Threonine Phosphorylation of the β₃ Integrin Cytoplasmic Tail, at a Site Recognized by PDK1 and Akt/PKB in Vitro, Regulates Shc Binding*

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The mechanism of outside-in signaling by integrins parallels that for growth factor receptors. In both pathways, phosphorylation of a cytoplasmic segment on tyrosine generates a docking site for proteins containing Src homology 2 (SH2) and phosphotyrosine binding domains. We recently observed that phosphorylation of a threonine (Thr-753), six amino acids proximal to tyrosine 759 in β₃ of the platelet specific integrin α₁bβ₃, inhibits outside-in signaling through this receptor. We hypothesized that the presence of phosphothreonine 753 either renders β₃ a poor substrate for tyrosine kinases or inhibits the docking capabilities of the tyrosyl-phosphorylated form of β₃. The first alternative was tested by comparing the phosphorylation of β₃ model peptides by the tyrosine kinase pp60c-src and we found that the presence of a phosphate group on a residue corresponding to Thr-753 did not detectably alter the kinetics of tyrosine phosphorylation. However, the presence of phosphate on this threonine inhibited the binding of Shc to tyrosyl-phosphorylated β₃ peptide. The inhibitory effect of the phosphate group could be mimicked by substituting an aspartic acid for Thr-753, suggesting that a negative charge at this position modulates the binding of Shc and possibly other phosphotyrosine binding domain- and SH2-containing proteins. A survey of several protein kinases revealed that Thr-753 was avidly phosphorylated by PDK1 and Akt/PKB in vitro. These observations suggest that activation of PDK1 and/or Akt/PKB in platelets may modulate the binding activity and/or specificity of β₃ for signaling molecules.

The integrins are a family of heterodimeric proteins that play important functional roles in hemostasis. Integrins are essential for the two basic platelet responses, adhesion and aggregation, by which primary hemostasis is maintained by limiting blood loss at sites of vascular injury. During adhesion, circulating platelets anchor to subendothelial matrix proteins and matrix-absorbed proteins, while during aggregation they adhere to other platelets. These interactions depend, in part, upon the affinity of platelet integrins, such as α₁bβ₃ and α₂β₁, for specific adhesive proteins (1). The binding of adhesive molecules to integrins has two consequences. One is it allows anchored platelets to withstand shear forces experienced in arteries and veins (1), and the other is it initiates intracellular biochemical processes necessary for post-adhesive and post-aggregatory events (2).

The molecular mechanisms by which integrin-mediated signals are communicated to intracellular targets are partly understood. Recent studies support a model analogous to that for growth factor receptors; phosphorylation of tyrosine residues leads to the tethering of signaling molecules to receptor or non-receptor proteins, forming complexes that initiate signaling cascades (3–5). Growth factor responses are initiated by the binding of a ligand to specific transmembrane receptors, leading to the activation of the receptor's tyrosine kinase activity and the phosphorylation of tyrosyl residues. The phosphorylated tyrosines are recognized as part of specific sites for protein-protein interactions mediated by Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains. Examples of phosphotyrosine-targeted binding proteins include adapter proteins such as Shc and GRB2 and enzymes such as PI3-kinase and phospholipase Cγ (6, 7). In an analogous fashion, activation of the integrins α₂β₁ or α₃β₃ results in the phosphorylation of focal adhesion kinase on tyrosyl residues; this creates docking sites for a variety of signaling molecules that includes GRB2-SOS (a guanine nucleotide exchange factor for activating Ras) and PI3-kinase (8, 9). In addition, phosphorylation of the integrin itself on tyrosyl residues also may directly initiate waves of signaling (10, 11) in fashion analogous to growth factor receptor signaling pathways (12, 13).

A common feature of several integrin β subunits (β₁, β₃, β₆, β₉) is the presence of conserved tyrosine residues whose sequence context resembles that of known PTB recognition sites, NXXY (14). These sequences are necessary for the proper functioning of α₁bβ₃ integrin in platelets (15) and HEL cells (16), α₇β₃ integrin in K562 cells (10), and α₃β₄ integrin when expressed in GD25 fibroblasts (17). Several lines of evidence support a role for tyrosine phosphorylation of these sites in a biological response. The phosphorylation of β₃ on tyrosine in activated platelets (11) and in α₃β₄-transfected K562 cells (18) correlates with the binding of these cells to specific ligands. Furthermore, phosphorylation of Tyr-747 and Tyr-759 in β₃ integrins has been demonstrated to generate docking sites for signaling molecules. Both Shc and GRB2 co-precipitate with synthetic peptides that model the tyrosyl-phosphorylated carboxyl-terminal tail of β₃ (11), whereas co-association with

* This work was supported by Grant MCB9816832 from the National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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The abbreviations used are: SH2, Src homology 2; cdk, cyclin-dependent kinase; cdc2, cyclin-dependent kinase 1; GRB2, growth factor receptor-binding protein 2; MAP, mitogen-activated protein; PAGE, polyacrylamide gel electrophoresis; HRP, horse radish peroxidase; PDK1, phospholipid dependent kinase 1; PI3-kinase, phosphatidylinositol 3 kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PMA, phorbol myristate acetate; PTB, phosphotyrosine binding; SOS, son-of-sevenless.
GRB2 in intact cells correlates with tyrosyl phosphorylation of β3 (18). The presence of Tyr-747 also is necessary for other outside-in signaling events, including focal adhesion kinase and paxillin phosphorylation (19). Expression of mutated forms of αb3β3 and αaβ3 in which the cytoplasmic tyrosine residues in β3 are replaced by phenylalanines blocks outside-in signaling linked to the formation of stable platelet aggregates and clot retraction (10, 15). Similarly, mutating these tyrosine residues in β3 integrins results in decreased migration of cells upon vitronectin, fibronectin, and laminin matrices (17).

In light of the aforementioned parallels between growth factor receptor signaling and signaling through integrins, similar mechanisms may participate in regulating their activities. Signaling by many growth factors is attenuated by the phosphorylation of their cognate receptors on serine and threonine residues by several protein kinases, including PKC and MAP kinases (4). Recently, we reported that β3 is stoichiometrically phosphorylated on Thr-753 following treatment of platelets with calycin A, a membrane-permeable inhibitor of protein serine/threonine phosphatases (20). Intriguingly, this treatment also inhibits outside-in signaling events. Thr-753 is located within the stretch of residues that separates the two PTB recognition sites in β3, and is immediately adjacent to a serine residue that is mutated to proline in a variant of Glazmann’s thrombocytopenia (21). A consequence of this mutation is decreased outside-in signaling through β3-containing integrins, implying that this region of the molecule plays a regulatory role.

The phosphorylation of β3 on Thr-753 may inhibit outside-in signaling events by preventing the tyrosine kinases from phosphorylating β3 or, alternatively, by interfering with the binding of signaling molecules to tyrosyl-phosphorylated β3. Here we report that threonine phosphorylation of β3 on residue 753 blocks recruitment of Shc, suggesting that threonine phosphorylation of β3 may be an important modulator of integrin function in vivo. Moreover, Thr-753 exists in a consensus sequence for phospholipid-dependent kinases (PKDs), raising the possibility that calycin A-induced phosphorylation of β3 via the Akt/PKB pathway. Calycin A treatment of platelets, as well as treatment with a variety of agonists, leads to increased Akt/PKB activation, providing a physiological role for this phosphorylation event in the cascade of events involved in platelet responses.

**Experimental Procedures**

**Materials**—Prostaglandin E2, aprotinin, leupeptin, benzamidine, Nonidet P-40, EDTA, avidin-Sepharose, phenylmethylosulfonyl fluoride, sodium vanadate, PKA catalytic subunit, kemptide, histones (type III), myelin basic protein, casein (dephospho-form), phosphatidylinerine, and diithiothreitol were obtained from Sigma. 1,2-Dioleoylglycerol, casein (dephospho-form), phosphatidylinerine, Nonidet P-40, EDTA, avidin-Sepharose, phenylmethylsulfonyl fluoride, and dithiothreitol were obtained from Source Bio-Tech. Casein and Diacylglycerol were obtained from Biomol (Philadelphia, PA). Myelin basic protein was obtained from Calbiochem (La Jolla, CA). Human thrombin was supplied by Dr. Walter Kiesiel (University of New Mexico, Albuquerque, NM).

**Platelet Preparations**—Human blood (10 parts) was drawn into acid citrate/dextrose (1 part). Platelet-rich plasma was obtained after centrifugation at 210 × g for 10 min at 21 °C. Following addition of prostaglandin E2 (0.4 μM) and aprotinin (10 units/ml), platelets were isolated as described previously (20) and suspended in a modified Tyrode’s buffer containing 10 mM Hepes, pH 7.4, 140 mM NaCl, 1 mM MgCl2, 2 mM KCl, 5.5 mM glucose, and 12 mM NaHCO3 at a concentration of 0.5–2 × 10^9/ml.

**Platelet Precipitation**—Peptide precipitations were conducted as described by Law et al. (11). In brief, lysates, prepared by solubilizing nonstimulated platelets (1 × 10^9/ml) with 1% Nonidet P-40, 140 mM NaCl, 20 mM Tris-HCl, pH 8.2, 2 mM EDTA, 1 mM sodium vanadate, 1 mM phenylmethylosulfonyl fluoride, 40 μM leupeptin, and 0.15 units/ml aprotinin were incubated with biotinylated peptides (10 μM) for 90 min at 4 °C. Three peptides were used for these studies: a monotyrosyl-phosphorylated peptide (biotin-KEATSTFTNITyRGT; peptide 1), a doubly threonyl-tyrosyl-phosphorylated peptide (biotin-KEATSTFTNITyRGT; peptide 2), and a peptide in which an aspartic acid replaced Thr-753 in the monotyrosyl-phosphorylated peptide (biotin-KEATSTFTNITyRGT; peptide 3). Following addition of avidin-Sepharose, proteins, complexed to each peptide, were separated by PAGE using 10% acrylamide gels and Shc identified by Western analysis.

**Protein Tyrosine Kinase Assays**—The effect of phosphorylated Thr-753 on phosphorylation of Tyr-759 was assessed by measuring the phosphorylation of synthetic peptides by preparations of active pp60^{c-src}. Kinase assays were conducted by incubating 1 unit of pp60^{c-src} in a mixture containing 25 mM Tris-HCl, pH 7.2, 10 mM MgCl2, 2 mM MnCl2, 0.2 mM diethiothreitol, 75 μM [γ-32P]ATP (NEN, 5000 Ci/mmol), and varying concentrations of non-phosphorylated β3 peptide (RR^46LFKEA^746TFTNIT; peptide 1), or the threonine-phosphorylated form of the synthetic peptide (RR^46LFKEA^746TFTNITyRGT; peptide 2). Identical results were obtained using peptides that contained Tyr-747. Although two tandem arginine residues were added to the NH2-terminal end of each peptide, they failed to bind phosphocellulose filter papers. Thus, ^32P incorporation into peptides was quantified by an SDS-PAGE-based method. Reactions were quenched by the addition of SDS gel buffer and peptides separated from [γ-32P]ATP by electrophoresis on 20% acrylamide gels (bisacrylamide:acylamide, 1:32). For Lineweaver-Burk analysis, reactions incubated for 30 min. Phosphorylated peptides were visualized by autoradiography and radioactivity incorporated determined by Cerenkov counting of excised peptide bands. Prior to these studies, peptides were suspended in 10 mM Hepes, pH 7.0, and concentrations of stock peptide solutions were determined using the Pierce BCA protein assay system and γ-globulin as the standard. The data were plotted in a linear form using Lineweaver-Burk reciprocal plots (shown in Fig. 1). Hanes-Woolf plots (data not shown), or Edie-Schacetard plots (data not shown). Linear regression analysis was conducted using SlideWrite Plus (Advanced Graphics Software, Encinitas, CA) or Fg P software (BioSoft, Ferguson, MO).

**Protein Serine/Threonine Kinase Assays**—The ability of various serine/threonine protein kinases to phosphorylate Thr-753 was determined using the following synthetic peptides: RR^46LYKEAT^746TFTNITyRGT (peptide 1), RR^46LYKEAT^746TFTNIT (peptide 2), and RR^46LYKEAT^746TFTNITyR (peptide 3). Synthetic peptide 3 was incubated with kinase preparations conducted using 0.5 mM peptide and concentrations of enzyme recommended by the source: PKA (1 unit/reaction), PKC (25 mg/reaction), cdc2 (25 mg/reaction), Akt/PKB (25 mg/reaction), PKA (100 mg/reaction), and casein kinase 2 (1:100 dilution). PKC reactions were conducted in the presence of diacylglycerol (0.05 mg/ml) and phosphatidylinerine (0.5 mg/ml). PKD reactions were conducted using an active enzyme in the absence of added lipid cofactors. Reactions were quenched at 30 °C for varying amounts of time and quenched by adding SDS gel buffer. Reactions were separated using 10% PAGE and visualized by staining with Coomassie Blue R-250. Peptides were excised from wet gels and the amount of ^32P incorporated assessed using an LKB scintillation counter. For kinetic analysis, the amount of peptide 1 ranged from 1 μM to 0.5 mM. As control, filter paper assays were conducted using proteins or synthetic peptides known to be substrates for each enzyme (kemptide (0.1 μM), PKA; histones (1 mg/ml) and myelin basic protein (1 mg/ml), PKC and cdc2 kinase (5 mg/ml), casein kinase 2; Akt/PKB peptide substrate (100 μM), Akt/PKB).

**Platelet Aggregation**—300 microliters of a platelet suspension (0.5 × 10^9 platelets) were treated with buffer, collagen (5 μg/ml), PMA (0.25 μM), and thrombin (0.2 units/ml) or calyculin A (0.1–37 °C) while stirring at 800 rpm in a Chronolog Lumiaggregometer. Reactions were quenched by the addition of SDS-PAGE gel buffer, and the samples were heated at 100 °C for 2 min and subjected to SDS-PAGE. In some cases, platelets were treated with wortmannin (25 or 500 nM) prior to the addition of agonists.

**SDS-PAGE and Western Analysis**—Platelet proteins associated with
biotinylated β2 peptides and those from detergent-solubilized cells were separated on 10% SDS-polyacrylamide gels followed by transfer to nitrocellulose membranes for 1 h at 100 V. Membranes were blocked with Tween 20-containing Tris-buffered saline containing 5% nonfat dried milk and incubated overnight at 4 °C with a polyclonal anti-Shc antibody (1:1000), a monoclonal anti-Akt/PKB antibody (1:1000) against the nonactive form of the enzyme, or a polyclonal anti-phospho-Akt/PKB antibody (1:1000). Following incubation with an HRP-coupled secondary antibody (HRP-labeled goat anti-rabbit or mouse IgG), the membranes were developed using an ECL detection kit (Amersham Pharmacia Biotech). To quantitate relative changes in reactivity, densitometry measurements were attained using an IS-1000 Digital Imaging System from Alpha Innotech Corp. (San Leandro, CA).

RESULTS

Using synthetic peptides modeled after residues 746–760 of β2, LFKEATSTFTNYR, we explored the hypothesis that phosphorylation of Thr-753 in the integrin β2 modulates the effect of phosphorylation at Tyr-759. Prior studies showed that pp60src phosphorylates tyrosine residues in β2 (11). As shown in Fig. 1, pp60src phosphorylates a β2 peptide with indistinguishable kinetic parameters (Fig. 1, B and C) regardless of whether the residue corresponding to Thr-753 is phosphorylated or not. Independent mathematical transformations of the data were used to compare the $V_{\text{max}}$ and $K_m$ values of pp60src and the parameters obtained were similar for both peptides. The $V_{\text{max}}$ values of pp60src phosphorylating the non-phosphorylated and threonine-phosphorylated peptides were 44 ± 11 and 34 ± 3 fmol of phosphate incorporated/min (mean ± S.E., $n = 3$), respectively. The apparent $K_m$ values for peptide 1 were calculated to be 68 ± 20 and 113 ± 7 μM (mean ± S.E., $n = 8$) using Lineweaver-Burk (Fig. 1C) and Hanes-Woolf (data not shown) plots, respectively. Similarly, the $K_m$ values for peptide 2 are 48 ± 16 and 118 ± 13 μM (means ± S.E., $n = 8$) using these plots, respectively. Thus, these experiments suggest that threonine phosphorylation of β2 does not appear to influence subsequent phosphorylation of β2 on tyrosine.

We next asked whether phosphorylation at Thr-753 affected the binding of phosphotyrosine-759 to other signaling partners. To address this, binding studies were performed using biotinylated peptides that contain either a single phosphorylated residue (Tyr(P)-759) or two phosphorylated residues (Thr(P)-753 and Tyr(P)-759) (Fig. 2A). Platelets contain three isofoms of the binding partner Shc with molecular masses of 64, 52, and 46 kDa that are present in a ratio of 1:10:3, respectively. When a platelet lysate was incubated with avidin-Sepharose and the peptide containing phosphorylated Tyr-759, both the 52- and 46-kDa isoforms bound. In contrast, when a peptide that was phosphorylated on both Thr-753 and Tyr-759 was used as bait, Shc binding decreased to control levels (Fig. 2B). This interference with Shc binding is most likely due to the presence of the negative charge in the threonine position. A tyrosyl-phosphorylated peptide containing an aspartic acid in place of Thr-753 binds neither isoform of Shc (Fig. 2B). Thus, the binding of signaling molecules via the PTB recognition motif in β2 can be regulated by the phosphorylation of one conserved threonine (753 in β2).

Having established that threonine phosphorylation limits the recruitment of PTB-containing proteins, we attempted to identify protein kinases that might be responsible for Thr-753 phosphorylation in vivo. Since incubation with calyculin A led to increased phosphorylation of β2, we asked whether this treatment was accompanied by the activation of a potential β2 kinase. Assays of PKA, PKC, casein kinase 2, and Ca2+-dependent kinases showed that none of these enzymes were activated following calyculin A treatment (22–24), nor could activation of MAP kinase be detected ($n = 7$, data not shown). The phenylalanine located adjacent to Thr-753 is suggestive of a potential site of phosphorylation for PDK1 or, albeit less likely, Akt/PKB (25, 26). Since activation of PDK1 is a prerequisite for activation of Akt/PKB, we tested whether calyculin A stimulated Akt/PKB activity in platelets. Using an antibody that recognizes the active, phosphorylated form of the enzyme,
Western blots revealed that concentrations of calyculin A that cause phosphorylation of β3 (20) increase Akt/PKB activity in platelets (data not shown). As seen in Fig. 3, exposure to calyculin A (0.1 μM) increased Akt/PKB activity in platelets by approximately 150% (Fig. 3). The levels of Akt/PKB activation observed were comparable to those induced by agents that promote activation such as collagen (5 μg/ml), thrombin (0.2 units/ml), or PMA (0.25 μM) (Fig. 3B). Generally, Akt/PKB activation in platelets is wortmannin-sensitive (27), indicating a dependence on PI3-kinase activity. By contrast, Akt/PKB activation by calyculin A was only partially blocked by wortmannin (Fig. 3), implying that calyculin A activates Akt/PKB in both a PI3-kinase-sensitive and -insensitive fashion.

We next examined the ability of purified preparations of both Akt/PKB and PDK1 to phosphorylate the carboxyl-terminal segment of β3. Their ability to phosphorylate the unphosphorylated peptide was compared with their ability to phosphorylate (i) a peptide in which Thr-753 was replaced with a phosphothreonine (rendering this residue not phosphorylatable in the assays), or (ii) a peptide in which all threonine and serine residues, except Thr-753, were replaced by alanine residues. Both Akt/PKB and PDK1 phosphorylated those peptides that contained a phosphorylatable Thr-753 residue (Fig. 4). The $K_m$ value of Akt/PKB for phosphorylating the native β3 peptide was 14.5 mM ($n = 2$), which is 1000-fold higher than the $K_m$ for phosphorylating an Akt-like peptide, which is 18 μM (data not shown). The estimated $K_m$ value of PDK1 for the native β3 peptide was 100 ± 17 μM (mean ± S.D., $n = 3$). Neither enzyme phosphorylated to a significant degree the peptide that lacked an available Thr-753 (Fig. 4). This behavior indicates that Thr-753 is specifically targeted by both Akt/PKB and PDK1 in vitro. In contrast, two other protein kinases, PKC and cdc2, phosphorylated all three peptides, indicating that they do not preferentially target Thr-753 (Fig. 4). Moreover, the presence of a phosphate group on Thr-753 rendered the peptide a better substrate for cdc2. PKA and casein kinase 2 did not detectably phosphorylate any of the peptides (data not shown).

**DISCUSSION**

The precise role of threonyl phosphorylation of β3 is not yet completely defined. Evidence exists that threonyl phosphorylation of β3 differentially affects α4β1β3 integrin functions; it promotes inside-out signaling leading to the exposure of fibrinogen/von Willebrand factor binding sites (28) but inhibits outside-in integrin signaling linked to cell spreading and cytoskel-
et al. rearrangements (20). Integrin engagement leads to the activation of signaling cascades, including the MAP kinase pathway, and redistribution of structural and signaling molecules to cell-matrix contact points (29, 30). Recent studies indicate that the mechanism by which integrins initiate signaling events may be a consequence of phosphorylated tyrosine residues in NXXY motifs that are conserved in $\beta_1$, $\beta_2$, $\beta_3$, and $\beta_7$ subunits (11). Phosphorylation of the tyrosine in these domains creates a docking site for proteins that contain PTB domains, including Shc, an adapter protein that may facilitate recruitment of GRB2- guanine nucleotide exchange proteins. This binding sets in motion events that culminate in MAP kinase activation (8) and cytoskeletal rearrangements in the platelet (31).

The involvement of threonine residues in regulating the platelet potential of these NXXY motifs may provide a mechanism by which outside-in signaling is finely regulated, analogous to the way in which threonyl phosphorylation of cyclin-dependent kinases (cdks) prevents premature activation of newly formed cdk-cyclin complexes (32). These phosphorylations assure that premature activation of the enzyme does not occur until conditions are optimal for cells to progress through the cell cycle. In the present study, attempts were made to determine how phosphorylation of Thr-753 modulates outside-in signaling using synthetic peptides based on the carboxyl-terminal sequence in $\beta_3$. Our data argue that phosphorylation of Thr-753, which is conserved in many $\beta$ subunits, reduces the ability of PTB-containing proteins to bind the NXXpY motif in $\beta_3$. The mechanism by which threonine phosphorylation acts in controlling $\beta_3$ function is not known. Modeling for secondary structure using Chou-Fasman (33) algorithms predicts that aspartic acid substitution for Thr-753 (to mimic the negative charge of a phosphate group) results in changes in secondary structure, which may be a mechanism explaining how integrin function is modulated by phosphorylation. A similar change in secondary structure is predicted when a proline substitutes for Ser-752; this mutation has been observed in vivo and inhibits both inside-out and outside-in signaling (16, 34).

Although the effects of phosphorylation of Thr-753 serves a regulatory role in signal transduction via $\beta_3$, the mechanism platelets use to regulate this phosphorylation is not clear. The inhibitory effects of membrane-permeable inhibitors of serine/threonine phosphatases (e.g. calyculin A) on platelet function have been known for approximately a decade (22, 35). However, the mechanisms by which calyculin A affects platelets are still unclear. Since protein phosphorylation represents the Akt/PKB pathway in intact platelets in a manner similar to platelet agonists such as thrombin, collagen, and PMA. Although the exact molecular mechanism by which calyculin A activates Akt/PKB remains to be determined, this represents the first demonstration of the activation of an enzyme in platelets upon exposure to calyculin A. These studies are interesting in light of reports that show the rapid activation of PDK1 and Akt/PKB by platelet agonists (27, 36). Based on these observations, we speculate that treatment of platelets with either calyculin A or agonists leads to the activation of a common kinase, resulting in phosphorylation of Thr-753 in $\beta_3$, although the residue in $\beta_2$ that becomes phosphorylated following agonist treatment remains unknown. However, the observation that Thr-753 was specifically targeted by Akt/PKB and PDK1 in peptide substrates is highly suggestive. PKC is also activated in platelets but fails to phosphorylate Thr-753 in the peptide substrate. In $\beta_1$, it does phosphorylate Ser-756, a residue not conserved in $\beta_3$ (37).

Parallels may exist between calyculin A effects and antagonists that act via PKA. However, several observations do not support this consideration; PKA does not phosphorylate the $\beta_3$ peptide in vitro, and calyculin A does not (i) lead to a rise in intracellular cAMP levels (22) or (ii) result in phosphorylation of substrates common to PKA (23).

Our results suggest a novel mechanism by which integrin action is controlled; transient phosphorylation of integrin molecules temporally controls outside-in signaling. Being that Thr-753 in $\beta_3$ is conserved in other $\beta$ subunits, this model may be a common mechanism by which integrin function is regulated. In addition, this model may explain, in part, why there is controversy over whether $\beta_3$ becomes phosphorylated on threonine residues following the exposure of platelets to agonists (28, 38). The inconsistency may be attributable to high levels of protein serine/threonine phosphatases in platelets (22) and that integrins are phosphorylated when their effective concentrations increase upon clustering. In support of these possibilities, stable phosphorylated forms of $\beta_3$ can be isolated in the presence of protein seryl/threonyl phosphatase inhibitors, okadaic acid and calyculin A (20) and the $K_m$ for phosphorylation of a $\beta_3$ peptide by Akt/PKB was in the millimolar range.

In conclusion, this report links phosphatase inhibition to kinase activation in platelets. In particular, PDK1 and Akt/PKB activation may be targeting $\beta_3$ and this may have considerable impact on the regulation of integrin function in platelets.

Acknowledgments—We thank Drs. Tony Hunter, Victor Fried, Pete Kennelly, and Susan Olson for invaluable discussions throughout these studies and Drs. Dave Litchfield, Debbie Law, Dave Phillips, and Walter Kisiel for reagents indicated above. We also thank Anne Marie Snow for help in preparation of the figures and Drs. Kennelly and Fried for a critical review of the manuscript.

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