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Demonstration That the Leukocyte Common Antigen CD45 Is a Protein Tyrosine Phosphatase†

Nicholas K. Tonks,* Harry Charbonneau, Curtis D. Diltz, Edmond H. Fischer, and Kenneth A. Walsh

Department of Biochemistry, University of Washington, Seattle, Washington 98195

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ABSTRACT: It has been proposed on the basis of amino acid sequence homology that the leukocyte common antigen CD45 represents a family of catalytically active, receptor-linked protein tyrosine phosphatases [Charbonneau, H., Tonks, N. K., Walsh, K. A., & Fischer, E. H. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7182–7186]. The present study confirms that CD45 possesses intrinsic protein tyrosine phosphatase (PTPase) activity. First, a mouse monoclonal antibody to CD45 (mAb 9.4) specifically eliminated, by precipitation, PTPase activity from a high M_r fraction containing CD45, prepared by gel filtration (Sephacryl S200) of a Triton X-100 extract of human spleen. Second, PTPase activity was demonstrated in a highly purified preparation of CD45 that was eluted with a high pH buffer from an affinity column, constructed from the same antibody. Third, on sucrose density gradient centrifugation, PTPase activity was only found in those fractions that contained CD45 as determined by Western analysis. When CD45 was caused to aggregate, first by reacting it with mAb 9.4 and then adding a secondary, cross-linking anti-mouse mAb, the PTPase activity shifted to the same higher M_r fractions that contained CD45. No shift in CD45 or PTPase was observed following addition of a control IgG_{2a}. On this basis, it is concluded that CD45 is a protein tyrosine phosphatase.

The phosphorylation of proteins on tyrosyl residues is a requirement for neoplastic transformation by oncogene products such as src (Synder et al., 1985), mos (Hannink & Donoghue, 1985), and fps (Weinmaster et al., 1986). It is also an essential component in the mediation of many of the effects of insulin (Ellis et al., 1986; Chou et al., 1987; Morgan & Roth, 1987) and growth factors such as EGF (Chou et al., 1987; Moolenaar et al., 1988) in a variety of cell types. Clearly, the state of phosphorylation of a protein reflects the relative activities of its kinases and phosphatases. Thus the characterization of protein tyrosine phosphatases (PTPases) may facilitate an understanding of mechanisms involved in controlling both normal and neoplastic cell growth.

Recently, the most abundant PTPases of human placenta have been purified to apparent homogeneity (Tonks et al., 1987, 1988a). These enzymes are active as monomeric units of ≈35 kDa. They appear to be specific for phosphotyrosyl residues in proteins and exhibit both a high affinity for substrate and a high V_{max} (Tonks et al., 1988b). Thus, one would expect that these enzymes would keep the level of protein phosphotyrosine within the cell under tight control.

The determination of the amino acid sequence of the major soluble and particulate enzyme, designated PTPase 1B, is currently nearing completion. From this study, it has become apparent that the sequence of PTPase 1B is not related to those of the protein Ser/Thr phosphatases or the acid and alkaline phosphatases; however, it is homologous to the cytoplasmic domains of the leukocyte common antigen CD45 (Charbonneau et al., 1988).

CD45 (L-CA, T200, B220, or Ly-5) is a family of structurally related, high M_r (180 000–220 000) membrane-spanning molecules found in all hematopoietic cells except mature red cells and their immediate progenitors (Thomas et al., 1985, 1987a). Their heterogeneity in size and antigenicity, which is cell type specific, has been attributed both to differences in primary structure at the amino terminus (the result of alternative mRNA splicing) and to differences in the pattern

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* To whom correspondence should be addressed.

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of glycosylation (Saga et al., 1986; Ralph et al., 1987; Barclay et al., 1987; Thomas et al., 1987b; Streuli et al., 1987). The molecule can be considered in terms of three distinct segments: a cysteine-rich extracellular segment (≈400 residues) heavily O- and N-glycosylated and bearing much of the carbohydrate found in lymphoid cells (Thomas et al., 1985); a single 22-residue hydrophobic segment with the characteristics of a membrane-spanning structure; and a cytoplasmic, C-terminal, segment of ≈700 residues comprising two tandem, internally homologous domains each of ≈300 residues. Similar structural organization is observed in human, rat, and mouse CD45. There is a striking conservation of sequence among the cytoplasmic segments [up to 90% identity (Thomas et al., 1987a)], clearly implying conservation of function. However, until now, neither an enzyme activity nor a detailed functional role has been ascribed to the CD45 molecule.

A 157-residue segment from PTPase 1B shows 40% and 33% sequence identity with corresponding regions of cytoplasmic domains I and II of CD45, respectively (Charbonneau et al., 1988); further data available at this time suggest that this homologous relationship extends over almost the entire length of the PTPase 1B molecule. On this basis, it was proposed that CD45 represents a family of catalytically active, receptor-linked protein tyrosine phosphatases, with each of its two cytoplasmic domains corresponding to a single PTPase 1B molecule. The present study tests this hypothesis and demonstrates that CD45 possesses intrinsic protein tyrosine phosphatase activity.

Experimental Procedures

Materials

Reagents for SDS–polyacrylamide gel electrophoresis, horseradish peroxidase (HRP) conjugated, affinity-purified goat anti-mouse IgG, and HRP color development reagent were from Bio-Rad. Nitrocellulose sheets were from Schleicher and Schuell, Keene, NH, sucrose (ultrapure) was from ICN, triethylamine was from Pierce Chemical Co., and protein A–Sepharose was from Pharmacia and Sigma. Other materials were as described in Tonks et al. (1988a). Mouse anti-CD45 mAb 9.4 (IgG_{2a}) (Cobold et al., 1987) was kindly donated by Jim Eselstyn and Ed Clark, Department of Microbiology and Regional Primate Center, University of Washington. Anti melanoma antigen p97 mAb 96.5 (IgG_{2a}) (Ledbetter et al., 1988) and rat anti-mouse κ-specific (187.1) (Ware et al., 1984) and γ-2-specific (2A96) mAbs (Yelten et al., 1981) were generously provided by Jeff Ledbetter, Oncogen Corp., Seattle, WA. An antibody affinity column for CD45 was prepared by coupling 56 mg of 9.4 anti-CD45 mAb to protein A–Sepharose, at a ratio of 10–12 mg of mAb/mL of packed gel, according to the procedure of Schneider et al. (1982).

Methods

Preparation of Tissue Extracts. Human spleens were obtained from local hospitals, stored on ice during transport, and processed within 2–3 h of surgical excision. Subsequent procedures were carried out at 4 °C. Each spleen was cut into slices of ≈50 g and washed in 10 mM imidazole hydrochloride (pH 7.2) and 150 mM NaCl to remove blood clots. The washed tissue was homogenized in a Waring Blendor at high speed for four 15-s intervals in 2.5 volumes of buffer A [10 mM imidazole hydrochloride (pH 7.2), 5 mM EDTA, 1 mM [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA), 0.1% (v/v) β-mercaptoethanol, 0.002% (w/v) phenylmethanesulfonyl fluoride (PMSF), 1 mM benzamidine, 2 μg/mL leupeptin] containing 0.25 M sucrose and then centrifuged at 6000*g* for 20 min to sediment the nuclei, which were discarded. The supernatants were further centrifuged at 48000*g* for 60 min, and this high-speed supernatant (soluble fraction) was collected for assay. The membrane pellet was washed with buffer A (50–100 mL/100 g of starting material) by resuspending it with five strokes in a glass Dounce homogenizer. This suspension was recentrifuged as above; the supernatants were again collected for assay, and at this point the membrane pellets could be stored frozen in aliquots at −70 °C following resuspension in buffer A containing 20% (v/v) glycerol.

Fresh, or thawed frozen, membranes were washed further with aqueous buffer. The membrane pellet was then homogenized as above in buffer A containing 0.5% (v/v) Triton X-100, and the solution was stirred on ice for 30–60 min and then centrifuged at 48000*g* for 60 min. This supernatant was collected and stored on ice. The pellet was reextracted in buffer A containing 0.5% (v/v) Triton X-100 and 2% (w/v) deoxycholate, as described for Triton alone. This final extract was retained for assay and the pellet discarded.

SDS–Polyacrylamide Gel Electrophoresis and Western Analysis. These procedures utilized a Bio-Rad Mini Protean II gel and blotting apparatus. SDS–polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). Following electrophoresis the gels were submerged in 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol (pH 8.3) for 15 min and the proteins transferred electrophoretically onto nitrocellulose in the same buffer at 80-V constant voltage for 90–120 min. The nitrocellulose filters were washed at room temperature in TBS [20 mM Tris, 500 mM NaCl (pH 7.5)] for 10 min, blocked with TBS containing 1% (w/v) bovine serum albumin for 60 min, washed twice for 10 min each time with TBS plus 0.05% (v/v) Tween 20, and then incubated in the same buffer in the presence of 1% (w/v) BSA and a 1:3000 dilution of 0.43 mg/mL 9.4 anti-CD45 mAb for 2 h. After being washed with TBS plus 0.05% (v/v) Tween 20 and further incubated with HRP-conjugated goat anti-mouse IgG as described for the primary antibody, the blots were developed according to the procedure described in the Bio-Rad immunoblot assay kit.

Assay of PTPase Activity. PTPase was assayed by using phosphorylated reduced, carboxamidomethylated, and maleylated (RCM) lysozyme as substrate at a final concentration of 5 μM phosphotyrosyl protein, according to the method of Tonks et al. (1988a). One unit of PTPase activity is defined as that amount which releases 1 nmol of phosphate/min at 30 °C.

Results

Extraction of PTPase Activity from Human Spleen. Fifty to sixty percent of the activity is solubilized with aqueous buffer and exhibits a low apparent M_r (≈50 000) on Sephacryl S200 (Table I and Figure 1A). Approximately 40% is extracted with Triton X-100, but only ≈30–35% of this, i.e., <15% of the total PTPase, appeared in a high M_r fraction that also contained CD45 (Table I and Figure 1B). It should be noted that, after storage for 3 days at 4 °C, the activity of the major ≈80-kDa form in the Triton X-100 extract was enhanced by around 3-fold. This was accompanied by the appearance of a new peak of ≈35–40 kDa, suggestive of a proteolytic activation mechanism. No such activation of the fraction containing CD45 was detected (data not shown). Therefore, care must be taken to assay samples as quickly as possible in the presence of proteinase inhibitors to avoid underestimating the contribution of the CD45 fraction to the total cellular PTPase activity. Less than 5% of the total PTPase and no further

Table I: PTPase Activity in Extracts of Human Spleen[a]

fraction	preparation A units	%	preparation B units	%
soluble	10400	34	21800	49
membrane washes in				
(i) aqueous buffer	5700	19	3670	8
(ii) Triton X-100	12730	42	17980	41
(iii) Triton X-100 + deoxycholate	1500	5	920	2
total	30330	100	44370	100

[a] Human spleen was fractionated and assayed for PTPase activity as described under Experimental Procedures. Preparations A and B represent different tissue samples of 44 and 148 g, respectively. Activity is expressed both as total units, obtained from duplicate assays at three different dilutions of extract, and as the percentage of the total combined extracted activity. The final pellet following extraction with Triton X-100 plus deoxycholate contained essentially no PTPase.

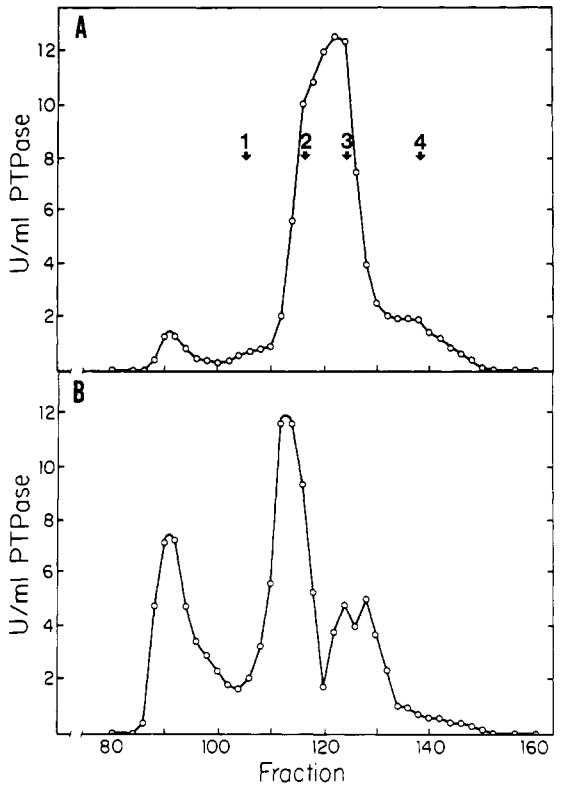

FIGURE 1: Gel filtration of human spleen extracts on Sephacryl S200. Aliquots (25 mL) of an aqueous extract (A) or Triton X-100 extract (B) were applied to a 5 × 85 cm column of Sephacryl S200 equilibrated in 25 mM imidazole hydrochloride (pH 7.2), 50 mM NaCl, 1 mM EGTA, 0.1% (v/v) β-mercaptoethanol, 1 mM benzamidine, 0.002% (w/v) PMSF, and 0.5% (v/v) Triton X-100. Fractions of 7.5 mL were collected at a flow rate of 60 mL/h. Western analysis indicated the presence of significant CD45 in fractions 86–96 of (B) only. The arrows denote the positions at which the marker proteins were eluted: 1, γ-globulin (169K); 2, bovine serum albumin (68K); 3, ovalbumin (45K); 4, carbonic anhydrase (29.5K).

CD45 (as assessed by Western analysis; data not shown) were solubilized by further extracting the membranes with Triton X-100 containing deoxycholate.

There are indications that if CD45 were a PTPase, its specific activity would have to be low. The total PTPase activity measured in human spleen extracts was ≈500000 units/kg of tissue (mean of three preparations). Eighty to ninety percent of this activity is present in low M_r forms (Figure 1). Thus, if one assumes the same specific activity as for the low M_r placenta enzyme (≈20000 units/mg under these assay conditions), one could expect 5000–10000 molecules per cell [125 fL/cell (Williams & Barclay, 1986)] and only half this value if both domains were active. However, lymphocytes are said to contain up to 100000 CD45 molecules per cell (Williams & Barclay, 1986). Thus either CD45 would have to possess less than 10% of the specific activity of PTPase 1B or its activity would have to be predominantly masked under our assay conditions. In view of this potential difficulty, the main problem in trying to detect PTPase activity in CD45 is to ensure that there is no contaminating or occluded low M_r enzyme.

Immunoadsorption of PTPase Activity onto Anti-CD45 mAb. Aliquots (100 μL) of the high M_r PTPase fraction, from gel filtration on Sephacryl S200 (Figure 1B), were incubated with 20 μg of either 9.4 anti-CD45 or a nonspecific IgG_{2a}, 96.5, as a control. Comparison of the activity in the immunosupernatants showed that 60–80% of the PTPase activity was specifically lost from the sample treated with the anti-CD45 mAb relative to the control mAb. However, significant activity could not be detected in the immune pellet. It should be noted that the addition of antibodies to a CD45-containing fraction did not by itself affect PTPase activity.

In an alternative approach, the high M_r PTPase was subjected to chromatography on an affinity column constructed by covalently cross-linking 9.4 anti-CD45 to protein A–Sepharose. As expected, the majority of the activity was retained on the column, as was CD45, and neither could be eluted with high salt. Upon being washed with pH 11 buffer, a fraction greatly enriched in CD45 (≈90% of total protein) was eluted (Figure 2). This contained PTPase activity (17% of the load), a low recovery of which probably resulted from exposure to the extreme pH.

The specific activity of the PTPase in the antibody affinity column eluate was 750 units/mg, with a K_m of ≈3 μM [values approximately 100 times lower and 10 times higher, respectively, than the placenta enzyme (Tonks et al., 1988b)]. Treatment with potential ligands such as lentil lectin or ConA as well as incubation with casein kinase II, PK-A, insulin and EGF receptors, and $pp60^{src}$ in the presence of Mg^{2+}- or Mn^{2+}-ATP was without effect. Limited trypsinolysis produced a 2–3-fold stimulation, however not nearly enough to elevate the specific activity to the levels of PTPase 1B.

Sucrose Density Gradient Centrifugation of Cross-Linked CD45. While these data strongly suggest that expression of PTPase activity is an intrinsic feature of CD45, there remained the possibility that the activity associated with the antibody-purified CD45 was due to trace contamination by a low M_r enzyme that was resistant to alkali treatment.

To eliminate this possibility, an experiment was performed in which CD45 was first complexed with mAb 9.4 and then with either anti-mouse κ (light chain) or γ-2 (heavy chain) specific secondary mAbs that would induce the formation of higher aggregates; these were separated from nonaggregated material on the basis of size. The effect of this treatment on CD45 was then compared with the effect on PTPase activity. Figure 3 indicates that, following sucrose density gradient centrifugation of an untreated sample of the high M_r PTPase fraction from Sephacryl S300, PTPase activity was only detected in the fractions that contained CD45. As expected, when 9.4 anti-CD45 was added and further cross-linked with the secondary antibodies, CD45 was observed by Western analysis to migrate at higher densities in the sucrose gradient. This change in aggregation was accompanied by a simultaneous shift in PTPase activity to the same fraction that now contained CD45. Again, this effect was specific for the anti-CD45 mAbs; in the controls, IgG_{2a} induced neither a shift

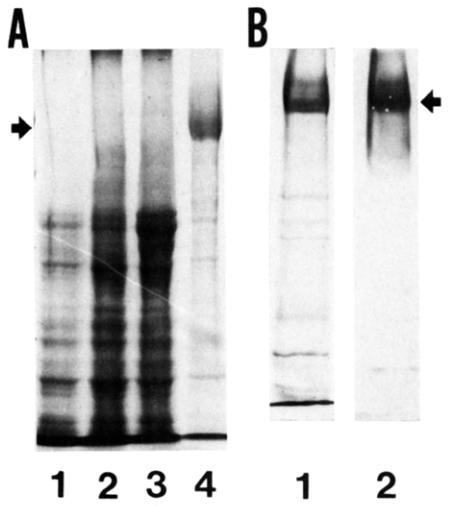

FIGURE 2: Chromatography of high M_r PTPase on an anti-CD45 antibody affinity column. The high M_r PTPase (30 mL/165 units) obtained from gel filtration of the Triton X-100 solubilized extract (Figure 1B) was applied to a 5-mL column of anti-CD45 mAb 9.4 covalently cross-linked to protein A–Sepharose, equilibrated in 20 mM imidazole hydrochloride (pH 7.2), 2 μg/mL leupeptin, 1 mM EGTA, 1 mM benzamidine, 0.002% (w/v) PMSF, 0.1% (v/v) β-mercaptoethanol, and 0.5% (v/v) Triton X-100. The breakthrough fraction was collected (30 mL, containing 52 units of PTPase) and the column washed with 30 mL of equilibration buffer containing 0.3 M NaCl. The salt wash contained 13 units of PTPase. Protein retained on the column was eluted at 4 °C within 3–4 min with 50 mM triethylamine (pH 11), 0.1% (v/v) β-mercaptoethanol, and 0.5% (v/v) Triton X-100. Three aliquots of 5 mL were collected into 0.5 g each of solid glycine to reduce the pH immediately. The eluates were then desalted by using Pharmacia PD10 columns equilibrated in the same buffer as the antibody affinity column. The desalted eluates were combined for a total volume of 21 mL, containing 27 units of PTPase. (A) Analysis of fractions by SDS–polyacrylamide gel electrophoresis. Prior to loading on the gel, the samples were concentrated by addition of 5 volumes of methanol and frozen at −70 °C for 20 min, and the precipitate was collected by centrifugation at 10000*g* for 5 min. In lanes 1–3, the protein concentration was high and the pellet resisted solubilization in the SDS sample buffer, which may explain small differences in staining intensity between lanes. Lane 1, 0.2 mL of antibody column load; lane 2, 0.2 mL of breakthrough material; lane 3, 0.2 mL of salt wash; lane 4, 2 mL of pH 11 eluate. (B) Analysis of high pH eluate by SDS–polyacrylamide gel electrophoresis and Western blotting. Two 1-mL samples were concentrated by addition of trichloroacetic acid to 5%, and precipitates were collected by centrifugation at 10000*g* for 5 min. This reduced the aggregation of CD45 experienced after methanol precipitation. After electrophoresis, the gel was sliced into two pieces. Lane 1 was stained for protein with Coomassie Brilliant Blue; lane 2 was subjected to Western analysis as described under Experimental Procedures. The arrows denote the positions of the marker protein myosin (200K).

in PTPase activity nor a shift in CD45 in the presence of the cross-linking antibodies.

On the basis of these data, we conclude that CD45 contains intrinsic protein tyrosine phosphatase activity.

Discussion

The protein sequence data presented by Charbonneau et al. (1988) established a homologous relationship between a low M_r human placenta PTPase, PTPase 1B, and each of the two tandem, internally homologous domains of CD45. From the data in the present paper, it can be concluded that at least one, if not both, of these domains is catalytically active. However, CD45 dephosphorylates RCM lysozyme with a specific activity ≈100-fold lower than that of PTPase 1B. Why should this be?

Within families of homologous proteins, there is precedent for finding lower specific activity in integral membrane enzymes than in soluble forms. Although the data are limited, the protein tyrosine kinases display this characteristic. The most potent protein tyrosine kinase is the soluble enzyme v-abl with a specific activity (nanomoles of ^{32}P incorporated into substrate per minute per milligram of enzyme) approaching 3000 (Foulkes et al., 1985). Another soluble protein tyrosine kinase recently purified from spleen has a specific activity estimated at ≈500 (Kong & Wang, 1987). In contrast, that of the insulin receptor is reported to be in the range of 5–80 (Kasuga et al.,, 1983; Nemenoff et al., 1984; Petruzzelli et al., 1984). Furthermore, when the catalytic domain of the human insulin receptor was expressed as a soluble enzyme, free of the restraint of the ligand binding structures, the specific activity was still only 20 nmol min^{-1} mg^{-1} (Herrera et al., 1988). Thus it is possible that this lower specific activity is an intrinsic feature of the receptor-linked enzyme, and one may speculate that a similar situation may hold in the comparison of receptor-linked and soluble protein tyrosine phosphatases.

Alternatively, RCM lysozyme may be a poor substrate for the class of transmembrane PTPases for which CD45 is a prototype. It is possible that optimal phosphatase activity of CD45 may require a specific orientation on the cell membrane or a particular phospholipid environment, neither of which was duplicated in our detergent-solubilized preparation. Therefore, this surface antigen might exhibit higher specific activity toward membrane-bound molecules such as insulin and EGF receptors or cell surface antigens such as CD3 (Klausner et al., 1987) that are known to be phosphorylated on tyrosyl residues.

The fact that CD45 possesses a large cytoplasmic segment suggests that it may interact with and dephosphorylate other structural elements at the membrane–cytoplasm interface. It has been reported to be associated with the cytoskeletal protein fodrin (Bourguignon et al., 1985), recently shown to be phosphorylated on tyrosyl residues of both its A and B subunits by a highly purified nonreceptor-associated kinase from bovine spleen (Wang et al., 1988). Phosphorylation of fodrin did not alter its ability to bind actin or calmodulin, but it did double its calmodulin-dependent stimulation of smooth muscle actomyosin ATPase. It is not known whether protein tyrosine phosphorylation modulates the function of fodrin in membrane-associated phenomena, but if this should be the case, CD45 might play an important regulatory role in the process.

Recently, Ledbetter et al. (1988) have presented evidence that CD45 can modulate signal transduction in T and B lymphocytes by interacting with cell surface receptor molecules. Formation of homoaggregates of CD3, CD2, and CD28 on the T cell surface by the use of cross-linking mAbs stimulated the release of Ca^{2+} into the cytosol. However, when these same surface antigens were cross-linked to CD45, the increase in cytoplasmic free Ca^{2+} was abolished. In contrast, the increase in intracellular free Ca^{2+} induced by the formation of homoaggregates of CD4 was strongly *amplified* when this receptor was coupled to CD45. T cell proliferation, induced by immobilized anti-CD3 antibodies, was also inhibited by immobilizing anti-CD3 and anti-CD45 together on the cell surface. It has been reported that the ζ chain of CD3 is phosphorylated on tyrosyl residues (Samelson et al., 1986) and that the CD4 receptor is associated with a protein tyrosine kinase, pp58 (Rudd et al., 1988). It is possible that CD45 would exert its effects by dephosphorylating these proteins, which should be considered as potential substrates for the enzyme.

It is now of crucial importance to identify the modulators of CD45 activity. Its topographical features are consistent with it being a receptor molecule. Although there is no de-

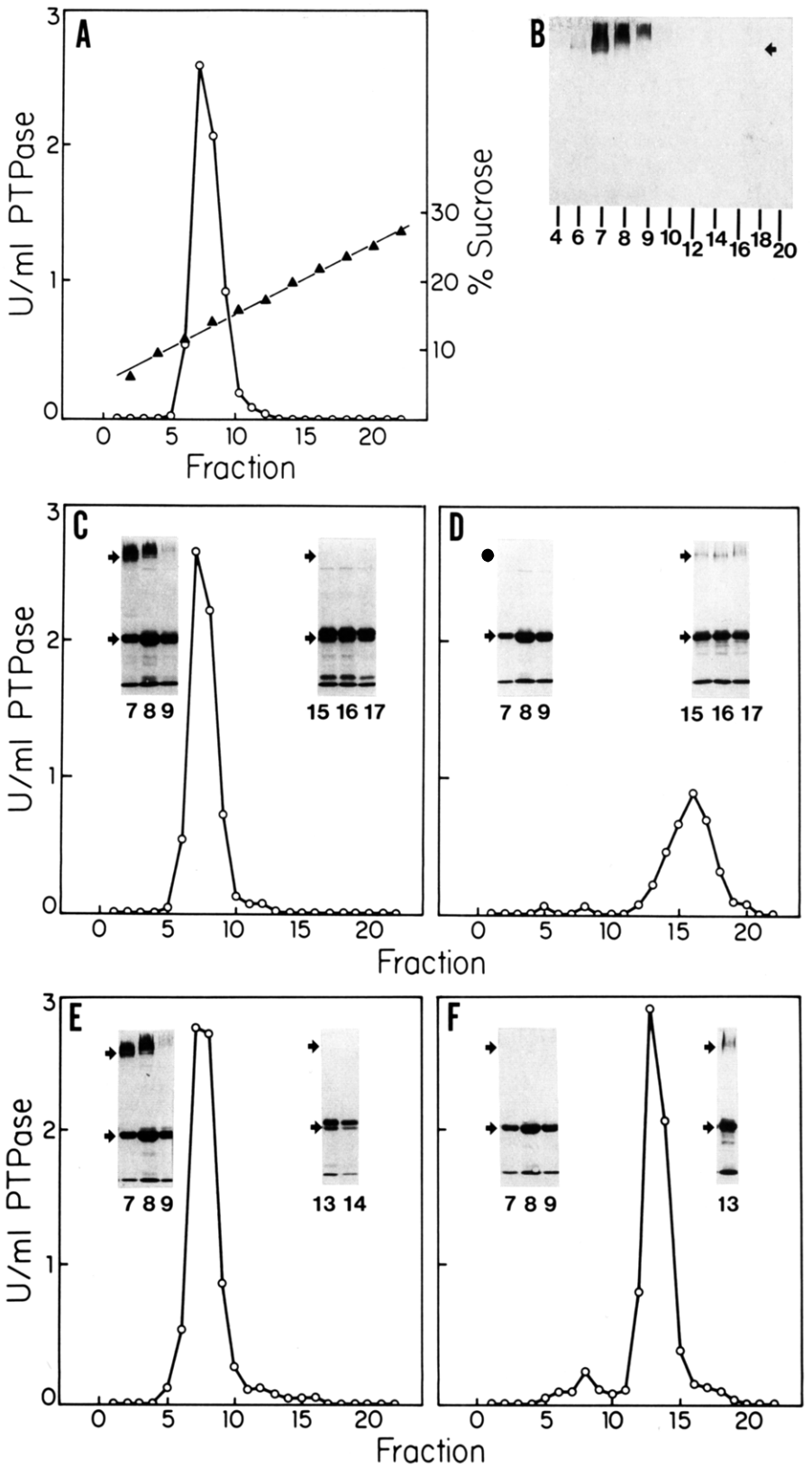

FIGURE 3: Sucrose density gradient centrifugation of CD45-containing fractions. A 500-μL aliquot of a high M_r, CD45-containing fraction from gel filtration on Sephacryl S300 was incubated on ice overnight with 35 μL of either PBS, control IgG_{2a} 96.5 (15 μg), or anti-CD45 mAb 9.4 (15 μg). Higher aggregates were formed by addition of secondary antibodies 2A96 or 187.1 in a 4:1 excess by weight over the primary antibody (60-μL volume). An equal volume of PBS was added to the non-antibody-treated control. Incubation was continued on ice overnight. Aliquots of 500 μL from each fraction were subjected to centrifugation on 5–25% sucrose density gradients for 15 h at 200000g. Fractions of 0.5 mL were collected off the top of the gradient by pumping 2 M sucrose in at the bottom of the tube at a flow rate of 1 mL/min. Samples were assayed for PTPase activity (open circles); CD45 was detected by Western analysis of 100-μL aliquots of each fraction [(B) and insets in (C–F)]. Arrows denote the positions of the marker protein myosin (200K, upper arrow) and the mAb heavy chain (lower arrow); sucrose concentration (closed triangles) was estimated by using a Bausch & Lomb hand refractometer. Identical gradients were observed in each tube. (A) Control without antibody; (B) Western blot of fractions in (A); treatment with mAbs 96.5 and 2A96 (C), mAbs 9.4 and 2A96 (D), mAbs 96.5 and 187.1 (E), and mAbs 9.4 and 187.1 (F).

tectable sequence homology, the structural analogy with the EGF receptor is intriguing. The ligand binding domain of the EGF receptor is of similar size and cysteine content to the extracellular segment of CD45 (Thomas et al., 1985), raising the possibility that the PTPase may itself be controlled by ligand binding. Whether this would result in activation or inhibition remains to be established, but the absence of a suitable ligand in our experiments may explain the low specific activity observed.

The variations in antigenicity of the CD45 family are confined to their extracellular segments. Thus it would appear that the conserved PTPase domains may be coupled to several alternative ligand binding domains, the specificity of which could select different sets of interacting molecules at the extracellular surface. The identification of such ligands, on accessory cells or among low M_r factors, should clarify the physiological function of CD45.

The receptors for hormones and growth factors that express protein tyrosine kinase activity are widely distributed. One would therefore predict that receptor-linked PTPases will not be restricted to cells of the hematopoietic lineage. The CD45 family of proteins is defined by a series of antibodies directed against their extracellular segments. Thus if analogous structures exist in other cells, their cytoplasmic segments would have to be linked to structurally unrelated extracellular domains. The demonstration of PTPase activity in these proteins could facilitate the search for homologues in other tissues.

The existence of two pools of PTPase, the low M_r cytoplasmic enzymes and the high M_r receptor-linked forms, raises the question of their differential role, particularly in view of the apparent discrepancy in their specific activities. The cytoplasmic forms may be constitutive housekeeping enzymes serving scavenging roles, acting, for instance, in the down-regulation of internalized membrane structures or surface receptors. In contrast, the activity of CD45 might be restricted to those membrane-bound proteins with which it would come into direct contact.

The existence of two homologous cytoplasmic domains in CD45 raises the interesting possibility of differential regulation of two discrete active centers. Domain II contains a unique sequence rich in Asp and Glu (11 out of 18 residues) interspersed with 6 serines (Thomas et al., 1985). This sequence has the hallmarks of a phosphorylation site for casein kinase II, by which the activity of domain II could be specifically regulated. The protein is known to be phosphorylated by protein kinase C (Shackelford & Trowbridge 1986; Autero & Gahmberg, 1987). Preferential phosphorylation of one domain over the other may provide a mechanism for selective activation or for altering their individual affinities for separate proteins. Precedent for such a double-headed arrangement is seen in the S6 kinase of *Xenopus* oocytes, which comprises two evolutionarily related but distinguishable kinase domains (Jones et al., 1988).

This study provides direct evidence that CD45 is a protein tyrosine phosphatase, confirming the prediction of Charbonneau et al. (1988). The demonstration of intrinsic PTPase activity in this transmembrane protein strengthens the hypothesis that CD45 molecules are prototypes for receptor-linked PTPases that participate directly in a novel mechanism of signal transduction involving the dephosphorylation of target proteins. Further characterization of CD45 should provide new insights into the physiological role of protein tyrosine phosphorylation.

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A DNA Helicase from *Xenopus laevis* Ovaries†

E. H. A. Poll‡ and R. M. Benbow*,‡,§

Department of Zoology and Nucleic Acid Facility, Iowa State University, Ames, Iowa 50011-3223

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ABSTRACT: A DNA helicase was extensively purified from *Xenopus laevis* ovaries. The most purified fraction was free of DNA topoisomerase, DNA polymerase, and nuclease activities. The enzyme had a Stokes radius of 54 Å and a sedimentation coefficient of 6–7.3 S, from which a native molecular weight of 140000–170000 was calculated. DNA helicase activity required Mg^{2+} or Mn^{2+} and was dependent on hydrolysis of ATP or dATP. Monovalent cations, K^+ and Na^+, stimulated DNA unwinding with an optimum at 130 mM. DNA-dependent ATPase activity copurified with the *X. laevis* DNA helicase. Double-stranded and single-stranded DNA were both cofactors for the ATPase activity, but single-stranded DNA was more efficient. The molecular weight, monovalent cation dependence, cofactor requirements, and elution from single-stranded DNA–cellulose suggest that the *X. laevis* DNA helicase is different from previously described eukaryotic DNA helicases.

Unwinding of duplex DNA is essential for DNA replication, recombination, and repair. The importance of DNA unwinding is highlighted by the identification of seven distinct DNA helicases in *Escherichia coli* (Lahue & Matson, 1988). The only viral protein required for SV40 DNA replication, the large tumor antigen (T-antigen), also is a DNA helicase (Stahl et al., 1986; Dean et al., 1987). DNA helicases contain an intrinsic DNA-dependent ATPase activity that supplies energy for unidirectional translocation along one strand of duplex DNA. This translocation results in DNA unwinding.

The exact physiological role of most DNA helicases is unknown. Some DNA helicases stimulate DNA synthesis by DNA polymerases (Scott & Kornberg, 1978; Sugino et al., 1986). The dnaB protein in *E. coli* has been shown to be the major replicative DNA helicase (Lebowitz & McMacken, 1986). Other *E. coli* DNA helicases have been postulated to function in DNA repair (Matson & George, 1987), DNA recombination (Amundsen et al., 1986), and DNA transfer during bacterial conjugation (Abdel-Monem et al., 1983).

Eukaryotic DNA helicases have been purified from lily, calf thymus, yeast, and mouse (Hotta & Stern, 1978; Hübscher & Stalder, 1985; Sugino et al., 1986; Sung et al., 1987b; Seki et al., 1987, 1988) by first isolating the major DNA-dependent ATPases and then assaying for DNA helicase activity. Most DNA helicases, except the lily enzyme, require single-stranded DNA adjacent to the duplex region to be unwound. Assays using short oligonucleotides hybridized to longer single-stranded DNA were used to identify DNA helicases in calf thymus, yeast, and mouse (Hübscher & Stalder, 1985; Sugino et al., 1986; Seki et al., 1987).

Xenopus laevis ovary has been used for the purification of many DNA replication proteins [for review, see Kaiserman et al. (1989)]. The recently developed cell-free DNA replication systems from *X. laevis* eggs (Lohka & Masui, 1983; Blow & Laskey, 1986; Newport, 1987) combined with the availability of these purified enzymes make *X. laevis* an excellent model system to study eukaryotic DNA replication. Lacking, however, are purified *X. laevis* DNA unwinding proteins.

In this study we describe the detection of a DNA helicase activity from *X. laevis* ovaries in relatively crude enzyme fractions using a partial duplex substrate and the subsequent

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* Address correspondence to this author at the Nucleic Acid Facility.
‡ Department of Zoology.
§ Nucleic Acid Facility.

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