FEBS 21059 FEBS Letters 438 (1998) 225–230

Protein kinase C-δ activation and tyrosine phosphorylation in platelets

Mitra Moussazadeh, Beatrice Haimovich*

Department of Surgery, MEB Room 434, Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA

Received 31 July 1998; received in revised form 18 September 1998

Abstract **Several protein kinase C (PKC) isoforms are expressed in human platelets. We report that PKC-δ is tyrosine phosphorylated within 30 s of platelet activation by thrombin. This correlated with a 2–3-fold increase in the kinase activity of PKC-δ relative to unstimulated platelets. The tyrosine phosphorylated PKC-δ isoform was associated with the platelet particulate (100 000 × g insoluble) fraction. $\alpha_{IIb}\beta_3$ integrin mediated platelet adhesion to fibrinogen did not significantly affect PKC-δ activity. Tyrosine phosphorylation of PKC-δ was similarly not detected in fibrinogen adherent platelet lysates. Treatment of the platelets with mAb 7E3 prior to the addition of thrombin blocked aggregation having no effect on the thrombin induced PKC-δ activation. We conclude that PKC-δ is activated in platelets by an $\alpha_{IIb}\beta_3$ independent pathway.**

Key words: Protein kinase C-δ; Platelet; $\alpha_{IIb}\beta_3$ integrin

1. Introduction

Protein kinase C (PKC) is a family of differentially expressed serine/threonine kinases implicated in a diverse array of cellular functions. The extended PKC family currently includes 12 structurally related isoenzymes [1–3]. Based on function and allosteric requirements, PKC isoforms are subdivided into conventional/classical (cPKC; α, βI/βII, and γ), novel/new (nPKC; δ, ε, η, and θ) and atypical (aPKC; ζ, λ, and μ) [4,5]. The cPKC are primarily activated by Ca^{2+}, diacylglycerol, phospholipids, or phorbol esters. The nPKC lack the Ca^{2+} binding domain and are, therefore, Ca^{2+} insensitive. The aPKC are not affected by diacylglycerol, phorbol esters, or Ca^{2+}.

In platelets, PKC regulates multiple agonist stimulated responses including platelet shape change, secretion and aggregation [6,7]. The platelet $\alpha_{IIb}\beta_3$ integrin mediates platelet spreading on fibrinogen and the induction of tyrosine phosphorylation of the focal adhesion kinase, pp125FAK. The involvement of PKC in this process has been demonstrated in two ways. First, two pharmacological PKC inhibitors abrogated both pp125FAK phosphorylation and platelet spreading on fibrinogen [8]. PKC inhibitors similarly prevented pp125FAK phosphorylation in Chinese hamster ovary (CHO) cells [9]. Second, platelet spreading and pp125FAK phosphorylation are inhibited by several types of pharmacological agents including apyrase [10]. Treatment of the platelets with phorbol 12-myristate 13-acetate (PMA), to directly activate PKC, effectively restored platelet spreading and pp125FAK phosphorylation [10]. In contrast with these results, Ca^{2+} chelators failed to affect either pp125FAK phosphorylation or platelet spreading on fibrinogen, raising the possibility the $\alpha_{IIb}\beta_3$-associated PKC is a Ca^{2+} insensitive PKC isoform [8].

PKC-δ is the primary Ca^{2+} insensitive PKC isoform identified in human platelets [11]. Baldassare et al. [11] have shown that in thrombin stimulated platelets, PKC-α, -β, and -ζ, but not PKC-δ, translocated from the soluble to the particulate fraction. From these data it was not possible to conclude whether or not PKC-δ was activated in platelets. The goal of this study was, therefore, to examine whether thrombin and/or the $\alpha_{IIb}\beta_3$ integrin mediate PKC-δ activation in platelets. We report that PKC-δ is activated and tyrosine phosphorylated in thrombin stimulated platelets, but not in platelets adherent to fibrinogen. Furthermore, $\alpha_{IIb}\beta_3$ receptor blockade did not affect the induction of PKC-δ phosphorylation. We therefore conclude that PKC-δ activation in thrombin stimulated platelets is regulated by an $\alpha_{IIb}\beta_3$ integrin independent pathway.

2. Materials and methods

2.1. Platelet preparation

Human platelets were purified from freshly drawn blood by gel filtration as previously described [10]. For adhesion studies, polystyrene plates were coated with fibrinogen (100 μg/ml; from Sigma). Where indicated, the platelets were treated for 10 min with phorbol ester (PMA) (10 nM; from Sigma) or with mAb 7E3 (10 μg/ml), an inhibitor of fibrinogen binding to $\alpha_{IIb}\beta_3$ and platelet aggregation, kindly provided by Dr. Barry Coller (Mount Sinai School of Medicine, New York).

2.2. Immunoprecipitation and immunoblotting

Suspended and adherent platelets were lysed for 20 min in Triton lysis buffer (0.02% Triton X-100, 1 mM EGTA, 50 mM Tris pH 8.0, 1 mM sodium vanadate, and 0.5 mM PMSF). The lysates were clarified by centrifugation at 15 000 × g for 10 min at 4°C, and were subsequently analyzed for protein content using the Pierce BCA reagents. Equal amounts of protein were then subjected to immunoprecipitation. The lysates were first pre-cleared for 1 h with protein A/G plus agarose (from Santa Cruz) and then incubated with the antiserum to PKC-δ (nPKC-δ C-20 from Santa Cruz, cat. # sc-937) for at least 2 h. The immune complexes were precipitated with protein A/G plus agarose. The immunoprecipitates prepared for Western blot analysis were washed 4 times with RIPA buffer and eluted in Laemmli sample buffer. Immunoprecipitates used for the kinase assay were processed as described below. Immunoprecipitated proteins were examined by immunoblotting with mAb 4G10 (from Upstate Biotechnology) which specifically interacts with tyrosine phosphorylated residues or the PKC-δ antiserum.

Total cell lysates were prepared by lysing the platelets in sample buffer (66 mM Tris-HCl, pH 7.4, 2% SDS) at 90°C. Lysates containing equal amounts of protein were subjected to immunoblot analysis with mAb 4G10 or the PKC-δ antiserum. Immunoreactivity was detected using the ECL kit from Amersham and autoradiography.

Cytosolic and particulate fractions were generated as previously

*Corresponding author. Present address: Department of Surgery, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 0885-5635, USA; Fax: (1) (732) 235-4825. E-mail: haimovic@umdnj.edu

Abbreviations: PMA, phorbol ester; PKC, protein kinase C; mAb, monoclonal antibody

PII: S0014-5793(98)01302-7

described [11]. Suspended platelets were stimulated with 5 U/ml thrombin for 30 s to 20 min with stirring. At the indicated time the platelets were lysed by the addition of a fifth volume of buffer containing 125 mM Tris-HCl pH 7.5, 10 mM EDTA, 25 mM EGTA, 5 mM dithiothreitol, 10 mM PMSF, 0.1% Triton X-100, and 5 mM vanadate (final concentration 25 mM Tris-HCl pH 7.5, 2 mM EDTA, 5 mM EGTA, 1 mM dithiothreitol, 2 mM PMSF, 0.02% Triton X-100, and 1 mM vanadate). All samples were sonicated with 4×10 s pulses. Intact platelets and insoluble material were removed by centrifugation at $12\,000\times g$ for 2 min. Lysates were subsequently spun at $100\,000\times g$ for 60 min. Supernatants ($100\,000\times g$ soluble) were recovered and a fraction of each was subjected to immunoblotting analysis. The pellets ($100\,000\times g$ insoluble) were resuspended in sample buffer and were similarly subjected to immunoblotting analysis.

2.3. PKC-δ immune complex kinase assay

PKC-δ was immunoprecipitated as described above and the immune complex kinase assays were performed using the kinase assay kit from Upstate Biotechnology. Immune complexes bound to the protein A/G agarose were washed once with 1 ml of buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.1% 2-mercaptoethanol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 1 mM sodium vanadate, and 1 mM PMSF), once with 1 ml buffer A containing 0.5 M NaCl, and finally with 1 ml of assay dilution buffer (ADB) (20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EGTA, 1 mM sodium vanadate, 1 mM PMSF, and 1 mM dithiothreitol). Following the last wash, the pelleted beads were resuspended in 30 μl of ADB. The kinase reaction was initiated with the addition of myelin basic protein (20 μg/ml) and a magnesium/ATP cocktail (75 mM $MgCl_2$ and 0.5 mM ATP) containing 300 Ci/mmol [γ-^{32}P]ATP (purchased from ICN Biochemicals). A control sample included in each experiment contained the immune complex and the kinase reaction components with the exception of the substrate myelin basic protein. The reaction mixtures were briefly vortexed and incubated at 30°C for 20 min with occasional mixing. To terminate the kinase reaction, the beads were pelleted, and 15 μl of the supernatant was spotted onto individual phosphocellulose filters. The filters were air-dried for 5 min, washed 3 times in 0.75% of phosphoric acid and once in acetone, and subjected to scintillation counting. Duplicate or triplicate samples were used for each data point. The [γ-^{32}P]ATP cpm counts bound to the filter in the absence of myelin basic protein were taken to represent background and were accordingly subtracted from the values obtained for all other samples. Data are expressed as a percent increase in cpm counts relative to the unstimulated platelet lysate.

3. Results

3.1. Activation of platelet PKC-δ by thrombin

Thrombin is a potent agonist that activates multiple signaling pathways in platelets. We examined the induction of PKC-δ activation in response to treatment of suspended platelets for 30 s to 30 min with 5 U/ml thrombin. The platelets were subsequently lysed and lysates containing an equal protein amount were divided into two fractions; one fraction containing 30–40 μg protein was analyzed by immunoblotting. The second fraction containing 400–500 μg protein was immunoprecipitated with the antiserum to PKC-δ. Shown in Fig. 1A,B are the results of an immunoblot of total cell lysates probed with mAb 4G10, specific for phosphotyrosine residues, and the PKC-δ antiserum. Enhanced tyrosine phosphorylation of a protein that migrated with an electrophoretic mobility similar to that of PKC-δ was seen within 30 s of thrombin addition. Furthermore, as shown in Fig. 1C,D, PKC-δ immunoprecipitated from platelet lysates activated with thrombin for 30 s to 30 min was also tyrosine phosphorylated. Densitometric scanning of the gel shown in Fig. 1C revealed two peaks of reactivity at 3 min and 20 min, respectively. In contrast, no tyrosine phosphorylation signal was associated with PKC-δ immunoprecipitated from unstimulated platelet lysates (Fig. 1C,D, first and last lanes).

PKC-δ activation was measured using an immune complex kinase assay with myelin basic protein as a substrate. Data were expressed as a fold increase relative to PKC-δ activity detected in unstimulated platelet lysates. As shown in Fig. 1E, PKC-δ activity increased about 2-fold within 30 s of thrombin stimulation and remained elevated for 30 min. A biphasic response such as the one shown in Fig. 1E was observed in a total of four independent experiments, and is consistent with the biphasic tyrosine phosphorylation pattern resolved through densitometric analysis.

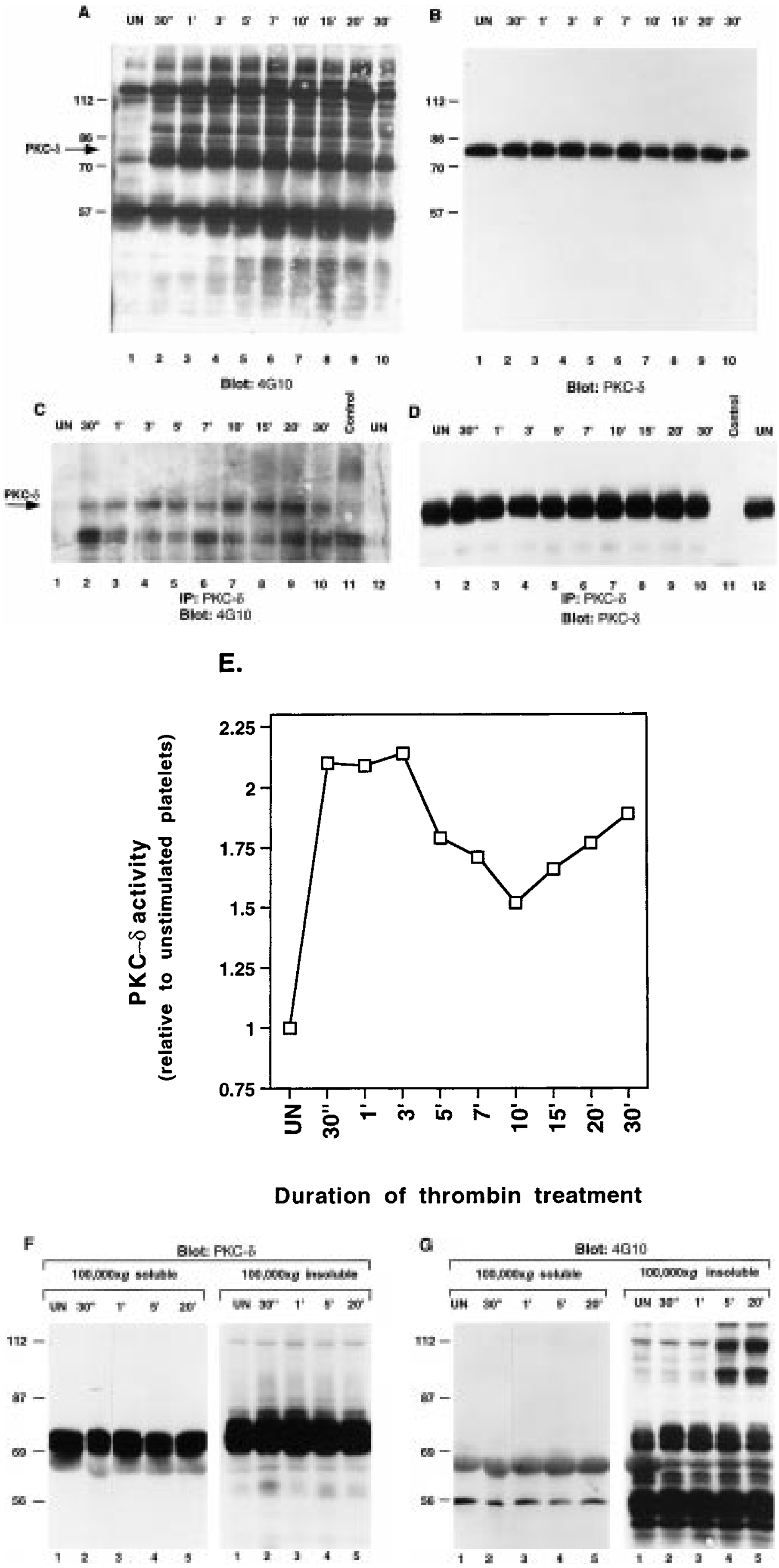
Baldassare et al. [11] have shown that when unstimulated platelets are lysed in a low detergent containing buffer (0.02% Triton X-100), about 90% of the total platelet PKC-α, β, and ζ isoforms are released into the soluble fraction. In thrombin stimulated platelets, a small part of each isoform redistributes from the soluble to the particulate ($100\,000\times g$ insoluble) fraction [11]. In contrast, about half of the total PKC-δ population was shown to be associated with the particulate fraction both in resting and in thrombin activated platelets [11]. Data shown in Fig. 1F confirm these observations, namely, that a substantial portion of the total platelet PKC-δ population was associated with the particulate fraction whether the platelets were stimulated or not. Furthermore, as shown in Fig. 1G, tyrosine phosphorylation of a protein that co-migrated with PKC-δ was restricted to the particulate fraction. Tyrosine phosphorylation of PKC-δ may be limited to the membrane fraction. Alternatively, PKC-δ may undergo a rapid translocation from the cytosol to the membrane fraction once tyrosine phosphorylated while the overall distribution of the PKC-δ population remains unchanged.

3.2. Differential effect of thrombin and fibrinogen on PKC-δ tyrosine phosphorylation and activation

Having established that thrombin stimulates the induction of PKC-δ tyrosine phosphorylation and activation, we next

→

Fig. 1. Time course of tyrosine phosphorylation and activation of PKC-δ in thrombin activated platelets. Purified suspended platelets were untreated (UN; lane 1) or stirred in suspension with 5 U/ml thrombin for the times indicated. Platelets were then lysed in Triton X-100 buffer. In A and B, unstimulated (lane 1) or thrombin stimulated (lanes 2–10) total platelet lysates containing 30–40 μg protein were analyzed by immunoblotting with mAb 4G10 (A), or the antiserum to PKC-δ (B). The arrow on the left indicates the position of PKC-δ. In C and D, unstimulated (lanes 1 and 12) or thrombin activated platelet lysates containing 400–500 μg protein were immunoprecipitated with the antiserum to PKC-δ (lanes 1–10 and 12) or a control antiserum (lane 11). The immunoprecipitated proteins were Western blotted and probed with mAb 4G10 (C) or the antiserum to PKC-δ (D). Data are representative of three experiments. In E, platelet lysates containing 400–500 μg protein were immunoprecipitated with the antiserum to PKC-δ. The immunoprecipitated proteins were subjected to an in vitro kinase reaction with myelin basic protein as substrate. The phosphorylated substrate was then spotted onto phosphocellulose filters, washed, and subjected to scintillation counting. Data are expressed as fold increase relative to unstimulated platelets. Shown is an average of two experiments, with duplicate samples for each data point. In F and G, cytosol ($100\,000\times g$ soluble) and particulate ($100\,000\times g$ insoluble) fractions of unstimulated (UN; lane 1) or platelets activated with thrombin for the indicated time (30 s to 20 min; lanes 2–5) were separated by high-speed centrifugation. Fractions were analyzed by immunoblotting with the antiserum to PKC-δ (F) or mAb 4G10 (G).


A
UN 30" 1' 3' 5' 7' 10' 15' 20' 30'
112
86
PKC-δ
70
57
1 2 3 4 5 6 7 8 9 10
Blot: 4G10
B
UN 30" 1' 3' 5' 7' 10' 15' 20' 30'
112
86
70
57
1 2 3 4 5 6 7 8 9 10
Blot: PKC-δ
C
UN 30" 1' 3' 5' 7' 10' 15' 20' 30' Control UN
PKC-δ
1 2 3 4 5 6 7 8 9 10 11 12
IP: PKC-δ
Blot: 4G10
D
UN 30" 1' 3' 5' 7' 10' 15' 20' 30' Control UN
1 2 3 4 5 6 7 8 9 10 11 12
IP: PKC-δ
Blot: PKC-δ
E.
PKC-δ activity
(relative to unstimulated platelets)
2.25
2
1.75
1.5
1.25
1
0.75
UN 30" 1' 3' 5' 7' 10' 15' 20' 30'
Duration of thrombin treatment
F
Blot: PKC-δ
100,000xg soluble
100,000xg insoluble
UN 30" 1' 5' 20'
UN 30" 1' 5' 20'
112
87
69
56
1 2 3 4 5
1 2 3 4 5
G
Blot: 4G10
100,000xg soluble
100,000xg insoluble
UN 30" 1' 5' 20'
UN 30" 1' 5' 20'
112
87
69
56
1 2 3 4 5
1 2 3 4 5

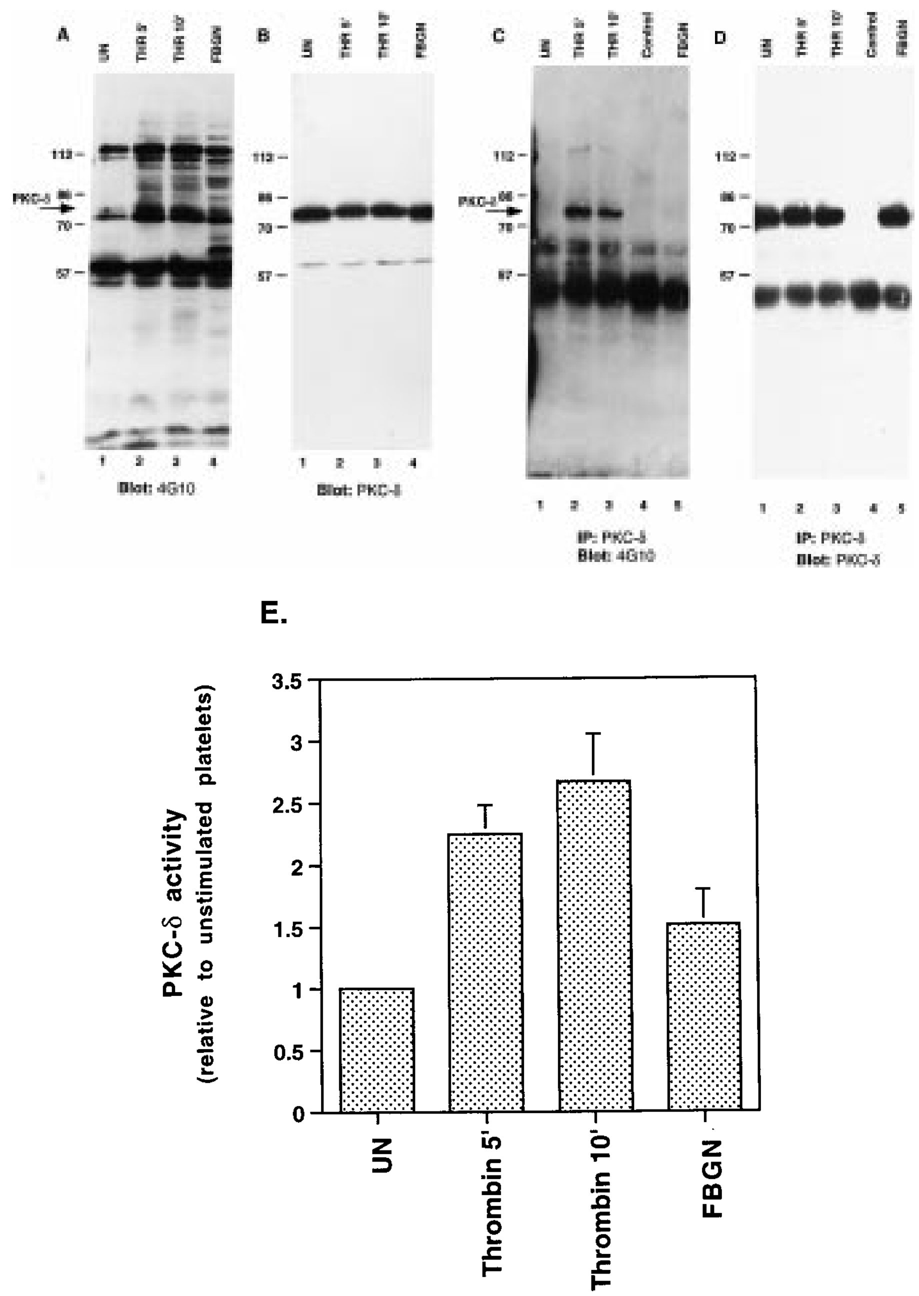


Fig. 2. Differential activation and tyrosine phosphorylation of PKC-δ by thrombin or immobilized fibrinogen. Platelets were kept in suspension untreated (UN), stirred in suspension with 5 U/ml thrombin for 5 min or 10 min (THR 5′ and THR 10′, respectively), or were added to fibrinogen (FBGN) coated plates for 30 min. In A and B, equal total protein amounts were analyzed by Western blotting with mAb 4G10 (A) or the antiserum to PKC-δ (B). The arrow on the left indicates the location of PKC-δ. In C and D, platelet lysates containing 400–500 μg protein were immunoprecipitated with the antiserum to PKC-δ (lanes 1–3 and lane 5) or a control antiserum (lane 4). The immunoprecipitated proteins were immunoblotted and probed with mAb 4G10 (C) or the antiserum to PKC-δ (D). In E, platelet lysates containing 400–500 μg protein were immunoprecipitated with the antiserum to PKC-δ. The immunoprecipitated proteins were subjected to an in vitro kinase reaction and analyzed as described in the legend to Fig. 1. The data shown are an average of at least five experiments with duplicate or triplicate samples for each data point.

examined the induction of PKC-δ activation in platelets adherent to fibrinogen. Platelets were either stirred in suspension with 5 U/ml thrombin for 5 or 10 min (in some experiments the platelets were stimulated with 1 U/ml thrombin yielding similar results), or were plated onto fibrinogen-coated plates for 30 min, the shortest time needed for all platelets to adhere to the surface. The platelets were lysed and the lysates were analyzed by Western blotting, immunoprecipitation, and immune complex kinase assay as described above. As shown in Fig. 2A–D, platelet adhesion to fibrinogen failed to trigger the induction of PKC-δ tyrosine phosphorylation. The activity of PKC-δ isolated from fibrinogen adherent platelets was not statistically different from the activity measured in unstimulated platelet lysates with an average of 1.5 ± 0.27 ($n = 7$; $P = 0.08$) (Fig. 2E). The values for the platelets stimulated with thrombin for 5 and 10 min, respectively, were

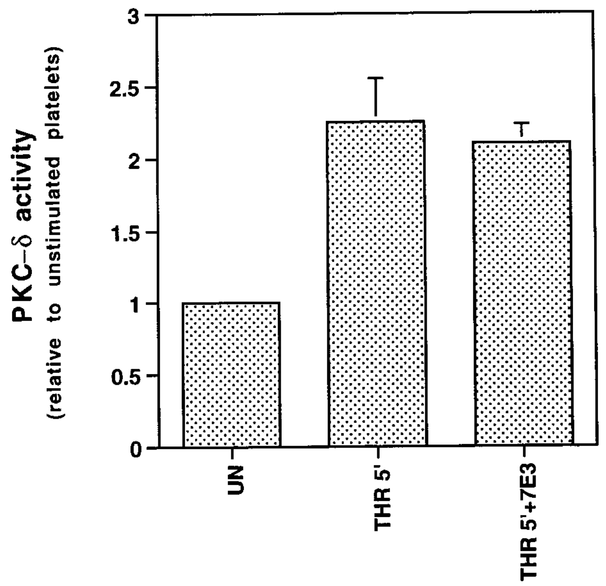


Fig. 3. Effect of $\alpha_{IIb}\beta_3$ inhibitors on thrombin induced PKC-δ activation. Platelets were kept in suspension untreated (UN), or were stirred in suspension with 5 U/ml thrombin for 10 min. Prior to the addition of thrombin, the samples were treated for 10 min with or without mAb 7E3 (10 μg/ml). Platelets were lysed in Triton X-100 buffer and were subjected to an in vitro kinase reaction as described in the legend to Fig. 1.

2.03 ± 0.22 ($n = 7$, $P = 0.004$) and 2.67 ± 0.38 ($n = 4$, $P = 0.002$). These data indicated the platelet adhesion to fibrinogen has a very modest, if any, effect on the induction of PKC-δ activity.

3.3. *Effect of an anti-$\alpha_{IIb}\beta_3$ antibody on PKC-δ activity*

Although $\alpha_{IIb}\beta_3$ appeared to be a poor mediator of PKC-δ activation we next asked whether $\alpha_{IIb}\beta_3$ contributes to thrombin mediated PKC-δ activation. For these studies, platelets were treated with mAb 7E3, an inhibitor of fibrinogen-$\alpha_{IIb}\beta_3$ interaction and platelet aggregation [12]. Treatment of the platelets with mAb 7E3 prior to the addition of thrombin blocked aggregation and pp125FAK phosphorylation [13], having no effect on the thrombin induced PKC-δ activation (Fig. 3); the PKC-δ activity isolated from platelets treated with mAb 7E3 prior to thrombin addition was 2.1 ± 0.13 ($n = 3$) and statistically insignificant relative to platelets treated with thrombin alone (2.25 ± 0.3). Thus, $\alpha_{IIb}\beta_3$ appears not to regulate PKC-δ activation either on its own or when thrombin is the stimulating agonist.

4. Discussion

In this study we demonstrate that PKC-δ is activated and tyrosine phosphorylated in thrombin stimulated platelets. We also show that thrombin induced PKC-δ activation is independent of the $\alpha_{IIb}\beta_3$ mediated signaling pathway. Baldassare et al. [11] have shown that in thrombin stimulated platelets, PKC-α, β, and ζ, but not PKC-δ, translocated from the soluble to the particulate fraction. These data provided no direct evidence on whether PKC-δ was activated or not. Data presented in this study clearly demonstrate that PKC-δ is activated as well as tyrosine phosphorylated within 30 s of platelet activation by thrombin. The tyrosine phosphorylated PKC-δ isoform was associated with the platelet particulate ($100\,000 \times g$ insoluble) fraction.

In contrast with the effect of thrombin on PKC-δ activation, platelet adhesion to fibrinogen had a minor, if any, effect on the activity of PKC-δ. Tyrosine phosphorylation of PKC-δ was similarly not detected in lysates of fibrinogen adherent platelets. In Swiss 3T3 cells, Rho-A stimulated the recruitment of both pp125FAK and PKC-δ to focal adhesion plaques [14]. However, PKC-δ was not localized to the focal adhesion plaques either in fibrinogen or phorbol ester treated fibrinogen adherent platelets (unpublished data). Finally, pretreatment of the platelets with mAb 7E3, an inhibitor of $\alpha_{IIb}\beta_3$-fibrinogen interaction and as a consequence platelet aggregation [12] as well as pp125FAK phosphorylation [13], did not affect PKC-δ activation in response to thrombin. Taken together, these data suggested that $\alpha_{IIb}\beta_3$ does not mediate PKC-δ tyrosine phosphorylation or activation.

A tyrosine phosphorylated PKC-δ was identified in several model systems including c-Ha-ras transformed fibroblasts [15,16], salivary gland epithelial cells [17], and phorbol ester stimulated 32D cells (a murine interleukin-3 dependent myeloid progenitor line) that overexpress PKC-δ [18]. The nonspecific tyrosine kinase inhibitor, genistein, prevented PKC-δ tyrosine phosphorylation [17,18], while treatment of the 32D PKC-δ overexpressing cells with growth factors known to trigger protein tyrosine phosphorylation, including insulin, and EGF, failed to trigger PKC-δ tyrosine phosphorylation suggesting that this tyrosine phosphorylation event is specific and tightly regulated [17]. Src and, to a lesser extent, fyn have been implicated in the induction of PKC-δ tyrosine phosphorylation [19–21]. The relationship between tyrosine phosphorylation of PKC-δ and its activation is not clear. Inhibitors that block PKC autophosphorylation and thus its activation did not prevent PKC-δ phosphorylation on tyrosine. These data suggested that PKC-δ tyrosine phosphorylation and activation represent independent events [16,19]. Studies in keratinocytes, and v-src transformed fibroblasts, suggested that tyrosine phosphorylation may attenuate the activity of PKC-δ [20,21]. In contrast, studies with a baculovirus derived PKC-δ which was phosphorylated on tyrosine by different tyrosine kinases, as well as studies with PDGF stimulated 32D cells, suggested that its kinase activity was increased as a result of the phosphorylation [16].

The physiological role of the PKC-δ isoform is also unclear at the present time. PKC-δ overexpression in NIH 3T3 cells decreased the growth rate and prevented anchorage independent cell growth in soft agar [22]. Other studies have proposed a role for PKC-δ in macrophage [23,24], PC12 cell [25], and keratinocyte differentiation [20], and more recently in relationship to apoptosis [26]. Based on these data it is possible that PKC-δ activation plays a critical role during megakaryocyte differentiation, having a secondary role, if any, relative to platelet activation.

In summary, we found that platelet activation with thrombin results in rapid induction of tyrosine phosphorylation and activation of PKC-δ that is mediated via an $\alpha_{IIb}\beta_3$ integrin independent pathway. Further studies are required to establish the role of PKC-δ in platelets.

Acknowledgements: This work was supported by a grant from the foundation of UMDNJ and the National Institute of Health (HL54101). We thank Dr. Barry Coller for mAb 7E3.

References

[1] Blobe, G.C., Stribling, S., Obeid, L.M. and Hannun, Y.A. (1996) Cancer Surv. 27, 213–248.
[2] Hofmann, J. (1997) FASEB J. 11, 649–669.
[3] Jaken, S. (1996) Curr. Opin. Cell Biol. 8, 168–173.
[4] Nishizuka, Y. (1992) Science 258, 607–614.
[5] Johannes, F.-J., Prestle, J., Eis, S., Oberhagemann, P. and Pfizenmaier, K. (1994) J. Biol. Chem. 269, 6140–6148.
[6] Toullec, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E., Loriolle, F., Duhamel, L., Charon, D. and Kirilovsky, J. (1991) J. Biol. Chem 266, 15771–15781.
[7] Siess, W. (1989) Physiol. Rev. 69, 58–177.
[8] Haimovich, B., Kaneshiki, N. and Ji, P. (1996) Blood 87, 152–161.
[9] Vuori, K. and Ruoslahti, E. (1993) J. Biol. Chem. 268, 21459–21462.
[10] Haimovich, B., Lipfert, L., Brugge, J.S. and Shattil, S.J. (1993) J. Biol. Chem. 268, 15868–15877.
[11] Baldassare, J.J., Henderson, P.A., Burns, d., Loomis, C. and Fisher, G.J. (1992) J. Biol. Chem. 267, 15585–15590.
[12] Coller, B.S. (1985) J. Clin. Invest. 76, 101–108.
[13] Lipfert, L., Haimovich, B., Schaller, M., Cobb, B.S., Parsons, J.T. and Brugge, J.S. (1992) J. Cell Biol. 1199, 905–912.
[14] Barry, S.T. and Critchley, D.R. (1994) J. Cell Sci. 107, 2033–2045.
[15] Borner, C., Guadagno, S.N., Hsiao, W.W.-L., Fabbro, D., Barr, M. and Weinstein, I.B. (1992) J. Biol. Chem. 267, 12900–12910.
[16] Li, W., Yu, J.-C., Michieli, P., Beller, J.F., Ellmore, N., Heidaran, M.A. and Pierce, J.H. (1994) Mol. Biol. Cell 14, 6727–6735.
[17] Soltoff, S.P. and Toker, A. (1995) J. Biol. Chem. 270, 13490–13495.
[18] Li, W., Mischak, H., Yu, J.-C., Wang, L.-M., Mushinski, J.F., Heidaran, M.A. and Pierce, J.H. (1994) J. Biol. Chem. 269, 2349–2352.
[19] Gschwendt, M., Kielbassa, K., Kittstein, W. and Marks, F. (1994) FEBS Lett. 347, 85–89.
[20] Denning, M.F., Dlugosz, A.A., Threadgill, D.W., Magnuson, T. and Yuspa, S.H. (1996) J. Biol. Chem. 271, 5325–5331.
[21] Zang, Q., Lu, Z., Curto, M., Barile, N., Shalloway, D. and Foster, D.A. (1997) J. Biol. Chem. 272, 13275–13280.
[22] Mischak, H., Goodnight, J., Kolch, W., M-B, G., Schaechtle, C., Kazanietz, M.G., Blumberg, P.M., Pierce, J.H. and Mushinski, J.F. (1993) J. Biol. Chem. 268, 6090–6096.
[23] Mischak, H., Pierce, J.H., Goodnight, J., Kazanietz, M.G., Blumberg, P.M. and Mushinski, J.F. (1993) J. Biol. Chem. 268, 2001–20115.
[24] Wang, Q.J., Acs, P., Goodnight, J., Giese, T., Blumberg, P.M., Mischak, H. and Mushinski, J.F. (1997) J. Biol. Chem. 272, 76–82.
[25] O'Driscoll, K.R., Tng, K.K., Fabbro, D., Greene, L.A. and Weinstein, I.B. (1995) Mol. Biol. Cell 6, 449–458.
[26] Sawai, H., Okazaki, T., Takeda, Y., Tashima, M., Sawada, H., Okuma, M., Kishi, S., Umegara, H. and Domae, N. (1997) J. Biol. Chem. 272, 2452–2458.