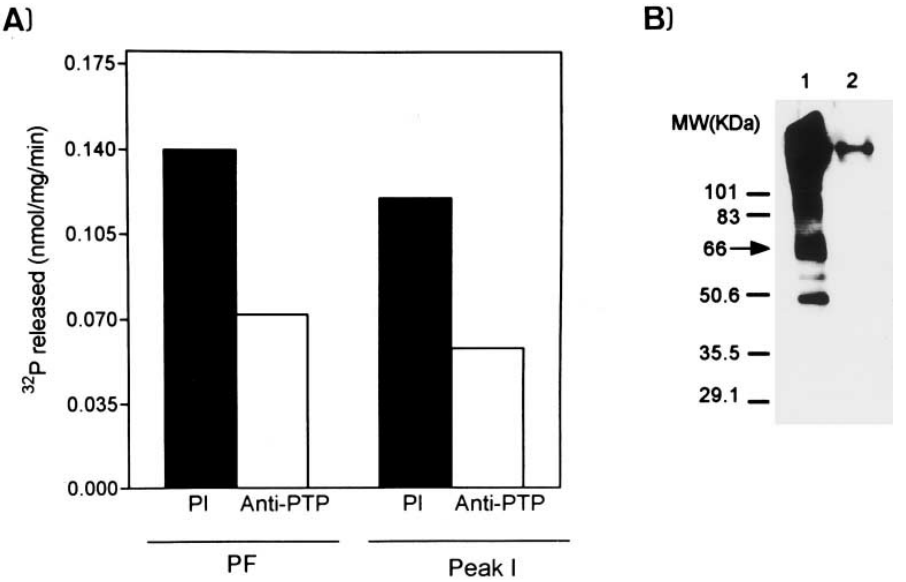

Fig. 3. PTPase activity in SHP1 immunodepleted solubilized. A: Solubilized particulate fraction (PF) (500 μg) was immunoprecipitated with anti-SHP1 antibody (anti-PTP) or with preimmune control serum (PI) as described in Section 2. Supernatants of both immunoprecipitates were collected and were assayed for PTPase activity. Both supernatants were also incubated with concanavalin A and PTPase activity was measured in the unbound fractions (peak I). This figure is representative of three experiments. B: Immunoprecipitate (lane 1) and immunoprecipitation supernatant (lane 2) were subjected to SDS-PAGE, transferred to nitrocellulose membranes and Western-blotted using anti-SHP1 antibody as described in Section 2.

activity, the solubilized particulate fraction was immunoprecipitated with an anti-SHP1 antibody and the PTPase activity present in the original and immunodepleted solubilized fraction was measured. As shown in Fig. 3A, the immunodepletion of SHP1 produced a loss of 51.4% of the PTPase activity. The loss of PTPase activity was very similar when both fractions were incubated with concanavalin A and the PTPase activity was measured in the fractions (peak I) unbound to the lectin. The absence of SHP1 in the immunodepleted solubilized fraction was tested by immunoblot (Fig. 3B, lane 2). This enzyme was present in the immunoprecipitated fraction (Fig. 3B, lane 1) as probed by immunoblotting.

In the particulate fraction and in peak I but not in peak II (Fig. 2), anti-SHP1 antibody also recognized a major band at 63 kDa and a minor band at 50 kDa. The 50 kDa band could be due to PTP1B, another ubiquitously expressed non-receptor PTPase, which could also contribute peak I PTPase activity. Given the homology between various PTPases, it is possible that the SHP1 antibodies cross-react with PTP1B. Thus, peak I was also subjected to immunoblotting using an anti-PTP1B antibody. The results obtained showed that the amount of PTP1B was very low (data not shown). We could also think that the 63 and 50 kDa band could correspond to proteolytic fragments of SHP1. To examine this possibility, immunoblotting, using anti-SHP1 antibodies, was next carried out on the pellet and supernatant obtained after centrifugation of the particulate fraction and on the cytosolic fraction. As shown in Fig. 4A, three immunoreactive bands of 66, 63 and 50 kDa were observed in the particulate and cytosolic fractions. The corresponding scanning densitometry of immunoreactive bands (Fig. 4B) allowed for estimating the relative contents of all three bands in the different fractions. In the particulate fraction, the 66 kDa form was the predominant one, with the intensity pattern $66 > 63 \gg 50$ kDa. However, in the cytosolic fraction the relative contents of the three bands were very similar. After centrifugation of the particulate fraction, a relocalization of the three forms from the particulate to the soluble fraction was observed. The 63 and 50 kDa bands were no longer present in the pellet, being located in the supernatant, whereas the 66 kDa band was present in both fractions but its amount was twofold higher in the soluble fraction than in the pellet. Centrifugation of the particulate fraction also resulted in activation of prostatic PTPase activity. About 50% of the PTPase activity was recovered in the pellet, the remainder being in the supernatant. The enzyme present in the supernatant exhibited a higher specific PTPase activity: 1.30 nmol/mg/min in the supernatant and 0.19 nmol/mg/min in the pellet. Limited digestion of the particulate fraction with trypsin increased the PTPase activity by approximately twofold but had little effect on the PTPase activity in the cytosolic fraction (data not shown).

The presence of SHP1 and PTP1B mRNA in rat ventral prostate was demonstrated using reverse transcription PCR (Fig. 5). It is clear that the expression of SHP1 mRNA was higher than that of PTP1B, according with the amount of both proteins detected by immunoblotting.

SHP1 expression in the rat prostate gland was examined using immunohistochemistry of paraffin-embedded sections. The prostatic epithelium showed positive SHP1 immunostaining (Fig. 6b,c). The SHP1 immunoreactivity was restricted to the epithelial cells of the prostate and blood vessels and was not found in the stromal cells or elsewhere in the prostate gland.

4. Discussion

In the last decade, besides the identification of PTPases as enzymes distinct from other classes of protein phosphatases by their specificity and their high activity towards protein phosphotyrosyl residues, it has become evident that all

PNPP phosphatases in animal tissues may act upon P-Tyr protein. In the case of the human prostate, it has been observed that highly purified preparations of acid phosphatase show dephosphorylating activity on Tyr-phosphorylated proteins [17–19]. In this study, we show that the rat prostate possesses a PTPase activity different from that associated with PAcP. SHP1 is one of the principal factors responsible for this activity. This was supported by several observations: we detected the presence of SHP1 by Western blotting in fractions depleted of PAcP; immunodepletion of SHP1 resulted in a considerable loss of PTPase activity; mRNA for SHP1 was detected by reverse transcription PCR; and finally, we found that SHP1 is localized within the epithelial cells, mainly in the apical zone. Thus, the SHP1 detected is specifically expressed in rat prostate gland and is not due to contaminating blood cells. Other PTPases can contribute to this activity but probably to a lesser extent than SHP1.

Li et al. [17] have described that the predominant form of active PTPase in human prostate is due to acid phosphatase and that during the purification processes, no PTPase activity could be detected other than that exhibiting optimum acidic

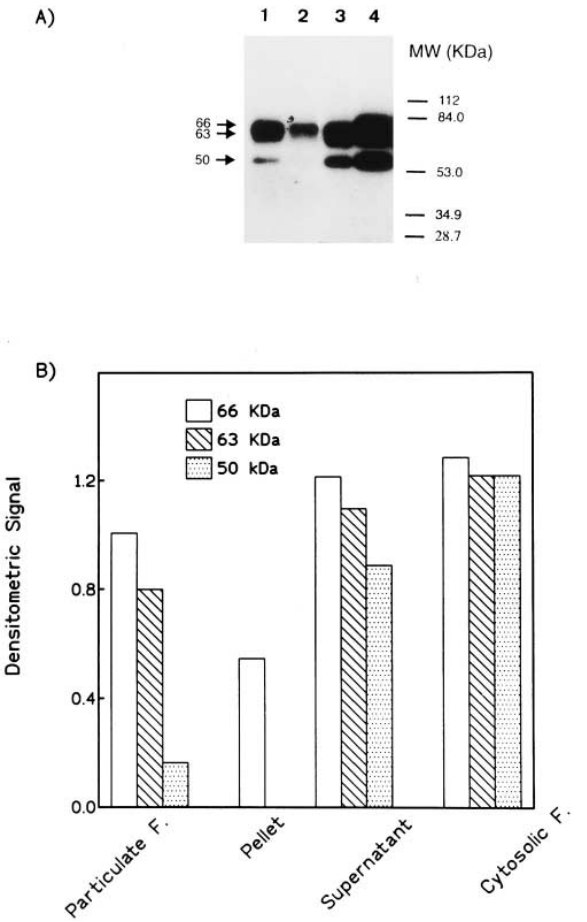

Fig. 4. Detection of SHP1 by Western immunoblotting following centrifugation of the particulate fraction. A: Equal amounts of extract (25 μg) were subjected to SDS-PAGE and analyzed by Western blotting using anti-SHP1 antibody. Revelation by an enhanced chemiluminescence-Western-immunoblotting detection system is shown. Lanes 1, 2, 3 and 4 correspond to the particulate fraction, pellet and supernatant obtained following centrifugation of the particulate fraction and the cytosolic fraction, respectively. This figure is representative of three experiments. B: Videodensitometric analysis of the immunoblot.

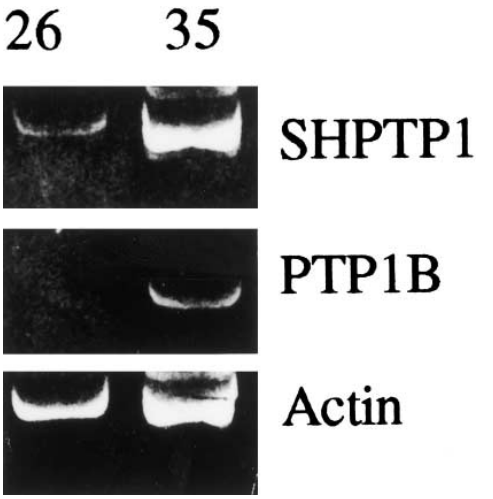

Fig. 5. Espression of PTP1B and SHP1 mRNA in rat ventral prostate. PCR was performed on total RNA as described in Section 2 and the products were revolved on a 7.5% polyacrylamide gel. Numbers 26–35 refer to PCR cycle number.

pH and sensitivity to inhibition by L(+) tartrate. Similar results were obtained by Lin and Clinton in human seminal plasma [18]. It is possible that the differences observed with our results may be related to the different conditions for the measurement of PTPase activity or to the species used.

In addition to SHP1, the anti-SHP1 antibody also recognized two other proteins of different molecular sizes: 63 and 50 kDa, which probably correspond to proteolytic fragments of the 66 kDa form. The three molecular forms were present in the particulate and cytosolic fractions derived from rat prostate. However, the three forms were more abundant in the cytosolic fractions than in the particulate fraction. A relocalization of the immunoreactive forms from the particulate to the soluble fraction, with an increase in the amount of the lower molecular size immunoreactive forms, was observed following centrifugation of the particulate fraction. This was associated with an increase in PTPase activity. The limited proteolysis of the particulate fraction also resulted in a considerable increase in catalytic activity. This indicates that both trypsinization and centrifugation generated soluble, smaller and more active SHP1 forms. In a recent study, Zhao et al. [25] reported that a fragment containing 41 amino acids can be cleaved from the C-terminus of SHP1, leaving a trypsin-resistant core of 63 kDa. This truncation was accompanied by an increase in catalytic activity. A similar increase in activity of the pancreatic PTPase [22] and PTP1B [4] was observed following truncation of a C-terminal fragment of these enzymes. The fractions of the C-terminal regions of non-transmembrane PTPases are largely unknown. The C-terminal region of PTP1B has recently been shown to anchor this protein at the membrane of the endoplasmic reticulum [26]. T-cell PTP has been shown to bind DNA in vitro through its C-terminal region [27]. In the case of SHP1, the C-terminal segment seems to play a regulatory role. Recent experiments have reported an increase in the catalytic activity of SHP1 following phosphorylation of Tyr^{538} in the C-terminal region in response to growth factors [28,29]. The low efficiency of trypsin on PTPase activity in the cytosolic fraction is striking, although the amount of SHP1 was similar to that of the particulate fraction. In vitro studies have shown that anionic

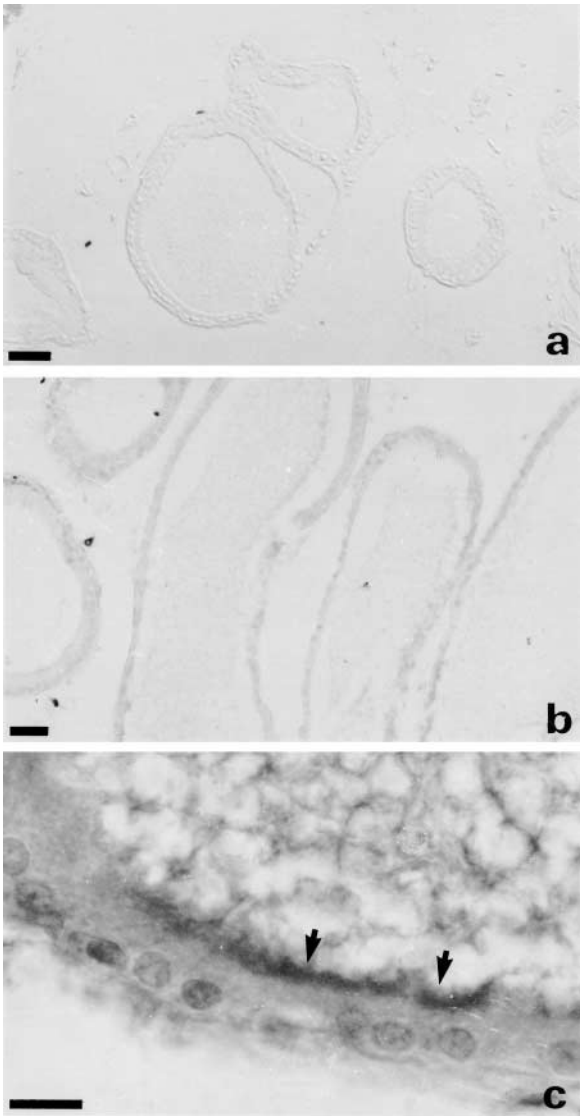

Fig. 6. Immunohistochemical localization of SHP1. a: Control section. No immunoreactive product is observed in the different rat prostatic ducts; ×200. b: The epithelial layer of the rat prostatic duct shows a general immunopositive reaction; ×200, bar = 30 μm. c: A stronger immunopositive reaction is observed in the apical zone (arrows) of the epithelial prostatic cells; ×1100, bar = 10 μm.

phospholipids increase SHP1 activity and its susceptibility to proteases [30]. This may explain our results since the presence of SHP1 in the particulate fraction may render this pool uniquely susceptible to proteolytic degradation. It has been shown that the enzyme can be translocated to the membrane, causing a significant change in the conformation of the enzyme [25,30]. This conformational change can affect the enzymatic activity and increase the susceptibility of the enzyme to tryptic attack. Further work will be necessary to determine the role of proteolytic cleavage with respect to activation, degradation and relocalization to cellular compartments of SHP1. In this way, the regulation of SHP1 could be reminiscent of that of PKC, whose activity is regulated by translocation to the membrane, autophosphorylation and proteolysis [31].

Although SHP1 was initially reported to be predominantly expressed in hematopoietic cells [8,13], recent studies have revealed that SHP1 is also expressed in many non-hematopoietic cells, notably in malignant epithelial cell lines [7,14] and in tissues that are actively replenished from stem cells [32], suggesting that this enzyme plays a role in cell growth or differentiation. Such a role has been recently demonstrated in interleukin-3-dependent hematopoietic cells [13] and fibroblasts [33] in which the overexpression of SHP1 reduced the cell growth rate.

In conclusion, our study shows, for the first time, the presence of a PTPase activity different from that associated with PAcP in the rat prostate gland. This PTPase activity is due, at least in part, to SHP1. The two PTPase activities can be separated by affinity chromatography using concanavalin A. The identification of SHP1 in the prostate gland makes it necessary to analyze the importance of its activity in vivo and its relation with the prostatic growth and differentiation processes.

Acknowledgements: This work was supported in part by Grant PM92-44 from the Dirección General de Investigación Científica y Técnica and by the Universidad de Alcalá.

References

[1] T.D. Hunter, J.D. Cooper, Annu. Rev. Biochem. 54 (1985) 97–930.
[2] E.M. Fisher, H. Charbonneau, N.K. Tonks, Science 253 (1991) 401–406.
[3] K.M. Walton, J.E. Dixon, Annu. Rev. Biochem. 62 (1993) 101–120.
[4] L.J. Mauro, J.E. Dixon, Trends Biochem. Sci. 19 (1994) 151–155.
[5] S.H. Shen, L. Bastien, B.I. Posner, P. Chretien, Nature 352 (1991) 736–739.
[6] J. Plutzky, B.G. Neel, R.D. Rosenberg, Proc. Natl. Acad. Sci. USA 89 (1992) 1123–1127.
[7] T. Yi, J.L. Cleveland, J.N. Ihle, Mol. Cell. Biol. 12 (1992) 836–846.
[8] R.J. Matthews, D.B. Bowne, E. Flores, M.L. Thomas, Mol. Cell. Biol. 12 (1992) 2396–2405.
[9] L.A. Perkins, I. Larsen, N. Perrimon, Cell 70 (1992) 225–236.
[10] R.M. Freeman, J. Plutzky, B.G. Neel, Proc. Natl. Acad. Sci. USA 89 (1992) 11239–11244.
[11] G.S. Feng, C.C. Hui, T. Pawson, Science 259 (1993) 11611–11614.
[12] T. Pawson, G.D. Gish, Cell 71 (1992) 359–362.
[13] T. Yi, A.L.F. Mui, G. Krystal, J.N. Ihle, Mol. Cell. Biol. 13 (1993) 7577–7586.
[14] T. Uchida, T. Matozaki, K. Matsuda, T. Suzuki, S. Matozaki, O. Nakano, K. Wada, Y. Konda, C. Sakamoto, M. Kasuga, J. Biol. Chem. 268 (1993) 11845–11850.
[15] R. Tatoud, F. Desgrandchamps, A. Degeorges, A. Degeorges, F. Thomas, Path. Biol. 41 (1993) 731–740.
[16] H. Lee, T.M. Chu, S.S.L. Li, C. Lee, Biochem. J. 277 (1991) 759–765.
[17] H.C. Li, J. Chernoff, L.B. Chen, A. Kirschonbaum, Eur. J. Biochem. 138 (1984) 45–51.
[18] M.F. Lin, G.M. Clinton, Biochem. J. 235 (1986) 351–357.
[19] M.F. Lin, G.M. Clinton, Mol. Cell. Biol. 8 (1988) 5477–5485.
[20] R.L. Van Etten, R. Davidson, P.E. Stevis, H. MacArthur, D.L. Moore, J. Biol. Chem. 266 (1991) 2313–2319.
[21] F.S. Sharier, H. Lee, M.M. Leuderman, A. Lundwall, L.L. Deaven, C. Lee, S.S.L. Li, Biochem. Biophys. Res. Commun. 160 (1989) 79–86.
[22] B. Colás, C. Cambillau, L. Buscail, M. Zeggari, J.P. Esteve, V. Lautre, F. Thomas, N. Vaysse, C. Susini, Eur. J. Biochem. 207 (1992) 1017–1024.
[23] U.K. Laemmli, Nature 227 (1970) 680–685.
[24] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[25] Z. Zhao, S. Shen, E.H. Fisher, Proc. Natl. Acad. Sci. USA 91 (1994) 5007–5011.
[26] J.V. Frangioni, P.H. Beahm, V. Shifrin, C.A. Jost, B.G. Neel, Cell 68 (1992) 545–560.

[27] V. Radha, S. Kamatkar, G. Swarup, Biochemistry 32 (1993) 2194–2201.
[28] P. Bouchard, Z. Zhao, D. Banville, F. Dumas, E.H. Fisher, S.H. Shen, J. Biol. Chem. 30 (1994) 19585–19589.
[29] T. Uchida, T. Matozaki, T. Noguchi, T. Yamao, K. Horita, T. Suzuki, Y. Fujioka, C. Sakamoto, M. Kasuga, J. Biol. Chem. 269 (1994) 12220–12228.
[30] Z. Zhao, S.H. Shen, E.H. Fisher, Proc. Natl. Acad. Sci. USA 90 (1993) 4251–4255.
[31] H. Hugh, T.F. Sarre, Biochem. J. 291 (1993) 329–343.
[32] M. Zeggari, J.P. Esteve, I. Rauly, C. Cambillau, H. Mazarguil, M. Dufresne, L. Pradayrol, J.A. Chayvialle, N. Vaysse, C. Susini, Biochem. J. 303 (1994) 441–448.
[33] T. Matozaki, T. Uchida, Y. Fujioka, M. Kasuga, Biochem. Biophys. Res. Commun. 28 (1994) 874–881.