Phosphorylation of the Cytoplasmic Domain of the Integrin CD18 Chain by Protein Kinase C Isoforms in Leukocytes*

Susanna Fagerholm‡§, Nick Morrice‡, Carl G. Gahmberg§§, and Philip Cohen‡

From the ²MRC Protein Phosphorylation Unit, MSU/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, United Kingdom and §Division of Biochemistry, Department of Biosciences, PB 56, University of Helsinki, 00014 Helsinki, Finland

The CD11/CD18 (β2 integrins) are leukocyte-specific adhesion receptors, and their ability to bind ligands on other cells can be activated by extracellular stimuli. During cell activation, the CD18 chain is known to become phosphorylated on serine and functionally important threonine residues located in the intracellular C-terminal tail. Here, we identify catalytic domain fragments of protein kinase C (PKC) δ and PKCβII as the major protein kinases in leukocyte extracts that phosphorylate a peptide corresponding to the cytoplasmic tail of the integrin CD18 chain. The sites phosphorylated in vitro were identified as Ser-745 and Thr-758. PKCα and PKCγ also phosphorylated these residues, and PKCα additionally phosphorylated Thr-760. Ser-745, a novel site, was shown to become phosphorylated in T cells in response to phorbol ester stimulation. Ser-756, a residue not phosphorylated by PKC isoforms, also became phosphorylated in T cells after phorbol ester stimulation. When leukocyte extracts were subjected to affinity chromatography on agarose to which residues 751–761 of the CD18 chain phosphorylated at Thr-758 were bound covalently, the only proteins that bound specifically were identified as isoforms of 14-3-3 proteins. Thus, PKC-mediated phosphorylation of CD18 after cell stimulation could lead to the recruitment of 14-3-3 proteins to the activated integrin, which may play a role in regulating its adhesive state or ability to signal.

The CD11/CD18 integrins (β2 integrins) are leukocyte-specific members of the integrin superfamily of heterodimeric cell surface receptors involved in adhesion to the extracellular matrix and to cells. The four different CD11/CD18 integrins (1–2) share a common β chain (CD18) but have different α chains (CD11α–d) and different cell distribution and ligands. CD11/CD18 integrins are unable to bind their ligands, the intercellular adhesion molecules (ICAMs) in resting cells, but instead need an activating signal for conversion to an adhesive state (2, 3). In addition to extracellular activation with divalent cations (4), ligands (5–7), or certain monoclonal antibodies to the extracellular domains (8–10), the integrins can also be activated by intracellular signaling, so-called inside-out signaling. This is induced by triggering the T cell receptor (3, 11) or other leukocyte surface receptors (11–13), or by direct activation of PKC by tumor-promoting phorbol esters (14, 15). The molecular basis for activation is still poorly understood; however, it is thought to involve changes in avidity via surface redistribution of the integrin (16–19) and perhaps signaling mediated conformational changes of the integrin extracellular domain (20). Several signaling pathways have been implicated in the activation process, including those that modulate protein kinase C (PKC) (21), phosphoinositide 3-kinase (PI 3-kinase) (22), mitogen-activated protein kinase (23), the small GTP-binding protein Rap1 (21), protein phosphatases (24, 25), and the calcium-binding proteins calpain (26) and calmodulin (27). The cytoplasmic domain of the CD18 chain is necessary for the regulation of adhesion (16, 28, 29), and interestingly, both phorbol esters and CD3 ligation induce CD18 phosphorylation on Ser (30–32) and Thr (25) residues. Thr phosphorylation is more transient than Ser phosphorylation. The Thr phosphorylation may regulate the adhesive state of the integrin, because mutation of three consecutive threonines in the integrin chain (Thr-758, Thr-759, and Thr-760) reduce integrin binding to ICAM-1 (29) and cytoskeletal association (16) of the integrin molecules. Additionally, phosphorylated integrin molecules preferentially partition with the actin cytoskeleton (33), indicating that phosphorylation of integrins could regulate integrin-cytoskeleton interactions.

In addition to their adhesive properties, the integrins are also involved in activating intracellular signaling pathways and can thus mediate bi-directional signaling across the plasma membrane. In fibroblasts they form focal adhesion complexes with cytoskeletal elements and a wide range of signaling molecules (34). In leukocytes these signaling complexes are believed to be more transient, because leukocytes are involved in relatively short lived interactions with other cells. Several cytoskeletal and signaling molecules have been shown to interact with the CD18 cytoplasmic tail, including the actin-binding proteins talin (33, 35), filamin (36), and α-actinin (37, 38), the adaptor proteins Rack1 (receptor for activated PKC) (39) and cytohesin (40), and the transcription factor-Jun activation domain-binding protein (41).

PKC, protein kinase C; Rack1, receptor for activated C kinase; PDBu, phorbol 12,13-dibutyrate; PI 3-kinase, phosphoinositide 3-kinase; JAB1, Jun activation domain-binding protein 1; PKI, the specific peptide inhibitor of CAMP-dependent protein kinase (TYYADFIASRTGRNRNAHID); PEG, polyethylene glycol; PVDF, polyvinylidene difluoride; BisTris, 2-[bina(2-hydroxyethyl)amino]-2-[hydroxyethyl]propane-1,3-diol.

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‡ Recipient of a postgraduate research studentship from the Oskari Huttunen Foundation. To whom correspondence may be addressed: Dept. of Biosciences, Division of Biochemistry, P. O. Box 56 (Vitikkaari 5), FIN-00014 University of Helsinki, 00014 Helsinki, Finland. Tel.: 358-9-191-59029; Fax: 358-9-191-59068; E-mail: susanna.fagerholm@helsinki.fi.

§ To whom correspondence may be addressed: Dept. of Biosciences, Division of Biochemistry, P. O. Box 56 (Vitikkaari 5), FIN-00014 University of Helsinki, 00014 Helsinki, Finland. Tel.: 358-9-191-59029; Fax: 358-9-191-59068; E-mail: carl.gahmberg@helsinki.fi.

The abbreviations used are: ICAM, intercellular adhesion molecule;
In this study, we present evidence that PKC isoforms are the major protein kinases that phosphorylate the C terminus of the integrin CD18 chain in leukocytes. Ser-745 is identified as a novel phosphorylation site in the integrin cytoplasmic domain. Additionally, we show that a Thr-758-phosphorylated integrin peptide can interact with 14-3-3 proteins in leukocyte lysates and thus potentially initiate signaling complex formation “downstream” of the phosphorylated integrin molecules.

EXPERIMENTAL PROCEDURES

Materials—PKI, the specific peptide inhibitor of cAMP-dependent protein kinase (TTYADFIASGTRGNAIHD), was synthesized by F.B. Caudwell in the MRC Protein Phosphorylation Unit and the other peptides by G. Bloomberg (University of Bristol, UK). Hitrap Q, Hitrap heparin, MonoQ, and gel filtration columns, protein G-Sepharose, and [32P]-orthophosphate were purchased from Amersham Biosciences. Vivysulfone-activated agarose and the calcium ionophore A23187 were from Sigma. Phosphocellulose P-81 paper was from Whatman. Okadaic acid, phorbol 12,13-dibutyrate (PDBu), Ro 318220, Go-6983, W-7, trifluoroacetic acid, phorbol 12-myristate 13-acetate, and 14-3-3 protein were from Calbiochem. PKC isozyme-specific antibodies were from Transduction laboratories (Lexington, KY). The broadly reactive 14-3-3 (K-19) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA).

Protein Kinase Assay—Protein kinases were assayed using a peptide corresponding to the integrin CD18 C terminus (RRIPEKKLSQWNND PLFKPSATTTT), where pS is phosphoserine, with an N-terminal cysteine added for coupling, and conjugated to keyhole limpet hemocyanin as in Ref. 42. The complex was injected into sheep at the Scottish Antibody Production Unit (Carluke, Scotland). The antiserum was passed through agarose to which the relevant keyhole limpet hemocyanin as in Ref. 42. The complex was injected into sheep at the Scottish Antibody Production Unit (Carluke, Scotland). The antiserum was passed through agarose to which the relevant

Antibodies—Two phosphopeptides were synthesized corresponding to residues 740–751 and 751–761 of the integrin CD18 chain (CREK-LKpSQWNND and CNPLFpKpSATTTT, where pS is phosphoserine), with an N-terminal cysteine added for coupling, and conjugated to keyhole limpet hemocyanin as in Ref. 42. The complex was injected into sheep at the Scottish Antibody Production Unit (Carluke, Scotland). The antiserum was passed through agarose to which the relevant phosphorylated peptide had been coupled covalently, and the phosphospecific antibodies were eluted with 0.1 M glycine, pH 2.4, immediately adjusted to pH 8.0 with Tris-HCl, and stored at 4 °C. This was carried out by Dr. J. Leitch and C. Clark in our laboratory. The CD18 integrin antibodies R7E4 and R2E7B have been described previously (14, 43). The monoclonal antibody OKT3, which reacts with CD3, was used in the form of ascites fluid produced by hybridoma cells (clone CRL 8001, American Type Culture Collection, Manassas, VA). PKC isozyme-specific antibodies were from Transduction laboratories (Lexington, KY). The broadly reactive 14-3-3 (K-19) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA).

FIG. 2. Protocol for the purification of the major CD18 kinase activities in leukocyte lysates. The specific activity and overall yield from Hitrap Q pH 7.5 is shown.

 FIG. 3. Purification of the integrin kinase activity. A, fractions from the final gel filtration column were assayed for CD18 C-terminal kinase activity (open circles). The elution position of the standard marker proteins bovine serum albumin (66 kDa) and ovalbumin (44 kDa) are shown. B, aliquots of indicated fractions from A were subjected to SDS-PAGE and stained with Coomassie Blue (left-hand panels) or transferred to nitrocellulose membranes and immunoblotted with an antibody raised against the PKCα catalytic domain (right-hand panels). The bands identified as PKCβ and PKCγ are indicated.
...protein) were fractionated from 5 to 20% polyethylene glycol 6000 phosphate, 0.27 M sucrose, 2 M NaCl, which was developed with a linear salt gradient to 1 M NaCl. The most active fractions, eluting at 0.3 M NaCl were pooled and applied to a 1-ml MonoQ column, which was developed with a linear salt gradient to 1 M NaCl. One major activity was detected eluting at 0.3 M NaCl in buffer B and 0.35 M in buffer C. The activity was then chromatographed on a 2-ml protamine-agarose column equilibrated in buffer A. The column was washed with 5 ml of buffer A plus 200 mM NaCl and then with buffer A plus 1 M NaCl. Four fractions, each of 6 ml, were collected.

Identification of Proteins by Mass Spectrometry and Phosphoamino Acid Analysis—Proteins of interest were excised from SDS-polyacrylamide gels, digested with trypsin, and identified using a Perspective Biosystems (Framingham, MA) Elite STR matrix-assisted laser desorption-time of flight-mass spectrometer as described previously (46). Phosphoamino acid analysis of integrin CD18 C terminus was also performed as described (47).

Isolation of CD11/CD18 Integrins from Lymphocyte Lysates—Integrins were affinity-purified from human leukocytes using R7E4 columns as described previously (48) and were at least 90% pure as monitored by SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining.

Subcellular Fractionation—Fractionation of cells into soluble and cytoskeletal fractions was done after lysis in the presence of Triton X-100 as described (27).

Peptide Affinity Chromatography—The phosphorylated and unphosphorylated forms of the peptides CNPLFKpSATTTV and CLFKSApTTRMV, were coupled to vinylsulfone-activated agarose via the N-terminal cysteine residue. The cytosol and solubilized cytoskeletal fractions (0.5–1 mg of protein) were then subjected to affinity chromatography on each peptide-agarose column as described (27).

**Table I**

| Mass measured | Theoretical | Residue no. | Peptide sequence |
|---------------|-------------|-------------|-----------------|
| 727.3920      | 727.3779    | 265-299     | (R)FELYR(A)     |
| 771.4979      | 771.4980    | 313-319     | (K)VLLGELK(G)   |
| 788.4502      | 788.4459    | 549-554     | (K)IHPFFK(T)    |
| 801.4859      | 801.4834    | 541-548     | (R)LGVTGNIK(I)  |
| 806.4185      | 806.4048    | 257-293     | (K)IVGESSK(C)   |
| 827.4313      | 827.4003    | 322-328     | (R)GEYFAIKA     |
| 940.5059      | 940.5005    | 393-399     | (K)GREFLYR(A)   |
| 951.4622      | 951.4536    | 447-454     | (K)ENIFGESR(A)  |
| 984.4967      | 984.4903    | 512-519     | (R)VDTPHYPRW     |
| 1019.5520     | 1019.5526   | 532-539     | (K)LFREPTKR(R)  |
| 1040.5577     | 1040.5529   | 320-328     | (R)KGReFYAIKA   |
| 1117.6279     | 1117.6257   | 555-563     | (K)TNWTLLEKR(R) |
| 1139.6608     | 1139.6465   | 322-331     | (K)GEYFAIKA(L)(K) |
| 1139.6608     | 1139.6689   | 565-573     | (K)LREPFPRK(V)  |
| 1175.6549     | 1175.6537   | 532-540     | (K)LFREPTKR(K)  |
| 1175.6537     | 1175.6537   | 532-540     | (K)LFREPTKR(L)  |
| 1279.7272     | 1273.7268   | 284-303     | (K)CNINNFPIHK(V) |
| 1306.6292     | 1306.6287   | 423-433     | (R)LDKLNVLDDR(D)(D) |
| 1313.7294     | 1313.7419   | 426-438     | (K)LDNVLLRDGHIIK(I) |
| 1313.7294     | 1313.7419   | 426-438     | (K)LDNVLLRDGHIIK(I) |
| 1507.8137     | 1507.8137   | 579-591     | (R)DVVLIDVECTMVK(R) |
| 1507.8137     | 1507.8137   | 579-591     | (R)DVVLIDVECTMVK(R) |
| 1648.7255     | 1648.7131   | 429-433     | (K)CTKVAGEĐMDQSNGTYGK(I) |
| 1648.7255     | 1648.7131   | 429-433     | (K)CTKVAGEĐMDQSNGTYGK(I) |
| 1873.7811     | 1873.8283   | 268-286     | (K)IADFGMKENIFGESR(A) |
| 1873.7811     | 1873.8283   | 268-286     | (K)IADFGMKENIFGESR(A) |
| 1879.8483     | 1879.8669   | 333-345     | (R)DVVLIDVECTMVK(R) |
| 2035.9658     | 2035.9680   | 333-345     | (R)DVVLIDVECTMVK(R) |
| 2086.9836     | 2087.0094   | 400-417     | (R)DVVLIDVECTMVK(R) |
| 2103.0381     | 2103.0043   | 400-417     | (R)DVVLIDVECTMVK(R) |
| 2417.2460     | 2417.2903   | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |
| 2417.2460     | 2417.2903   | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |
| 286.4313      | 286.4303    | 322-328     | (R)GEYFAIKA     |
| 303 (K)CNINNFIFHK(V) | 303 (K)CNINNFIFHK(V) | 322-328 | (R)GEYFAIKA     |
| 303 (K)CNINNFIFHK(V) | 303 (K)CNINNFIFHK(V) | 322-328 | (R)GEYFAIKA     |
| 328 (K)GRGEYFAIK(A) | 328 (K)GRGEYFAIK(A) | 328-329 | (R)GRGEYFAIK(A) |
| 328 (K)GRGEYFAIK(A) | 328 (K)GRGEYFAIK(A) | 328-329 | (R)GRGEYFAIK(A) |
| 331 (R)GEYFAIKALK(K) | 331 (R)GEYFAIKALK(K) | 331-332 | (R)GRGEYFAIK(A) |
| 348 (K)DVVLIDDVECTMVEKR(V) | 348 (K)DVVLIDDVECTMVEKR(V) | 348-349 | (R)DVVLIDVECTMVK(R) |
| 349 (K)DVVLIDDVECTMVEKR(V) | 349 (K)DVVLIDDVECTMVEKR(V) | 349-350 | (R)DVVLIDVECTMVK(R) |
| 349 (K)DVVLIDDVECTMVEKR(V) | 349 (K)DVVLIDDVECTMVEKR(V) | 349-350 | (R)DVVLIDVECTMVK(R) |
| 350-370 | 350-370     | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |
| 350-370 | 350-370     | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |
| 350-370 | 350-370     | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |
| 350-370 | 350-370     | 350-370     | (K)RLTLaAENPFLTHLTCTFQTK(D) |

* Methionine sulfone derivative is indicated.

**Table I**

Identification of the 42-kDa band in Fig. 3, B and D, as the catalytic domain of PKCα

Tryptic peptides from the 42-kDa autophosphorylating bands in Fig. 3, B and D, were analyzed on a Perspective Biosystems Elite STR matrix-assisted laser desorption-time of flight mass spectrometer as described under “Experimental Procedures.” The tryptic ions were scanned against the Swiss-Prot and Geneppe databases using the SF-FIT program of Protein Prospector. The table summarizes the peptides from the integrin kinase that matched the PKC sequence.
noncovalently to protein G-Sepharose. Bound proteins were eluted with 1% SDS, then subjected to polyacrylamide gel electrophoresis, and transferred to nitrocellulose. The phosphorylated integrin was immunoblotted and detected using the ECL chemiluminescence detection system (Amersham Biosciences). The phosphorysensitive antibody that recognizes CD18 phosphorylated at Ser-756 was used at 1:5000 dilution. The anti-PKC β antibody, which recognizes phosphorylated and unphosphorylated CD18 equally, was used at 1:1000. An antibody that recognizes all 14-3-3 isoforms was used at 1:5000 dilution. The antibody against the PKC β catalytic domain, which also reacts with PKC β1 isoform of PKC, was used at 1:1000.

### RESULTS

**Identification of the Major Protein Kinases in Human Leukocyte Lysates That Phosphorylate the C Terminus of the Integrin CD18 Chain**—To identify the major CD18 kinases, human leukocyte lysates were initially fractionated on MonoQ. All the activity was retained by the column and eluted as one major peak (Fig. LA), which was pooled and chromatographed on heparin-Sepharose. At this step, 80% of the activity was not retained by the column, whereas 20% was bound. The activity present in both fractions was then further purified as described under “Experimental Procedures.” A flow chart of the overall purification protocol is shown in Fig. 2.

The activity that was retained by heparin-Sepharose eluted as a single peak with an apparent molecular mass of 40 kDa at the final gel filtration step (Fig. 3A). SDS-PAGE revealed two major proteins, whose elution position correlated with activity (Fig. 3B). When the fractions were incubated with Mg[y-32P]ATP, both proteins became phosphorylated (Fig. 3C), suggesting that they might be protein kinases capable of autophosphorylation. The bands were excised and subjected to tryptic mass fingerprinting, which revealed that they both corresponded to the β isoform of PKC. All the peptides detected were located in the catalytic domain of PKCβ (Table I). This observation and the apparent molecular mass of the purified protein (which is much smaller than full-length PKCβ) indicated that it represented an active proteolytic fragment. Such fragments are known to be active in the absence of phospholipids and diacylglycerol, which are required for the activity of full-length PKCβ.

The activity that was not retained by heparin-Sepharose was purified by chromatography on MonoQ at two different pH values and finally on protamine-agarose. SDS-PAGE of the activity initially eluted at 1 M NaCl (accounting for 46% of the activity) showed five protein-staining bands (Fig. 3D) of which the three most rapidly migrating (apparent molecular masses, 173, 204, and 238 kDa) indicated that it represented an active proteolytic fragment. Such fragments are known to be active in the absence of phospholipids and diacylglycerol, which are required for the activity of full-length PKCβ. This explains why these active fragments were detected, because the assays did not contain the cofactors essential for the activation of PKCβ isoforms.

### Table II

Identification of the proteins in Fig. 3D as the catalytic domain of PKCβ/βII

| PKCβ (pool 1 upper band) | 570.2999 | 570.3040 | 455–458 | R(F)FFTR(H) | K(E)HAFR(Y) |
|--------------------------|----------|----------|--------|------------|------------|
| 806.4152                 | 806.3949 | 419–424  |        | R(N)AEFDR(F) |
| 865.3986                 | 865.3804 | 448–454  |        | R(L)CGFGER(D) |
| 974.4582                 | 974.4366 | 467–415  |        | R(GLMTRHKGR) |
| 984.5165                 | 984.5301 | 397–405  |        | R(EIQPPFYKPA) |
| 1099.6324                | 1099.5301| 434–443  |        | R(YIDWKEKER) |
| 1126.6154                | 1162.5744| 313–322  |        | R(E)NWDGVT(T) |
| 1227.7244                | 1227.7101| 434–443  |        | R(E)NPDGPYKPA |
| 1251.6493                | 1251.6374| 425–433  |        | R(YIDWKEKER) |
| 1397.7617                | 1397.7503| 162–173  |        | R(K)TDNFMLVGLK(G) |
| 1413.7627                | 1413.7452| 162–173  |        | R(K)TDNFMLVGLK(G) |
| 1470.7443                | 1470.7263| 292–304  |        | R(K)LDNLMDSEGHIK(F) |
| 1826.9299                | 1826.9222| 289–304  |        | R(K)LDNLMDSEGHIK(F) |
| 2229.0171                | 2229.1338| 1–20     |        | –(−)DKANLVPNMDPGNSDPYVK(L) |
| 2623.2489                | 2623.2692| 239–260  |        | R(GLYVMETGNYGLMLYHIQVGF(F) |
| 2648.2569                | 2648.2641| 239–260  |        | R(GLYVMETGNYGLMLYHIQVGF(F) |
| 2656.3370                | 2656.3744| 216–238  |        | R(V)LALPGKPLQFLHSCFQTMDR(L) |

| PKCβII (pool 1 lower band) | 570.2999 | 570.3040 | 632–635 | R(F)FFTR(H) |
|----------------------------|----------|----------|--------|------------|
| 806.4152                   | 806.3949 | 596–601  |        | K(E)HAFR(Y) |
| 865.3986                   | 865.3804 | 625–631  |        | R(N)AEFDR(F) |
| 974.4582                   | 974.4366 | 584–592  |        | R(L)CGFGER(D) |
| 984.5165                   | 984.5301 | 574–582  |        | R(GLMTRHKGR) |
| 1099.6324                  | 1099.6152| 612–620  |        | R(EIQPPFYKPA) |
| 1126.6154                  | 1162.5744| 490–499  |        | R(E)NWDGVT(T) |
| 1162.6154                  | 1162.6009| 593–601  |        | R(K)IKEHIFR(Y) |
| 1227.7244                  | 1227.7101| 611–620  |        | R(E)NPDGPYKPA |
| 1251.6493                  | 1251.6374| 602–610  |        | R(YIDWKEKER) |
| 1397.7617                  | 1397.7503| 339–350  |        | R(K)TDNFMLVGLK(G) |
| 1413.7627                  | 1413.7452| 339–350  |        | R(K)TDNFMLVGLK(G) |
| 1470.7443                  | 1470.7263| 469–481  |        | R(K)LDNLMDSEGHIK(F) |
| 1826.9299                  | 1826.9222| 466–481  |        | R(K)LDNLMDSEGHIK(F) |
| 2229.0171                  | 2229.1338| 416–437  |        | R(GLYVMETGNYGLMLYHIQVFR(F) |
| 2623.2489                  | 2623.2652| 416–437  |        | R(GLYVMETGNYGLMLYHIQVFR(F) |
| 2648.2569                  | 2648.2641| 393–415  |        | R(V)LALPGKPLQFLHSCFQTMDR(L) |

* A methionine-sulfone derivative is indicated.
recognizes the catalytic domains of PKCa and PKCb.

More prolonged elution of the protamine-agarose column with 1 M NaCl eluted 54% of the activity. This fraction contained a single protein of apparent molecular mass 42 kDa that was identified as the catalytic domain of PKC8 by tryptic mass fingerprinting (Table I). This indicated that PKC8 was only partially retained by heparin-Sepharose.

To establish that PKC isoforms accounted for all the activity eluted from the initial MonoQ column (Fig. 1A), the following additional experiments were performed. First, the activity from this column was shown to be inhibited by 100 nM Ro 318220, a potent inhibitor of conventional PKC isoforms, as well as several other protein kinases (49). Second, the activity was strongly suppressed by a pseudosubstrate peptide, which is believed to be a specific inhibitor of PKC (Fig. 1B).

Identification of Residues in the C Terminus of Integrin CD18 Phosphorylated by PKC Isoforms—The C-terminal peptide was phosphorylated with the purified catalytic domain of PKC8 and digested with trypsin. The resulting phosphopeptides were then chromatographed on a Vydac C18 column equilibrated in 0.1% (v/v) trifluoroacetic acid and the column developed with an acetonitrile gradient (straight line). The flow rate was 1 ml/min, and 0.5-ml fractions were collected and analyzed for 32P radioactivity (see "Experimental Procedures"). The phosphopeptides T1B and T2 were sequenced by Edman degradation using an Applied Biosystems 42A protein sequencer (Fig. 2A). The C-terminal peptide was phosphorylated for 10 min with the catalytic domain of PKCα, -βI, -βII, -γ, and a PKC- substrate peptide for PKCδ-C, ε, and -η in 1 min. Lipids and calcium ions were included as recommended by the supplier. The phosphorylated peptides were subjected to SDS-PAGE, transferred to PVDF membranes, and subjected to autoradiography. The phosphorylated substrates were excised from the PVDF membrane, partially hydrolyzed in 6 M HCl, and phosphoamino acids resolved by thin layer chromatography (see "Experimental Procedures"). PS, phosphoserine; PT, phosphothreonine.

Phosphorylation of the CD18 C Terminus in Vivo—It has been shown previously (25, 30–32) that PDBu in conjunction with...
the phosphatase inhibitor okadaic acid induces phosphorylation of the integrin CD18 chain in T cells, whereas the CD11 chain is phosphorylated constitutively. We confirmed these findings in the present study and also found that the phosphorylation of CD18 was prevented by 1 μM Ro 318220 and 0.5 μM of a related compound, Go-6983 (data not shown).

To identify the residues at the C terminus of CD18 whose phosphorylation is induced by PDBu, we raised phospho-specific antibodies capable of recognizing CD18 only when phosphorylated at Ser-745. We also raised phospho-specific antibodies that should recognize CD18 when phosphorylated at Ser-756, because this site has also been reported to become phosphorylated in response to PDBu, as judged by phosphopeptide mapping (44) and mutagenesis (29). We have been unable, thus far, to generate phospho-specific antibodies that recognize CD18 phosphorylated at Thr-758 and Thr-760 and which are sufficiently sensitive to detect the phosphorylation of these residues in cells. However, based on phosphopeptide mapping, it has been reported previously that two of the three threonine residues Thr-758, Thr-759, and Thr-760 become phosphorylated after stimulation with PDBu and okadaic acid (25, 44).

The antibody raised against the peptide 740–751 phosphorylated at Ser-745 was tested for recognition of the phosphopeptide immunogen and the unphosphorylated peptide in the presence of unphosphorylated peptide to block any antibodies present that recognize both the phosphorylated and unphosphorylated forms of the peptide. These experiments demonstrated that, under these conditions, the antibody only recognized the phosphopeptide and not the unphosphorylated peptide (Fig. 7A). Additionally, it only recognized the CD18 protein after phosphorylation by PKC in vitro (Fig. 7B). Recognition by the antibody was prevented by preincubation with the phosphopeptide immunogen but not by the unphosphorylated form of the peptide or the Ser-756 phosphopeptide (Fig. 7B). The specificity of the antibody raised against the peptide comprising residues 751–761 phosphorylated at Ser-756 was established in an analogous manner. However, as protein kinases capable of phosphorylating CD18 at Ser-756 have not yet been identified, specificity was established using the phosphopeptide immunogen (Fig. 8A). The phospho-specific antibody toward Ser-756 did not recognize the CD18 chain phosphorylated at Ser-756 (Fig. 8B).

These antibodies were then used to demonstrate that Ser-745 (Fig. 7C) and Ser-756 (Fig. 8B) both become phosphorylated when T cells are exposed to PDBu. No other unspecific bands were seen on the gels (not shown). Phosphorylation of Ser-745 could be detected in the presence of PDBu alone, but the phosphorylation was increased when cells were stimulated with PDBu and okadaic acid in the presence of OKT3, a stimulating antibody raised against the CD3 component of the T cell receptor. Phosphorylation of either site was inhibited by 1 μM Ro 318220 (Figs. 7C and 8D). The phosphorylation of Ser-756 could be detected readily after stimulation with high (200 nM) PDBu and OKT3 in the absence of okadaic acid, but OKT3...
alone in the presence or absence of okadaic acid did not induce Ser-756 phosphorylation (Fig. 8B). Ser-756 phosphorylation can also be induced to similar levels by low (10 nM) PDBu in the presence of the calcium ionophore A12387 (Fig. 8C). Because no PKC isoform tested was able to phosphorylate CD18 at Ser-756 in vitro, this suggested that the phosphorylation of Ser-756 was likely to be catalyzed by another protein kinase activated directly or indirectly by a PKC isoform. To try and identify the signaling pathway in which this putative kinase was located, we examined the effect of inhibitors of other protein kinases on the phosphorylation of Ser-756. Interestingly, the phosphorylation of Ser-756 was found to be suppressed by W-7, a calmodulin antagonist (Fig. 8D), but not by PD 98059, an inhibitor of the classical mitogen-activated protein kinase pathway, or by calpeptin, an inhibitor of the calcium-dependent proteinase calpain. Trifluoperazine, another calmodulin antagonist, was also found to suppress phorbol ester-induced Ser-756 phosphorylation (data not shown). Both the phosphorylation of Ser-756 induced by high concentrations of phorbol ester alone and the phosphorylation induced by low levels of phorbol ester in conjunction with the calcium ionophore A23187 were greatly suppressed by W-7 as well as the PKC inhibitor Ro 318220 (Fig. 8E). These observations indicate that calmodulin may be involved in regulating Ser-756 phosphorylation in vivo.

A Thr-758-phosphorylated CD18 Peptide Binds 14-3-3 Proteins from Leukocyte Lysates—To identify potential functions for the phosphorylation of the CD18 C terminus, we investigated whether proteins in T cell lysates were capable of binding to the C terminus when phosphorylated at particular sites. These experiments showed that two proteins of apparent molecular mass 30 and 28 kDa bound specifically to the C-terminal peptide phosphorylated at Thr-758. In contrast, these proteins did not bind to the unphosphorylated C-terminal peptide or to the peptide phosphorylated at Ser-756 (Fig. 9A). The 30- and 28-kDa bands were excised and identified by tryptic mass fingerprinting as 14-3-30β and 14-3-3δ, respectively (Table III). These results were confirmed by immunoblotting with an antibody that recognizes all 14-3-3 isoforms (Fig. 9B). The presence of 14-3-3 proteins binding to the C-terminal peptide could also be detected in the cytoskeletal fraction of leukocytes, where the major part of the phosphorylated integrins resides (Fig. 9C).

**DISCUSSION**

The phosphorylation of integrin cytoplasmic domains has been proposed as a way of regulating integrin activity and/or interaction with cytoplasmic proteins and cell signaling. For example, tyrosine phosphorylation of the integrin β3 cytoplasmic tail leads to association with Shc (52), an adaptor protein involved in activation of the classical mitogen-activated protein kinase cascade. Tyrosine phosphorylation of the integrin β2 cytoplasmic tail might also regulate its binding to cytoskeletal elements (53). On the other hand, threonine phosphorylation of β2 integrins is reported to prevent Shc from binding to the tyrosine-phosphorylated integrin (54).

In contrast to the β1 and β3 integrins, the CD18 integrin polypeptide (also called the β2 integrin) lacks two of the three tyrosines in the conserved NPXY motifs found in the integrin β1 and β3 chains. However, the CD18 integrin chains have been shown to become phosphorylated on serine and threonine residues in cells after stimulation with phorbol ester (30–32) or T cell receptor engagement (25). The role of these phosphorylation events is not yet understood.

To identify the protein kinases that phosphorylate CD18, we purified and identified the major activities in T cell extracts that phosphorylate a synthetic peptide corresponding to most of the cytoplasmic domain of the integrin CD18 chain. The protein kinases detected were found to be active proteolytic fragments of PKCβ and PKCδ, which are known to be cleaved intracellularly from the native enzymes by proteases, such as calpain (55, 56). The native forms of these and other PKC isoforms were presumably not detected because they are only active in the presence of one or more cofactors, i.e. calcium ions, phospholipids, and diacylglycerol or phorbol esters (57, 58), and it is possible that other PKC isoforms are cleaved proteolyti-
cally to a lesser extent than PKCβ and PKCd and thus not
detected under our assay conditions. This led us to discover
that many of the known PKC isoforms are capable of phos-
phorylating CD18 in vitro, and indeed two of them, PKCβ
and PKCd, appeared to be very active toward CD18 (Fig. 4).

![Image](https://i.imgur.com/3Q7yJ.png)

**Fig. 4.** Ser-756 phosphorylation is induced by low concentrations of PDBu in the presence of calcium ionophore and inhibited by
the calmodulin antagonist W-7. A, the phosphorylated and unphosphorylated forms of the peptide comprising residues 751–761 of CD18
(phosphorylated at Ser-756) were conjugated to keyhole limpet hemocyanin and spotted onto nitrocellulose membranes in the amounts indicated.
The membranes were then immunoblotted with an antibody raised against the phosphorylated peptide that had been incubated without additions
(none), with the unphosphorylated form of the peptide 751–761, or with the phosphorylated form of the same peptide (each at 100 μg/ml).
The figure shows that the antibody only became phospho-specific after incubation with the unphosphorylated peptide. All subsequent experiments with this
antibody were therefore performed in the presence of excess unphosphopeptide (25 μg/ml). B, the experiment was carried out as in Fig. 7B except
that the anti-pSer-756 antibody replaced the anti-phospho-Ser-745 antibody. T cells were preincubated with or without 1.5 μM okadaic acid before
activation with 1:200 dilution of OKT3 or 200 nM PDBu. C, same as B, except that T cells were stimulated with or without 10 or 200 nM PDBu in
the presence or absence of 500 nM A23187. D, same as B, except that the T cells were stimulated in the presence or absence of PDBu with or without
Ro 318220 (5 μM, Ro), PD 98059 (50 μM, PD), W-7 (50 μM), or calpeptin (100 μg/ml). E, same as B, except that the T cells were preincubated with
or without the indicated concentrations of W-7 or Ro 318220 and stimulated with 10 nM PDBu and 500 nM A23187 or with 200 nM PDBu.

However, the PDBu-induced phosphorylation of CD18 at Ser-
756 is prevented by inhibitors of PKC (Fig. 8), suggesting that
Ser-756 is phosphorylated by a protein kinase that is activated
by a PKC isoform. Alternatively, a PKC isoform may inhibit a
protein phosphatase that dephosphorylates Ser-756 in cells.

Interestingly, we found that the phosphorylation of Ser-756
induced by low concentrations of PDBu only occurred in the
presence of calcium ionophore (Fig. 8) and was reported previ-
ously (59) for total CD18 phosphorylation. Moreover, the
phosphorylation of Ser-756 induced by low concentrations
of PDBu and A23187, or high concentrations of PDBu in
the absence of A23187, was suppressed by the calmodulin
antagonist W-7. This raises the possibility that a PKC-acti-
vated Ser-756 kinase might also be dependent on calcium ions
and calmodulin. The phosphorylation of Ser-756 does not seem
to be important in adhesion, because its mutation to alanine

![Image](https://i.imgur.com/3Q7yJ.png)
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has no effect on phorbol ester-induced binding of CD18 to ICAM-1, a major ligand of the integrin (29). However, we have shown previously that calmodulin antagonists are strong suppressors of PDBu-induced T cell aggregation (27). Because a calmodulin antagonist also reduces Ser-756 phosphorylation, it is possible that Ser-756 phosphorylation plays a role in these events.

Based on phosphopeptide mapping, it has been reported previously (44) that two of the three threonine residues Thr-758, Thr-759, and Thr-760 become phosphorylated when T cells are stimulated with PDBu, although the protein phosphatase inhibitor okadaic acid also had to be added to the cells. The cytoplasmic domain also becomes phosphorylated on a threonine residue(s) when the T cell receptor is activated (25). In the present study, we have shown that Ser-745 becomes phosphorylated, albeit somewhat weakly, when T cells are stimulated with PDBu alone, and that phosphorylation could be increased in the presence of OKT3, an antibody that binds to the CD3 component of the T cell receptor, plus okadaic acid. Because PKC isoforms are the major protein kinase activities in leukocyte lysates responsible for the phosphorylation Ser-745, and the phosphorylation of Ser-745 is inhibited by Ro 318220 in vivo, it would appear that a PKC isoform mediates the phosphorylation of CD18 at Ser-745 in cells. However, since Ro 318220 and Go-6983, which both inhibit CD18 phosphorylation, are not exclusively specific PKC inhibitors, it cannot be completely excluded that another unknown phorbol ester-activated kinase is mediating the phosphorylation of CD18 at Ser-745. It is also possible that Ser-745 becomes phosphorylated more strongly in response to other signals or combinations of signals that have yet to be identified.

The mutation of Ser-745 to Ala, like the mutation of Ser-756 to Ala, has no effect on phorbol ester-induced binding of CD11/CD18 to ICAM-1 (29), indicating that these phosphorylation events may play a different role. Ser-745 is not conserved in the β1 or β2 integrins, and it may thus play a role in CD11/CD18-specific signaling events. The stoichiometry of CD18 integrin phosphorylation in vivo after phorbol ester treatment in the presence of okadaic acid has been determined as 0.92 mol per mol of protein (44). Because Ser-756, Ser-745, and Thr-758/Thr-759/Thr-760 phosphorylation take place under these conditions, each of these sites is clearly phosphorylated to only a low stoichiometry. However, this does not exclude them playing important roles physiologically if, by analogy with receptor tyrosine phosphorylation, their function is to recruit other signaling molecules to the plasma membrane.

PKC has been implicated previously in the regulation of integrin function. PKCα associates with βi integrins and regulates their internalization (60), whereas PKCε in the regulation of integrin-dependent cell spreading (61, 62). Interestingly, the PKC isoforms α, βII, βII, and δ, but not PKCζ, activate integrin-mediated adhesion to ICAM-1 in a model system (21). Moreover, the receptor for activated PKC (Rack1), a PKCβ-interacting protein that is believed to regulate its localization and substrate specificity (63, 64), interacts with the membrane-proximal part of the integrin CD18 cytoplasmic tail in phorbol ester-activated leukocytes (39). An attractive hypothesis is that the binding of Rack1 to the integrin cytoplasmic tail could recruit active PKCβ to the integrin and allow it to phosphorylate Ser-745 and Thr-758.

To investigate the functions of the C-terminal phosphorylation on CD18, we initially studied whether proteins present in the cytoplasm of T cells bound to the C-terminal peptide when it was phosphorylated at particular sites. This led us to find that the C terminus of CD18 binds specifically to 14-3-3 proteins when it is phosphorylated at Thr-758. The 14-3-3 proteins were recruited both from the soluble and, importantly, from the cytoskeletal fractions of leukocytes, where most of the phosphorylated integrins have been shown to reside (33). In contrast, the 14-3-3s did not bind to the unphosphorylated C-terminal peptide or the peptide phosphorylated at Ser-756. One additional protein of 16 kDa bound to the C-terminal peptide (Fig. 9A), but this was independent of phosphorylation. In coprecipitation studies, we failed to detect 14-3-3 binding to CD18 in activated cells, but this could be due to the low stoichiometry of threonine phosphorylation under these circumstances. Alternatively, the phosphorylation of Ser-756, Thr-759, and/or Thr-760 may interfere with binding of 14-3-3s to the Thr-758-phosphorylated integrins. Further work is clearly needed to evaluate whether 14-3-3 binding to CD18 occurs in vivo.

14-3-3s are adaptor proteins that bind to phosphoserine- and phosphothreonine-containing motifs (65) and are known to be involved in regulating a number of signaling molecules (66). They have been shown to dimerize and could thus recruit other signaling proteins to form complexes. The optimal consensus sequence for 14-3-3 binding is RXRxPSpTXP (65), which does not conform to the sequence surrounding Thr-758. However, other phosphorylated sequences have been shown to interact with 14-3-3 proteins (67, 68). Indeed, recently, 14-3-3β was identified in a yeast two-hybrid screen with β1 integrins, but

FIG. 9. 14-3-3 proteins bind to a Thr-758-phosphorylated integrin C-terminal peptide but not to a Ser-756-phosphorylated peptide. Human leukocyte lysates (A and B) or cytoplasmic and cytoskeletal fractions (C) were subjected to affinity chromatography on agarose to which the peptides indicated below had been attached covalently (see “Experimental Procedures”). The protein bound to each column was eluted with SDS, subjected to SDS-PAGE, and either stained with Coomassie Blue (A) or transferred to nitrocellulose and immunoblotted with an antibody that recognizes all 14-3-3 isoforms (B and C). A, lane 1, leukocyte lysate; lane 2, eluate from a control protein G-Sepharose column; lane 3, eluate from agaro to which the peptide 751–761 had been bound; lane 4, eluate from agarose to which the peptide 751–761 phosphorylated at Ser-756 had been bound; lane 5, eluate from agarose to which the unphosphorylated peptide 753–763 had been bound; lane 6, eluate from agaro to which the peptide 753–763 phosphorylated at Thr-758 had been bound. B, fractions 3–6 from A were electrophoresed and immunoblotted with an anti-14-3-3 antibody. C, same as B, except that cytoplasmic (lanes 1 and 2) and cytoskeletal (lanes 3 and 4) fractions were subjected to affinity chromatography on agarose to which the unphosphorylated form of the peptide 753–763 (lanes 1 and 3) or the same peptide phosphorylated at Thr-758 (lanes 2 and 4) had been bound.
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Tryptic peptides from the bands marked 14-3-3 in Fig. 9A were analyzed by mass fingerprinting. The table summarizes the peptides from the bands that matched the 14-3-3 sequences.

| Mass measured | Theoretical | Residue no | Peptide sequence |
|---------------|-------------|------------|-----------------|
| 14-3-3αβ | 816.441 | 12-18 | (KLAEQAER)Y |
| | 907.5347 | 42-49 | (RNLLSVAYK)N |
| | 948.4249 | 121-127 | (KMKEDYYTR)Y |
| | 1124.5695 | 159-167 | (KEMQPTHPIR)L |
| | 1189.6738 | 213-222 | (KIDSTLIMQLLR)D |
| | 1205.6717 | 213-222 | (KIDSTLIMQLLR)D |
| | 1252.6594 | 158-167 | (KEMOPTHPIR)L |
| | 1279.6678 | 128-139 | (RYLAEVAAGDDKK)G |
| | 1304.6990 | 104-115 | (KFILPNASQAESKV) |
| | 1548.7507 | 28-41 | (KSVTEGGAESNEE)N |
| | 2041.0707 | 140-157 | (KGGIVDQSQQYQAEISKK) |
| | 2132.0076 | 194-212 | (KTAFDEAELDTLSEESYKD) |
| | 2169.0848 | 159-173 | (KTAFDEAELDTLSEESYKD) |
| | 2137.1906 | 168-187 | (RILGLALNSVFFYELNSPEK)A |
| | 3302.6657 | 194-222 | (KTAFDEAELDTLSEESYKDSTLIMQLLR)D |
| | 3318.7238 | 194-222 | (KTAFDEAELDTLSEESYKDSTLIMQLLR)D |

* A methionine-sulfoxide derivative is indicated.

The interaction was not thought to be phosphorylation-dependent (69).

The TTT motif (Thr-758, Thr-759, and Thr-760) appears to play a pivotal role in integrin regulation. Mutation of these threonines singly or in combination decreases binding to ICAM-1 in response to PDBu (29). In addition, an activating mutation (L732R) that induces phorbol ester responsiveness of CD11a/CD18 integrins in K562 cells that normally do not respond to phorbol esters is abolished by the mutation of Thr-758 to Ala (70). The mutation of the TTT motif also causes defects in post-receptor signaling events, whereby the integrin receptor interacts with the actin cytoskeleton and induces cell spreading (16), and phosphorylated integrins have been shown to associate with the actin cytoskeleton preferentially (33). It is therefore tempting to speculate that threonine phosphorylation of CD18 recruits 14-3-3 proteins to the plasma membrane-cytoskeleton connection and that the 14-3-3s in turn recruit further proteins to regulate cell spreading and/or integrin signaling.

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REFERENCES

1. Springer, T. A. (1990) *Nature* **346**, 425–434
2. Gahnberg, C. G., Tolvanen, M., and Rotevour, P. (1997) *Eur. J. Biochem.* **245**, 215–222
3. Dastin, M. L., and Springer, T. A. (1989) *Nature* **341**, 619–624
4. Dransfield, I., Cahanas, C., Craig, A., and Hogg, N. (1992) *J. Cell Biol.* **116**, 219–226
5. Cahanas, C., and Hogg, N. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 5838–5842
6. Li, R., Nortamo, P., Kantor, C., Kovanen, P., Timonen, T., and Gahnberg, C. G. (1993) *J. Biol. Chem.* **268**, 21474–21477
7. Rotevour, P., Pessa-Morikawa, T., Kotevour, P., Nortamo, P., and Gahnberg, C. G. (1999) *J. Immunol.* **162**, 6613–6620
8. Keizer, G. D., Visser, W., Vliem, M., and Figdor, C. G. (1988) *J. Immunol.* **140**, 1393–1400
9. Landis, R. C., Bennett, R. I., and Hogg, N. (1993) *J. Cell Biol.* **120**, 1519–1527
10. Robinson, M. K., Andrew, D., Rosen, H., Brown, D., Ortlepp, S., Stephens, P., and Butcher, E. C. (1992) *J. Immunol.* **148**, 1080–1085
11. Van Kosyk, Y., van de Wiel, P., van de Kemanede, P., Woder, P., Kuijpers, T. W., and Figdor, C. G. (1999) *Nature* **342**, 811–813
12. Axelsson, B., Youssefi-Etemad, R., Hammarsrom, S., and Perlmann, P. (1988) *J. Immunol.* **141**, 2912–2917
13. Koopman, G. van Kosyk, Y., de Graaff, M., Meyer, C. J. L., Figdor, C. G., and Pais, S. T. (1990) *J. Immunol.* **145**, 3589–3593
14. Patarno, M., Beatty, P. G., Fabre, J. W., and Gahmberg, C. G. (1985) *Scand. J. Immunol.* **22**, 171–182
15. Rothlein, R., and Springer, T. A. (1986) *J. Exp. Med.* **163**, 1132–1149
16. Pardi, R., Inverardi, L., Rugarli, C., and Binder, J. R. (1992) *J. Cell Biol.* **116**, 1211–1220
17. Stewart, M. P., Cahanas, C., and Hogg, N. (1995) *J. Immunol.* **156**, 1810–1817
18. Van Kosyk, Y., van de Vliet, S. J., and Figdor, C. G. (1999) *J. Biol. Chem.* **274**, 26689–26677
19. Lu, C., Foray, M., Takagi, J., and Springer, T. A. (2001) *J. Immunol.* **166**, 5629–5637
20. Katagiri, K., Hottori, M., Minato, N., Irie, S.-K., Takatsu, K., and Kinashi, T. (2000) *Mol. Cell. Biol.* **20**, 1569–1569
21. Nagel, W., Zeitmann, L., Schlicher, P., Geiger, C., Kolanus, J., and Kolanus, W. (1998) *J. Biol. Chem.* **273**, 14853–14861
22. Rouche, M. A., Shao, H., and kaye, J. (1998) *J. Immunol.* **161**, 5800–5803
23. Hedman, H., and Landgren, E. (1999) *J. Immunol.* **162**, 2295–2299
24. Valmu, L., and Gahnberg, C. G. (1999) *J. Immunol.* **165**, 1175–1183
25. Stewart, M. P., McDowall, A., and Hogg, N. (1998) *J. Cell Biol.* **140**, 699–707
26. Fagerholm, S., Prescott, A., Cohen, P., and Gahmberg, C. G. (2001) *FEBS Lett.* **491**, 131–136
27. Hibbs, M. L., Xu, H., Stacke, S. A., and Springer, T. A. (1991) *Science* **251**, 1611–1613
28. Hibbs, M. L., Jakes, S., Stacke, S. A., Wallace, R. W., and Springer, T. A. (1991) *J. Exp. Med.* **174**, 1227–1238
29. Haru, T., and Fu, M. S. (1985) in *Leukocyte Typing II* (Reinherz, E. L., Haynes, B. F., Nadler, L. M., and Bernstein, I. D., eds) Vol. 3, pp. 77–84, Springer-Verlag Inc., New York
30. Chatila, T. A., Gehr, E. R., and Arnaout, M. A. (1989) *J. Cell Biol.* **109**, 3435–3444
31. Boyun, J. P., Slade, S. G., Reisman, J., Abramson, S. B., Phillips, M. R., Weissman, G., and Winchester, R. (1990) *J. Immunol.* **144**, 191–197
32. Valmu, L., Fagerholm, S., Suula, H., and Gahmberg, C. G. (1999) *Eur. J. Immunol.* **29**, 2107–2118
33. Schoenwaelder, S., and Burridge, K. (1999) *Curr. Opin. Cell Biol.* **11**, 274–286
34. Rupfer, A., Burn, P., and Singer, S. J. (1990) *J. Mol. Cell. Immunol.* **4**, 317–325
36. Sharma, C. P, Ezzell, R. M., and Arnaout, M. A. (1995) J. Immunol. 154, 3461–3470
37. Pavalko, F. M., and LaRoche, S. M. (1993) J. Immunol. 151, 3795–3807
38. Sampath, R., Gallagher, P. J., and Pavalko, F. M. (1998) J. Biol. Chem. 273, 33588–33594
39. Lilientahl, J., and Chang, D. D. (1998) J. Biol. Chem. 273, 2379–2383
40. Kolanus, W., Nagel, W., Shiller, B., Zeitlmann, L., Godar, S., Stockinger, H., and Seed, B. (1996) Cell 86, 233–242
41. Bianchi, E., Denti, S., Granata, A., Bossi, G., Geginat, J., Villa, A., Rogge, L., and Pardi, R. (2000) Nature 404, 617–621
42. Harlow, E., and Lane, D. (eds) (1988) Antibodies: A Laboratory Manual, pp. 82–83, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
43. Nortamo, P., Patarroyo, M., Kantor, C., Suopanki, J., and Gahmberg, C. G. (1998) Scand. J. Immunol. 28, 537–546
44. Valmu, L., Hilden, T., van Willigen, G., and Gahmberg, C. G. (1999) Biochem. J. 339, 119–125
45. Deleted in proof
46. Woods, L. Y., Rena, G., Morrice, N., Barthel, A., Becker, W., Guo, S., Unterman, T. G., and Cohen, P. (2001) Biochem. J. 355, 597–607
47. Lawler, S., Fleming, Y., Goedert, M., and Cohen, P. (1988) Curr. Biol. 8, 1387–1390
48. Kantor, C., Suomalainen-Nevanlinna, H., Patarroyo, M., Osterlund, K., Bergman, T., Jornvall, H., Schroder, J., and Gahmberg, C. G. (1988) Eur. J. Biochem. 170, 653–659
49. Davies, S. P., Reddy, H., Caiiano, M., and Cohen, P. (2000) Biochem. J. 351, 95–105
50. Steeves, D., Campbell, D. G., Nakielny, S., Hikada, H., Leevers, S. J., Marshall, C., and Cohen, P. (1993) EMBO J. 11, 3985–3994
51. Olivier, A. R., and Parker, P. J. (1991) Eur. J. Biochem. 200, 805–810
52. Cowan, K. J., Law, D. A., and Phillips, D. R. (2000) J. Biol. Chem. 275, 36423–36429
53. Jenkins, A. L., Nannizzi-Alaimo, L., Silver, D., Sellers, J. R., Ginsberg, M. H., Law, D. A., and Phillips, D. R. (1998) J. Biol. Chem. 273, 13878–13885
54. Kirk, R. I., Sanderson, M. R., and Lerea, K. M. (2000) J. Biol. Chem. 30901–30906
55. Suzuki, K., Saido, T. C., and Hirai, S. (1992) Ann. N. Y. Acad. Sci. 674, 218–227
56. Pontremoli, S., and Melloni, E. (1989) Rev. Biol. Cell. 20, 161–177
57. Hug, H., and Sarre, T. F. (1993) Biochem. J. 291, 329–343
58. Mellor, H., and Parker, P. J. (1998) Biochem. J. 332, 281–292
59. Valmu, L., Autero, M., Siljander, P., Patarroyo, M., and Gahmberg, C. G. (1991) Eur. J. Immunol. 21, 2857–2862
60. Ng, T., Shima, D., Squire, A., Bastiaens, P. I. H., Gecheimeisser, S., Humphries, M., and Parker, P. J. (1999) EMBO J. 18, 3909–3923
61. Haller, H., Lindau, C., Maesch, C., Othoff, H., Kurscheid, D., and Luz, F. C. (1998) Circ. Res. 82, 157–165
62. Berrier, A. L., Magrangelo, A. M., Downward, J., Ginsberg, M., and LaFlamme, S. E. (2000) J. Biol. Chem. 275, 36423–36429
63. Ron, D., Chen, C.-H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 839–843
64. Ron, D., Jiang, Z., Ya, L., Vgts, A., Diamond, I., and Gordon, A. (1999) J. Biol. Chem. 274, 27039–27046
65. Muslin, A. J., Tanner, J. W., Allen, P. M., and Shaw, A. S. (1996) Cell 84, 889–897
66. Baldin, V. (2000) in Progress in Cell Cycle Research (Meijer, L., Jezequel, A., and Ducommun, B, eds) Vol. 4, pp. 49–60, Kluwer Academic, Plenum Publishers, New York
67. Yaffe, M. B., Rittinger, K., Volinia, S., Caron, P. R., Aitken, A., Jeffers, H., Gamblin, S. J., Enrodn, S. J., and Castley, L. C. (1997) Cell 91, 961–972
68. Liu, Y.-C., Liu, Y., Elly, C., Yoshida, H., Lipkowitz, S., and Altman, A. (1997) J. Biol. Chem. 272, 9979–9988
69. Han, D. C., Rodriguez, L. G., and Guan, J.-L. (2001) Oncogene 20, 346–357
70. Bleijs, D., van Duinphoven, G. C. F., van Vliet, S. J., Thijsen, A. P. H., Figdor, C. G., and van Kooyk, Y. (2001) J. Biol. Chem. 276, 10338–10346
Phosphorylation of the Cytoplasmic Domain of the Integrin CD18 Chain by Protein Kinase C Isoforms in Leukocytes
Susanna Fagerholm, Nick Morrice, Carl G. Gahmberg and Philip Cohen

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