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Use of a Novel Method to Find Substrates of Protein Kinase C Delta Identifies M2 Pyruvate Kinase

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Abstract

Protein kinase C (PKC) family members have been implicated in numerous cellular processes. However, identifying the substrates of each PKC isozyme remains a challenge. Here, we describe a method using two dimensional (2-D) isoelectric focusing gel electrophoresis to identify substrates of delta PKC (δ PKC) in MCF-7 breast carcinoma cells. We show that M2 pyruvate kinase is a substrate of δ PKC, and further characterize the interaction between M2 pyruvate kinase and δ PKC in MCF-7 cells by immunoprecipitation. δ PKC activation in vitro or in cells did not appear to alter the enzyme activity or polymerization of M2 pyruvate kinase.

Keywords

Protein Kinase C; Signal transduction; M2-type pyruvate kinase; Heat shock protein 27

1. Introduction

δ PKC has a role in multiple cellular processes, including apoptosis (Zhong et al., 2002), metastasis (Kiley et al., 1999), and tumor suppression (Perletti et al., 1999; Reddig et al., 1999). However, other PKC isozymes are also involved in these processes, possibly with opposite roles to that of δ PKC (Chen et al., 2001; Hornia et al., 1999; Mischak et al., 1993). Individual isozymes can have differing substrates in vivo (Jideama et al., 1996; Maizels et al., 1998). Additionally, because most pharmacological activators and inhibitors of PKC affect multiple isozymes and/or other proteins, clarifying the role of individual PKC isozymes in many processes remains a challenge.

In breast cancer specifically, δ PKC has been implicated in several traits, including sustained migration (Kruger & Reddy, 2003), metastasis (Kiley et al., 1999), proliferation (Shanmugam

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Competing Interests: Daria Mochly-Rosen is the founder of KAI Pharmaceuticals, Inc., a company that plans to bring PKC regulators to the clinic. However, none of the work described in this study is based on or supported by the company. Stefan Siwko declares no competing interests.

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et al., 2001), and response to DNA damage (Abbas et al., 2004). However, at present it is not clear whether δ PKC plays an oncogenic or a tumor suppressive role.

We hypothesized that identifying the specific substrates of δ PKC in breast cancer cells will highlight its role in these cells. Since classical approaches to substrate identification do not adequately account for isozyme specificity or the role of scaffolding proteins in controlling substrate access in vivo, we took a novel approach to identify substrates of δ PKC in MCF-7 cells. We used $\psi\delta$ RACK, an 8-amino acid (aa) peptide derived from δ PKC that is a specific δ PKC activator (Chen et al., 2001; Disatnik et al., 2002) to selectively activate δ PKC in MCF-7 cells. Proteins that changed to a more acidic isoelectric point on 2-D gel electrophoresis (indicative of phosphorylation) upon δ PKC activation were identified as potential δ PKC substrates. We further characterized one candidate substrate, M2 pyruvate kinase, for its interaction with δ PKC and for functional effects of this interaction.

2. Materials and Methods

2.1. Materials

Phorbol 12 myristylate, 13 acetate was purchased from Alexis Biochemicals (San Diego, CA), alkaline phosphatase from Sigma (St. Louis, MO), and antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) except for 2B9 anti-phosphoserine antibody (Immunex, Seattle, WA) and DF-4 anti-M2 pyruvate kinase antibody (Sche Bo Biotech, Germany). $\psi\delta$ RACK, a selective δ PKC activator (δ PKC aa 74-81 linked to a carrier peptide (Antennapedia aa 43-58)) (Chen et al., 2001), as well as the Antennapedia carrier peptide alone were synthesized by the Stanford Protein and Nucleic Acid facility (Stanford, CA). The sources of other reagents are indicated below.

2.2. Cell Culture

MCF-7 cells were obtained from Dr. James Ford (Stanford University) and cultured in RPMI 1640 media with 5% fetal calf serum (Invitrogen-Gibco, Carlsbad, CA), L-glutamine, penicillin, and streptomycin. Cells were plated at 10^5 cells/ml and cultured at 37° in 6% CO_2 until ~50% confluence, followed by culturing in serum-free medium for one day prior to the experiment.

2.3. 2-D isoelectric focusing (IEF) gel electrophoresis

MCF-7 cells were washed in PBS buffer and lysed in 10 mM Tris pH 7.4, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4 , 50 mM β -glycerophosphate, 2.5 μM microcystin LR, 17 $\mu\text{g}/\text{mL}$ PMSF, 25 $\mu\text{g}/\text{mL}$ aprotinin, 25 $\mu\text{g}/\text{mL}$ leupeptin, and 20 $\mu\text{g}/\text{mL}$ soy bean trypsin inhibitor. Cells were lysed by trituration and centrifuged for 30 min. at $100\,000 \times g$. The supernatant was concentrated using a centricon 10 filter (Millipore, Bedford, MA). Protein concentration was determined by Bradford Assay (Bio-Rad, Hercules, CA) and 100 μg total protein was precipitated using the PAGE-perfect system (Geno-Tech, St. Louis, MO). Pellets were resuspended by sonication in urea solution (8 M urea, 2% CHAPS, 2.8 mg/mL dithiothreitol, 0.5 % IPG buffer (Amersham)) and proteins were separated on an Immobiline pH 3-10 Dry Strip (Amersham) using an IPGphor flatbed IEF electrophoresis apparatus (Pharmacia) according to manufacturer's instructions. Strips were equilibrated in SDS buffer (50 mM Tris, pH 8.8, 6 M urea, 30% glycerol, 2% SDS) and separated in the second dimension on an SDS 10% polyacrylamide gel followed by Western blot analysis.

2.4. Phosphatase treatment

MCF-7 cells were lysed as described above, except in the absence of phosphatase inhibitors (Na_3VO_4 , β -glycerophosphate, microcystin LR). Following supernatant concentration, two

lysate samples (100 µg total protein each) were taken from each condition, and 660 ng Histone (Sigma) and MgCl₂ (10 mM final) were added to both samples. White potato acid phosphatase (7 units) or phosphatase inhibitors (1 mM Na₃VO₄, 50 mM β-glycerophosphate, 2.5 µM microcystin LR) were added to the respective samples, and both were incubated 30 minutes at 37°C, followed by protein precipitation and analysis by 2-D IEF.

2.5. M2 pyruvate kinase purification

A baculovirus expression construct for rat M2 pyruvate kinase (a gift of Dr. Tamio Noguchi, Fukui Medical University, Japan) was used to express M2 pyruvate kinase in Hi-5 insect cells. Cells were lysed and M2 pyruvate kinase was purified according to Ikeda & Noguchi (1998).

2.6. PKC kinase activity assay

Purified PKC isozymes (Panvera, Madison, WI) were incubated with purified M2 pyruvate kinase or myelin basic protein (Sigma) in 20 mM Tris HCl pH 7.4, 20 mM MgCl₂, 20 µM ATP (26 nCi/mL γ-³²P ATP (Amersham)), 0.24 mg/mL phosphatidylserine (Avanti, Alabaster, AL), 0.04 mg/mL 1,3-sn diolethylglycerol (Avanti) for 20 minutes at 25° C and then analyzed by SDS-PAGE and autoradiography.

2.7. Pyruvate kinase activity assay

Purified M2 pyruvate kinase was incubated in a PKC assay (as above, but in the absence of γ-³²P ATP). Following this, pyruvate kinase activity was measured in a coupled lactate dehydrogenase assay, as described in Gutmann & Bernt (1977).

2.8. Immunoprecipitation

MCF-7 cells were lysed as for the IEF 2-D gel protocol with the addition of 25 mM NaCl and 0.1 % NP-40 to the lysis buffer. Lysate supernatants from three identically treated plates were pooled together and run on a 15 mL DEAE column (DE52, Whatman, Clifton, NJ). The peak pyruvate kinase activity fraction (500 µg) was precleared with protein G beads (Invitrogen, Carlsbad, CA) for 1 hour, and then was incubated with 40 µg of the DF-4 anti-M2 pyruvate kinase antibody and protein G beads rotating at 4° C overnight. Beads were washed four times with lysis buffer, then boiled in SDS sample buffer and analyzed by SDS-PAGE followed by Western blotting.

2.9. Gel filtration

Protocol was the same as Mazurek et al., (2001) except as noted. In brief, MCF-7 cell lysates were ultracentrifuged 30 minutes at 100 000 × g at 4 degrees. Supernatants were filtered through a 0.22 micron filter, then loaded on a 2 × 85 cm column of Sephacryl S-200 beads pre-equilibrated with sodium phosphate buffer (72 mM Na₂HPO₄, 28 mM NaH₂PO₄, 1 mM DTT, 1 mM β-mercaptoethanol, 10% glycerol, 1 mM Na₃VO₄, 50 mM β-glycerophosphate, 2.5 µM microcystin LR, 17 µg/mL PMSF, 25 µg/mL aprotinin, 25 µg/mL leupeptin, 20 µg/mL soy bean trypsin inhibitor). Fractions were collected every 20 minutes and assayed for total protein (UV absorbance) and pyruvate kinase activity (as above). Approximately 2 × 10⁷ MCF-7 cells were used per filtration.

3. Results

3.1. Verification of 2-D IEF method using a known substrate of δPKC

The heat shock protein HSP 27 is known to be phosphorylated by δPKC (Maizels et al., 1998), and therefore was used to verify that 2-D IEF can detect changes in phosphorylation of established δPKC substrates. Treatment of MCF-7 cells with 3 nM PMA, a potent activator of classical (α, β, γ) and novel (δ, ε, θ, and η) PKC isozymes, shifted the mobility of HSP 27 to

a lower pI (Figure 1A), indicative of HSP 27 phosphorylation, possibly by PKC directly. The further shift of HSP27 to a more acidic pI upon co-treatment with PMA and the specific δ PKC activator peptide, $\psi\delta$ RACK (Figure 1B), suggests that δ PKC causes this phosphorylation-induced change in mobility. A shift similar to 3 nM PMA was observed following treatment with $\psi\delta$ RACK alone (Figure 2). Importantly, phosphatase treatment decreased the lowest pI forms of HSP 27 by approximately 50% (Figure 3), confirming that phosphorylation of HSP 27 was responsible for the pI change. These data are consistent with the interpretation that δ PKC activation induces the phosphorylation of a known δ PKC substrate, HSP 27, and demonstrates the use of 2-D IEF for this analysis.

3.2. Identification of M2 pyruvate kinase as a potential substrate of δ PKC

To identify new potential substrates of δ PKC, MCF-7 cells were treated with $\psi\delta$ RACK to specifically activate δ PKC and then analyzed by 2-D IEF. The resulting blots were probed with a phospho-specific antibody and examined for either spots which shifted to lower pI upon δ PKC activation, or the appearance of new spots. One protein (dashed circle, upper panel) that shifted to a more acidic pI upon treatment with $\psi\delta$ RACK (solid circle, lower panel) is shown in Figure 4A. MALDI mass spectroscopy identified this potential δ PKC substrate as human M2 pyruvate kinase (Figure 4B). An antibody that specifically recognizes M2 pyruvate kinase (Schneider et al. 2002, Schneider et al. 2000, Eigenbrodt et al. 1992; Supplementary Figure 1) further confirmed this identification (Figure 4A vs. 4C). Quantitation of the distribution of M2 pyruvate kinase (excluding the lowest pI spot, whose intensity was highly variable between experiments) showed an increase in the proportion of acidic pI spots (solid circle in Figure 4C) from $39 \pm 8\%$ in control to $65 \pm 7\%$ upon $\psi\delta$ RACK treatment ($n=3 \pm \text{SE}$; $P<0.05$), indicating phosphorylation of M2 pyruvate kinase upon δ PKC activation.

3.3. δ PKC phosphorylates purified M2 pyruvate kinase in vitro

We next determined whether δ PKC phosphorylates M2 pyruvate kinase in vitro. Upon activation by phosphatidyl serine and diacylglycerol, purified δ PKC directly phosphorylated purified M2 pyruvate kinase in vitro (Figure 5A, compare lanes 2 and 3), and the degree of phosphorylation increased in proportion to the amount of δ PKC used (Figure 5A lanes 3-5). In addition, α PKC, but not ϵ PKC, phosphorylated M2 pyruvate kinase in vitro (Figure 5B).

Somewhat surprisingly, phosphorylation by δ PKC in vitro did not alter either the initial rate (data not shown) or the steady state activity of M2 pyruvate kinase (Supplementary Figure 2). Further, there was no difference in the activity of δ PKC-treated enzyme versus the non-treated enzyme when phosphoenolpyruvate was limiting (100 μ M). These data indicate that phosphorylation of M2 pyruvate kinase by δ PKC does not significantly alter pyruvate kinase activity in vitro, but in vivo effects on M2 pyruvate kinase stability or degradation are certainly possible (see Discussion).

3.4. Association of δ PKC with M2 pyruvate kinase in cells

The above data demonstrate that M2 pyruvate kinase is phosphorylated in response to the specific activation of δ PKC (Figure 4), and that δ PKC phosphorylates M2 pyruvate kinase in vitro, suggesting that M2 pyruvate kinase is a direct substrate of δ PKC in MCF-7 cells. We next determined whether M2 pyruvate kinase and δ PKC interact in cells. We observed a selective immunoprecipitation of δ PKC by anti-M2 pyruvate kinase antibodies from lysates of untreated MCF-7 cells (on average $242 \pm 24\%$ versus IgG control (which shows limited background binding of δ PKC to the immunoprecipitation beads), $n=3 \pm \text{SE}$; Figure 6A lanes 1 vs. 3). Importantly, neither α PKC nor ϵ PKC coimmunoprecipitated with M2 pyruvate kinase (Figure 7), indicating that the interaction between PKC and M2 pyruvate kinase in MCF-7 cells is specific for δ PKC. (Note that the reverse experiment where δ PKC immunoprecipitate was probed for M2 pyruvate kinase did not detect M2 pyruvate kinase in the

immunoprecipitate, which may indicate that the δ PKC epitope is not available when δ PKC is complexed with M2 pyruvate kinase.) In cells treated with 100 nM PMA the ratio of δ PKC/M2 pyruvate kinase in the immunoprecipitate was significantly decreased (8.5 ± 1.0 in vehicle-treated vs. 1.6 ± 0.3 in PMA-treated cells (arbitrary units), $n=2 \pm \text{SD}$; Fig. 6A-B), suggesting that, upon activation, δ PKC phosphorylates M2 pyruvate kinase and then rapidly releases its phosphorylated substrate.

3.5. δ PKC activation and M2 pyruvate kinase activity

We found no significant difference in pyruvate kinase activity in lysates of cells treated with $\psi\delta$ RACK (500 nM, Figure 8), or with PMA (10 nM, not shown), as compared to control. M2 pyruvate kinase activity may also be controlled through regulation of its multimeric form (Eigenbrodt et al., 1992). However, when we examined the multimerization of pyruvate kinase by gel filtration, we observed no significant changes in the distribution of pyruvate kinase between dimeric and tetrameric forms upon treatment with 500 nM $\psi\delta$ RACK (Table 1).

4. Discussion

We describe a method to detect substrates of particular isozymes of protein kinase C, using a selective δ PKC activator peptide $\psi\delta$ RACK followed by 2-D gel electrophoresis. We detected a pI shift in HSP 27, a known substrate of δ PKC, in response to PKC activation. We identified an additional substrate of δ PKC, M2 pyruvate kinase, that is phosphorylated in cells treated with $\psi\delta$ RACK. We also showed that δ PKC phosphorylates M2 pyruvate kinase in vitro. M2 pyruvate kinase interacts with δ PKC (but not with α PKC or ϵ PKC) as demonstrated by co-immunoprecipitation in quiescent MCF-7 cells, and this interaction decreased upon PKC activation by PMA (Figure 6). However, the effect of δ PKC-mediated phosphorylation of M2 pyruvate kinase was not identified in this study.

We chose to use a phospho-specific antibody to identify new substrates of δ PKC for several reasons. Foremost is the high sensitivity of immunostaining as compared to other methods of protein detection. We used a relatively low level of δ PKC activators, therefore we expected only a fraction of the amount of a given substrate to become phosphorylated. This places a premium on the need for sensitive detection. Furthermore, protein modifications other than phosphorylation could cause a pI shift; we hoped to improve the likelihood that observed protein shifts were indeed due to phosphorylation. Finally, to complement the use of a phospho-specific antibody we also silver stained 2D IEF gels and searched for spots that shifted upon δ PKC activation; we did not observe any additional potential δ PKC substrates by silver staining than those observed using the phospho-specific antibody approach.

Pyruvate kinase is a key glycolytic enzyme, catalyzing the ATP-generating conversion of phosphoenolpyruvate to pyruvate. Importantly, the M2 isoform is overexpressed in multiple types of cancer (Luftner et al., 2000; Newsholme & Board, 1991), and has been proposed to regulate tumor metabolism by switching between its highly active, tetrameric form and its less active, dimeric form (Mazurek et al., 2002). Transformation by either pp60v-src or the human papillomavirus E7 oncoprotein shifts M2 pyruvate kinase from the tetrameric to the dimeric form due to a direct interaction with the transforming oncoprotein (reviewed in Mazurek et al. (2005)). Regulation of M2 pyruvate kinase by δ PKC phosphorylation could, therefore, have important consequences on tumor metabolism.

Several studies have examined whether M2 pyruvate kinase is phosphorylated by PKC, with conflicting results. Chicken PKC phosphorylates M2 pyruvate kinase, without altering pyruvate kinase activity (Noda et al., 1986). In contrast, Mieskes et al. (1987) did not observe phosphorylation of M2 pyruvate kinase by PKC in vitro. A limitation of these studies is that

they did not examine specific isozymes of PKC, which we found to differ widely in their ability to phosphorylate M2 pyruvate kinase (Figure 5B).

We found a selective interaction of δ PKC, but not of α PKC or ϵ PKC, with M2 pyruvate kinase in MCF-7 cells. PKC isozymes bind to different anchoring proteins; this has been proposed to regulate the substrates available to each isozyme (reviewed in Mochly-Rosen (1995)). Therefore, it is likely that mechanisms regulating PKC isozyme localization in vivo act to confer isozyme specificity on δ PKC to phosphorylate M2 pyruvate kinase. In addition, the ratio of immunoprecipitated δ PKC/M2 pyruvate kinase decreased upon PKC activation by PMA, suggesting that δ PKC activation results in δ PKC dissociation from M2 pyruvate kinase, a type of interaction seen in other kinase:substrate pairs (e.g., ϵ PKC and its substrate calsequestrin (Rodriguez et al., 1999)).

While we did not detect a significant effect of δ PKC-mediated phosphorylation on pyruvate kinase activity, we cannot rule out that δ PKC-mediated phosphorylation of M2 pyruvate kinase affects M2 pyruvate kinase in vivo. A connection between δ PKC and M2 pyruvate kinase regulation was demonstrated in a proteomics study of δ PKC knockout mice (δ PKC^{-/-}) (Mayr et al., 2004). δ PKC^{-/-} mice had a two-fold decrease in M2 pyruvate kinase levels as compared to δ PKC^{+/+} mice, suggesting that phosphorylation by δ PKC may regulate M2 pyruvate kinase protein stability or degradation, which were not addressed here.

In conclusion, we present a method for identifying substrates of individual PKC isozymes, using an isozyme-selective activator peptide for δ PKC. This method is extensible to other isozymes with the availability of additional isozyme-specific activator peptides (Schechtman & Mochly-Rosen, 2002). Through finding substrates of individual PKC isozymes, this method can contribute towards understanding the role of PKC in biological processes, and towards finding new targets for drug discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

2-D, two-dimensional; aa, amino acid; IEF, isoelectric focusing; IgG, immunoglobulin G; MALDI, matrix assisted laser desorption ionization; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; pI, isoelectric point; PKC, protein kinase C; PMA, phorbol 12 myristylate 13 acetate; RACK, receptor for activated C-kinase; SDS, sodium dodecylsulfate.

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Fig 1.

δ PKC activation increases phosphorylation of HSP 27.

A. MCF-7 cells were treated as indicated with 3 nM PMA alone (5 minutes) or with the specific δ PKC activator peptide $\psi\delta$ RACK (500nM) for 15 minutes followed by the addition of 3nM PMA for 5 minutes, lysed, and chromatographed by 2-D IEF gel electrophoresis. Blots were probed with an antibody to HSP 27. Note the shift to lower pI (spots A and B) that occurs in response to PKC activation, suggesting phosphorylation of HSP 27. Quantitation of HSP 27 is provided below each spot as percent of total HSP 27.

B. Quantitation from four independent experiments of the mean percent of total HSP 27 with one or more phosphorylations, as indicated by a shift from spot C to spots A and B (mean $\pm$ SE; * $P < 0.05$).

Fig 2.

$\psi\delta$ RACK peptide alone induces HSP 27 phosphorylation.

MCF-7 cells were treated with 500 nM Antennapedia carrier peptide (control, top panel) or 187 nM $\psi\delta$ RACK peptide (bottom panel) for 20 minutes, lysed, and chromatographed by 2-D IEF electrophoresis. Blots were probed with an antibody to HSP 27. Quantitation of HSP 27 as a percent of total is below each spot.

Phosphatase

Fig 3.

Phosphatase treatment shifts HSP 27 to a higher isoelectric point.

MCF-7 cells were treated with DMSO + 1 μM Antennapedia carrier peptide (top two panels) or 3 nM PMA + 1 μM ψδRACK peptide (bottom two panels) as in Figure 1 followed by lysis. Two lysate aliquots (100 μg total protein) from each condition were incubated at 30° C for 60 minutes in the presence or absence of alkaline phosphatase (14 U), followed by 2D IEF. Resulting Western blots were probed for HSP 27, and the distribution of HSP 27 was quantitated (shown beneath each spot). N=2 (second experiment gave a similar result using λ protein phosphatase).

Fig 4.

M2 pyruvate kinase is phosphorylated upon δ PKC activation.

A. MCF-7 cells were treated with 500 nM $\psi\delta$ RACK or with control peptide (carrier alone) for 20 minutes, lysed, chromatographed by 2-D IEF gel electrophoresis, and probed with 2B9 anti-phosphoserine antibody. The spot in the dashed circle (upper panel) shifted to lower pI upon PKC activation (solid circle; lower panel).

B. Sequence of human M2 pyruvate kinase; underlined sequences were identified in the trypsin digest fragments from the spot in panel A (dashed circle) by MALDI mass spectroscopy.

C. The blots shown in panel A were stripped and re-probed with an antibody to M2 pyruvate kinase.

Fig 5.

δ PKC and α PKC phosphorylate M2 pyruvate kinase in vitro.

A. Autoradiogram (top panel) showing [32 P] incorporation into purified M2 pyruvate kinase in the absence of PKC (lane 1), in the presence of purified δ PKC and the absence of PKC activators phosphatidylserine and diacylglycerol (lane 2), or in the presence of both PKC activators and increasing amounts of δ PKC (lanes 3-5). The bottom panel shows the same blot probed with an anti-M2 pyruvate kinase antibody.

B. Preferential phosphorylation of M2 pyruvate kinase by δ and α PKC. An in vitro PKC assay was performed as above, using 200 ng of δ PKC, ϵ PKC, or α PKC. Duplicate lanes were loaded on an SDS gel, and the resulting western blot was autoradiographed (top) or probed for M2 pyruvate kinase (bottom). Representative blots of two (A) or three (B) experiments.

Fig 6.

δ PKC interacts with M2 pyruvate kinase in MCF-7 cells.

A. MCF-7 cells treated for 5 minutes with 100 nM PMA (lane 2) or vehicle control (lane 3) were lysed and immunoprecipitated with the anti-M2 pyruvate kinase antibody (lanes 2-3) or with control mouse IgG (lane 1). Blots were probed for δ PKC. Quantitation of δ PKC (relative to IgG control lane) is shown below each lane. n=3 (vehicle treatment) or 2 (PMA treatment). B. The blot from panel A was stripped and re-probed for M2 pyruvate kinase. Quantitation of M2 pyruvate kinase (relative to amount in PMA-treated lane) is shown below each lane. Lane 0 is purified M2 pyruvate kinase serving as control for the western blot. The strong band labeled IgG is the heavy chain of the anti-M2 pyruvate kinase antibody used in the co-immunoprecipitation, which cross-reacts with the secondary antibody in the western blot.

Fig 7.

α PKC and ϵ PKC do not co-immunoprecipitate with M2 pyruvate kinase.

A. MCF-7 cells were lysed and immunoprecipitated with the anti-M2 pyruvate kinase antibody (lane 2) or control mouse IgG (lane 3); lane 1 is input lysate. Shown is a blot stripped and reprobed for α PKC (top panel) and ϵ PKC (bottom panel).

B. MCF-7 cells treated for 5 minutes with 100 nM PMA (lanes 2 and 4) or vehicle control (lanes 1, 3, and 5) were lysed and immunoprecipitated with the anti-M2 pyruvate kinase antibody (lanes 3 and 4) or control mouse IgG (lane 5). Shown is a blot stripped and reprobed for ϵ PKC. Representative of 2 independent experiments.

Fig 8.

No significant effect of δ PKC on in vivo pyruvate kinase activity.

MCF-7 cells were treated as indicated for 20 minutes, lysed, and lysate pyruvate kinase activity was determined. Total pyruvate kinase activity (relative to untreated control) is shown as the mean $\pm$ SD of five independent experiments.

Table 1

Multimeric form distribution of MCF-7 pyruvate kinase activity

M2 pyruvate kinase distribution unchanged following δ PKC activation. MCF-7 cells were treated as indicated and the distribution of M2 pyruvate kinase (expressed as a percentage of total pyruvate kinase activity) between its low activity, dimeric form and its highly active, tetrameric form was determined by size exclusion chromatography. $n = 6 \pm \text{SE}$.

Treatment	Dimeric Form (%)	Tetrameric Form (%)
Control	84 ± 5	16 ± 5
$\psi\delta$ RACK peptide	87 ± 3	13 ± 3