Translocation of Protein Phosphatase 1 Catalytic Subunits during 1,25-Dihydroxyvitamin D3-induced Monocytic Differentiation of HL-60 Cells

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ABSTRACT

To elucidate the roles of protein phosphatases type 1 (PP1) and type 2A (PP2A) in 1,25-dihydroxycholecalciferol [1,25(OH)2D3]-induced differentiation of HL-60 cells into monocytes, we examined the enzyme activity and the protein and gene expressions of PP1 and PP2A in these cells. Calyculin-A augmented the 1,25(OH)2D3-induced differentiation of the cells. Treatment of the cells with 1,25(OH)2D3 led to a decrease in PP1-like activity in the cytosol fraction, with a concomitant increase in the membrane and nuclear PP1-like activity, as determined when protein phosphatase activity was assayed using myosin light chain as substrate in the presence of 5 nM okadaic acid. Western blot analysis with antibodies specific for PP1 catalytic subunit isoforms (PP1α, PP1γ, and PP1δ) showed that all three PP1 isozymes were expressed but were differentially distributed in each cellular fraction. Subcellular redistribution of PP1-like activity during 1,25(OH)2D3-induced differentiation was mainly attributed to PP1γ and PP1α proteins. In contrast, the localization of PP1β and PP2A catalytic and regulatory subunits were not significantly affected by 1,25(OH)2D3 treatment. The gene expressions of PP1α and PP1γ appeared to be constant during processes of monocytic differentiation. The correlation between phenotypic and functional changes of HL-60 cells on the one hand and subcellular redistribution of PP1-like activity on the other suggest that the translocations of PP1α and PP1γ isozymes may contribute to the 1,25(OH)2D3-induced monocytic differentiation of HL-60 cells.

INTRODUCTION

HL-60 cells differentiate along the granulocytic or the monocytic pathway when treated with the respective inducer molecules (1). This characteristic feature of HL-60 cells attracted researchers’ interest, and this cell line has become a frequently described model system for cell differentiation in vitro (1).

1,25(OH)2D3 induces human myelogenous leukemic HL-60 cells, as well as normal human bone marrow cells, to differentiate terminal into mature monocytes (1, 2). Differentiation with this agent is coupled to inhibition of proliferation. The intracellular mechanisms leading to terminal differentiation have yet to be clarified (2). Because retinoic acid has proved to be effective in differentiation therapy of acute promyelocytic leukemia, analogues of 1,25(OH)2D3 also are currently being investigated as putative differentiation-inducing agents to treat patients with leukemia (3).

Kinases and phosphatases modulate the protein phosphorylation "status" of a cell, which in turn directly regulates numerous fundamental biological phenomenon such as cell division and development (4, 5). Accordingly, protein phosphatases may also be involved in the process of HL-60 cell differentiation (6). The major serine/threonine protein phosphatase catalytic subunits of mammalian cells comprise four forms which have been designated as PP1, PP2A, PP2B (calcineurin), and PP2C, based on a classification system proposed by Cohen (7) and Mumby and Walter (8). PP1 and PP2A do not have an absolute requirement for divalent cations, whereas protein phosphatases PP2B and PP2C are Ca2+/calmodulin- and Mg2+-dependent, respectively. OKA, a recently discovered tumor promoter, is a polyehter fatty acid with IC50 of 60–200 nM for PP1 and 1–10 nM for PP2A (9); therefore, it can distinguish between PP1 and PP 2A. Cal-A is a spiroroketal fatty acid with IC50 of 2 nM for PP1 and 1 nM for PP2A (10). While the regulatory subunits of PP2A have been purified and cloned, much less is known of the regulatory subunits of PP1. Three different but highly similar isoforms of PP1 catalytic subunits (PP1α, PP1γ, and PP1δ) with an 85–95% amino acid sequence identity have been identified by cDNA cloning, and their mRNAs are expressed in a wide range of mammalian tissues (11). PP1 isoforms have been reported to be identified in both soluble and particulate cellular fractions and in various forms (12). The glycosgen-associated form of PP1, termed PP1G, consists of the catalytic subunit complexed to a M, 124,000 G subunit which targets PP1 to glyco GN particles (13). Recently, a myosin-bound PP1 holoenzyme in smooth muscle cells was isolated and was found to consist of a catalytic subunit, M, 130,000 and M, 20,000 regulatory subunits (13). In leukemic cells, including HL-60 cells, the regulation of PP1 is not well understood. PP1 and PP2A are involved not only in glycercol metabolism and muscle contraction but also regulate processes such as the cell division cycle (13). We reported that OKA and Cal-A augmented the granulocytic differentiation of HL-60 cells induced by retinoic acid (14), and we also found that retinoic acid induces down-regulation of the PP2A catalytic subunit associated with granulocytic differentiation (15–17). To further elucidate the involvement of protein phosphatases in HL-60 cell differentiation, we investigated changes in phosphatase activity and expression patterns of PP1 and PP2A proteins and mRNA expressions of PP1 isozymes during the monocytic differentiation induced by 1,25(OH)2D3.

MATERIALS AND METHODS

Reagents. 1,25(OH)2D3 purchased from Solvay-Duphar B.V. (Weesp, the Netherlands) was dissolved in ethanol (10−3 m) and stored at −20°C with protection from light. CAL-A and OKA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Determination of Cell Differentiation. Procedures used for the maintenance of HL-60 cells and determination of viable cell counts were done as described elsewhere (18, 19). The extent of differentiation was assessed by morphology, the ability to produce superoxide as monitored by the reduction of NBT (Sigma Chemical Co.), nonspecific esterase positivity, and surface antigen analysis. For morphological assessment, cytosin was prepared and stained with May-Giemsa. Differential cell counting was done on 200 to 400 stained cells from at least two preparations for each experimental point. Surface antigens were examined by flowcytometry in a FACSscan (Becton Dickinson, Mountain View, CA) using monoclonal antibodies, including...
CD11b (OKM1; Ortho Diagnostic System, Inc., Raritan, NJ), CD11c (LeuM5; Becton Dickinson), and CD14 (My4; Coulter Immunology, Hialeah, FL).

Preparation of Cellular Fractions. HL-60 cells (1 × 10⁶ cells for each sample) were harvested by centrifugation and washed twice with ice-cold Tris-HCl-buffered saline, pH 7.4. All preparative procedures were carried out at 4°C; then cells were homogenized in a buffer containing 20 mM Tris-HCl (pH 7.4), 2 mM DTT, 2 mM EDTA, 2 mM EGTA, 0.25% sucrose, and a cocktail of protease inhibitors (18) by a glass-to-glass Potter-Elvehjem homogenizer. The homogenates were immediately centrifuged at 1,000 × g for 10 min, and the resulting pellet was used as the crude nuclear fraction. The supernatant was collected and centrifuged at 100,000 × g for 1 h to separate cytosol and membrane fractions. The membrane and nuclear fractions were rinsed with the homogenization buffer and subsequently resuspended in homogenization buffer followed by glass-to-glass homogenization. By further centrifugation at 100,000 × g for 30 min, the membrane fraction was obtained.

Measurement of Phosphatase Activity. 32P-phosphorylated myosin light chains were used as substrates of the phosphatase assay because they are good substrates for both mammalian PP1 and PP2A (14). Phosphatase activity was determined by the liberation of 32P from the substrate (32P-labeled M₉, 20,000 myosin light chain) at 30°C, according to the methods of Pato and Kerc (20). The extent of dephosphorylation was restricted to below 10%. Under these conditions, rates of dephosphorylation were linear with respect to time and enzyme dilution.

Immunoblot Analysis. Antisera against PP1 catalytic subunit isoforms, PP2A-C, A, and B regulatory subunits were obtained by immunizing a rabbit with the synthesized fragments of PP1 (11) or PP2A (21, 22). HL-60 cells were treated with 1,25(OH)₂D₃ (10⁻¹⁰ to 10⁻⁷ M), and the membrane fractions were used as described and subjected to SDS-PAGE using a 12.5% running gel followed by electrophoretic transfer onto Immobilon polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was reacted with specific antibodies for PP1 and PP2A, and immunoreactive proteins were stained using the avidin-biotin peroxidase complex method (Vector Laboratories, Burlingame, CA). Prestained SDS-PAGE standards (Bio-Rad, Richmond, CA) were used as molecular weight standards. Quantitative estimation of the level of PP1 isozymes and PP2A-C was carried out densitometrically with a Molecular Dynamics scanning densitometer (Sunnyvale, CA) in conjunction with the ImageQuant program run on a Dell Personal Computer (Austin, TX) by scanning the immunoreactive band after immediately photographing the visualized band. The area of an individual peak was measured above background in densitometric tracings and was expressed as arbitrary unit (count). The signal was then compared with that of known amounts of the purified recombinant PP1α, PP1γ, PP18, and PP2A-C. Routinely, at least four different amounts of each enzyme were included as standards to quantify the level of each enzyme. The immunoreactive signals determined by this method were proportional to increasing amounts of each enzyme (Fig. 1). If the signal of the sample was off the limit of linearity shown in Fig. 1, the loaded quantity of the sample was adjusted until a signal in the linear range was obtained. The level of PP1 isozymes and PP2A-C were estimated accordingly (Table 1).

RNA Isolation and Northern Blot Analysis. Total cellular RNA was extracted using the guanidium isothiocyanate technique (23). Twenty μg of total RNA of HL-60 cells was electrophoresed through 0.8% agarose with 18% formaldehyde and transferred to Nytran membranes (Schleicher and Schuell, Keene, NH). After baking at 95°C for 2 h under vacuum, blots were hybridized at 42°C in 50% formamide, 5X Denhardt’s solution, 5X SSPE (1X SSPE = 0.15 M NaCl, 10 mM NaH₂PO₄, and 0.1 mM EDTA), 0.5% SDS, 200 μg/ml of denatured salmon sperm DNA, and 32P-labeled cDNA probe. The probes used were the cDNA probes of the rat PP1α and PP1γ (12, 22, 24). They were labeled with [α-32P]dCTP using a multiprime labeling system kit (Amersham, Buckinghamshire, England). Hybridized blots were finally washed with 0.1X SSC, (1X SSC = 0.15 M NaCl-15 mM trisodium citrate) and 0.5% SDS at room temperature and autoradiographed.

RESULTS

Effect of Cal-A on 1,25(OH)₂D₃-induced Cell Differentiation. 1,25(OH)₂D₃ suppressed the proliferation of HL-60 cells and induced these cells to differentiate terminally into monocytic cells. Morphological examination of May-Giemsa stained cytospin preparations showed that untreated HL-60 cells retained a blast-like phenotype throughout all of the experiments, whereas the 1,25(OH)₂D₃-treated cells gradually acquired a differentiated pattern indicated by surface markers (CD11b, CD11c, and CD14), ability to reduce NBT, and nonspecific esterase positivity after 4 days of treatment (Fig. 2). Treatment of HL-60 cells with 1,25(OH)₂D₃ resulted in a time-dependent increase in NBT-positive and nonspecific esterase-positive cells and an increase in reactivities of the cells with CD11b, CD11c, and CD14 (Fig. 2), although the kinetics of each differentiation marker acquisition was not always identical. The reactivities of HL-60 cells with CD11b and CD14 reached a plateau more rapidly than that of CD11c. The reduction of NBT and the positivity of nonspecific esterase provide functional markers for monocytic differentiation of HL-60 cells. Cal-A at concentrations of 1 nM or lower had no effect on proliferation (14). As shown in Fig. 3, the addition of Cal-A in combination with 1,25(OH)₂D₃ led to further maturation when compared to findings with Cal-A alone. Cal-A alone led to no increase in the numbers of NBT- and nonspecific esterase-positive cells. 1,25(OH)₂D₃-induced differentiation was dose dependent at concentrations of 1.25(OH)₂D₃ from 2 to 100 nM. Exposure to Cal-A shifted the dose-response curve by 1.25(OH)₂D₃ of monocytic differentiation of HL-60 cells to the left. However, Cal-A did not facilitate the differentiation induced by 100 nM 1,25(OH)₂D₃. The addition of OKA in combination with 1,25(OH)₂D₃ did not augment the 1,25(OH)₂D₃-
induced differentiation, and higher concentrations of OKA could not be applied in cultures because of toxicity (data not shown).

**Phosphatase Activity during 1,25(OH)_2D_3-Induced Monocytic Differentiation.** The activity of serine/threonine protein phosphatase in HL-60 cells was assayed in the absence of divalent cations, using 32P-phosphorylated MLC of smooth muscle as substrate for both mammalian PP1 and PP2A (15). Alteration of phosphatase activity was examined in cytosolic, nuclear, and membrane fractions following treatment of 1,25(OH)_2D_3 (Fig. 4). Table 1 shows protein phosphatase activities of cytosol, nuclear and membrane fractions, in untreated HL-60 cells. In HL-60 cells, approximately 60–65% of total activity of MLC phosphatase was present in the cytosol fraction. The exposure of exponentially growing cells to 1,25(OH)_2D_3 resulted in a gradual decrease in cytosolic phosphatase activity, and the activity reached the lowest level (approx. 40% decrease relative to that in untreated HL-60 cells) 4 days later. Concomitant with the decrease in the cytosolic MLC phosphatase activity in these 1,25(OH)_2D_3-treated HL-60 cells, there was a significant increase in this activity in both the nuclear and membrane fractions (Fig. 4). Treatment with 20 nM 1,25(OH)_2D_3 for 4 days resulted in 1.8- and 1.9-fold increases in the nuclear and membrane fractions, respectively. These data suggest that most of the lost activity can be accounted for by association with the nuclear and membrane activity during the 1,25(OH)_2D_3-induced differentiation. A decrease in phosphatase activity appeared to coincide with increases in the positivity of the surface marker CD11c and the nonspecific esterase positivity.

To elucidate which type of phosphatase is associated with the decrease of MLC phosphatase activity in the cytosol of HL-60 cells after treatment of 1,25(OH)_2D_3, inhibition assay with 5 nM OKA was also done (Fig. 4). While PP2A is inhibited mostly by 5 nM OKA, PP1 is almost not affected at this 5 nM concentration (9). The influence of PP2B and PP2C which absolutely require divalent cations (8) was little, since a sufficient amount of EGTA was present in the MLC phosphatase assay mixture. MLC phosphatase activity in the presence of 5 nM OKA is thought to be mostly PP1-like activity. The phosphatase activity, subtracting the PP1-like activity from total MLC phosphatase activity is thought to be PP2A-like activity. Fig. 4 shows that 60% of the phosphatase activity in the cytosol fraction, 46% in the
nuclear fraction, and 98% in the membrane fraction, respectively, show PP1-like activity in untreated HL-60 cells. There appears to be very little PP2A-like activity in the membrane fraction of HL-60 cells. Fig. 4 indicates that the PP1-like activity was gradually decreased in the cytosol fraction and increased in the nuclear and membrane fractions during 1,25(OH)_{2}D_{3}-induced differentiation of HL-60 cells. On the other hand, PP2A-like activity was relatively constant in the cytosol and nuclear fractions during 4 days after addition of 1,25(OH)_{2}D_{3}. These data suggest that PP1-like activity can translocate from the cytosol to the nuclear and membrane fractions after treatment of HL-60 cells with 1,25(OH)_{2}D_{3}.

Expressions of PP1 Isozymes and PP2A during 1,25(OH)_{2}D_{3}-induced Differentiation of HL-60 Cells. To further clarify the selective decrease in PP1-like activity during 1,25(OH)_{2}D_{3}-induced differentiation, protein expressions of PP1 isozymes and PP2A in cellular fractions were investigated by Western blot analysis using polyclonal antibodies specific against PP1 isozyme catalytic subunits (PP1α, PP1γ, and PP1β) and PP2A-C, A-, and B-regulatory subunits of PP2A. As shown in Fig. 5, the antibodies against PP1α, PP1γ, and PP1β isozymes detected major immunoreactive bands with Mr 36,000, Mr 36,000, and Mr 37,000, respectively, in each cellular fraction of HL-60 cells. PP2A-C was detected as a major single band of Mr 35,000, the A-regulatory subunit as a major band of Mr 65,000, and the Bα-regulatory subunit as a major band of Mr 55,000 (Fig. 6). Bβ- and B′-regulatory subunits of PP2A proteins were not detectable in HL-60 cells. These results obtained were even better illustrated by densitometer scanning as shown in Fig. 5B and Fig. 6B. We determined the protein amounts of PP1 catalytic subunits and PP2A-C in the cytosol, nuclear and membrane fractions of HL-60 cells, using purified recombinant proteins as standards (Table 1). We could not estimate the amounts of A- and Bα-regulatory subunits because of not having the purified subunits. We found that each isozyme of PP1 catalytic subunits was distributed differentially in subcellular fractions of HL-60 cells, although all isoforms were present in each cellular fraction. The nuclear fraction mainly contained PP1γ, whereas the membrane fraction contained mostly PP1α. PP1β was a minor isotype in all cellular fractions. Most of PP2A-C was detected in the cytosol fraction. The addition of 1,25(OH)_{2}D_{3} led to a dramatic decrease in the levels of PP1γ and PP1α in cytosol fraction, with a concomitant increase of PP1γ and PP1α in nuclear and membrane fractions. After 4 days of incubation with 1,25(OH)_{2}D_{3}, approximately 90% of cytosol PP1γ had been redistributed to both the nuclear and membrane fractions. The doublet in nuclear fractions for PP1γ and PP1α might be proteolytic degradation. In comparison, the decrease in cytosol PP1α protein was slower than that of PP1γ, and PP1α appeared to be redistributed mostly to the membrane fraction. Thus, treatment of HL-60 cells with 1,25(OH)_{2}D_{3} led to a redistribution of PP1 isozymes from cytosol to nuclear and membrane fractions, and each PP1 isozyme appeared to be differentially translocated during the 1,25(OH)_{2}D_{3}-induced differentiation. The distribution of PP2A-C, A-, and Bα-regulatory subunits of PP2A among the subcellular fractions were unchanged, consistent with the finding that PP2A-like activity is unchanged during 1,25(OH)_{2}D_{3}-induced differentiation of HL-60 cells (Fig. 4). In the membrane fraction, A-regulatory subunit could not be detected until we applied a triple protein amount for immunoblot analysis. It may explain why PP2A-like activity was very low in the membrane fraction. PP2A-C and B-regulatory subunits were detected, but PP2A-C/A complex formation was probably little for PP2A activity, because of the relatively small amount of A-regulatory subunit.

To determine whether the 1,25(OH)_{2}D_{3}-induced reduction of PP1α and PP1γ proteins in the cytosol fraction is reflected at the reduced level of RNA transcripts, Northern blot analysis was done on total cellular RNA prepared from HL-60 cells, before and after treatment with 1,25(OH)_{2}D_{3} (Fig. 7). The PP1γ and PP1α probes detected major bands of 1.8 and 1.6 kilobases, respectively, in HL-60 cells. Expressions of both PP1γ and PP1α transcripts in HL-60 cells were relatively unaltered following treatment with 1,25(OH)_{2}D_{3}. Rehybridization of these blots with the glyceraldehyde-3-phosphate dehydrogenase cDNA probe confirmed that relatively equal amounts of RNA were loaded in each lane. These results provide further support that cytosolic PP1γ and PP1α proteins were translocated to the membrane and nuclear fractions during 1,25(OH)_{2}D_{3}-induced differentiation.
Fig. 5. Time course of changes in immunoreactive PP1α, PP1γ, and PP1β catalytic subunits of HL-60 cells during 1,25(OH)₂D₃-induced differentiation. HL-60 cells (1 x 10⁶ cells) were treated with 20 nM 1,25(OH)₂D₃, and each cellular fraction was obtained as described in “Materials and Methods.” A, the proteins of cytosol, nuclear, and membrane fractions were analyzed by immunoblot analysis using antisera specific for PP1γ, PP1α, and PP1β isozymes as described in “Materials and Methods.” Ten μg of protein sample were applied to each lane, while 20 μg were applied from nuclear fraction for PP1α and membrane fraction for PP1β. B, graphic display of PP1 isozymes (A), as quantitated by densitometry (● PP1γ; ▲ PP1α; △ PP1β). Results are expressed as the percentage of the mean control value in untreated HL-60 cells. These data are from one representative experiment, but similar results were obtained in two other experiments.

Fig. 6. Time course of changes in immunoreactive PP2A catalytic and regulatory subunits of HL-60 cells during 1,25(OH)₂D₃-induced differentiation. HL-60 cells (1 x 10⁶ cells) were treated with 20 nM 1,25(OH)₂D₃, and each cellular fraction was obtained as described in “Materials and Methods.” A, 10 μg protein of cytosol, nuclear, and membrane fractions were analyzed by immunoblot analysis using antisera specific for PP2A catalytic (PP2A-C) and A- and B- regulatory subunits, as described in “Materials and Methods.” Twenty μg were applied from nuclear and membrane fractions for PP2A-C, and 30 μg protein of membrane fraction were applied for the A-regulatory subunit. B, graphic display of PP2A catalytic and regulatory subunits (A), as quantitated by densitometry (● PP2A-C; ○ A-regulatory subunit; □ B-regulatory subunit). Results are expressed as the percentage of the mean control value in untreated HL-60 cells. These data are from one representative experiment, but similar results were obtained in two other experiments.
Total RNA (20 μg) was isolated from HL-60 cells at the indicated periods of time below (first lane) and PPα (third lane) cDNA probes. The same RNA blots were hybridized to the corresponding lanes. Northern blots of total RNA were hybridized to 32P-labeled PPα'y stimulated with 1,25(OH)2D3. HL-60 cells (2 × 10^6 cells) were treated with 1,25(OH)2D3 to glyceraldehyde-3-phosphate dehydrogenase eDNA probe for assessment of RNA independent experiments.

**DISCUSSION**

Although a number of phosphatases have been isolated and characterized and some functions clarified, data regarding their role in cellular differentiation, especially related to leukemia, are scanty. The present studies were designed to define PPα and PPα'y expressions in HL-60 cells, to examine the effects of 1,25(OH)2D3 treatment on the phenotype and function of these cells, and to correlate them with the effects on enzymatic activity of PPα and PPα'y and on expression of immunoreactive proteins of PPα and PPα'y. Using antibodies specific for each of three PPα catalytic subunit isozymes, α, γ, and δ and for PPα-C and A- and B-regulatory subunits of PPα, we followed changes in individual PPα species at the protein level.

1,25(OH)2D3 is reported to exert its effects on the cells in two ways, by rapidly stimulating membrane phosphoinositide breakdown, thereby activating PKC (25), and by binding to its nuclear hormone receptor (26). The 1,25(OH)2D3 receptor is classified as a member of nuclear receptor family with sequences in common with those for the retinoic acid receptor (26). 1,25(OH)2D3 receptor regulation as a transcription factor is considered to be governed by phosphorylation of the serine 51 residue by PKC (27). 1,25(OH)2D3-induced monocytic differentiation of HL-60 cells is associated with an increase in PKCα activity and expression of PKCα and PKCβ, as well as the steady-state levels of PKCα and PKCβ mRNA expressions (6). On the other hand, the levels of both PKCα activity and the expression PKCα, PKCβ, and PKCγ isofoms have also been shown to increase during granulocytic differentiation of HL-60 cells induced by DMSO and retinoic acid (6). These studies suggest that monocytic and granulocytic inducers share PKC as a target molecule and that the activation and/or induction of PKC is a common mechanism for bringing about terminal differentiation of HL-60 cells, irrespective of the terminal cellular phenotype. The biochemical activation of PKC alone is apparently insufficient to account for the differentiation responses to monocytic or granulocytic phenotypes, and additional elements probably contribute to each differentiation pathway.

We reported that Cal-A, a potent inhibitor of PPα and PPα'y, augmented the retinoic acid induced granulocytic differentiation but not the phorbol ester induced macrophage-like differentiation of HL-60 cells (14). Moreover, PPα'y is down-regulated during retinoic acid-induced granulocytic differentiation, while PPα is unchanged (15–17). The finding that Cal-A also augments 1,25(OH)2D3-induced monocytic differentiation of HL-60 cells is evidence for the involvement of protein phosphatases. Treatment of HL-60 cells with 1,25(OH)2D3 led to a decrease in cytosolic PPα'y-like activity with a concomitant increase in PPα-like activity of membrane and nuclear fractions. Reduction of cytosolic PPα-like activity correlated with the increased expression of certain differentiation markers related to monocytes. Subcellular redistribution of PPα-like activity induced by 1,25(OH)2D3 was mainly due to the selective translocation of cytosolic PPα'y and PPαα proteins to membrane and nuclear fractions, whereas the distribution of PPα18 and PPα22 was relatively constant. Interestingly, translocation of PPα'y was more rapid than that of PPαα, hence individual PPα isozymes might be differentially regulated. PPα was shown to be colocalized with chromosomes in mammalian cells at mitosis (28). Intracellular distribution of PPα activity may be an important aspect of regulation related to the differentiation of HL-60 cells. Induction of c-fos mature transcripts and an increase in AP-1 activity have been noted in cases of 1,25 (OH)2D3-induced inhibition of proliferation and monocytic differentiation (29). 1,25(OH)2D3 was found to cause a decrease in c-myc mRNA expression in HL-60 cells (30). These nuclear events may be related to the increased PPα'y activity in the nucleus, facilitating the activated state of definite transcription factors, including 1,25(OH)2D3 receptor, and affecting processes of differentiation. It has been shown that PPα activity is associated with glycogen particles and SR membranes and is enriched in other particulate fractions and organelles such as microsomes, ribosomes, nuclei, and myofibrils (5). The increase of PPα'y and PPαα protein in the membrane fraction translocating from cytosol fraction may be indicative of the changing targeting specificity. The decrease in immunoreactive PPα'y and PPαα isoforms of cytosol fraction during 1,25(OH)2D3-induced differentiation was not associated with a modified expression of PPα'y and PPαα genes. Chronic 1,25(OH)2D3 treatment has no significant effect on the expressions. Thus, the modulation of PPα isozymes may be involved in the regulation of 1,25(OH)2D3-induced differentiation. Although PKC is up-regulated by retinoic acid and 1,25(OH)2D3 (6), protein phosphatase dynamics differ possibly in association with different pathways of differentiation. Cal-A augmented both retinoic acid-induced granulocytic differentiation (14) and 1,25(OH)2D3-induced monocytic differentiation; however, during retinoic acid-induced granulocytic differentiation, the down-regulation of PPαα-C occurred (15, 16), whereas during the 1,25(OH)2D3-induced monocytic differentiation, translocation and redistribution of PPα isozymes occurred. On the other hand, Cal-A did not augment the phorbol ester-induced monocytic differentiation, and there was neither down-regulation nor translocation of PPα and/or PPα22 (14–16). When these results of our studies are considered all together, it becomes clear that each differentiation pathway has its specific phosphatase enzyme dynamics, and the translocation-redistribution of PPα isozymes during 1,25(OH)2D3-induced monocytic differentiation that we have reported in this study is specific for this pathway and not a general phenomenon.
The mechanisms underlying the different redistribution of PP1 isoforms in response to 1,25(OH)2D3 treatment are not clear. PP1 consists of a multimeric structure composed of a catalytic subunit complexed to a number of regulatory components that play important roles in regulating phosphatase activity or targeting it to specific subcellular locations (8). The free catalytic subunit has not been detected in cells. The amino acid sequences of amino-terminal and central regions of all PP1-catalytic subunit isoforms are highly conserved, and differences are mainly in the carboxy-terminal regions (8). The PP1γ, PP1θ, and PP1α proteins expressed in Escherichia coli in HL-60 cells, although physiological importance of this redistribution patterns of individual PP1 isoforms during monocytic differentiation. However, further studies of regulatory subunits should be performed in HL-60 cells, although physiological importance of this redistribution is unknown at present.

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