Journal of Cellular Biochemistry 42:71–81 (1990)

Preliminary Characterization of Phosphotyrosine Phosphatase Activities in Human Peripheral Blood Lymphocytes: Identification of CD45 as a Phosphotyrosine Phosphatase

R.J. George and C.W. Parker

Howard Hughes Medical Institute, Division of Allergy and Immunology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

A preliminary characterization of the protein phosphotyrosine phosphatase (PTPase) activity in human peripheral blood lymphocytes (PBL) has been made using two tyrosine-containing peptides and the epidermal growth factor receptor from A-431 cells as substrates. High PTPase activity with a pH optimum near 7.4 was observed in both the membrane and the cytosolic fractions. At least three distinct fractions with PTPase activity were separated on DEAE cellulose columns, indicating that the enzyme is heterogeneous. Vanadate, molybdate, and salts of zinc, copper, and mercury were all effective enzyme inhibitors, although the inhibition was generally incomplete and showed some variation with the enzyme preparation. The difficulty in completely inhibiting PTPase activity in lymphocytes may help explain the variation between laboratories in studies of tyrosine phosphorylation in these cells. Studies with highly purified T lymphocytes obtained by filtration of PBL through nylon wool columns indicated that the activity is present in T cells. Absorption with agarose containing anti-HLe-1, a mouse monoclonal IgG1 antibody specific for the leukocyte-specific surface protein T-200 (CD45), removed up to 40% of the PTPase activity. Enzyme activity was recovered on the immunoadsorbent after extensive washing, confirming that the enzyme was being bound to the beads. Immunoabsorbents containing other mouse IgG1 antibodies failed to bind PTPase activity, indicating that the binding to beads with anti-HLe-1 antibody is specific. Further characterization of the CD45 and PTPase activities in lymphocytes may provide a better under standing of the role of protein tyrosine phosphorylation in the regulation of proliferation and differentiation in these cells.

Key words: T200, DC-3, RRSRC, proliferation, PTPase

The observation that many oncogenes and growth factor receptors possess tyrosine kinase activity has led to the hypothesis that tyrosine phosphorylation is an important regulatory mechanism in cell growth and proliferation [for reviews, see 1–3]. Elimina-

Received March 28, 1989; accepted August 10, 1989.

tion of the kinase activity by single site mutation abolishes the ability of this class of oncogenes to transform cells [4–6] and of growth factors to initiate calcium mobilization, receptor internalization, and activation of gene transcription [7–9].

Lymphocytes have been a major model for studies of control of cellular proliferation and differentiation. A number of lymphocyte proteins have been reported to be phosphorylated on tyrosine, including p56 tyrosine kinase [10,11], zeta chain of CD3 complex [12], and unknown proteins of various molecular weights [13–19]. How the phosphorylation is controlled in lymphocytes is not clear. Unlike cell types in which the kinase is the receptor for the primary mitogenic stimulus, the tyrosine kinase activity described in lymphocytes [10,11,18–21] is not an inherent part of the antigen receptor or any of the receptors for known lymphocyte-specific growth factors. The level of phosphotyrosine in cells is the net result of both the tyrosine kinase and phosphotyrosine phosphatase (PTPase) activities. The implication from studies of oncogene-induced cellular transformation is that this balance is upset when the kinase is overproduced. Although there have been a number of studies of lymphocyte protein tyrosine kinases [19,21–25], evidence that the kinase is stimulated by mitogens, as might be expected based on apparent correlations between tyrosine phosphorylation and cellular growth in other tissue, is not available. Little attention has been given to phosphatases [23–25]. Identification and characterization of PTPases in lymphocytes will be essential to understand the role of tyrosine phosphorylation in these cells. If tyrosine phosphorylation is an important factor in controlling cellular proliferation, the activity of phosphatases must normally exceed that of the kinases to prevent unregulated growth. Regardless of their potential regulatory roles, phosphatases are a potential problem when lymphocytes are broken in an attempt to isolate phosphorylated proteins. As with other cell types, attempts to identify lymphocyte proteins phosphorylated on tyrosine have had limited success. Often, few proteins other than the autophosphorylated kinase have been identified, and these proteins are not consistently observed. A better understanding of the PTPase and the control of their activities may be essential if important phosphotyrosine-containing proteins are to be identified. We present a preliminary characterization of the PTPase activities present in human peripheral blood lymphocytes (PBL).

MATERIALS AND METHODS

Substrates

Two peptides (DC-3 and RRSRC) and a membrane preparation from A431 cells were phosphorylated and used as substrates in these studies. DC-3 is a 14-amino-acid peptide based on a sequence of amino acids 136–148 (DDAQYSHLGGNWAR) from the cytoplasmic domain of the delta chain of the T-3 complex [26]. In unpublished experiments, we observed that the delta chain of T3 is heavily phosphorylated on tyrosine in vitro in human lymphocyte membranes. RRSRC is a modified sequence of the autophosphorylation site of v-src pp60, developed by Casnellie et al. [27]. Both peptides were synthesized by the Protein Chemistry Facility at Howard Hughes Medical Institute, St. Louis. A431 membranes were prepared as described [28].

Phosphorylation of Substrates

The two peptide substrates were phosphorylated with an A431 membrane preparation of the epidermal growth factor (EGF) receptor kinase at 4°C for 90 min. The reaction mixture contained 1 mCi [γ^{32}P]ATP (6,000 Ci/mmol), 2 mM $MnCl_2$, 0.1 M

Hepes 7.4, 0.2 mM Na_2VO_4, 20 μg/ml EGF, 100 μg peptide, and 66 μg protein of the kinase preparation. At the end of the reaction, the A431 membranes were removed by centrifugation. The peptides were isolated by thin-layer electrophoresis at pH 1.9 for 60 min at 1,000 V on 250 μm silica G plates. Areas on the plates containing the labeled peptides were localized by autoradiography, scraped, and extracted with water, and eluate was dried on a Speed Vac. By phosphoamino acid analysis greater than 95% of ^{32}P incorporated into DC-3 and 100% of the ^{32}P incorporated into RRSRC was in phosphotyrosine (data not shown). After pelleting, the membrane preparation used to phosphorylate the peptides was washed twice with 10% trichloroacetic acid (TCA), resuspended in 1 mM NaOH, and dialyzed against 4 liters of 5 mM Hepes 7.4, 1 mM EDTA. Acid hydrolysis showed phosphotyrosine to be the predominant ^{32}P-labeled phosphoamino acid. This preparation was also used as a substrate.

PTPase Assay

Lymphocyte-rich mononuclear cells (PBLs) were obtained from the peripheral blood of healthy adult human volunteers by venous puncture and isolated by isopycnic centrifugation on Ficoll-Hypaque [29]. Enriched T cells obtained by filtration of mononuclear cells through nylon columns also were studied [29]. The cells were subsequently maintained in RPMI-1640 supplemented with L-glutamine, nonessential amino acids, and 10% fetal calf serum at 37°C in a CO_2 incubator at an initial cell density of 1×10^6 cells/ml until use. Cells were harvested by centrifugation, washed with 0.15 M NaCl, 0.01 M phosphate, pH 7.4 (PBS) and disrupted at 4°C in 50 mM Hepes, pH 7.4, 0.5% Triton X-100 containing 0.001% (W/V) leupeptin and 0.002% PMSF (w/v) at a density of 5×10^7 cells/ml. The lysate was cleared of nuclei and insoluble material by centrifugation at 400*g* for 5 min. Lysate from 0.5×10^6 cells was incubated with 8.5 fmoles (0.33 nM final concentration, estimated 4,800 cpm/fmol) ^{32}P-labeled substrate in a volume of 25 μl at 4°C for 10 min. The reaction was stopped by addition of an equal volume of 3% TCA at 4°C and the samples were centrifuged in a Microfuge at maximal velocity for 5 min. Eighty percent of the supernate was spotted into P81 phosphocellulose paper, which was subsequently washed with 0.5% (v/v) phosphoric acid. Radioactivity remaining in the labeled substrate bound to the P81 paper was quantitated by counting Cerenkov radiation.

Subcellular Fractionation

Cells were harvested, washed twice with PBS and once with 20 mM Hepes, pH 7.4, 5 mM $MnCl_2$ (20/5 buffer). The cells were swollen in 20 mM Hepes/5 mM Mn^{2+} for 30 min on ice and then homogenized. Nuclei and unbroken cells were removed by centrifugation at 400*g* for 5 min. A plasma-membrane rich fraction was prepared by centrifugation at 16,000*g* for 20 min. The membrane-rich pellet was resuspended in 20/5 buffer, recentrifuged, and resuspended again in the original volume of 20/5 buffer with 0.5% Triton X-100. Triton X-100 was added to the 16,000*g* supernatant, microsomal/cytosolic fraction, to a final concentration of 0.5%. Both fractions were studied. In some experiments, cells were broken by sonication using a Kontes microcell disrupter and subsequently fractionated as described. No differences in enzyme activity were observed as a result of the method of cell breakage.

T200 (CD45) Precipitation

Lymphocytes were harvested and lysed with detergent as described above. Solubilized proteins in the lysate were reacted for 1 hr at 4°C with mouse IgG_1 monoclonal

antibodies (anti-CD45, HLe-1; anti-CD3, Leu4, anti-IgM; Becton Dickinson) preabsorbed to agarose containing covalently bound goat antimouse IgG_1 antibody (Sigma). After removal of the agarose beads by centrifugation, the lysate was assayed for PTPase activity. The agarose–antibody–antigen complex was washed three times with lysis buffer and resuspended in the same buffer before assay.

RESULTS

Detergent lysates of lymphocytes were tested for PTPase activity using the ^{32}P-labeled peptides and labeled A431 membranes as substrates. Incubation of the lysate with either peptide resulted in a time- and temperature-dependent loss of ^{32}P from the peptide (Fig. 1). In a typical experiment at a 330 pM substrate concentration, 30% of the substrate is hydrolyzed over a 5 min period. To confirm that the decrease in ^{32}P bound to the P81 phosphocellulose paper was not due to proteolysis, supernates from the phosphatase reaction were analyzed by thin-layer electrophoresis at pH 1.9 (Fig. 2). The supernate from incubated samples contained a single additional ^{32}P-labeled spot that migrated with the same Rf as inorganic phosphate. Heat inactivation of the lysate or pretreatment with 3% trichloroacetic acid resulted in no release of free $^{32}PO_4$. In subsequent experiments, pretreatment with TCA was routinely used as a zero time point in kinetic assays. Loss of phosphate from the peptide substrates was linear over the range of cell equivalents tested. As is shown in Figure 3, the detergent lysate of lymphocytes was also able to release ^{32}Pi from proteins in labeled A431 membranes. Since the A431 membrane substrate contained both ^{32}P-labeled phosphoserine and phosphotyrosine, PTPase was determined as the difference between the activity in the presence of sodium fluoride alone (to inhibit phosphoserine phosphatase activity) and the activity in the presence of both sodium fluoride and sodium vanadate (to inhibit phosphoserine and phosphotyrosine phosphatase activities). Gel electrophoresis studies showed that radioactivity was lost selectively from the 160 Kd EGF receptor band (not shown).

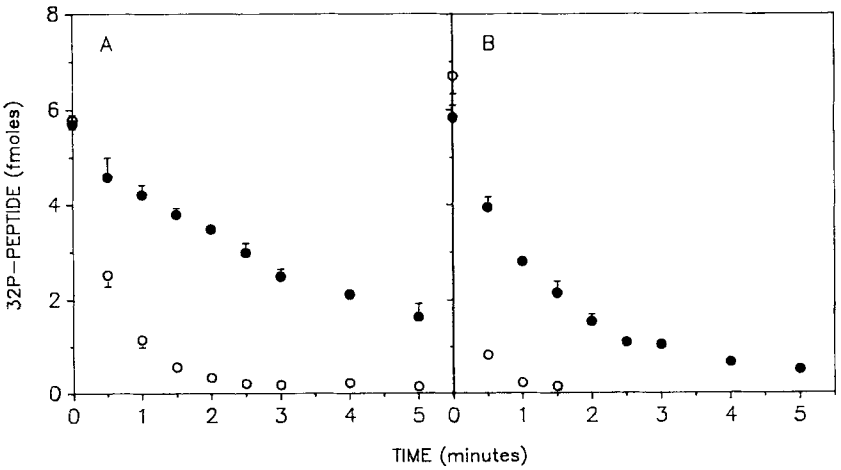

Fig. 1. Time- and temperature-dependent loss of ^{32}P from peptide substrates. **A:** DC-3. **B:** RRSRC. Detergent lysate from 0.5×10^6 lymphocytes was incubated with ^{32}P-labeled peptide for the times indicated at either 4°C (closed circles) or 22°C (open circles). The reaction was terminated with TCA, and counts remaining in the labeled peptide substrate were determined as described in Materials and Methods. The bars indicate the standard deviation of triplicate values.

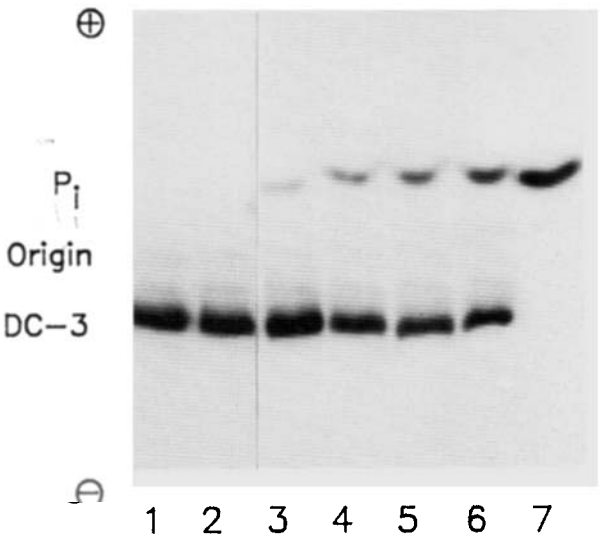

Fig. 2. Loss of ^{32}P from the peptide substrate is the result of the action of a phosphotyrosine phosphatase. Labeled DC-3 was incubated with detergent lysate from 0.5×10^6 cells for varying lengths of time at 4°C. After the reaction was terminated with TCA, the reaction mixture was applied to thin-layer silica plate, electrophoresed at 1,000 V for 30 min at pH 1.9 (formic acid:acetic acid:H_2O, 50:156:1,794), and autoradiographed. **Lane 1.** DC-3 standard. **Lane 2.** Zero time. **Lane 3.** 1 min. **Lane 4.** 2.5 min. **Lane 5.** 5 min. **Lane 6.** 10 min. **Lane 7.** ^{32}P-inorganic phosphate standard.

A number of phosphatases have been described that specifically or nonspecifically dephosphorylate phosphotyrosine [23–25,30–41]. To characterize better the phosphotyrosine phosphatase activity in lymphocyte lysates, we have examined the pH optima for the activity (Fig. 4) and the ability of various reagents known to influence the activity of other phosphatases to either potentiate or inhibit the enzyme activity using DC-3 as the substrate (Table I). As is shown in Figure 4, the enzyme activity is maximal at neutral pH and rapidly drops off below pH 6.5 or above pH 8. Vanadate and molybdate, inhibitors of both tyrosine-specific phosphatases and acid phosphatases are effective inhibitors of the enzyme. Also effective are salts of zinc, copper, and mercury. Although these substances are capable of inhibiting the enzyme, it should be noted that the inhibition is generally incomplete and can vary with the enzyme preparation. A number of known phosphatase inhibitors are inactive: They include 1) inhibitors of alkaline phosphatase (tetramisole and levamisole) and acid phosphatase (tartrate, EDTA, PNPP, and fluoride); 2) Mg^{2+}, Mn^{2+}, and a variety of nucleotides that influence the activity of many serine phosphatases; 3) lithium ion, a phospholipid phosphatase inhibitor; 4) reagents that interact with calcineurin, such as calcium, EGTA, and trifluoroperazine. Moreover, purified calcineurin failed to dephosphorylate the peptide substrates under our usual assay conditions (not shown). Reagents that interact with sulfhydryl groups do influence activity. p-Hydroxymercuriphenyl sulfonic acid is immediately inhibitory, whereas dithiothreitol (DTT) prevents a time-dependent loss of enzyme activity. Addition of DTT to partially inactivated preparations can partially restore the level of phosphatase activity. Nonionic detergent is stimulatory. For example, Triton X-100 in amounts up to 0.5% (V/V) increases phosphatase activity. Sodium pyrophosphate, β-glycerol phosphate, and high concentration of NaCl moderately inhibit enzyme

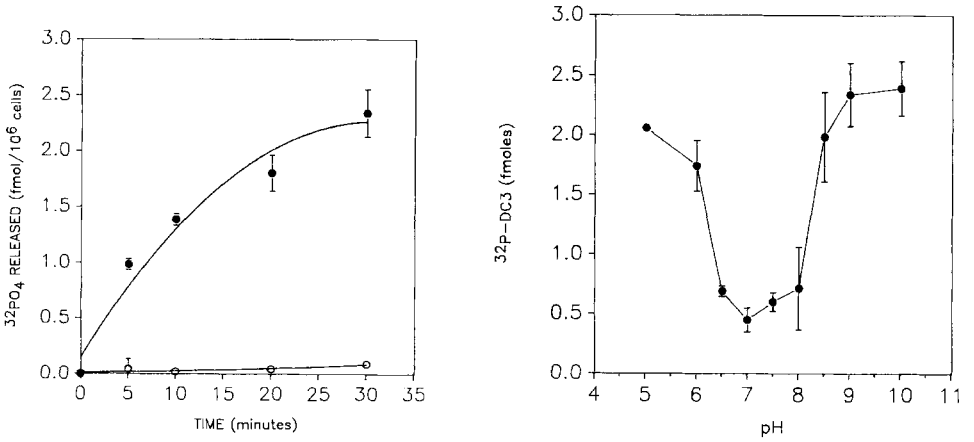

Fig. 3. Dephosphorylation of ^{32}P-labeled A431 membranes. Inactivated,^{32}P-labeled A431 membranes (6 fmoles $^{32}PO_4$) were incubated with detergent lysate from 0.5 x 10^6 lymphocytes for varying lengths of time at 4°C in the presence or absence of various inhibitors to determine the amino acid specificity of the phosphatase activity. The reaction was terminated by addition of an equal volume of 20% TCA, the membranes were removed by centrifugation, and the amount of phosphate released into the supernate was quantitated. Closed circles, 10 mM sodium fluoride: open circles, 10 mM sodium fluoride with 0.1 mM sodium vanadate.

Fig. 4. The optimum pH of the PTPase activity. Phosphatase activity was assayed for 10 min at the designated pH at 4°C with the DC-3 substrate. Buffers used at a concentration of 50 mM were: sodium acetate, pH 5.1; MES, pH 6.0, 6.5; Hepes, pH 7.0, 7.5, 8.0; and Tris, pH 8.5, 9.0, and 10.0. Residual radioactivity in DC-3 is shown. Error bars indicate standard deviation of triplicate values.

activity. Less extensive studies with RRSRC and A-431 membranes have given similar results (see, e.g., Fig. 3).

The subcellular distribution (Fig. 5) of the PTPase activity was determined in supernatant and pellet fractions of homogenates centrifuged at 16,000*g*. The membrane fraction contains approximately 60% of activity when determined on a per cell basis and about three-fourths of the total activity when determined on a per milligram of protein basis.

Fractionation of the phosphatase activity in detergent lysate by ion-exchange chromatography reveals at least three peaks of phosphatase activity (Fig. 6). Although partial proteolysis of PTPases or aggregation might explain some of this heterogeneity, it is probably due to the presence of multiple PTPases.

Our primary interest in the PTPase is as a potential regulator of protein phosphotyrosine levels in T lymphocytes. Our usual lymphocyte-rich preparations contain 10–15% monocytes and 1–2% polymorphonuclear leukocytes in addition to 60–70% T cells and 10–15% B cells. PTPase activity has previously been described in polymorphonuclear cells and in the promyelocytic leukemia cell line HL-60 [23–25]. To determine if the activity we observed was indeed mostly due to lymphocytes rather than to contaminating cells, essentially pure T cells were obtained by filtering lymphocytes through nylon wool columns. As is shown in Figure 7A on a per cell basis, the enriched T cells have slightly greater activity than less purified lymphocyte preparations. PTPase is also present in a variety of T-cell lines. The activity in HUT102, HSB2, and CTLL lines was found to be approximately equal to unstimulated PBL on a per cell basis (Fig. 7B).

TABLE I. Effect of Possible Inhibitors on Tyrosine Phosphatase Activity*

Experimental condition	mM	Residual radioactivity in substrate (% Inhibition)
TCA-treated control		100
Heat-inactivated control		100
No addition		0
F^-	25	0
Mg^{2+}	25	0
Mn^{2+}	25	1.5
Ca^{2+}	1	1
VO_4^{-2}	0.1	90
MbO_4^{-2}	0.1	62
Zn^{2+}	0.1	85
Cu^{2+}	0.1	88
Hg^{2+}	0.1	96
MPSA	0.1	86
EDTA	5	0
EGTA	5	0
ATP	5	0
ADP	5	0
AMP	5	0
PPi	20	39
B glycerol PO_4	100	32
PNPP		0
Tartrate	20	2
Levamisole	1	0
Tetramisole	1	0
NaCl	250	40

*Phosphatase activity was assayed on the DC-3 substrate as described in Materials and Methods. The reaction was terminated after 20 min at 4°C by adding an equal volume of 3% TCA. Heat-inactivated tissue or lysate precipitated with TCA prior to addition of substrate was used as control. Anions were added as sodium salts, cations as chlorides. MPSA, p-hydroxymercuriphenylsulfonic acid.

Sequence homology search of DNA data banks has revealed a high degree of homology of a tyrosine phosphatase isolated from human placenta with the T-200 leukocyte common antigen [42], and T-200 has recently been demonstrated to possess phosphatase activity [41]. Precipitation of T-200 with HLe-1 [43], an antibody specific for the lower-molecular-weight forms of T-200, both removes activity from the lysate and precipitates phosphatase activity. Addition of excess antibody to the detergent lysate removes approximately 40% of the phosphatase activity (Fig. 8A). Assay of the antigen–antibody–agarose complex (Fig. 8B) reveals that HLe-1 antibody specifically precipitates PTPase activity. The level of phosphatase activity demonstrated in the T-200–antibody complex is approximately 12% of what can be removed by the anti-T-200 antibodies. This decrease in activity is probably due to the removal of the T-200 from its lipid environment and an inability of the solid-phase T-200 to orient properly in the detergent micelle.

DISCUSSION

We have identified the presence of multiple PTPase species in PBL. Phosphatase activity is found in both the 16,000*g* plasma membrane fraction and the microsomal/

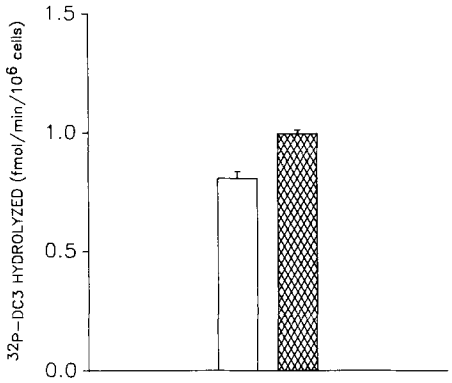

Fig. 5. Subcellular distribution of PTPase activity. PBLs were homogenized, and 16,000*g* plasma membrane (cross-hatched bar) and microsomal/cytosolic (open bar) fractions were prepared as described in Materials and Methods. Quantities corresponding to 0.5×10^6 cells were assayed for phosphatase activity at 4°C.

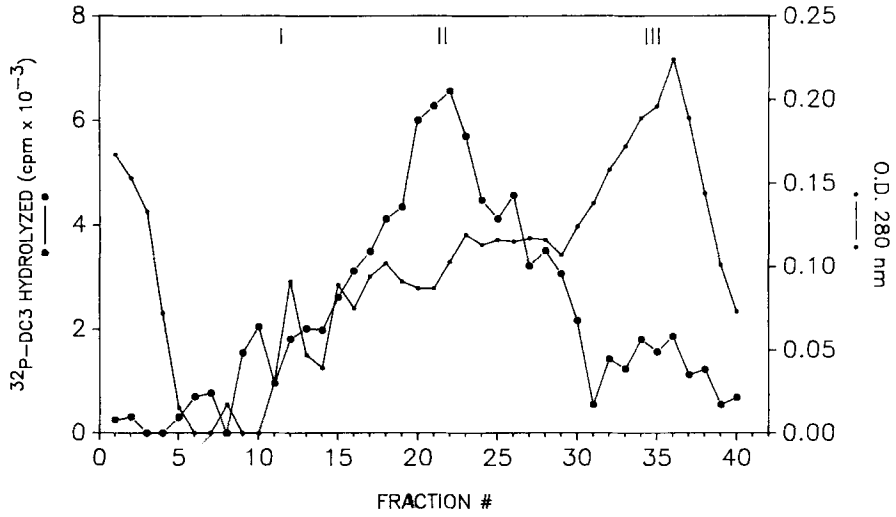

Fig. 6. Fractionation of phosphatase activity by ion exchange chromatography. PBLs were solubilized in 50 mM Hepes, 1% octyl glucoside, 0.001% leupeptin, and 0.002% PMSF. After removal of detergent-insoluble material and dilution of the sample to a concentration of 0.5% octyl glucoside, the equivalent of 7.5×10^7 cells was applied to a 3×0.7 cm column of DEAE-Sephal equilibrated in 50 mM Hepes, 0.5% octyl glucoside, and 0.001% leupeptin. The column was developed with a linear gradient of 0–0.5 M NaCl at a flow rate of 0.25 ml/min. A 5 μl aliquot from each 1 min fraction was assayed for phosphatase activity.

cytoplasm fraction. As much as 40% of the total phosphatase activity may be accounted for by the T-200 (CD45) membrane antigen. The remaining cytosolic and non-T-200 membrane activities suggest that at least three species exist in the PBL population. Fractionation of the phosphatase activity by ion exchange chromatography also suggests at least three species. The possibility that any of these species are unique to a single cell type within the PBL population seems unlikely; T-cell–enriched populations and T-cell lines have equivalent levels of activity. The presence of multiple forms of PTPase in

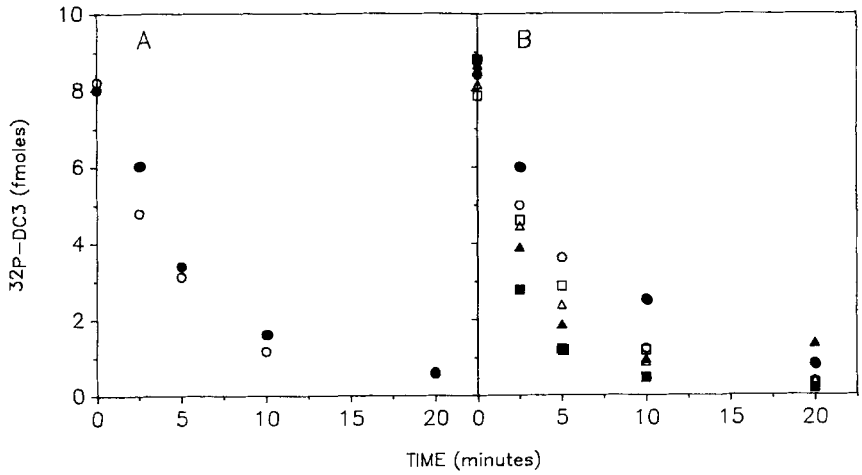

Fig. 7. PTPase activity of T cells. Detergent lysates for equal number of nylon wool-enriched T cells (A) (●) or T-cell lines (B), HSB2 (●), CTLL (▲), and HUT 102 (■) were compared to PBLs prepared from three individuals (open symbols).

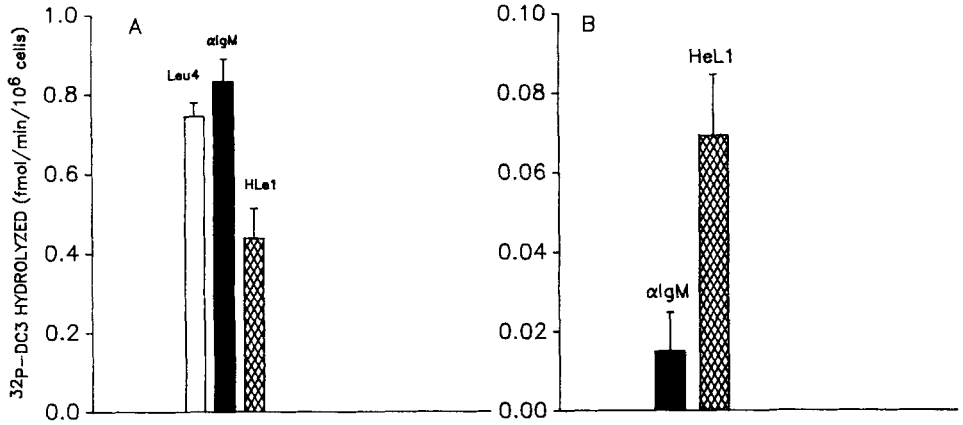

Fig. 8. T-200 immunoprecipitation. **A:** Removal of T-200 from PBL detergent lysate. HLe-1 antibody (1 $\mu g/10^6$ cell equivalents) or IgG_1 control antibodies Leu4 and αIgM prebound to goat antimouse IgG1 agarose were incubated with solubilized PBL proteins as described in Materials and Methods. After removal of the antigen–antibody complex, an aliquot of the lysate equal to 0.5×10^6 cells was assayed for phosphatase activity for 5 min at 4°C. **B:** Phosphatase activity in T-200 immunoprecipitate. PBL detergent lysate was incubated with HLe1 or αIgM antibody–agarose complex at a ratio of 0.5 μg IgG/10^6 cell equivalents for 1 hr at 4°C. The antibody complexes were washed and resuspended in the original volume of lysis buffer, and an aliquot equal to 0.5×10^6 cells was assayed for phosphatase activity for 20 min at 4°C.

lymphocytes is similar to other tissues in which phosphotyrosine-specific phosphatase have been examined. Three populations of enzymes have been purified from rabbit kidney [37,38]; and at least two species of PTPase have been demonstrated in human placenta [39].

At least one additional PTPase, calcineurin, a calmodulin-dependent phosphatase with equal affinity for both phosphoserine and phosphotyrosine and previously shown to

be present in lymphocytes [40] is not detected by the DC-3 peptide assay system. Inhibitors to calcineurin have no effect on the activities identified in this study and purified calcineurin, although readily hydrolyzing PNPP, is unable to dephosphorylate DC-3. The inability of calcineurin to dephosphorylate DC-3 may result from the low concentration of DC-3, approximately 1 nM, in the assay. The reported kinetic constant of calcineurin for phosphotyrosyl residues of myosin light chain is 0.9 μM [44]. Estimates of the affinity constant of the activity in the lymphocyte lysate using DC-3 is approximately 0.02 μM. Although the DC-3 assay system may not detect calcineurin, it does provide a simple specific assay for PTPase activity.

PTPase activity has recently been identified in CD45 precipitates [47] consistent with amino acid sequence homology between CD45 and smaller molecular weight PTPases in human placenta [39]. Antibodies to T-200 are effective in removing only part of the phosphatase activity from the cell lysate. Since CD45 is a prominent surface protein on leukocytes [45] and anti-CD45 antibodies modulate T-cell function [46,47], the PTPase activity of this group of proteins is of considerable interest. The presence of an extracellular domain on CD45 obviously provides an opportunity for selectively regulating enzyme activity in response to extracellular stimuli and may be the mechanism underlying the modulation of T-cell function through this receptor. We have shown that multiple PTPase are present in lymphocytes. At least one additional membrane and one soluble enzyme are suggested by the data. If the soluble activity is the product of proteolysis of a membrane species, as suggested for the soluble enzyme in rabbit kidney [31], or a distinct enzyme is unknown. Gel filtration studies have not revealed a low-molecular-weight activity as might be expected if the soluble activity were a product of proteolysis. This possibility, however, is not excluded. Purification and characterization of the PTPase activities are in progress.

ACKNOWLEDGMENTS

The authors thank Ms. Carolyn Davinroy for her assistance in preparing the manuscript.

REFERENCES

1. Yarden Y: Annu Rev Biochem 57:443–478, 1988.
2. Hunter T, Cooper JA: Annu Rev Biochem 54:897–930, 1985.
3. Bishop JM: Annu Rev Biochem 52:301–354, 1983.
4. Snyder MA, Bishop JM, McGrath JP, Levinson AD: Mol Cell Biol 5:1772–1779, 1985.
5. Hannink M, Donoghue DJ: Proc Natl Acad Sci USA 82:7894–7898, 1985.
6. Weinmaster G, Zoller MJ, Pawson T: EMBO J 5:69–76, 1986.
7. Bertics PJ, Chen WS, Hubler L, Lazar CS, Rosenfeld MG, Gill GN: J Biol Chem 263:3610–3617, 1988.
8. Glenney JR Jr, Chen WS, Lazar CS, Walton GM, Zokas LM, Rosenfeld MG: Cell 52:675–684, 1988.
9. Chen WS, Lazar CS, Poenie M, Tsien RY, Gill GN, Rosenfeld MG: Nature 328:820, 1987.
10. Voronova AF, Buss JE, Patschinsky T, Hunter T, Sefton BM: Mol Cell Biol 4:2705–2713, 1984.
11. Marth JD, Peet R, Krebs EG, Perlmutter RM: Cell 43:393–404, 1985.
12. Samelson LE, Patel MD, Weissman AM, Harford JB, Klausner RD: Cell 46:1083–1090, 1986.
13. Fischer S, Fagard R, Gacon G, Genetet N, Piau JP, Blaineau C: Biochem Biophys Res Commun 124:682–689, 1984.
14. Morla AO, Schreurs J, Miyajima A, Wang JYJ: Mol Cell Biol 8:2214–2218, 1988.
15. Naldini L, Stacchini A, Cirillo DM, Aglietta M, Gavosto F, Comoglio PM: Mol Cell Biol 6:1803–1811, 1986.

16. Huhn RD, Posner MR, Rayter SI, Foulkes JG, Frackelton AR Jr: Proc Natl Acad Sci USA 84:4408–4412, 1987.
17. Wedner HJ, Bass G: J Immunol 136:4226–4231, 1986.
18. Trevillyan JM, Canna C, Maley D, Linna TJ, Phillips CA: Biochem Biophys Res Commun 140:392–398, 1986.
19. Trevillyan JM, Nordstrom A, Linna TJ: Biochim Biophys Acta 845:1–9, 1985.
20. Rudd CE, Trevillyan JM, Dasgupta JD, Wong LL, Schlossm SF: Proc Natl Acad Sci USA 85:5190–5194, 1988.
21. Marth JD, Lewis DB, Wilson CB, Gearn ME, Krebs EG, Perlmutter RM: EMBO J 6:2727–2734, 1987.
22. Veillette A, Horak ID, Horak EM, Bookman MA, Bolen JB: Mol Cell Biol 8:4353–4361, 1988.
23. Frank DA, Sartorelli AC: Biochem Biophys Res Commun 140:440–447, 1986.
24. Frank DA, Sartorelli AC: Cancer Res 48:4299–4306, 1988.
25. Kraft AS, Berkow RL: Blood 70:356–362, 1987.
26. Van Den Elsen P, Georgopoulos K, Shepley B-A, Orkin S, Terhorst C: Proc Natl Acad Sci USA 83:2944–2948, 1986.
27. Casnellie JE, Harrison ML, Pike LJ, Hellstrom KE, Krebs EG: Proc Natl Acad Sci USA 79:282–286, 1982.
28. Brautigan DL, Bornstein P, Gallis B: J Biol Chem 256:6519–6522, 1981.
29. Eisen SA, Wedner HJ, Parker CW: Immunol Comm 1:571–577, 1972.
30. Horlein D, Gallis B, Brautigan DL, Bornstein: Biochemistry 21:5577–5583, 1982.
31. Swarup G, Cohen S, Garbers DL: J Biol Chem 256:8197–8201, 1981.
32. Lau KHW, Farley JR, Baylink DJ: J Biol Chem 260:4653–4660, 1985.
33. Hammond KD, Wollbrandt RK, Gilbert DA: Int J Biochem 17:259–264, 1985.
34. Lin M-F, Clinton GM: Biochem J 235:351–357, 1986.
35. Motoyama N, Takimoto K, Okada M, Nakagawa H: J Biochem 101:939–947, 1987.
36. DeSeau V, Rosen N, Bolen JB: J Cell Biochem 35:113–128, 1987.
37. Shriner CL, Brautigan DL: J Biol Chem 259:11383–11390, 1984.
38. Rotenberg SA, Brautigan DL: Biochem J 243:747–754, 1987.
39. Tonks NK, Diltz CD, Fischer EH: J Biol Chem 263:6722–6730, 1988.
40. Chantler PD: J Cell Biol 101:207–216, 1985.
41. Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA: Biochemistry 27:8695–8701, 1988.
42. Charbonneau H, Tonks NK, Walsh KA, Fischer EH: Proc Natl Acad Sci USA 85:7182–7186, 1988.
43. Beverley PCL: Transplant Clin Immunol 11:87–94, 1980.
44. Chan CP, Gallis B, Blumenthal DK, Pallen CJ, Wang JH, Krebs EG: J Biol Chem 261:9890–9896, 1986.
45. Horejsi V, Bazil V: Biochem J 253:1–26, 1988.
46. Tighe H, Clark M, Waldmann H: Transplantation 44:818–823, 1987.
47. Martorell J, Vilella R, Borche L, Rojo I, Vives J: Eur J Immunol 17:1447–1451, 1987.