GP180 is one of the major transmembrane glycoproteins in mouse T-lymphoma cells. This molecule is an isoform of CD45 and is known to contain an intrinsic protein tyrosine phosphatase (PTPase) activity. Using several complementary biochemical techniques, we have found that fodrin (a spectrin-like protein) is preferentially co-isolated with CD45 (GP180), suggesting that a complex between CD45 (GP180) and the cytoskeleton exists in mouse T-lymphoma cells. Furthermore, we have determined that this CD45 (GP180)-fodrin complex is dissociated by high salt treatment. Using in vitro binding assays, we have shown that CD45 (GP180) binds directly and specifically to fodrin \( (K_d \approx 1.1 \text{nm}) \) or spectrin \( (K_d \approx 3.2 \text{nm}) \) in a saturable manner. Additional analyses indicate that a 48-kDa phosphopeptide of CD45 (GP180) contains the fodrin/spectrin increase in the amount of CD45. The binding of fodrin/spectrin to CD45 (GP180) is found to significantly stimulate the PTPase activity of CD45. Enzyme kinetic analysis indicates that fodrin and spectrin increase the \( V_{\text{max}} \) of CD45 (GP180)-mediated dephosphorylation by 7.5 and 3.2-fold, respectively, without significantly changing the \( K_m \) value. These results strongly suggest that the cytoskeletal proteins, fodrin and spectrin, play an important role in the regulation of the CD45 (GP180) PTPase activity during lymphocyte activation.

The leukocyte common antigen, CD45, consists of a family of structurally related transmembrane glycoproteins that are found exclusively on leukocytes and their hematopoietic progenitors (1). The CD45 family includes various isoforms that differ in molecular weight, ranging from 180,000 to 220,000. These isoforms are generated by alternate splicing of three exons \( (e.g. \text{exons 4, 5, and 6}) \) found in the primary mRNA transcript of a single CD45 gene \( (2-5) \). The alternate splicing of CD45 mRNA results in the generation of highly related isoforms, which are known to differ only in their extracellular domains \( (1, 6-10) \). The various isoforms of CD45 display a cell type-specific expression. For example, B cells express the 220-kDa form, whereas thymocytes express the 180-kDa form \( (1) \). As the pattern of expression of different isoforms is tightly regulated during lymphoid development, it is likely that the structural variations in the extracellular domain are of functional significance. In fact, CD45 shows a topological arrangement similar to that of a receptor where the external domain is involved in ligand binding. However, the identity of a CD45-specific ligand is still unknown \( (6, 11) \).

The CD45 molecule also contains a large cytoplasmic domain of 705 amino acids that is separated from the extracellular domain \( (542 \text{ amino acids}) \) by a single transmembrane domain \( (22 \text{ amino acids}) \) \( (1, 6) \). The cytoplasmic domain contains two repeat sequences of 240 amino acids, each of which shows a striking homology with human placental protein tyrosine phosphatase PTPase 1B \( (12-15) \). It is now well established that the cytoplasmic domain, which is common in all of the CD45 isoforms, possesses an intrinsic protein tyrosine phosphatase (PTPase) activity \( (16, 17) \).

Regulation of protein phosphorylation on tyrosine residues is believed to play a pivotal role in the signal-transducing cascade of biochemical events that leads to cell growth, cellular transformation, and oncogenesis \( (18-22) \). Therefore, both protein tyrosine kinases and PTPases are crucial for normal cell growth. Specifically, the PTPase activity of CD45 is thought to be involved in the regulation of transmembrane signaling by T-cell receptor, CD2, and B-cell antigen receptor \( (23-25) \). Moreover, lymphoid protein tyrosine kinases \( (e.g. \text{p56}^{\text{CSK}}) \) have been shown to be the cellular substrates for CD45 \( (26, 27) \). It is probable that CD45 regulates lymphocyte activation by dephosphorylating one or more of the common cellular substrates that play a crucial role in the transmembrane signaling cascade. Therefore, the demonstration that CD45 has PTPase activity considerably improves our understanding of lymphocyte activation. However, unlike receptor-protein tyrosine kinases whose activity is regulated by binding of a specific ligand, it is not known how the PTPase activity of CD45 is regulated. Nevertheless, it has been shown that \( \text{Ca}^{2+} \) influx and the intracellular redistribution of CD45 molecule may influence CD45 PTPase activity indirectly \( (28, 29) \).

We have previously demonstrated that the mouse T-lymphoma cell line, BW5147, expresses GP180, the 180-kDa isoform of CD45. Furthermore, CD45 (GP180) is found to be tightly associated with a cytoskeletal protein, fodrin \( (30) \). Fodrin is a heterodimer containing a 240- and a 235-kDa polypeptide \( (31) \). It is structurally and functionally related to spectrin, and both cytoskeletal proteins bind actin \( (32, 33) \). Our results indicate that receptor clustering (patching/capping) increases the amount of CD45 (GP180) in the membrane-associated cytoskeleton fraction along with a concurrent increase in the amount of fodrin in the same fraction. Furthermore, in this cytoskeletal fraction CD45 (GP180) is
found to be tightly associated with fodrin in a 20 S complex that binds actin (30, 34). We have concluded that the CD45 (GP180)–fodrin complex and its association with actin is of functional significance in ligand-induced patching and capping of CD45 (GP180) during lymphocyte activation. In the light of the newly discovered PTPase activity of CD45 (GP180), we believe that a direct association of CD45 (GP180) with fodrin may be involved in the regulation of CD45's PTPase activity.

We report that a significant amount of CD45 (GP180) is complexed to fodrin isolated from the plasma membrane fraction of mouse T-lymphoma cells. Additional analyses in vitro indicate that purified CD45 (GP180) binds directly to both fodrin and spectrin with high affinity. The fodrin/spectrin-binding domain of CD45 (GP180) is found within a 48-kDa phosphopeptide. Most interestingly, the binding of fodrin or spectrin to CD45 (GP180) significantly stimulates its PTPase activity.

MATERIALS AND METHODS

Reagents—[γ-32P]ATP (>3,000 Ci/mmol) and [35S]methionine were obtained from Du Pont–New England Nuclear. Wheat germ agglutinin (WGA) and soybean trypsin were purchased from Sigma. Fodrin was purified from rat brain as described by Glenny et al. (31). Spectrin was purified from human erythrocytes (35). A rat hybridoma clone secon-ducibly coupled to 1 g of cyanogen bromide-activated Sepharose serum or 10% fetal bovine serum, respectively, at 37 °C in 5% CO2, 95% air.

Cell Culture—The murine T-lymphoma BW5147 cells (an AKR/J mouse lymphoma) and human epidermoid carcinoma A431 cells were grown in Dulbecco's modified Eagle's medium containing 10% horse serum or 10% fetal bovine serum, respectively, at 37 °C in 5% CO2 and 95% air.

Plasma Membrane Isolation—Plasma membranes were isolated from T-lymphoma and A431 cells as described previously (30).

Purification of CD45 (GP180)–CD45 (GP180) was purified from lymphoma plasma membranes. Plasma membranes (2–3 mg of protein) were solubilized in 25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10 μg/ml each leupeptin and aprotinin, and 1 mM phenylmethylsulfonyl fluoride at 4 °C for 30 min. The supernatant was incubated with WGA-Sepharose beads at 4 °C for 1 h. CD45 (GP180) was purified from [35S]methionine-labeled plasma membranes as described above. [35S]Labeled CD45 (GP180, 5–10 mg of protein) was subjected to limited trypsin digestion with soybean trypsin (50 pg) at 37 °C for 10 min. The trypsin was inhibited by adding 0.1 μg of soybean trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride. An aliquot of the digested sample was solubilized in SDS sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. The remaining sample was diluted 10-fold in the binding buffer and incubated with 25 μl of fodrin and spectrin beads at 4 °C for 4 h in either presence or absence of 100 μg of spectrin. Following incubation, fodrin/spectrin beads were washed extensively and the bound peptides were solubilized in SDS sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

Preparation of A431 EGF-receptor Kinase—EGF receptor kinase was partially purified from the detergent-solubilized A431 plasma membranes by WGA-Sepharose chromatography as described previously (38). The kinase activity was assayed using poly(Glu.Tyr) (4:1) copolymer as described by Tonks et al. (12).

Phosphorylation of RCM-lysozyme—RCM-lysozyme was phosphorylated using A431 EGF-receptor kinase in presence of 4 mM [γ-32P]ATP (200 cpn/pmol) at 30 °C for 16–18 h as described by Tonks et al. (12). We have routinely obtained 0.5–0.7 mole of 32P incorporated/10 μg of RCM-lysozyme. The kinase activity was stored at 4 °C in phosphate assay buffer (25 mM imidazole-HCl, pH 7.2, 0.1% bovine serum albumin, and 0.1% 2-mercaptoethanol). After phosphorylation of RCM-lysozyme was not stoichiometric, substrate concentrations in the phosphate assay are expressed as phosphorylated RCM-lysozyme.

Phosphatase Assay—CD45 (GP180) was purified from lymphoma plasma membranes essentially as described above, except that in all buffers 20 mM imidazole-HCl, pH 7.2, replaced 25 mM Tris-HCl, pH 7.4. Furthermore, to avoid the loss of activity, CD45 (GP180) bound to anti-CD45 (GP180) immunobeads was not eluted from the column by SDS-polyacrylamide gel electrophoresis and autoradiography.

To study the effects of fodrin and spectrin on the PTPase activity, CD45 (GP180)-bound immunobeads (~10–20 ng of protein) were incubated with phosphotyrosyl RCM-lysozyme at a final concentration of 2–5 μM in phosphate assay buffer (200 μl final volume) at 30 °C for 10–15 min. The release of P, from phosphotyrosyl RCM-lysozyme was linear only up to 50%. Therefore, the reaction time and concentration of CD45 (GP180) used in these experiments were adjusted such that approximately 10–15% of P, was released from phosphotyrosyl RCM-lysozyme within 10–15 min of phosphate assays. Subsequently, the reaction was terminated by adding 40 μl of bovine serum albumin (25 mg/ml) and 800 μl of 10% (w/v) ice-cold trichloroacetic acid. The suspension was vortexed, allowed to stand for 10 min, and microcen-
washed in phosphatase assay buffer to remove the unbound cytoskeletal proteins. For controls, phosphoryrosyl RCM-lysozyme was incubated with fodrin or spectrin alone. The amount of Pi released in these samples was the same as that released in sample mixtures containing no CD45 (GP180) or fodrin/spectrin.

**RESULTS**

**Isolation of CD45 (GP180) from T-lymphoma Plasma Membranes**

We have previously shown that the mouse T-lymphoma cell line (BW5147) contains a transmembrane glycoprotein, GP180, which is the 180-kDa isoform of CD45. Subsequently, we have purified CD45 (GP180) from plasma membrane preparations of BW5147 lymphoma cells. Fig. 1A shows the Coomassie Blue staining pattern of the lymphoma plasma membrane proteins separated by SDS-polyacrylamide gel electrophoresis. Clearly, the plasma membrane contains a large number of polypeptides ranging in molecular weight from ≈300,000 to ≈17,000. We have previously identified the high molecular mass of doublet (240 and 235 kDa) as the actin-binding cytoskeletal protein, fodrin (30). The transmembrane glycoprotein CD45 (GP180) is not readily detected in this total plasma membrane preparation, probably because of its lack of staining by Coomassie Blue (30).

From Triton X-100-solubilized extracts of the lymphoma plasma membranes, we have purified CD45 (GP180) by sequential WGA-Sepharose and anti-CD45 (GP180) immunofinity chromatography. Fig. 1B shows the SDS-gel electrophoresis pattern obtained by silver staining of CD45 (GP180) preparation. Clearly, this CD45 (GP180) preparation contains both GP180 (an 180-kDa isoform of CD45) and fodrin. Since fodrin is not a glycoprotein, it should not bind WGA-Sepharose (30–32). Furthermore, we have confirmed that purified fodrin does not bind to the anti-CD45 (GP180) immunofinity column (data not shown). Analysis of this preparation by sucrose density gradient (7–28%) centrifugation reveals that a large fraction of the CD45 (GP180) is associated with fodrin in a 20 S complex. However, some CD45 (GP180) appears to stay in an unbound form (free of any associated fodrin) and displays a sedimentation value of 9 S as shown previously (34).

We next attempted to purify CD45 (GP180) free of associated fodrin. Since our previous results demonstrated that the CD45 (GP180)-fodrin complex can be dissociated in the presence of high salt (0.6 M NaCl) (30, 34), we have included a high salt wash during WGA-Sepharose chromatography prior to GlcNAc elution of the proteins bound to the lectin column. As shown in Fig. 1C, this procedure yields a single detectable protein, CD45 (GP180), which is free of the previously associated fodrin.

**Binding Interactions between CD45 (GP180) and Fodrin/Spectrin**

Since fodrin appears to be associated with CD45 (GP180) in vivo, we have determined in the following sets of experiments whether a direct binding interaction exists between these two proteins. We have also examined the possible binding interaction between CD45 (GP180) and spectrin, an erythrocyte cytoskeletal protein known to share a great deal of structural homology with fodrin (31). In these experiments, various concentrations of purified CD45 (GP180) (125I-labeled) were incubated with fodrin or spectrin-conjugated Sepharose beads in order to determine the binding affinities between CD45 (GP180) and fodrin/spectrin. As shown in Fig. 2A, 125I-labeled CD45 (GP180) bound both fodrin- and spec-

**Fig. 1. Purification of CD45 (GP180) from lymphoma plasma membranes.** CD45 (GP180) was purified from Triton X-100-solubilized plasma membrane extracts by sequential WGA-Sepharose and anti-CD45 immunoaffinity chromatography as described under "Materials and Methods." Lane A, Coomassie Blue staining of total lymphoma plasma membrane proteins separated on a 7.5% SDS-polyacrylamide gel. Lane B, silver staining of CD45 (GP180) purified according to the method described above. Note the co-isolation of fodrin (240/235 kDa). Lane C, silver staining of CD45 (GP180) purified as described above, except that a 0.6 M NaCl wash was included during WGA-Sepharose chromatography.

**Fig. 2. Binding of 125I-labeled CD45 (GP180) to fodrin/spectrin-conjugated Sepharose beads.** Various concentrations (10–400 ng/ml) of 125I-labeled CD45 (GP180) were incubated with 50 μl (≈5 μg of protein) of fodrin or spectrin-conjugated Sepharose beads at 4 °C for 4 h as described under "Materials and Methods." Nonspecific binding was estimated in presence of 150 μg of soluble spectrin and was subtracted. Results represent an average of triplicate determinations from the same experiments. Standard deviation was less than 5%. A, equilibrium binding between 125I-labeled CD45 (GP180) and fodrin (●) or spectrin (▲)-conjugated beads. B, Scatchard plot of the equilibrium binding data presented in panel A. Apparent dissociation constants (Kd) for 125I-labeled CD45 (GP180) binding to fodrin and spectrin are 1.1 and 3.2 nM, respectively.
trin-conjugated beads in a concentration-dependent manner that reached saturation under equilibrium binding conditions at approximately 400 ng/ml initial concentration of $^{125}$I-labeled CD45 (GP180). However, $^{125}$I-labeled CD45 (GP180) appeared to bind the fodrin-conjugated beads better than spectrin-conjugated beads. Fig. 2B shows the Scatchard plot analyses of the equilibrium binding data shown in Fig. 2A. The linear Scatchard plots indicate a single class of binding affinity between $^{125}$I-labeled CD45 (GP180) and fodrin or spectrin. The slopes of these Scatchard plots reveal some differences in the binding affinities of $^{125}$I-labeled CD45 (GP180) for fodrin and spectrin. The apparent dissociation constants ($K_d$) for binding of $^{125}$I-labeled CD45 (GP180) to fodrin and spectrin are 1.1 and 3.2 nM, respectively.

Mapping of Fodrin/Spectrin-binding Domain of CD45 (GP180)

Since fodrin is an intracellular protein, the observed interaction between CD45 (GP180) and fodrin must involve the cytoplasmic domain of CD45 (GP180). To identify the fodrin/spectrin-binding domains, CD45 (GP180) was phosphorylated in its cytoplasmic domain by metabolic labeling of lymphoma cells with $^{32}$P. As shown in Fig. 3A, CD45 (GP180) was phosphorylated in vivo, which is consistent with previous reports (39-42). Fig. 3B shows at least five phosphopeptides (molecular weights ranging from $\approx 100,000$ to $\approx 36,000$) generated by limited trypsin digestion of $^{32}$P-labeled CD45 (GP180). However, only a single phosphopeptide of 48 kDa bound to fodrin-conjugated beads (Fig. 3C). Binding of the 48-kDa phosphopeptide to fodrin-conjugated beads is specific, because it can be totally blocked in the presence of excess soluble fodrin (data not shown). The same phosphopeptide is also the only one that binds to spectrin-conjugated beads specifically (data not shown). These data indicate that the fodrin/spectrin-binding domain of CD45 (GP180) lies in a 48-kDa peptide. Since this 48-kDa peptide is phosphorylated in vivo, it is probably derived from the cytoplasmic domain of CD45 (GP180).

**Effect of Fodrin/Spectrin on the PTPase Activity of CD45 (GP180)**

The data presented above (Figs. 2 and 3) clearly indicate that both fodrin and spectrin bind specifically and with high affinity to the cytoplasmic domain of CD45 (GP180). In this set of experiments, we have determined the effect of fodrin or spectrin on the PTPase activity of CD45 (GP180) using two different substrates, phosphotyrosyl RCM-lysozyme and $^{32}$P-labeled EGF receptor. In all of the PTPase assays, the CD45 (GP180) used was purified by sequential WGA-Sepharose and anti-CD45 (GP180) immunoaffinity chromatographies. However, we have observed that exposure to high pH during elution from the immunoaffinity column significantly reduces (>60%) the PTPase activity of CD45 (GP180) (data not shown). Therefore, in all of the phosphatase assays we used CD45 (GP180) still bound to the immunoaffinity beads as the enzyme source.

**Effect of Fodrin/Spectrin on the Dephosphorylation of Phosphotyrosyl RCM-lysozyme by CD45 (GP180)**—It has been previously demonstrated that CD45 (GP180) dephosphorylates phosphotyrosyl RCM-lysozyme (16, 17). As shown in Fig. 4, our CD45 (GP180) preparation also dephosphorylated phosphotyrosyl RCM-lysozyme. Using a 2.5 μM substrate concentration, we have found that 3.3 pmol of P$_i$/min were released in the presence of CD45 (GP180). In this assay, the concentration of CD45 (GP180) was adjusted such that approximately 15% of the total phosphotyrosyl RCM-lysozyme added was dephosphorylated within 10 min. Fig. 4 also shows that preincubation of CD45 (GP180) with fodrin or spectrin increased CD45 (GP180)-mediated dephosphorylation of the substrate in a concentration-dependent manner. A maximum increase of 3.3-3.5-fold in the dephosphorylation of phosphotyrosyl RCM-lysozyme was observed in the presence of fodrin or spectrin. However, fodrin caused a greater increase in the dephosphorylation of phosphotyrosyl RCM-lysozyme than spectrin at all concentrations.

In the next series of experiments, we determined which of the CD45 PTPase kinetic parameters (e.g. $K_m$ and $V_{max}$) are modulated in the presence of fodrin and spectrin. CD45
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(GP180) preincubated in presence of fodrin or spectrin was used to dephosphorylate various concentrations of phosphotyrosyl RCM-lysozyme. As shown in Fig. 5, Lineweaver-Burk plots for the dephosphorylation of phosphotyrosyl RCM-lysozyme in the presence or absence of fodrin (Fig. 5a) or spectrin (Fig. 5b) were linear, indicating that fodrin or spectrin does not induce any cooperativity. The presence of fodrin and spectrin, however, decreased the slopes of these plots and intercepts on the y axis without influencing the x axis intercepts significantly. These observations indicate that both fodrin and spectrin increase the $V_{\text{max}}$ of the dephosphorylation reaction but cause little variation in $K_m$ values.

The kinetic parameters determined for the dephosphorylation of phosphotyrosyl RCM-lysozyme are shown in Table I. The apparent $K_m$ estimated for phosphotyrosyl RCM-lysozyme ($\sim 5 \mu M$) is similar to that reported previously (17), and it did not vary significantly in presence of fodrin or spectrin. However, fodrin and spectrin increased the $V_{\text{max}}$ of the dephosphorylation reaction in a concentration-dependent manner. Fodrin and spectrin at 65 nM concentration caused a 7.5- and 3.2-fold increase, respectively, in the $V_{\text{max}}$ of phosphotyrosyl RCM-lysozyme dephosphorylation by CD45 (GP180). These results indicate that the increase in the initial velocity of the phosphotyrosyl RCM-lysozyme dephosphorylation reaction is due to an increase in $V_{\text{max}}$ but not to a decrease in $K_m$. Furthermore, fodrin caused a greater increase in $V_{\text{max}}$ than spectrin.

Effect of Fodrin and Spectrin on the Phosphatase Activity

We also examined the effect of these two cytoskeletal proteins on the dephosphorylation of autophosphorylated EGF receptor (a natural substrate) by CD45 (GP180). As shown in Fig. 6, CD45 (GP180) alone dephosphorylated $^{32}$P-labeled EGF receptor, but preincubation of CD45 (GP180) with fodrin and spectrin increased the amount of dephosphorylation in a concentration-dependent manner. However, as noted for phosphotyrosyl RCM-lysozyme, fodrin induced a greater dephosphorylation of $^{32}$P-labeled EGF receptor by CD45 (GP180) than spectrin. At 30 nM concentration, fodrin caused a 4-fold increase in the dephosphorylation of $^{32}$P-labeled EGF receptor, whereas at a 60 nM concentration spectrin caused only a 3.2-fold increase. These results clearly indicate that the PTPase activity of CD45 (GP180) is stimulated by both fodrin and spectrin.

Table I

<table>
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<th>$K_m$ (nM)</th>
<th>$V_{\text{max}}$ (pmol/min)</th>
<th>$K_m$ (nM)</th>
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DISCUSSION

CD45 (GP180) represents a family of integral membrane proteins, which contain tyrosine phosphatase activity (PTPase) and consist of various isoforms (180–220 kDa) that differ only in the extracellular domain (1, 6). CD45 (GP180) dephosphorylates a variety of artificial and cellular proteins that contain phosphorylated tyrosine residues (17, 26, 27, 41, 43). Although CD45 (GP180) and its PTPase activity have been shown to be required for lymphocyte activation, very little is known about the regulation of this PTPase activity. Data presented in this study demonstrate that the association of the 180-kDa isoform of CD45 with the cytoskeleton proteins fodrin or spectrin leads to stimulation of the PTPase activity.

Fodrin and spectrin are known to bind actin (31–33). Both proteins are heterodimers consisting of polypeptide chains, 240/235 kDa (in the case of fodrin) and 240/220 kDa (in the case of spectrin), respectively. A significant homology exists between the 240-kDa polypeptide chains of fodrin and spectrin, whereas the second polypeptide (i.e. 235 versus 220 kDa) is unique for each protein (31). The fact that both fodrin and spectrin bind CD45 (GP180) (Figs. 1–3) and stimulate its PTPase activity (Figs. 4–6) is consistent with the notion that these two cytoskeletal proteins share a great deal of structural and functional homology in their membrane binding capability.

The fodrin/spectrin-binding domain of CD45 (GP180) lies in a 48-kDa phosphopeptide (Fig. 3). These results indicate that both fodrin and spectrin bind to CD45 (GP180) at a

![Fig. 5. Double-reciprocal analysis of dephosphorylation of phosphotyrosyl RCM-lysozyme by CD45 (GP180) in presence of fodrin or spectrin. CD45 (GP180) (10–20 ng of protein) incubated in presence or absence of fodrin/spectrin was used to dephosphorylate various concentrations (0.625–10 μM) of phosphotyrosyl RCM-lysozyme at 30 °C for 15 min. The initial velocity of the reaction (V) was defined as pmol of P, released/ min. Panel a and b, effects of fodrin (a) or spectrin (b) concentrations on phosphotyrosyl RCM-lysozyme dephosphorylation by CD45 (GP180).](image)

![Fig. 6. Effect of fodrin (●) or spectrin (▲) on dephosphorylation of $^{32}$P-labeled EGF receptor by CD45 (GP180). CD45 (GP180) (10–20 ng of protein) preincubated with various concentrations of fodrin or spectrin was incubated with $^{32}$P-labeled EGF receptor (20,000 cpm) at 30 °C for 15 min. $^{32}$P released due to dephosphorylation of EGF receptor was estimated.](image)
common domain in of CD45 (GP180). Since the 48-kDa peptide is phosphorylated in vivo, it is most likely this phosphopeptide is derived from the cytoplasmic domain of CD45 (GP180). Although under our experimental condition, 48 kDa appears to be the major fodrin-binding domain detected in the assay (Fig. 3), we are currently using other methods such as cyanogen bromide-generated peptide fragments and deletion mutation analyses to determine the precise fodrin-binding domain on CD45. Furthermore, CD45 (GP180) has been found not to bind ankynir, a membrane-associated cytoskeletal linker protein known to bind the lymphocyte adhesion molecule GP85 (Pgpl-1/CD44) (44). Therefore, these observations suggest that transmembrane receptors selectively interact with specific cytoskeletal proteins (45).

One of the most important findings of this work is that the binding of fodrin or spectrin significantly up-regulates the PTPase activity of CD45 (GP180). Furthermore, kinetics of PTPase reaction indicates that the cytoskeletal protein binding to CD45 (GP180) increases V_{max} of the reaction and has little effect on the K_{m} values. This suggests that: (a) in the presence of fodrin or spectrin, release of the dephosphorylated product from CD45 (GP180)-substrate (e.g. phosphotyrosyl RCM-lysosome) complex occurs at a higher rate; and (b) fodrin/spectrin binding site may be distinct from the catalytic center of the phosphatase domains. Therefore, it is possible that binding of fodrin or spectrin to CD45 (GP180) induces conformational changes in phosphatase domains such that the velocity of the dephosphorylation reaction increases. The classical estimates of binding constants (K_{b}) and enzyme kinetic parameters (K_{m} and V_{max}) require free solution interactions of identical molecules. In this study, because of operational needs and convenience, we have used chemically linked fodrin/spectrin-Sepharose or immunoabsorbed CD45 to conduct equilibrium binding and kinetic analyses. These methodological limitations, using immobilized proteins which may display different structures and/or activities and/or undefined concentrations, have prevented us from obtaining an "absolute" value of K_{b}, K_{m}, and V_{max}. Consequently, we have used the term "apparent" values (Fig. 2, A and B) and kinetic parameters (Fig. 5 and Table I).

With regard to the regulation of PTPase activity of CD45 (GP180), a small amount of information is available in the literature. For example, it has been shown that a Ca^{2+} ionophore, ionomycin, inhibits the PTPase activity of CD45 (GP180) (28). However, the cellular/molecular explanations for such down-regulation of PTPase activity of CD45 (GP180) are not available. Furthermore, it has been shown that CD45 (GP180) displays a high basal level PTPase activity in presence of certain substrates (e.g. phosphorylated myelin basic protein) (17). Under this in vitro condition, it is possible that myelin basic protein plays a role as an activator of CD45’s PTPase activity (17). However, it is not clear how a high basal level PTPase activity is achieved or how this enzyme is regulated in vivo. Currently, some hypotheses related to the regulation of CD45’s PTPase activity have been proposed. For example, it has been speculated that the PTPase activity of CD45 (GP180) may be down-regulated through the binding of putative ligand to CD45 (GP180) (17). Since the physiologically relevant ligand(s) for CD45 (GP180) has not been identified, this idea still remains to be tested.

Recently, it has been shown that CD45 (GP180)-mediated dephosphorylation of p56^Lck (an src family-related, lymphocyte-specific protein tyrosine kinase) activates p56^Lck’s protein tyrosine kinase activity during lymphocyte activation (23, 43, 46). Most interestingly, it has been shown that p56^Lck from CD45(–) mutants (lacking PTPase activities) display a significantly lower protein tyrosine kinase activity (26). Due to the reduction of both PTPase and protein tyrosine kinase activities, these CD45(–) mutants fail to conduct receptor-mediated signal transduction (23, 26, 43, 44). These findings strongly suggest that a higher PTPase activity of CD45 (GP180) (leading to a higher protein tyrosine kinase activity of p56^Lck) may be required for lymphocyte activation. In this study, we have found that the basal PTPase activity of CD45 (GP180) can be up-regulated by fodrin, which may be a physiological regulator in lymphoid cells.

Previously, we have shown that a small amount of CD45 is complexed with fodrin in vivo during the resting state (in the absence of externally added ligands) (30). When lymphocytes are stimulated by externally added ligands, such as mitogens or specific antibodies against CD45, there is an increasing amount of CD45-fodrin complex formed, which accumulates underneath lymphocyte receptor patching and capped structures as shown in our previous paper (30). Therefore, it is possible that the high affinity interaction between these two molecules is responsible for the effective recruitment of free CD45 and free fodrin into a “complexed state” during lymphocyte activation. In particular, the enhancement of CD45-fodrin complex formation upon external ligand addition may trigger an up-regulation of tyrosine phosphatase activity (in vivo), which may then directly or indirectly facilitate an efficient cross-linking of fodrin to actin filaments and the subsequent movement of actin/myosin sliding filaments to collect receptors into patches and/or caps during lymphocyte function.

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