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Translocation of the α- and β-isoforms of protein kinase C following activation of human T-lymphocytes

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We have analyzed how activation of human Jurkat T-cells by the mitogenic lectin, concanavalin A (Con A), may affect the cellular distribution of the α- and β-isoforms of protein kinase C (PKC) in T-cells. In non-stimulated cells almost all of the α- and β-PKC was localized to the cytoplasmic compartment. Stimulation with Con A caused a transient translocation of both α- and β-PKC from the cytoplasm to the cell membrane. The α-isoform appeared to be translocated to a somewhat greater extent and for a longer period of time than the β-form. Translocation was maximal between 1 and 5 min for both of the isoforms. 30 min after stimulation, β-PKC had returned to basal levels, whereas a substantial amount of α-PKC remained associated with the particulate fraction. We conclude that activation of human T-cells causes the translocation of at least two different isoforms of PKC, α-PKC and β-PKC.

Translocation; Isoform; Protein kinase C; T-lymphocyte

1. INTRODUCTION

Protein kinase C (PKC) is known to play a critical role in the process of T-cell activation [1–3]. Specific antigen, monoclonal antibodies or mitogenic lectins all act via the Ti/CD3 receptor complex [4] to stimulate phospholipase C to produce diacylglycerol which activates PKC [5,6]. There is also an increase in inositol triphosphate, leading to elevated levels of free intracellular Ca^{2+}, which act in synergy with diacylglycerol to translocate PKC from the cytosol to the plasma membrane, where it appears in the active state [7].

There are at least seven isoforms of PKC; they exhibit significant homologies, but also distinct differences, both in their biochemical properties and in their tissue distribution [8]. T-cells have so far been shown to contain at least two of these forms, α- and β-PKC (β-PKC consisting of two separate forms, βI and βII) [9,10]. Both the α- and the β-isoforms are translocated following stimulation with phorbol esters or diacylglycerols that directly bind to, and activate PKC [11]. The roles of the different PKCs are still poorly understood, as is the reason why most cells possess more than one type of PKC. In T-cells, it was recently shown that the proportions of α- and β-PKC vary between different cell lines, suggesting the possibility of isoenzyme-specific effects [12]. It is also possible that the isoforms of PKC are selectively activated in response to different stimuli. This was recently demonstrated in HeLa cells, where interferon-γ (which increases diacylglycerol production without affecting intracellular Ca^{2+} levels), but not epidermal growth factor (which increases both), selectively translocates β-PKC but not α-PKC [13].

The present study was carried out in order to elucidate which isoforms of PKC are translocated following stimulation with the mitogenic lectin, Con A, which acts via the Ti/CD3 receptor complex [14].

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; Con A, concanavalin A; PKC, protein kinase C

2. EXPERIMENTAL

2.1. *Cells*

The human leukemic T-cell line, Jurkat, was maintained in RPMI-1640 supplemented with 7.5% foetal calf serum, L-glutamine and penicillin/streptomycin.

2.2. *Chemicals*

PMA, Con A, PHA, biotinylated goat anti-rabbit antibody, alkaline phosphatase-conjugated avidin, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) and Nitro blue tetrazolium (NBT) were from Sigma, St. Louis, USA. The monoclonal anti-CD3 antibody, OKT3, was purified from hybridoma supernatant. The MC5 mouse monoclonal antibody to α/β-PKC was from Amersham (Buckinghamshire, UK). Rabbit antisera to the PKC α- and β-isoforms were kindly donated by Professor Yasutomi Nishizuka (Kobe, Japan). The α-serum is against the synthetic peptide QFVHPILQSAV and the β-serum is against the synthetic peptide FARKGALRQKNVHEVKNHKF.

2.3. *Separation of cytosolic and membrane-bound PKC*

Jurkat cells were stimulated with phorbol ester or Con A at a cell density of 10×10^6/ml at 37°C. Incubations were stopped by rapid centrifugation followed by the addition of ice-cold phosphate-buffered saline (PBS). Cytosolic and membrane-bound PKC were separated essentially as described by Pelech et al. with minor modifications [15]. Briefly, cells were permeabilized for 5 min at 4°C under constant whirl-mixing with 0.5 ml digitonin buffer (20 mM Tris, pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol with

0.025% digitonin). After centrifugation at 10000 × g for 10 min, 0.4 ml of the supernatant, containing cytosolic PKC, was removed. After this, 0.5 ml of Triton buffer (20 mM Tris, pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol with 0.2% Triton X-100) was added to the pellet and samples were whirl-mixed for 30 min at 4°C. After centrifugation for 10 min, 0.4 ml of the supernatant, containing membrane-bound PKC, was removed. The soluble proteins were precipitated with 10% TCA at 4°C for 30 min whereafter the samples were centrifuged for 10 min at 10000 × g. The precipitate was washed with acetone for 30 min. After lyophilization of the precipitate, Laemmli-buffer was added and proteins separated by electrophoresis.

2.4. *Detection of PKC and PKC isoforms*

Cytosolic and membrane samples were run on 7.5% SDS-PAGE under reducing conditions. Proteins were transferred to Immobilon membrane (Millipore, Bedford, MA, USA) and non-specific binding was blocked by incubation of membranes in PBS containing 3% bovine serum albumin and normal mouse serum at 1:100 dilution (not added to the MC5-antibody). After washing of the filters, antibodies were added at 1:300 dilution in PBS/3% bovine serum albumin. Specific binding was detected by using biotin-avidin-alkaline phosphatase with 5-bromo-4-chloro-3-indoyl phosphate (BCIP) and Nitro blue tetrazolium (NBT) as substrate. The blots were densitometrically scanned using a Microcomputer Imaging Device (Imaging Research, Toronto, Canada).

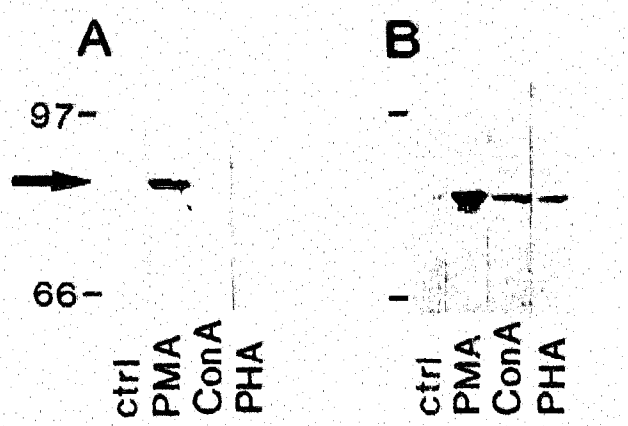

Fig. 1. Translocation of PKC in Jurkat cells. The Ti/CD3 receptor complex was down-regulated on Jurkat cells by overnight incubation with the anti-CD3 antibody, OKT3. CD3⁺ (A) and CD3⁻ (B) cells were then stimulated with PMA (100 nM, 10 min), Con A (20 μg/ml, 5 min) and PHA (20 μg/ml, 5 min) whereafter the cytosolic and membrane fractions were separated and PKC translocation analyzed as described in section 2. Only the membrane fractions are shown. The arrow shows the position of PKC. Molecular mass markers are shown in kDa.

3. RESULTS AND DISCUSSION

Using the MC5 antibody to both the α- and β-forms of PKC, we first analyzed the effect of the phorbol ester PMA on PKC translocation. In Jurkat cells, PMA caused a strong and sustained translocation of PKC (Fig. 1B). This is in agreement with previous findings, both in T-cells and in other systems. Stimulation of Jurkat cells with the mitogenic lectins Con A or PHA, both of which act via phospholipase C (PLC), also caused a readily detectable translocation of PKC (Fig. 1B). Downregulation of the CD3 receptor by incubation with OKT3 antibodies prevented the Con A and PHA stimulated translocation of PKC (Fig. 1A). When this antibody was used, no PKC was found translocated in unstimulated cells if the cells were lysed under calcium-chelating conditions. If the unstimulated cells were first permeabilized in digitonin without calcium-chelators, a substantial amount of PKC was found in the membranes (not shown). The relative effect of Con A or PHA stimulation on the PKC distribution was the same under both lysis conditions. This suggests that both Con A and PHA are able to produce translocation of PKC that exceeds the basal Ca^{2+}-dependent translocation. Since calcium-ionophore A23187 alone does not increase the enzymatic activity of PKC in the membrane fraction of Jurkat cells, as measured by histone phosphorylation [16]; the basal translocation does not necessarily imply activation.

We next analyzed the effects of PMA and Con A stimulation on the translocation of the α- and β-forms of PKC (more specifically, the α- and βI-forms, since Jurkat cells do not contain the βII-form) [10]. A small amount of α-PKC could be seen in the membrane fraction of unstimulated cells whereas no β-PKC was found (Fig. 2A and C). We could also confirm that Jurkat cells contain significantly more α- than β-PKC [10]. PMA caused a strong translocation of both isoforms. There was, however, a significant difference in the amount of PKC remaining in the cytosolic fraction after PMA stimulation. Whereas β-PKC translocation was almost total, a substantial amount of the α-form remained in the cytosol (approximately 30% as measured by densitometric scanning). This may be explained by a difference in the requirement of additional factors, e.g. Ca^{2+}, between the isoforms. Indeed, it has been shown that the α-form is highly sensitive to intracellular Ca^{2+} whereas the β-form is not [8]. When Jurkat cells were stimulated with Con A, both the α- and the β-isoforms of PKC were translocated as shown in Fig. 2A and C. No decrease in the cytosolic fraction was detected, which probably reflects the fact that only a relatively small proportion of the PKC is translocated following activation of these cells. By using densitometric scanning we could estimate the amount of translocated PKC to 25% for the α-form and approximately 10% for the β-form. The translocation was transient for both forms, with changes in the distribution of the β-form occurring somewhat more rapidly. Thus, the α-form peaked at 5 min and had not subsided to basal levels after 30 min, whereas the β-form peaked at 1 min and was down to basal levels within 30 min (Fig. 2B and D). These differences may be related to the differences in diacylglycerol and Ca^{2+} requirement.

Some recent reports have focused on the possible significance of the cellular specificity in the setup of PKC isoforms. The isoforms have different

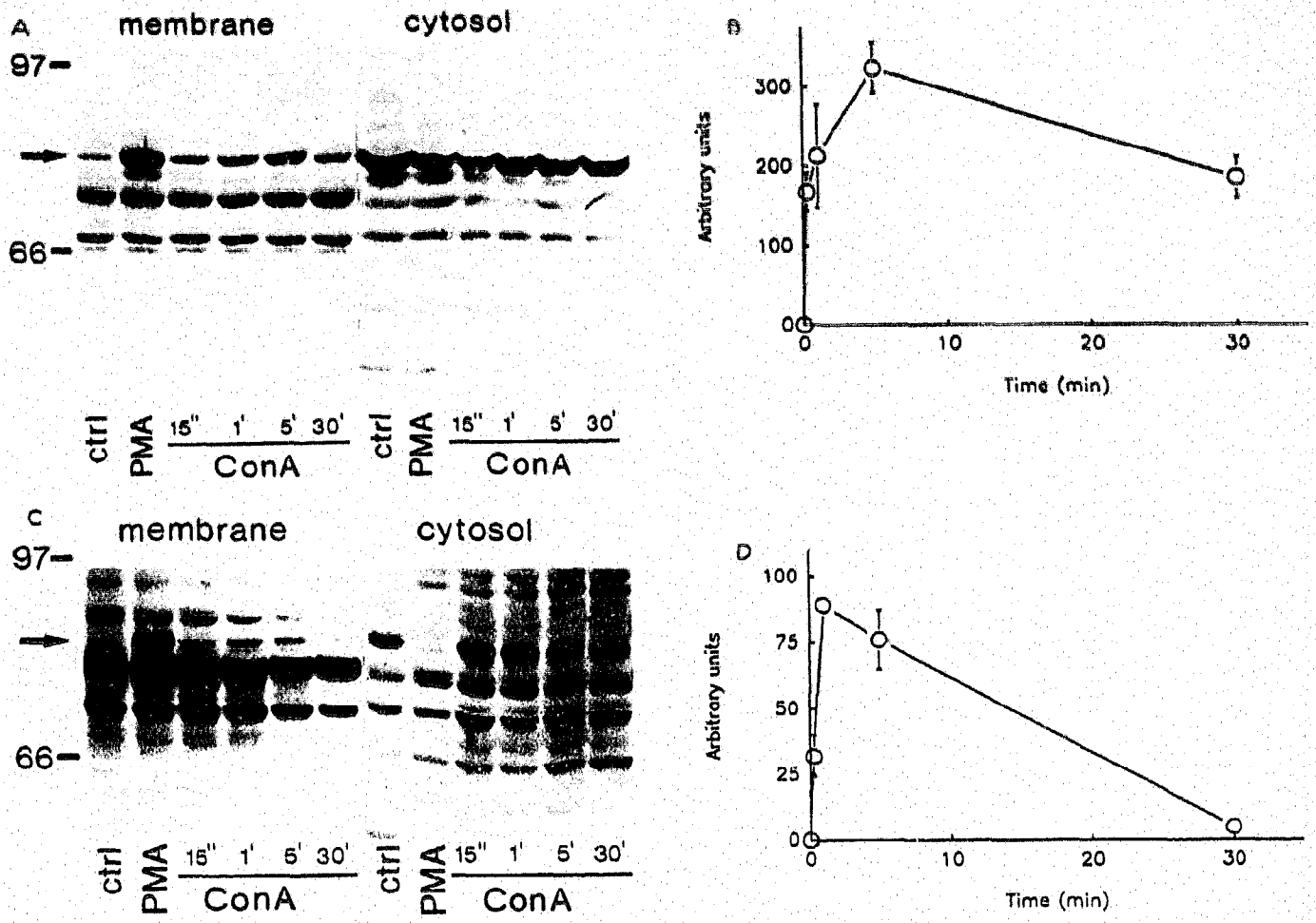

Fig. 2. Translocation of α- and β-PKC in Jurkat cells. Jurkat cells were stimulated with PMA (100 nM, 10 min) and Con A (20 μg/ml). After separation of cytosolic and membrane fractions, translocation of α- (A) and β-PKC (C) was detected as described in section 2. The arrow shows the position of PKC. Molecular weight markers are shown in kDa. Densitometric scanning of three independent experiments was done on α- (B) and β-PKC (D) translocation and the result expressed in arbitrary units ± SE. The intensity of the PKC band was corrected for variations in background staining.

biochemical characteristics [8], and differential activation may occur following stimulation with agonists that activate PKC through different mechanisms, e.g. phospholipase C and phospholipase D. It was recently shown that thyreotropin-releasing hormone, which activates phospholipase C, may translocate both the α- and the β-forms of PKC, thus resembling the present system [17]. However, it was also recently shown that epidermal growth factor, that also activates phospholipase C, does not translocate the β-form of PKC in HeLa cells [13] suggesting that activation of phospholipase C may not always be sufficient to translocate all PKC-isoforms. Another type of isoform-specific PKC action was recently described in HL-60 cells where the βII form of PKC was shown to be translocated to the nuclear membrane (as well as to the plasma membrane) by bryostatin but not by phorbol esters [18]. One possible explanation for the difference in kinetics between the α- and β-forms of PKC in Jurkat cells may be that they are translocated to separate subcellular membranes. Further studies will be required to analyze the possibility of selective translocation of different PKC isoforms in T-cells.

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