ABSTRACT

12-O-Tetradecanoylphorbol-13-acetate (TPA) induced decreases in the catalytic activity and immunoreactivity of protein kinase C (PK-C) in the soluble fraction, accompanied by increases in their activities in the particulate fraction, of a human myeloid leukemia cell line KG-1. TPA also caused a similar down-regulation and translocation of PK-C in KG-1a, a cloned subline shown to be resistant to the differentiating effect of TPA. The activity levels of enzyme in the soluble and particulate fractions from KG-1 cells, however, were about three times higher than those from KG-1a cells. Immunocytochemical studies showed that, when KG-1 cells were treated with 10 nM TPA for 30 min, PK-C was translocated to the plasma membrane in the adherent subpopulation of cells, whereas the enzyme remained largely in the cytoplasm and perinuclear area of the nonadherent cells. TPA, in contrast, caused a PK-C translocation primarily to the perinuclear region in KG-1a cells. Phosphorylation patterns of PK-C substrate proteins in the two cell lines were similar, except that phosphorylation of the M, 33,000 and 97,000 proteins were predominant in KG-1 and KG-1a cells, respectively. The present findings showed existence of certain differential effects of TPA on the PK-C system in the two leukemia cell lines, suggesting a molecular basis for the selective resistance of KG-1a cells to the differentiating action of TPA.

INTRODUCTION

Phorbol esters such as TPA
and PDBu are the most potent tumor promoters (1–3). They also exert pleiotropic actions on various types of cells including proliferation and differentiation (4, 5). The primary site of the actions of phorbol esters appears to be on the cell surface membrane (4, 5). It has been demonstrated that phorbol esters directly activate PK-C, both in vivo and in vitro (6), and that the enzyme is the major receptor for phorbol esters (7–10). In unstimulated cells, PK-C is largely present in the cytosolic (soluble) fraction as an inactive form (11). When TPA or PDBu is added to intact cells, PK-C is found in a form tightly associated with the particulate fraction (12). Translocation of PK-C has been demonstrated in several cell types and is considered to be an early event associated with TPA actions which finally lead to specific biological effects in cells (11–16). Translocation of the enzyme can also be induced by a number of endogenous agonists including interleukin 2 (17), interleukin 3 (18), gonadotropin-releasing hormone (19), thyrotropin-releasing hormone (20, 21), and adrenocorticotropin (22). TPA has been shown to induce terminal differentiation to macrophage-like cells in human promyelocytic leukemia line HL-60 (23, 24) and human acute myelogenous leukemia KG-1 (25).

However, it is ineffective for a HL-60 subline HL-60BII (26) and a KG-1 subline KG-1a (27). The molecular mechanisms underlying the responsiveness or resistance to the differentiating effect of TPA remain unclear. The density and affinity of cell surface receptors for phorbol esters appear to be uninvolved, because there are no significant differences in binding properties of [3H]PDBu between KG-1 and KG-1a cells (28) or between HL-60 and HL-60BII cells (26). We reported previously that TPA induced a rapid, marked, and sustained translocation of PK-C to the plasma membrane, as determined immunocytochemically, immunologically, and by assaying for the PK-C activity in the subcellular fractions (29). In comparison, TPA was found to cause a PK-C translocation to the perinuclear and nuclear structures in leukemia K562 cells and fibroblastic CHO and E7SKS cells (30), all of which are resistant to TPA-induced differentiation. Others also reported an association of PK-C translocation to the particulate fraction with susceptibility of human leukemic cells to TPA-induced differentiation (31).

In the present studies, we investigated in detail the PK-C system in KG-1 and KG-1a cells. We observed certain differences in the two cell lines regarding the levels of PK-C, translocation and down-regulation of PK-C in response to TPA, and phosphorylation of endogenous substrate proteins for PK-C in a cell-free system.

MATERIALS AND METHODS

Materials. Materials used for cell culture were obtained from Gibco (Grand Island, NY); fetal bovine serum was from J. R. Scientific (Woodland, CA); TPA was from LC Services (Woburn, MA); PS (bovine brain), lysine-rich histone (type III-S, calf thymus, corresponding to histone H1), diisopropylphosphorothioate, phenylmethylsulfonyl fluoride, 3,3'-diaminobenzidine tetrahydrochloride and DMSO were from Sigma Chemical C. (St. Louis, MO); Vectastain ABC kit (rabbit IgG) was from Vector Laboratories (Burlingame, CA); formaldehyde (Poly/LEM fixative, histology grade) and glutaraldehyde (8%, electron micrograph grade) were from Polysciences, Inc. (Warrington, PA); Lab-Tek Chamber slides were from Miles Scientific (Naperville, IL); 125I-protein A (>30 ¿¿ei/Mg) was from ICN (Irvine, CA).

Cell Culture. KG-1 and KG-1a cells were cultured in RPMI 1640 supplemented with penicillin G (10 units/ml), streptomycin sulfate (10 µg/ml), L-glutamine (0.3 mg/ml), and either 20% (for KG-1) or 10% (for KG-1a) heat-inactivated fetal bovine serum in a humidified incubator at 37°C in 5% CO2. Cell Adhesion. Adhesion was examined by plating 3 x 10⁴ KG-1 cells in 3 ml of culture medium in each flat glass tissue culture chamber (25). After incubation for 5 to 360 min at 37°C in the presence of DMSO (0.01%) or TPA (10 nM), the slides were washed twice with the warm culture medium, and the nonadherent cells were collected and counted in a Coulter Counter. Numbers of the adherent cells were determined by subtracting numbers of the nonadherent cells from the initial cell numbers plated.

Preparation of Soluble and Particulate Fractions. For PK-C assay, immunoblotting, and phosphorylation of endogenous proteins, 100 ml of cell suspension (10¹⁶/ml) were cultured in fresh medium overnight prior to experiments. The cells were then incubated with 0.01% DMSO
The activity level of the enzyme in KG-1 cells, however, was [36, 37], except for using 8% acrylamide in separating gels. Preparation of soluble and solubilized particulate fractions of cells was carried out as reported previously (29, 32), using a buffer consisted of 25 mM Tris-HCl (pH 7.5), 0.34 mM sucrose, 10 mM MgCl₂, 50 mM 2-mercaptoethanol, 1 mM PMSF, and 2 mM EGTA, without or with 0.3% Triton X-100.

PK-C Assay. The activity of PK-C was assayed as described previously (32, 33). Briefly, the standard reaction mixture contained, in a final volume of 0.2 ml, 5 μmol of piperazine-N,N'-bis(2-ethanesulfonic acid) (pH 6.5); 2 μmol of MgCl₂; 5 μg of PS; 40 μg of lysine-rich histone (histone H1); 0.04 μmol of EGTA; with or without 0.1 μmol of CaCl₂; 1 nmol of [γ-³²P]ATP (containing 0.5 to 1.5 x 10⁶ cpm); and appropriate amounts of the enzyme. All reactions were carried out at 30°C for 5 min and started by the addition of the radioactive ATP.

Immunocytochemical Localization and Immunoblotting of PK-C. Procedures were essentially the same as those described previously for HL-60 cells (29). Briefly, KG-1 and KG-1a cells (2.5 x 10⁶/ml), cultured in fresh medium for 24 h, were incubated with 0.01% DMSO (control) or varying concentrations of TPA (3-200 nM) for various periods of time (0-20 min) by centrifuging cells onto glass slides in a cytospin centrifuge at 700 rpm for 3 min. TPA-induced adherent KG-1 cells on slides were also similarly centrifuged. The cells on the slides were then fixed in 4% formaldehyde/0.1% glutaraldehyde in 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl (Tris-buffered saline) for 30 min at room temperature. The slides were then immersed in 0.2% Triton X-100 in Tris-buffered saline for 8 min followed by 20 min in 1% normal goat serum in Tris-buffered saline to block nonspecific binding sites. The cells were then incubated at 4°C with anti-PK-C antiserum or preimmune serum diluted 1:500. The slides were subsequently rinsed with Tris-buffered saline and stained using immunoperoxidase methods (Vectorstain ABC kit), as described previously (29).

For immunoblotting of PK-C, the soluble and particulate fractions of the two cell lines were prepared as described previously (29, 34), and 100 μg of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting using [125I]-protein A described previously (34). The polyclonal rabbit antiserum to PK-C used in the present studies was produced against the purified PK-C from pig brains. It has been demonstrated to be specific to PK-C (35) and cross-interacting with the M, 80,000 native enzyme as well as its fractions, notably the M, 67,000, 50,000, and 40,000 species, as determined by the immunoreactivity of the purified mono-specific antibodies toward the individual molecular species of the enzyme (34). Preimmune serum was used as controls for immunocytochemistry and immunoblottings; no significant immunoreactivity was observed in all cases.

Endogenous Protein Phosphorylation. Phosphorylation of endogenous proteins (25-75 μg) in soluble and particulate fractions was carried out in a reaction mixture (0.2 ml) containing 5 μmol of piperazine-N,N'-bis(2-ethanesulfonic acid) (pH 6.5); 2 μmol of MgCl₂; 0.04 μmol of EGTA; 2.6 nmol of [γ-³²P]ATP containing 1.5 x 10⁶ cpm; in the presence or absence of 0.042 μmol of CaCl₂; 3 μg of PS; 4 pmol of TPA; and 1.2 μg of pig brain PK-C purified through the Affi-Gel Blue step (35). Initiation or termination of reaction and subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography of phosphoproteins were carried out essentially the same as described (36, 37), except for using 8% acrylamide in separating gels.

RESULTS

Effects of TPA Concentrations on PK-C Translocation and Down-Regulation in KG-1 and KG-1a Cells. Under the unstimulated condition (i.e., in the absence of TPA), the PK-C activity was found to be nearly equally distributed in the soluble and particulate fractions of both KG-1 and KG-1a cells (Fig. 1). The activity level of the enzyme in KG-1 cells, however, was about 3-fold higher than that in KG-1a cells. TPA, at 3, 10, and 30 nm, in 30 min increased the particulate enzyme 20-30 and 4-100% in KG-1 and KG-1a cells, respectively, with a maximal membrane translocation seen at 3 nm for KG-1 cells and 30 nm for KG-1a cells (Fig. 1). The particulate enzyme decreased to the control levels at 200 nm for both cell lines. The soluble PK-C, on the other hand, progressively decreased as a function of TPA concentration in both cell lines, with a complete depletion seen at 200 nm for KG-1 cells but at a much lower concentration of 10 nm for KG-1a cells.

The time-dependent effects of a low concentration (20 nm) of TPA on the PK-C activity were examined (Fig. 2). There was little or no changes in the enzyme levels in the soluble and particulate fractions of both cell lines in the absence of TPA for the entire 1440-min incubation period. In the presence of 20 nm TPA, however, the particulate PK-C in KG-1 cells increased 20% in 5 min, returned to the control level in 120 min, and reached a value lower than the control value in 1440 min. Similar effects of TPA were also seen for KG-1a cells, except that the increase in the enzyme activity was higher (60-70%) and the duration of the increase was longer (up to 30 min). Decrease in the soluble enzyme in both cell lines was rapid, reaching a maximum in 10-30 min. At a higher concentration (200 nm) of TPA, little or no increase in the particulate enzyme was observed in both cell lines, but the decrease in the soluble enzyme was faster and more pronounced (Fig. 3). It seemed worth noting that a complete depletion of the soluble PK-C in KG-1 cells was achieved by TPA at 200 nm (Fig. 3) but not at 20 nm (Fig. 2); this, however, was noted in KG-1a cells at both TPA concentrations (Figs. 2 and 3).

Immunoblottings of PK-C were performed in order to reexamine the findings shown in Figs. 1-3. The M, 80,000 immunoreactivity (i.e., the native PK-C) in the soluble and particulate fractions from KG-1 cells was found to be comparable (Fig. 4). The similar subcellular distribution of the immunoreactivity...
PROTEIN KINASE C SYSTEM IN KG-1 AND KG-1a

Fig. 2. Time-dependent changes in PK-C activity in the soluble and particulate fractions of KG-1 and KG-1a cells incubated with a low concentration (20 nM) of TPA. PK-C activity in the soluble (S) and particulate (P) fractions of (A) KG-1 and (B) KG-1a cells was assayed after the cells were incubated with DMSO (0.01%) or TPA (20 nM) for various periods of time, as indicated. Points, means of three determinations; bars, SE. *, significantly different from the zero time samples (P < 0.01).

Fig. 3. Time-dependent changes in PK-C activity in the soluble (S) and particulate (P) fractions of (A) KG-1 and (B) KG-1a cells incubated with a high concentration (200 nM) of TPA. Experimental conditions and data presentations were as in Fig. 2 except that the cells were incubated with 200 nM TPA.

was also seen for KG-1a cells, except that the immunostaining was lower than that seen for KG-1 cells (Fig. 4). Upon treatment with 200 nM TPA for 30 min, we found that the M, 80,000 immunoreactivity was greatly reduced in the soluble fraction, accompanied by little or no change in the particulate fraction, of both cell lines (Fig. 4). The immunoreactive species with lower molecular weights were probably the fragments of the M, 80,000 PK-C (35).

Differentiation-related Subcellular Distribution of PK-C. About 30% of KG-1 cells were induced to differentiate (as indicated by cell adhesion) by incubation with 10 nM TPA for 30 min (Table 1). Longer treatments (up to 360 min) did not increase the proportion of the cells to commit to differentiation. KG-1a cells, in contrast, did not respond to the TPA effect (data not shown). The present findings confirmed those originally reported by Koehler et al. (25, 28). In all studies presented thus far, the catalytic activity and immunoreactivity of PK-C were analyzed using the entire cell population without addressing the problems in the adherent and nonadherent subpopulations of the cells after TPA treatment. Studies were conducted in order to define potential changes in the subcellular distribution of PK-C in these two cell subpopulations. We now observed that, after KG-1 cells were treated with 10 nM TPA for 30 min, the soluble PK-C was greatly reduced to a similar extent in both cell subpopulations (Table 2). The particulate PK-C was also decreased in both but, interestingly, the decrease in the adherent cells was much smaller than that in the nonadherent cells. Similar results were found for KG-1 cells treated for 30 min with a higher concentration (100 nM) of TPA (Table 2), suggesting a functional role for the particulate enzyme in cell adhesion and differentiation.

Immunocytochemistry of PK-C. Translocation of PK-C demonstrated in Figs. 1–3 was further investigated immunocytochemically. TPA (30 nM) caused a translocation of the enzyme to the plasma membrane in 20–30% of KG-1 cells in 30 min (Fig. 5). The effects, however, appeared to be less obvious at a lower concentration (3 nM) of TPA. The remaining population appeared to be a mixture of cells in which PK-C was already down-regulated or the response to TPA had yet to occur. PK-C translocation to the plasma membrane was also observed in

Fig. 4. Immunoblots of PK-C in KG-1 and KG-1a cells incubated with or without TPA. The cells were incubated for 30 min under the conditions as indicated. The amount of protein in both soluble (S) and particulate (P) fractions used for the determinations was 100 μg. See “Materials and Methods” for further experimental details. K, molecular weight in thousands.

Table 1 Adhesion of KG-1 cells treated with TPA

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>DMSO</th>
<th>TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adhesion (%)</td>
<td>Viability (%)</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.01</td>
<td>95</td>
</tr>
<tr>
<td>30</td>
<td>&lt;0.01</td>
<td>ND</td>
</tr>
<tr>
<td>120</td>
<td>&lt;0.01</td>
<td>ND</td>
</tr>
<tr>
<td>360</td>
<td>&lt;0.01</td>
<td>97</td>
</tr>
</tbody>
</table>

* ND, not determined.

Table 2 Subcellular Distribution of PK-C

KG-1 KG-1a

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Soluble (S)</th>
<th>Particulate (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>30</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>120</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>360</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 5. Immunocytochemistry of PK-C in KG-1 and KG-1a cells incubated with TPA. The cells were incubated for 30 min under the conditions as indicated. The amount of protein in both soluble (S) and particulate (P) fractions used for the determinations was 100 μg. See “Materials and Methods” for further experimental details. K, molecular weight in thousands.

KG-1 KG-1a

0.01% 200nM 0.01% 200nM
DMSO TPA DMSO TPA
S P S P S P S P

80K−
Table 2: PK-C activity of the soluble and particulate fractions of adherent and nonadherent KG-1 cells

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cell</th>
<th>Fraction</th>
<th>PK-C activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>Nonadherent</td>
<td>Soluble</td>
<td>148 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particulate</td>
<td>210 ± 12</td>
</tr>
<tr>
<td>TPA (10 nM)</td>
<td>Adherent</td>
<td>Soluble</td>
<td>43 ± 4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particulate</td>
<td>159 ± 4</td>
</tr>
<tr>
<td></td>
<td>Nonadherent</td>
<td>Soluble</td>
<td>32 ± 2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particulate</td>
<td>119 ± 6*</td>
</tr>
<tr>
<td>TPA (100 nM)</td>
<td>Adherent</td>
<td>Soluble</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particulate</td>
<td>176 ± 6</td>
</tr>
<tr>
<td></td>
<td>Nonadherent</td>
<td>Soluble</td>
<td>3 ± 3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particulate</td>
<td>98 ± 4*</td>
</tr>
</tbody>
</table>

* Significantly different from the DMSO control (P < 0.01).
* Significantly different from the adherent cells (P < 0.01).

KG-1 cells treated with higher concentrations (up to 200 nM) of TPA (data not shown).

In contrast, PK-C was found to localize in the perinuclear region (probably the Golgi apparatus and endoplasmic reticulum) in almost all cells of TPA-resistant subline KG-1a exposed to low concentrations (3 or 30 nM) of TPA (Fig. 5). This pattern of immunostaining was essentially the same as that of the control cells, although the TPA-treated cells exhibited a decreased cytoplasmic staining compared to the control cells, probably reflecting the down-regulation and/or translocation of cytoplasmic PK-C (Fig. 5). KG-1a cells, however, exhibited some plasma membrane translocation of PK-C when incubated with high concentrations (100–200 nM) of TPA (data not shown). Therefore, it seems that KG-1a cells were relatively resistant to TPA with respect to PK-C translocation to the plasma membrane, and this relative unresponsiveness was partially overcome by higher TPA concentrations.

Taking advantage of the fact that KG-1 cells become adherent when the cells have committed to differentiate, we investigated PK-C localization in the adherent and nonadherent subpopulations of the cells after KG-1 cells were incubated with 10 nM TPA for 30 min. We found that the enzyme appeared to localize in the plasma membrane of the adherent cells, whereas it was localized primarily in the perinuclear region of the nonadherent cells (Fig. 6). It should be noted that the adherent cells were smaller and more rigid than the nonadherent cells. In comparison, the immunostaining was found mainly in the cytoplasm of control cells treated with DMSO (0.01%) for 0 or 30 min or treated with TPA (10 nM) for 0 min (Fig. 6). The cells incubated with preimmune serum showed only a slight staining (Fig. 6).

Phosphorylation of Endogenous Proteins. Phosphorylation patterns of endogenous substrate proteins for PK-C were investigated in the soluble and particulate fractions of both cell lines preincubated with DMSO (0.01%) or TPA (200 nM) for 30 min. The soluble fraction of KG-1 cells treated with DMSO (control) revealed several major substrates (M, 97,000, 55,000, 50,000, and 19,000) and minor substrates (M, 81,000, 40,000, 33,000, and 24,000) (Fig. 7). Phosphorylation of these substrates was decreased in the soluble fraction of KG-1 cells treated with TPA but was partially restored by adding exogenous PK-C to the reaction mixture (Fig. 7). Phosphorylation of these substrate proteins in the particulate fraction from the TPA-treated cells appeared to be greater than that seen in the same fraction from the control cells, but addition of exogenous PK-C had practically no effect on phosphorylation of these...
proteins (Fig. 7). These findings were consistent with the data shown in Figs. 1–3 for KG-1 cells that TPA down-regulated PK-C in the soluble fraction which was accompanied by a slight increase in the particulate enzyme under the incubation conditions.

Phosphorylation patterns in the soluble fraction from the control KG-1a cells were similar to those shown earlier for KG-1 cells in Fig. 7, except that phosphorylation of the M, 97,000 protein was high whereas that of the M, 33,000 protein was low in KG-1a cells (Fig. 8). The phosphorylation patterns of the soluble fraction from the control KG-1a cells (Fig. 8) were found to be very similar to the particulate fraction of the same cells (Fig. 8) as well as to the particulate fraction of the control KG-1 cells shown in Fig. 7. As in KG-1 cells, phosphorylation of substrate proteins, especially the minor substrates (e.g., M, 40,000 and 33,000), was reduced slightly in the soluble fraction but slightly increased in the particulate fraction of KG-1a cells treated with TPA (Fig. 8). Addition of exogenous PK-C appeared to partially restore phosphorylation of these substrate proteins in the soluble fraction of the TPA-treated KG-1a cells (Fig. 8).

DISCUSSION

The present studies showed that TPA caused, in a dose- and time-dependent manner, a down-regulation of PK-C in the soluble fraction and an accompanying translocation of the enzyme to the particulate fraction in KG-1 cells. Immunocytochemical studies revealed that PK-C immunostaining was largely associated with the plasma membrane of the subpopulation of the cells which were responding to TPA and became adherent. In another subpopulation of the cells which were yet to respond to TPA and stayed nonadherent, however, the enzyme remained largely cytoplasmic and was associated with the perinuclear area, as in the unstimulated, control cells. The findings seemed to provide direct evidence to support a close relationship between the plasma membrane translocation of PK-C and cell adhesion, an initial event characteristic of the cells to commit to differentiate in response to TPA. A down-regulation of PK-C in the soluble fraction and its translocation to the particulate fraction, similar to KG-1 cells, were also noted for the TPA-resistant KG-1a cells treated with TPA. The apparent similarity between the two cell lines, however, ended here; immunocytochemical evidence showed that PK-C was translocated primarily to the perinuclear region in KG-1a cells after the TPA treatment. The present findings were consistent with our reports that TPA induced an “outward” redistribution of PK-C to the plasma membrane in TPA-responsive HL60 cells (29, 30), but an “inward” translocation of the enzyme to the perinuclear region or the nucleus in various cell types which are resistant to the differentiating effects of TPA, exemplified by leukemic K562 cells and fibroblastic CHO and E7SKS cells (30). However, it should be noted that our immunocytochemical studies rely on the recognition of PK-C by antibodies developed against purified PK-C (35). Thus, the observed localization of PK-C in various parts of cells may represent only certain types of the isozymes as demonstrated (38). Forsbeck et al. (39) and Wickremasinghe et al. (40) have reported that TPA produced a down-regulation and a translocation of PK-C in malignant hematopoietic cells sensitive or resistant to TPA. Homma et al. (31), on the other hand, have shown that the TPA-induced PK-C translocation and down-regulation did not occur in the
TPA-resistant variants of HL60 cells. In this respect, it is of interest to identify immunocytochemically the intracellular locations or structures of the "particulate" fraction to which PK-C has been shown to translocate. Blackshear et al. (41) have reported that TPA markedly down-regulated PK-C in the soluble fraction in 3T3-L1 cells. Down-regulation or depletion of soluble (cytosolic) PK-C probably is a common adaptive reaction of many cell types to TPA irrespective of the responsiveness of the cells to the differentiating effect of TPA. It appeared, therefore, that the directional intracellular translocations of PK-C might be a potential determinant for, or an event closely related to, the TPA-dependent cell differentiation. The membrane translocation of PK-C has been shown to be mediated by various agonists (16–22). However, further studies are required to determine whether the perinuclear or nuclear translocation of the enzyme is mediated by the endogenous signal transduction systems in KG-1a (this study) or in K562, CHO, and E7SKS cells (30) or is merely due to the structure of TPA and its consequential compartmentalization.

It seemed worth noting that the endogenous substrate proteins for PK-C in KG-1 and KG-1a cells were similar but distinct. Notably, the M, 97,000 proteins was predominant in KG-1a cells, whereas the M, 33,000 protein and other low molecular weight species were abundant in KG-1 cells. Because TPA appeared to be fully effective in down-regulating and translocating PK-C in both cell lines, the presumed molecular "defects" responsible for the TPA resistance in KG-1a cells might reside on steps beyond TPA interactions with the membrane receptors. It was attractive to speculate that altered substrate proteins in KG-1a cells might be functionally significant. In this regard, the M, 33,000 protein might be a "repressor" for this process. We observed recently that the TPA-stimulated phosphorylation of protein A (Mr 76,000; pl 5.1) was greater in KG-1a cells than that in KG-1 cells, whereas the opposite was observed for block I proteins (Mr 60,000–64,000; pl 5.45–5.65) (42).

The observed difference in the directions of PK-C translocation and substrate proteins might constitute a molecular basis for the TPA resistance characteristic of KG-1a cells.

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**Fig. 7.** Phosphorylation of endogenous proteins in the soluble and particulate fractions of M.II cells incubated with or without TPA. The cells were incubated with 0.01% DMSO or 200 nM TPA for 30 min. Preparation of soluble and particulate fractions (Fr.) and phosphorylation of endogenous proteins were as described in "Materials and Methods." When present, the additions were CaCl2 (10 mM), phosphatidylserine (PS, 3 µg/0.2 ml), TPA (20 nM), and the purified pig brain PK-C (1.2 µg). M, molecular weight in thousands.
**Fig. 8.** Phosphorylation of endogenous proteins in the soluble and particulate fractions of KG-1a cells treated with or without TPA. The conditions and procedures employed were as in Fig. 7 and “Materials and Methods.”

**REFERENCES**


Effects of Phorbol Ester on Translocation and Down-Regulation of Protein Kinase C and Phosphorylation of Endogenous Proteins in Human Acute Myeloid Leukemia Cell Line KG-1 and Its Phorbol Ester-resistant Subline KG-1a


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