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Expression of a human T-cell protein-tyrosine-phosphatase in baby hamster kidney cells

(phosphorylation/regulation/localization)

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ABSTRACT **A human T-cell cDNA encoding a 48-kDa protein-tyrosine-phosphatase (PTPase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) was cloned into a mammalian expression vector and introduced into baby hamster kidney cells, and stable colonies were isolated. The expressed PTPase was found to be associated with the particulate fraction of the cells, where it was essentially inactive in an *in vitro* assay unless first subjected to limited trypsinization; trypsin treatment generated an active fragment of 33 kDa by the removal of a carboxyl-terminal segment of the full-length enzyme. Gel filtration indicated that the expressed enzyme was associated with a complex of >600 kDa. Introduction of a premature stop codon into the T-cell cDNA at position 1012 resulted in the production of a fully active 37-kDa species that distributed between both the particulate and soluble fractions. The truncated form of the enzyme was readily solubilized by detergents and was eluted within its predicted molecular mass range. These results suggest that the carboxyl-terminal segment is important in determining the localization and regulation of the PTPase. The level of protein-tyrosine phosphorylation observed after 5 min of platelet-derived growth factor stimulation was reduced in cells overexpressing either form of the phosphatase, indicating that both are active *in vivo*. Overexpressing the truncated enzyme resulted in a growth rate that was approximately 50% of that observed in cells transfected with either the full-length PTPase cDNA or the vector alone.**

A human T-cell cDNA encoding a 48-kDa protein-tyrosine-phosphatase (PTPase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) has been isolated (1). Its predicted amino acid sequence exhibited 75% identity to that of a 37-kDa human placenta enzyme (PTPase 1B) in a 236-residue conserved core segment found in all PTPases identified thus far (2–4). However, the T-cell PTPase also contained an 11-kDa extension at the carboxyl terminus, suggesting that the placenta enzyme may have been isolated in a truncated, yet fully active, form. This was confirmed by the isolation of cDNAs encoding either the human placenta enzyme (5, 6) or an isoform from rat brain (7), both with predicted molecular masses of approximately 50 kDa.

On the basis of the primary structure of placenta PTPase 1B (8), a family of PTPases has been established that includes integral membrane proteins, such as CD45, with the structural features of receptor molecules (9–12). The nature of the ligands that might regulate the high molecular mass transmembrane PTPases is unknown. Likewise, nothing is known about the possible regulation of the low molecular mass class of PTPases; however, the fact that their carboxyl extensions display considerable sequence diversity, as compared with their conserved predicted catalytic core, might imply that this region plays an important role in regulation.

The physiological functions of the low molecular mass and receptor-linked PTPases remain unclear, though obviously they must be necessary to control the overall level of protein-tyrosine phosphorylation and thus would be required for regulating growth, differentiation, and transformation. In this present study, overexpression of the T-cell PTPase transfected into baby hamster kidney (BHK) cells was investigated to determine whether this would have adverse effects on normal cell growth, including cytotoxicity. In addition, a carboxyl-terminal truncated form of the enzyme closely resembling PTPase 1B was constructed. To clarify the possible function of the deleted segment, this was also expressed in BHK cells.

MATERIALS AND METHODS

Restriction and modifying enzymes were from Stratagene; ^{125}I-labeled protein A was from New England Nuclear; platelet-derived growth factor (PDGF)β (c-*sis*) was from Amgen Biologicals, and mouse monoclonal anti-phosphotyrosine antibody bound to agarose beads was from Oncogene Science (Manhasset, NY). Conjugated goat anti-rabbit IgG horseradish peroxidase and the substrate color reagent were purchased from Bio-Rad. Oligonucleotide primers and peptides were synthesized by the Howard Hughes Biopolymer Synthesis Facility at the University of Washington.

Plasmid Constructs and Site-Directed Mutagenesis. An *Eco*RI–*Hin*dIII fragment [1.328 kilobase pairs (kbp)], comprising the entire coding region of the PTPase cDNA and 60 and 22 bp of the 5′ and 3′ untranslated ends, respectively, were isolated from a human T-cell cDNA PTPase clone. Single-stranded ends of the cDNA fragment were treated with nuclease S1 and ligated to a 5.5-kbp *Sma* I fragment from the pNUT expression vector (provided by R. Palmiter of the University of Washington). The 5.5-kbp fragment encodes a dihydrofolate reductase cDNA under the regulation of a simian virus 40 (SV40) promoter and a Zn^{2+} metallothionein I promoter required for *in vivo* transcription of the newly inserted cDNA (13).

A mutation of the PTPase cDNA was performed as described (14). An oligonucleotide (5′-GGGAACAGATAGAAGAAG-3′) identical to nucleotides 1004–1025 within the T-cell PTPase cDNA except for a seven-base deletion was synthesized. It was used as a primer for *in vitro* DNA synthesis in a reaction with single-stranded phage M13 DNA containing the T-cell PTPase cDNA. A stop codon (TAG) was placed into the translation open reading frame following Arg-317. Selection of M13 phage that carry the altered cDNA was performed by *in situ* plaque filter hybridization with ^{32}P-labeled oligonucleotide. The filters were washed under

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Abbreviations: PTPase, protein-tyrosine-phosphatase; PDGF, platelet-derived growth factor.

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conditions requiring perfect duplex formation for stability (15). A 1.6-kbp (*Tha* I and *Ssp* I) fragment encoding the mutated cDNA was isolated from the purified M13 recombinant plasmid and ligated with the 5.5-kbp *Sma* I fragment from the pNUT expression vector described above. All plasmid constructs were verified by DNA sequence analysis as described (16).

Cell Culture and Transfections. BHK cells were routinely grown in Dulbecco's modified Eagle's medium containing 10% (vol/vol) heat-inactivated fetal calf serum. The cells were transfected with 10 μg of plasmid DNA by using the calcium phosphate precipitation method (17); after 24 hr, they were switched to selection medium containing 250 μM methotrexate. Stable colonies were isolated at about 14 days posttransfection. For all experiments using PDGF stimulation, cells were first treated overnight with 80 μM $ZnSO_4$, and then incubated for 48 hr in medium and 0.1% heat-inactivated fetal calf serum for 48 hr.

Cell Homogenization. Cells grown to confluency were treated with 80 μM $ZnSO_4$ for 12 hr, washed three times with phosphate-buffered saline (PBS), scraped, and collected by centrifuging at 800 × *g* for 5 min. The cell pellet was homogenized by using a Teflon Dounce homogenizer in low-salt buffer (LSB) (25 mM imidizole, pH 7.0/1 mM EDTA/1 mM EGTA/0.1% 2-mercaptoethanol/2 mM $MgCl_2$/0.002% phenylmethysulfonyl fluoride/0.1 mM benzamidine/1 μg of leupeptin per ml/250 mM sucrose) and centrifuged at 5000 × *g* for 5 min. The supernatant was recentrifuged at 100,000 × *g* for 30 min at 4°C. All fractions, including the 5000 × *g* pellet (5P), the 100,000 × *g* pellet (100P), and the 100,000 × *g* supernatant (100S) were assayed for PTPase activity. The 5P fraction was further extracted in LSB buffer containing either 0.5% Triton X-100 or 0.6 M KCl or a mixture of both solutions and was centrifuged in an Eppendorf centrifuge for 5 min; these supernatants were also assayed.

PTPase Assay. Activity measurements were performed as described by Tonks *et al.* with reduced, carboxyamidomethylated, and maleylated (RCM)-lysozyme as substrate (2). For those assays requiring trypsin, 20 μl of cell extract was diluted 1:2 in buffer and treated with 1 μg of trypsin for 5 min at 30°C. Trypsin digestion was stopped by adding 6 μg of lima bean trypsin inhibitor, followed immediately by 20 μl of substrate. One unit of PTPase activity is defined as that amount that releases 1 nmol of phosphate per min.

Preparation of Anti-peptide Antisera. Rabbit antibodies were raised to a synthetic peptide (Cys-Asn-Arg-Asn-Arg-Tyr-Arg-Asp-Val-Ser-Pro-Phe-Asp-His-Ser-Arg-Ile-Lys) derived from the amino-terminal region of placenta PTPase 1B (3) (corresponding to residues from Asn-43 to Lys-60 in PTPase 1B and from Asn-45 to Lys-62 in the T-cell PTPase) and purified as described (18). The peptide contains an additional cysteinyl residue to facilitate cross-linking to rabbit serum albumin as a carrier protein (18). The antibody still recognizes the T-cell enzyme even though two amino acid substitutions are found in this domain (tyrosine for Phe-52 and valine for Ile-57). Specificity of the antibody for PTPase was verified by peptide competition experiments. One anti-PTPase antibody (no. 8172) was not affinity-purified; instead, the serum was loaded onto a 1:1 Affi-Gel Blue/DE 52 column and eluted in 20 mM Tris, pH 8.0/20 mM NaCl.

Immunoprecipitation and Immunoblot (Western) Analysis with Anti-phosphotyrosine Antibodies. Serum-deprived cells were treated with 40 ng of PDGF per ml at 37°C for various times and then washed immediately with ice-cold PBS. Lysis buffer (1 ml) containing 50 mM Hepes (pH 7.5), 150 mM NaCl, 10% (vol/vol) glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 μg of aprotinin per ml, 2 μg of leupeptin per ml, 0.002% phenylmethysulfonyl chloride, 200 μM sodium orthovanadate, 10 mM sodium pyrophosphate, and 100 mM NaF was added to the plates, which were then incubated on ice for approximately 20 min. The lysates were centrifuged at 10,000 × *g* for 5 min at 4°C, and the protein concentrations were determined as described by Bradford (19). A suspension of agarose-linked monoclonal anti-phosphotyrosine antibody beads (30 μl) was added to equivalent amounts of lysate protein in each immunoprecipitation, and the mixture was rotated overnight at 4°C. The beads were collected by centrifugation, washed twice in 20 mM Hepes, pH 7.5/150 mM NaCl/0.1% Triton X-100/10% glycerol/200 μM orthovanadate, then twice again with the same buffer but with increasing salt concentration to 0.5 M NaCl, and finally with buffer containing 150 mM NaCl. The beads were boiled for 2 min in 30 μl of Laemmli sample buffer (20).

The immunoprecipitated protein was subjected to Western blot analysis (21) with an anti-phosphotyrosine antibody kindly provided by J. Schlessinger, New York University Medical School. To detect antibody binding, ^{125}I-labeled protein A (500,000 cpm/ml in 10 mM Tris, pH 7.4/150 mM NaCl/1% bovine serum albumin) was added to the blot for 2 hr and washed in 10 mM Tris, pH 7.4/150 mM NaCl/0.05% Triton X-100. The blot was then subjected to autoradiography for 2–5 days at room temperature.

RESULTS

Expression of a Full-Length 48-kDa Human T-Cell PTPase in BHK Cells. The T-cell PTPase cDNA was transfected into BHK cells to determine the enzymatic properties of the 48-kDa form and to examine the physiological consequences of its overexpression. Over 100 methotrexate-resistant stable colonies were observed of which 20 were selected and analyzed for PTPase expression. One cell line producing the highest level of protein, as determined by Western blot analysis, was then chosen for further study. These cells grew at the same rate as the controls transfected with the vector alone; their morphologies, as observed by phase-contrast microscopy, were also similar. These results suggested either that the enzyme was not cytotoxic when overexpressed or that the activity was low because of the presence of endogenous inhibitors. Alternatively, the PTPase could be expressed in an inactive form.

To examine the properties of the expressed enzyme, confluent cells, transfected with either the vector alone or the T-cell PTPase cDNA, were disrupted by using a Dounce homogenizer. The extracts were fractionated into a pellet from low-speed centrifugation (5P) and a pellet and supernatant from high-speed centrifugation (100P and 100S), respectively, and PTPase assays were carried out on the various fractions (Table 1). Although the level of enzyme in the transfected cells was found at first to be no greater than in the controls, it could be increased considerably upon limited trypsinization (6- and 20-fold in the 5P fraction for the control and transfected cells, respectively). Under these conditions, the total activity in the transfected cells was 10 times greater than that in the controls.

Essentially all of the endogenous and expressed PTPase activities sedimented with the 5P pellet from which they could be released by 0.5% Triton X-100/0.6 M KCl. Triton alone was only partially effective, and salts alone at high concentration were totally ineffective (Fig. 1). The low levels of activity and protein found in the 100P pellet was not considered further.

Inclusion of Overexpressed T-Cell PTPase Within a High Molecular Mass Complex. The possibility existed that the inactivity of the enzyme in cell extracts was due to the presence of protease-sensitive inhibitory proteins. Therefore, a 0.5% Triton X-100 extract was subjected to Superose 12 fast protein liquid chromatography (FPLC) gel filtration (Fig. 2 *A* and *B*). In both control and transfected cells, the PTPase activity emerged in a high molecular mass (>650 kDa) fraction. Western blot analysis of this material following

Table 1. PTPase activity in BHK cells

Fraction	Transfected plasmid	Total units		Units/mg		Fold trypsin stimulation	% total units	
		−	+	−	+		−	+
A Low-speed pellet (5 P)	Control	5.2	31.0	0.7	4.4	6.0	73	87
	TC.PTPase	11.3	320.0	1.6	31.0	19.0	84	97
	TCΔC11.PTPase	17.0	26.0	2.5	3.7	1.5	48	66
B High-speed pellet (100 P)	Control	0.4	0.5	0.6	2.6	4.5	5	2
	TC.PTPase	1.0	5.6	1.0	5.6	5.0	5	2
	TCΔC11.PTPase	3.4	3.9	4.3	4.9	1.0	9	10
C High-speed supernatant (100 S)	Control	1.6	4.0	0.4	1.1	3.0	22	11
	TC.PTPase	2.1	5.4	0.6	1.5	2.5	11	1
	TCΔC11.PTPase	12.5	7.5	3.4	2.1	0.6	43	24

BHK cells were fractionated (5P, 100P, or 100S fractions) by centrifugation as described. The PTPase activity was determined in cells expressing either the control plasmid (control) or the cDNA of the full-length 48-kDa T-cell PTPase (TC.PTPase) or the truncated form (TCΔC11.PTPase). Total units of activity have been standardized to a constant amount of protein in each fraction. The signs "−" or "+" indicate assays without or with 1 μg of trypsin in the assay, respectively. "% total units" represents the percentage of total cellular activity found in each fraction.

CCl_3 COOH precipitation and SDS/PAGE revealed the presence of a 48-kDa immunoreactive protein. Similar results were obtained when the cells were extracted in 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)/0.6 M KCl and then freed of detergent by dialysis against a low-salt buffer prior to gel filtration, suggesting that the formation of the high molecular mass complex was not due to the presence of detergents.

When the Triton-soluble extracts were treated with trypsin prior to gel filtration, the PTPase was eluted with an apparent molecular mass of approximately 35 kDa (Fig. 2*D*). Western blot analysis (Fig. 2*C*) also revealed a band at approximately 33 kDa, suggesting that cleavage had occurred at the carboxyl terminus, since the antibody used recognized a sequence near the amino terminus of the enzyme. Pretrypsinization of the extract from the control cells also resulted in a new peak of activity that was eluted in fractions containing low molecular mass proteins. This material could not be detected by Western blot analysis either because antibody 8172 does not recognize the endogenous enzyme or because the latter was present in too low a concentration. The above data indicate that removal of the carboxyl-terminal segment of the enzyme by trypsin treatment results in the formation of a water-soluble, low molecular mass, constitutively active enzyme.

Expression of a Constitutively Active Truncated (37-kDa) Form of T-cell PTPase. To express a molecule with properties similar to placenta PTPase 1B, a truncated form of the T-cell enzyme (TCΔC11.PTPase) in which an 11-kDa segment was deleted from the carboxyl end was generated as described. BHK cells selected from colonies transfected with this form of the PTPase exhibited only about 50% of the growth rate of the control cells or cells transfected with the wild-type enzyme.

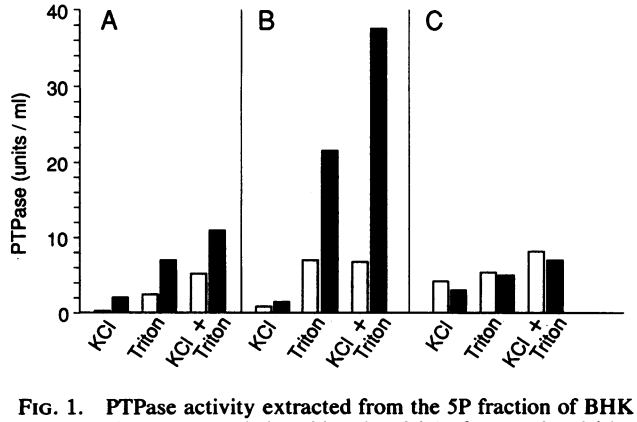

FIG. 1. PTPase activity extracted from the 5P fraction of BHK cells expressing the control plasmid or the 48-kDa form or the 37-kDa form of the T-cell PTPase. The pellets (5P) from the 5000 × *g* centrifugations were extracted with either KCl, Triton X-100, or KCl/Triton X-100, and the activity was determined for a constant amount of protein. (*A*) Control. (*B*) 48-kDa T-cell PTPase. (*C*) Truncated PTPase (TCΔC11.PTPase). Solid bars indicate pretreatment of extracts with trypsin.

When extracts of the TCΔC11.PTPase-transfected cells were fractionated as described above and assayed without prior trypsin treatment, only about 50% of the PTPase activity sedimented with the 5P fraction, while the remainder was in the 100S supernatant (Table 1). However, the enzyme present in the 5P pellet was fully active without trypsin treatment and could be extracted with either 0.5% Triton X-100 or 0.6 M KCl, indicating that it was not as tightly associated with the particulate fraction as was the full-length PTPase. Although the total phosphatase activity in the cells expressing the truncated enzyme was the same as that in the controls, it differed in that it was 8-fold higher in the 100S fraction. When BHK cells expressing the truncated PTPase were extracted with the Triton buffer and subjected to Superose 12 gel filtration, little of the activity distributed with the high molecular mass complex; indeed, it was detected only in low molecular mass fractions of approximately 35 kDa (Fig. 3) as confirmed by Western blot analysis (data not shown).

Overexpression of Wild-Type and Truncated PTPase Reduces Tyrosine Phosphorylation *in Vivo*. To determine the state of activity of the enzyme *in vivo*, cells expressing both forms of the T-cell PTPase were stimulated with PDGF, and changes in protein-tyrosine phosphorylation were investigated. Serum-deprived BHK cells were stimulated with PDGF and extracted at various times in the presence of vanadate, and proteins phosphorylated on tyrosyl residues were immunoprecipitated with anti-phosphotyrosine antibody. The precipitated proteins were subjected to SDS/PAGE and then analyzed in a Western blot with a second anti-phosphotyrosine antibody (Fig. 4). Autoradiography of the blot revealed that 2 min after PDGF stimulation, there was a dramatic increase in tyrosine phosphorylation in proteins of approximately 180, 140, 116, 92, and 60 kDa in the control cells. On the other hand, in cells overexpressing either the wild-type or truncated T-cell PTPase, there was a considerably lower level of phosphorylation in several protein bands, particularly those of 140, 116, and 60 kDa but not that of the 180-kDa protein assumed to be the PDGF receptor. The 116- and 60-kDa proteins appeared to undergo dephosphorylation in extracts from control cells within this time period, suggesting that an endogenous PTPase was activated. Similar phosphoproteins were detected in phosphotyrosine immunoprecipitates from quiescent fibroblasts treated with PDGF (22) or normal fibroblasts pretreated with vanadate (23).

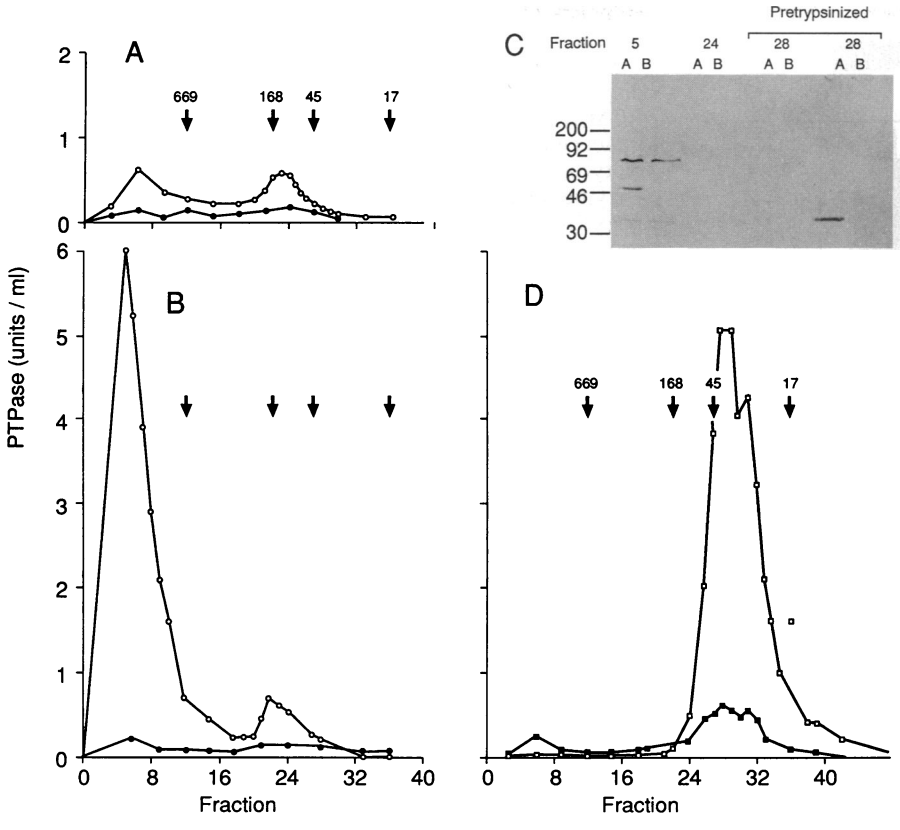

FIG. 2. Fast protein liquid chromatography gel filtration on Superose 12 of Triton X-100 extracts of BHK cells overexpressing T-cell PTPase activity. Equal amounts of protein from control cells (*A*) or from cells overexpressing the 48-kDa T-cell PTPase (*B*) were subjected to chromatography, and the fractions were assayed without (●) or with (○) trypsin pretreatment. Extracts were pretreated with trypsin prior to column chromatography (*D*). Fractions were assayed without trypsin pretreatment. ■, Control cells; □, 48-kDa T-cell PTPase-expressing cells. Arrows indicate elution positions of standard molecular mass markers in kDa: 669, thyroglobulin; 168, γ-globulin; 45, ovalbumin; and 17, myoglobin. (*C*) Western blot of CCl_3 COOH-precipitated proteins from peak-activity fractions 5, 22, and 28 from all three fractionations shown in *A*, *B*, and *D*. The blot was probed with an anti-PTPase antibody. Immune complex was detected with a secondary goat anti-rabbit antibody conjugated to horseradish peroxidase; visualization was with a substrate color reagent. Molecular mass markers in kDa (obtained from Bio-Rad) are indicated (200, myosin; 92, phosphorylase B; 69, bovine serum albumin; 46, ovalbumin; and 30, carbonic anhydrase).

DISCUSSION

Protein-tyrosine phosphorylation represents a crucial event in normal cell growth. Therefore, it was of interest to determine whether overexpression of a PTPase would interfere with this process. Stable BHK cells were obtained that expressed the full-length 48-kDa T-cell PTPase at 10-fold over background activity, as measured by using an artificial substrate in an *in vitro* assay. The enzyme was also active on endogenous phosphotyrosyl proteins. Despite this increase in activity, there was no effect on growth rate or morphology as compared with cells expressing the vector alone. These data could be interpreted by assuming that the enzyme is highly regulated within the cell or that it might be partitioned into a compartment(s) where its activity would be restricted to specific substrates.

The results in this study have shown that the overexpressed 48-kDa T-cell PTPase localizes to a particulate fraction that sediments at low speed. The enzyme is inactive *in vitro* toward the artificial substrate used unless the fraction is pretreated with trypsin. This behavior can be ascribed to the 11-kDa carboxyl-terminal segment of the molecule. A hydrophobicity plot (Fig. 5) indicates that this segment is essentially hydrophilic until approximately the last 20 residues, at which point the polypeptide chain becomes hydrophobic, with a hydrophobicity index approaching that of transmembrane segments or signal peptides. A similar distribution of hydrophilic and hydrophobic residues is also found in the carboxyl-terminal segments of low molecular mass human placenta and rat brain PTPases, even though the primary structure of these segments is more variable than within their conserved 236-residue core structure. Sucrose density centrifugation gave no evidence for association of the T-cell PTPase enzyme with the plasma membrane (data not shown). However, this PTPase might be interacting with

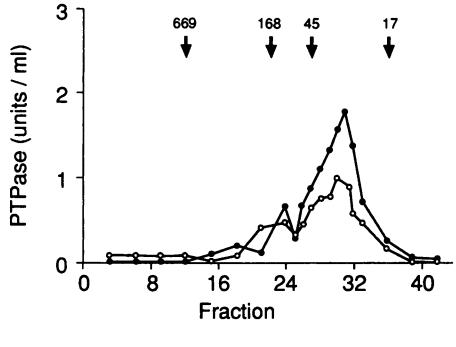

FIG. 3. Gel filtration on Superose 12 of extracts from cells expressing the truncated form of the T-cell PTPase (TCΔC11.PTPase). The activity was measured in each fraction without (●) or with (○) trypsin in the assay. Molecular mass markers in kDa are denoted by arrows: 669, thyroglobulin; 168, γ-globulin; 45, ovalbumin; and 17, myoglobin.

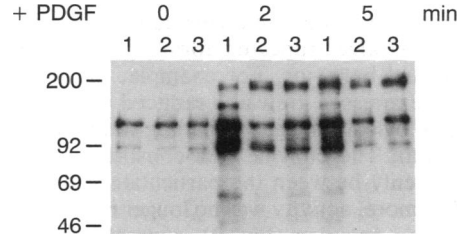

FIG. 4. Western blot of phosphotyrosine-immunoprecipitated proteins following PDGF stimulation. Phosphotyrosine-containing proteins and PDGF-stimulated cells were immunoprecipitated with an anti-phosphotyrosine antibody. A SDS/7.5% Laemmli gel was used to separate the precipitated proteins followed by Western blot analysis with an anti-phosphotyrosine antibody as a probe in the blot. Detection of binding was with ^{125}I-labeled protein A followed by autoradiography at room temperature for 5 days. Lanes: 1, control cells; 2, cells expressing the full-length 48-kDa T-cell PTPase; 3, cells expressing the truncated form. Times of PDGF stimulation following 48 hr of serum-deprivation are indicated (0, 2, or 5 min). Standard molecular mass markers in kDa (200, myosin; 92, phosphorylase B; 69, bovine serum albumin; and 46, ovalbumin) are also shown.

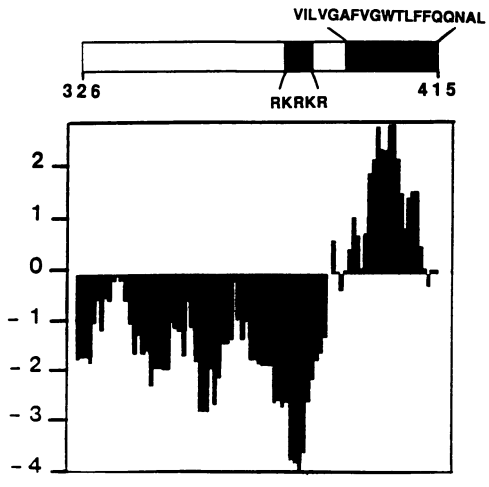

FIG. 5. A hydrophobicity plot of the carboxyl-terminal region in the T-cell PTPase. The hydrophobicity calculations of amino acid residues 326–415 in the carboxyl-terminal segment of the T-cell PTPase were determined as described (24). A computer-generated plot was obtained by using a default window of 7. The numbers above and below the 0 line represent hydrophobic and hydrophilic amino acids, respectively; the units in the *y* axis are defined as in ref. 24. The bar above the plot depicts a putative Arg-Lys-Arg-Lys-Arg nuclear recognition signal (25) between residues 377 and 381 (solid) and a hydrophobic terminal region (396–415) (solid; 19 residues shown in single-letter code) representing the carboxyl terminus of the molecule.

other cellular components (nucleus, Golgi, or endoplasmic reticulum) or the cytoskeleton, since both high salt concentration and detergents are required for solubilization.

The function of the carboxyl-terminal segment of the protein may be specific for each PTPase isoform. One hypothesis would be that, in directing the localization of the enzyme, including translocation in response to cell stimulation or to defined stages of the cell cycle, it may restrict substrate specificity. More directly, it may modulate activity possibly as an autoinhibitor, shielding the catalytic center from substrate as described for myosin light chain kinase (26) and calcineurin (27). Furthermore, it may serve as a site of interaction with regulatory subunits as may be found in the high molecular mass complex recovered from extracts of cells expressing the full-length enzyme (Fig. 2). Finally, it may itself be a site of posttranslational modification, such as phosphorylation, or indirectly may control the modification of the catalytic segment.

That the carboxyl-terminal segment is involved in the localization and regulation of enzyme activity is seen from the fact that the 37-kDa truncated form of the T-cell PTPase exhibits unusual behavior. For example, the BHK cells in which it was expressed grew at a reduced rate and displayed gross morphological changes including multinucleation (unpublished data). The enzyme was constitutively active and distributed evenly between the particulate and soluble fractions. Furthermore, activity was no longer recovered in a high molecular mass complex (Fig. 2), suggesting that what remained in the particulate fraction was no longer associated with the same elements that interacted with the 48-kDa form. Interestingly, similar levels of activity in cells expressing the truncated PTPase or the vector alone have consistently been detected when extracts were assayed in the presence of trypsin. It is only when the assays were performed in the absence of protease that enhanced activity in the cells expressing the truncated PTPase was detected (by approximately 5-fold). One possible explanation of this phenomenon would be that the expression of the endogenous trypsin-stimulated PTPase may be suppressed by the truncated T-cell enzyme.

Whereas the full-length PTPase and its truncated form display considerable differences in activity *in vitro* when RCM-lysozyme is used as substrate, this is not the case in intact cells. After 5 min of PDGF stimulation, the same extent of tyrosine dephosphorylation was observed with the two enzyme species, suggesting that both are equally active *in vivo*. Obviously it will be of importance to characterize the proteins involved in the regulation of the 48-kDa form of the enzyme. These results pertain solely to early phosphorylation events; however, it remains to be seen whether the two forms of the PTPase would recognize the same substrates upon prolonged PDGF stimulation.

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