

# Stimulation of synthesis *de novo* of NAD<sup>+</sup>-dependent 15-hydroxyprostaglandin dehydrogenase in human promyelocytic leukaemia (HL-60) cells by phorbol ester

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Human promyelocytic leukaemia (HL-60) cells were employed to study the induction of NAD<sup>+</sup>-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the key enzyme in controlling prostaglandin inactivation. Phorbol 12-myristate 13-acetate (PMA) stimulated 15-PGDH activity in a time- and concentration-dependent manner. Dimethyl sulphoxide (DMSO) also stimulated the enzyme activity, although a much delayed stimulation was observed. Western blot studies indicated that PMA increased significantly a 28 kDa immunoreactive protein characteristic of 15-PGDH. L-[<sup>35</sup>S]Methionine labelling of the PMA-treated cells showed a similar enhancement over the control cells. These studies indicate that PMA induced synthesis of 15-PGDH. Stimulation of 15-PGDH activity by PMA or DMSO appears to be mediated by protein kinase C activation, since an inactive analogue of PMA failed to induce the effect, and both staurosporine and H-7 blocked the stimulation. Stimulation by PMA was optimal at 10 nM and less effective at higher concentrations. Western blot studies indicated that a similar, if not greater, amount of enzyme protein was induced at high concentrations of PMA, suggesting that enzyme inactivation might be occurring. Possible enzyme inactivation by protein kinase C activation was further examined by incubating DMSO-treated cells with a high concentration of PMA (50 nM). Time-dependent inactivation of 15-PGDH within the first 1 h was observed and this inactivation was partially blocked by staurosporine and H-7. Pulse-chase experiments indicated that 15-PGDH had a rapid turnover rate ( $t_{1/2} = 47$  min), and PMA shortened the half-life of the enzyme ( $t_{1/2} = 33$  min), suggesting that PMA might have an additional effect on 15-PGDH degradation. The rapid turnover of 15-PGDH indicates that the enzyme activity depends on continued enzyme synthesis, and this could be susceptible to hormone and drug control mechanisms.

## INTRODUCTION

NAD<sup>+</sup>-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH, EC 1.1.1.141) catalyses oxidation of the 15(S) hydroxyl group of prostaglandins to the 15-oxo function, resulting in a substantial decrease in the biological activities of prostaglandins [1]. This enzyme has been considered to be the key enzyme in controlling the biological inactivation of prostaglandins. Several pharmacological agents, including thyroid hormones [2], non-steroidal anti-inflammatory drugs [3,4], anti-allergic drugs [5], acrolein [6] and anti-psychotic drugs [7] have been shown to inhibit or stimulate the enzyme directly. Some physiological and pathological conditions such as pregnancy [8], ontogenic development [9], hormone [10] and steroid [11] administration are also known to alter the enzyme activity although the mechanism of enzyme regulation remains to be elucidated.

Although the enzyme is ubiquitously present in mammalian tissues [12], the specific cell types responsible for prostaglandin catabolism are not clear. It would be desirable if a specific cell type could be identified and cultured to facilitate studies on the regulation of 15-PGDH. We have found that human promyelocytic leukaemia (HL-60) cells can be induced by phorbol esters to exhibit 15-PGDH activity. In the present investigation we focused attention on the induction and regulation of 15-PGDH by phorbol 12-myristate 13-acetate (PMA) in this HL-60 model system. A preliminary report of this study has been presented in an abstract form [13].

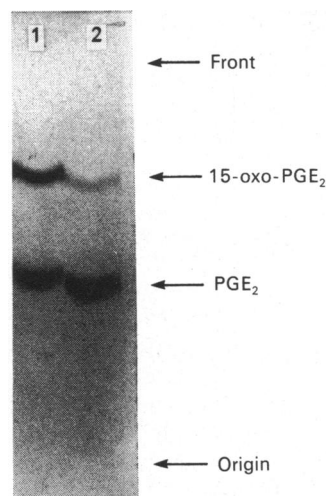
## EXPERIMENTAL

### Materials

PMA, 4 $\alpha$ -phorbol 12,13-didecanoate (PDD), dimethyl sulphoxide (DMSO), penicillin, streptomycin, RPMI 1640, NAD<sup>+</sup>, 2-oxoglutarate, dithiothreitol (DTT), 5-bromo-4-chloro-3-indolyl phosphate, Nitro Blue tetrazoleum, bovine liver glutamate dehydrogenase (40 units/mg), actinomycin D, cycloheximide, phenylmethanesulphonyl fluoride, Tween 20, Triton X-100, BSA, H-7 and biotinylated protein standards were obtained from Sigma Chemical Co. Biotinylated rabbit anti-mouse IgG and alkaline phosphatase-labelled streptavidin were supplied by Zymed Laboratories. PGE<sub>2</sub> and 15-oxo-PGE<sub>2</sub> were obtained from the Upjohn Company. *Staphylococcus aureus* cells (Pansorbin) were purchased from Calbiochem. Staurosporine was obtained from Boehringer-Mannheim, 8-well microwell strips were supplied by Costar, and a silica gel G plate was purchased from EM Science. Human promyelocytic leukaemia (HL-60) cells were obtained from the American Type Culture Collection. Trans-<sup>35</sup>S-label containing 85% L-[<sup>35</sup>S]methionine was purchased from ICN. Monoclonal antibodies to human placental 15-PGDH were prepared according to Tai *et al.* [14]. 15(S)-[15-<sup>3</sup>H]PGE<sub>2</sub> was prepared according to Tai [15]. [1-<sup>14</sup>C]PGE<sub>2</sub> was biosynthesized from [1-<sup>14</sup>C]arachidonic acid using sheep seminal vesicular microsomes according to a previous procedure [16].

Abbreviations used: PG, prostaglandin; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; DTT, dithiothreitol; DMSO, dimethyl sulphoxide; PMA, phorbol 12-myristate 13-acetate; PDD, 4 $\alpha$ -phorbol 12,13-didecanoate; HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; 12-oxo-HHT, 12-oxo-5,8,10-heptadecatrienoic acid.

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**Fig. 1.** Metabolism of [ $1\text{-}^{14}\text{C}$ ]PGE<sub>2</sub> by HL-60 cells treated with or without PMA

HL-60 cells were treated with or without PMA (10 nM) for 24 h, and then washed and sonicated. A homogenate equivalent to  $5 \times 10^5$  cells was incubated with [ $1\text{-}^{14}\text{C}$ ]PGE<sub>2</sub> (5.7 nmol, 30000 c.p.m.) and NAD<sup>+</sup> (1  $\mu$ mol) in 1 ml of 0.05 M-Tris/HCl buffer, pH 7.5, containing 1 mM-DTT. After 20 min of incubation at 37 °C the reaction mixture was acidified and extracted. The extract was chromatographed on a Silica gel G plate as described in the Experimental section. Lane 1, PMA treatment; lane 2, no PMA treatment.

### Cell culture

The human promyelocytic leukaemia (HL-60) cell line was maintained in RPMI 1640 medium containing 10% (v/v) fetal calf serum, 100 units or penicillin/ml and 100  $\mu$ g of streptomycin/ml at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. The cells were inoculated at about  $5 \times 10^5$  cells/ml into T-flasks, and cell differentiation was induced by the addition of PMA or DMSO. PMA and H-7 were dissolved in ethanol and staurosporine was dissolved in DMSO before additions to the culture medium. The organic solvent concentration was kept at less than 0.1% and the same amount of vehicle was added to the control culture.

### Preparation of HL-60 cell homogenate

HL-60 cells from the above culture were spun down at 1500 g for 5 min and washed once with saline. About  $1 \times 10^6$  cells were suspended in 1 ml of 0.05 M-Tris/HCl, pH 7.5, containing 1 mM-DTT and then sonicated in an ice bath for 15 s using an ultrasonic sonicator at setting 4. The crude homogenate was centrifuged at 1500 g for 5 min and the supernatant was used as an enzyme preparation.

### Determination of PGE<sub>2</sub> metabolism using t.l.c.

The oxidation of [ $1\text{-}^{14}\text{C}$ ]PGE<sub>2</sub> into [ $1\text{-}^{14}\text{C}$ ]15-oxo-PGE<sub>2</sub> by HL-60 cells was monitored by t.l.c. The incubation mixture contained [ $1\text{-}^{14}\text{C}$ ]PGE<sub>2</sub> (5.7 nmol, 30000 c.p.m.) NAD<sup>+</sup> (1  $\mu$ mol) and HL-60 homogenate in a final volume of 1 ml of 0.05 M-Tris/HCl, pH 7.5. The reaction was allowed to proceed at 37 °C for 20 min and terminated by acidification with 1 M-citric acid to pH 4. The mixture was extracted with 2 ml of chloroform/methanol (2:1, v/v) and 1 ml of water by vigorous mixing. The chloroform layer was removed, evaporated under a stream of nitrogen and chromatographed on a silica gel G plate (2 cm  $\times$  20 cm) developed in a solvent system of ethyl acetate/acetic acid/iso-octane/water (11:2:5:10, by vol; organic phase) to a height of 15 cm. The plate was dried and exposed to X-ray

film. Authentic PGE<sub>2</sub> and 15-oxo-PGE<sub>2</sub> standards (10  $\mu$ g each) were co-chromatographed to indicate the positions of PGE<sub>2</sub> and its metabolite by exposure to I<sub>2</sub> vapour.

### Enzyme assay

15-PGDH was routinely assayed by measuring the transfer of <sup>3</sup>H from 15(S)-[15-<sup>3</sup>H]PGE<sub>2</sub> to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously [15]. Briefly, the reaction mixture contained NH<sub>4</sub>Cl (5  $\mu$ mol),  $\alpha$ -oxoglutarate (1  $\mu$ mol), NAD<sup>+</sup> (1  $\mu$ mol), 15(S)-[15-<sup>3</sup>H]PGE<sub>2</sub> (1 nmol, 30000 c.p.m.), glutamate dehydrogenase (100  $\mu$ g), DTT (1  $\mu$ mol) and 15-PGDH enzyme preparation in a final volume of 1 ml of 0.05 M-Tris/HCl, pH 7.5. The reaction was allowed to continue for 10 min at 37 °C, and was terminated by the addition of 0.2 ml of 10% aqueous charcoal suspension.

After incubation for 5 min the mixture was centrifuged at 2000 g for 5 min. The radioactivity in the supernatant was determined by liquid scintillation counting. Calculation of the amount of PGE<sub>2</sub> oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(S)-hydroxyl group of 15(S)-[15-<sup>3</sup>H]PGE<sub>2</sub> as substrate. Enzyme activity of HL-60 sample was always assayed in duplicate.

### SDS/PAGE and immunoblotting of 15-PGDH

Proteins were fractionated by SDS/PAGE according to the method of Laemmli [17]. Electrophoretic transfer of proteins from the polyacrylamide gel to nitrocellulose paper was performed according to the method of Towbin *et al.* [18]. Non-isotopic visualization of antigenic protein using biotinylated rabbit anti-mouse IgG and streptavidin-alkaline phosphatase was carried out according to Knecht & Dimond [19].

### [<sup>35</sup>S]Methionine metabolic labelling of HL-60 cells and immunoprecipitation of 15-PGDH

HL-60 cells ( $5 \times 10^5$ /ml) were incubated in the presence or the absence of 10 nM-PMA for 16 h. The methionine-containing medium was then removed and replaced by methionine-free medium, followed by the addition of 5  $\mu$ Ci of L-[<sup>35</sup>S]methionine/ml and incubation for 4 h. The <sup>35</sup>S-labelled medium was removed and the cells ( $1 \times 10^7$ ) were washed twice with 0.9% NaCl and lysed with lysing buffer (0.01 M-phosphate-buffered saline containing 0.1% Triton X-100, 1 mM-phenylmethanesulphonyl fluoride and 0.01% EDTA). After centrifugation at 10000 g for 5 min the supernatant was incubated for 2 h with 2  $\mu$ g of monoclonal antibody against human placental 15-PGDH, followed by the addition of 20  $\mu$ l of a 10% (w/v) *S. aureus* cell suspension and incubation for 1 h. The mixture was briefly centrifuged (2000 g, 10 min) and the pellet was disrupted by heating in the sample buffer before analysis by SDS/PAGE. <sup>35</sup>S-labelled 15-PGDH on the gel was localized by autoradiography.

### Immobilization and quantification of [<sup>35</sup>S]methionine-labelled 15-PGDH in microwell strips (reverse immunoblot)

A microwell strip was first coated with 1  $\mu$ g of affinity-purified rabbit anti-mouse IgG per well in 200  $\mu$ l of 0.1 M-NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer, pH 9.6, at 4 °C overnight. Each well was then blocked by adding 100  $\mu$ l of 0.1% BSA in 0.1 M-phosphate-buffered saline and incubated at 22 °C for 2 h. The wells were then washed with washing buffer (0.01 M-phosphate buffer, pH 7.5, containing 0.05% Tween 20) three times before incubating with anti-15-PGDH antibody and <sup>35</sup>S-labelled enzyme. HL-60 cells were labelled with [<sup>35</sup>S]methionine as described above. Following lysis of the cells, supernatant equivalent of  $10^6$  cells and 1  $\mu$ g of monoclonal antibody to 15-PGDH were added to each well. Incubation was allowed to continue at 22 °C for 2 h. After washing the wells with washing buffer, each well was

broken away from the strip and the radioactivity was determined by liquid scintillation counting.

## RESULTS

PMA stimulated PGE<sub>2</sub> metabolism in HL-60 cells (Fig. 1). Untreated HL-60 cells metabolized [1-<sup>14</sup>C]PGE<sub>2</sub> insignificantly, whereas cells treated with PMA (10 nM) for 24 h metabolized [1-<sup>14</sup>C]PGE<sub>2</sub> substantially into [1-<sup>14</sup>C]15-oxo-PGE<sub>2</sub>, as revealed by t.l.c. In order to rapidly determine prostaglandin catabolic activity in HL-60 cells during treatment with PMA, 15-PGDH was assayed by using [15-<sup>3</sup>H]PGE<sub>2</sub> as a substrate and determining the transfer of <sup>3</sup>H from the substrate to glutamate by coupling 15-PGDH with glutamate dehydrogenase as reported previously [16]. The effects of the concentrations of PMA and PDD on stimulation of 15-PGDH activity are shown in Fig. 2(a). Maximal stimulation of 15-PGDH activity by PMA was observed at 10 nM; higher concentrations of PMA appeared to be less effective. PDD, an inactive phorbol ester, was found to be unable to stimulate 15-PGDH activity at the concentrations tested. The kinetics of stimulation of 15-PGDH activity by two different concentrations of PMA showed a biphasic profile (Fig. 2b). Initial maximal activity was observed at 24 h after the addition of PMA. A decrease in activity was found at 36 h, followed by a

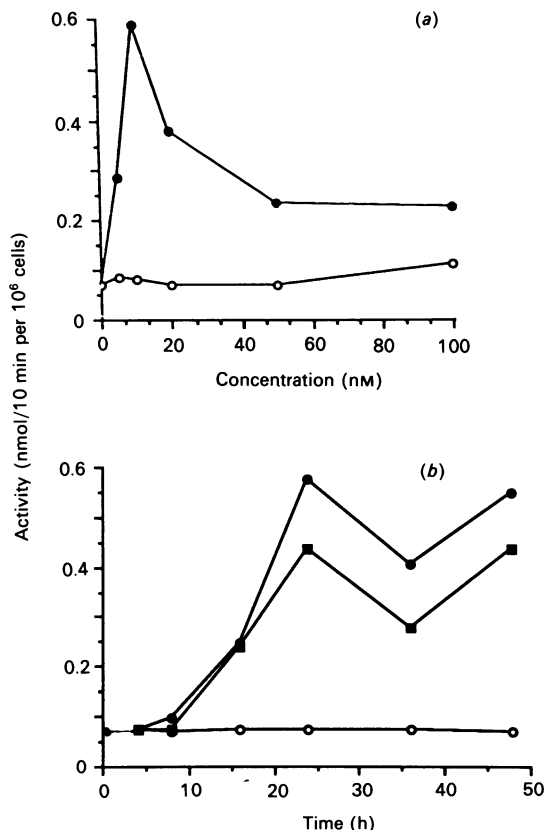


Fig. 2. Effect of PMA and PDD on 15-PGDH activity

(a) HL-60 cells were treated with indicated concentrations of PMA (●) or PDD (○) for 24 h. (b) HL-60 cells ( $5 \times 10^5$ /ml) were treated with 10 nM-PMA (●), 20 nM-PMA (■), or without PMA (○) for indicated lengths of time. Cells were washed and sonicated for determination of 15-PGDH activity as described in the Experimental section. Each point is the average of two determinations, and the Figure is representative of two separate experiments with qualitatively similar results.

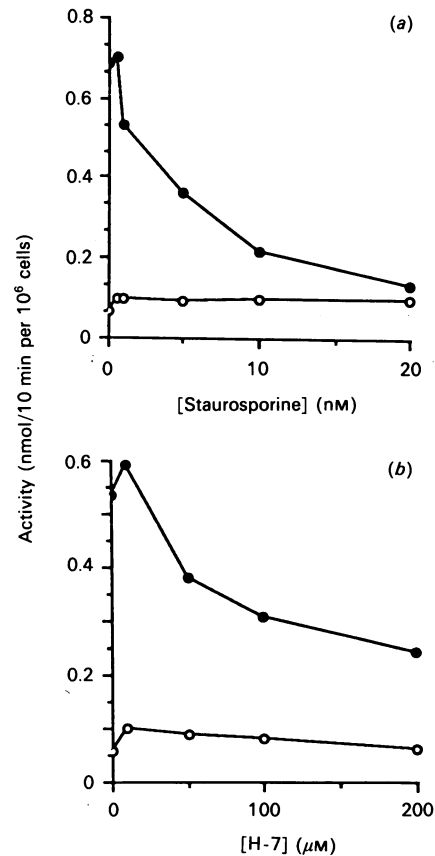


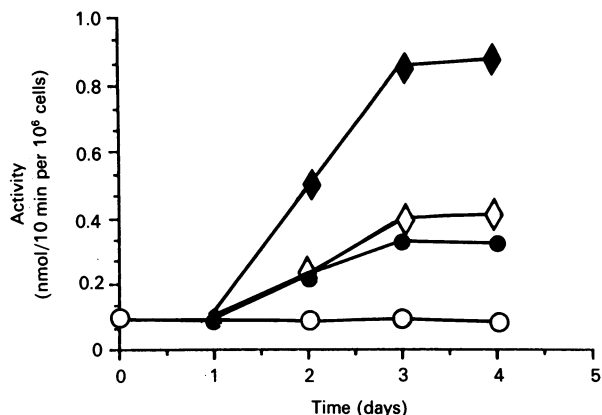
Fig. 3. Effects of staurosporine and H-7 on the induction of 15-PGDH activity by PMA

(a) HL-60 cells ( $5 \times 10^5$ /ml) were treated either with staurosporine (○) or with 10 nM-PMA plus staurosporine (●) at the indicated concentrations for 24 h. (b) HL-60 cells ( $5 \times 10^5$ /ml) were treated either with H-7 (○) or with 10 nM-PMA plus H-7 (●) at the indicated concentrations for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in the Experimental section. Each point is the average of two determinations, and the Figure is a representative of two separate experiments with qualitatively similar results. The viability of cells during the treatment with staurosporine or H-7 was greater than 90%.

'rebound' of activity at 48 h. The biphasic nature of the increased activity induced by PMA has been reproducibly observed.

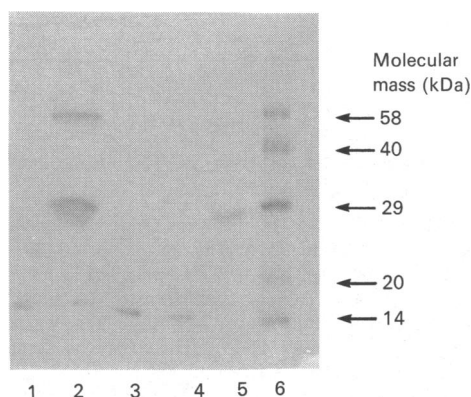
Stimulation of 15-PGDH activity by PMA was found to be blocked by the addition of the protein kinase C inhibitors staurosporine and H-7 [20,21]. Fig. 3 shows the effects of increasing concentrations of staurosporine or H-7 on PMA-induced stimulation of 15-PGDH activity. Almost total inhibition by staurosporine was observed at 20 nM, whereas about 60% inhibition by H-7 was seen at 200 μM. In addition to PMA, DMSO was also shown to stimulate 15-PGDH activity, albeit requiring a longer exposure time (Fig. 4). Stimulation of 15-PGDH activity by DMSO was found to be significantly inhibited by staurosporine at 10 nM or H-7 at 50 μM (Fig. 4). Although H-7 at 200 μM was clearly more effective, it is possible that this is due to more non-specific effects. Therefore H-7 was used at 50 μM.

The increase in 15-PGDH activity induced by PMA was apparently due to synthesis *de novo* of the enzyme protein, since the immunoreactive bands of 15-PGDH in the Western blot were abolished by cycloheximide and actinomycin D (Fig. 5). Cells treated with PMA for 24 h exhibited enhanced 28 kDa and 56 kDa band (Fig. 5, lane 2) compared with those without PMA



**Fig. 4.** Effects of staurosporine and H-7 on the induction of 15-PGDH activity by DMSO

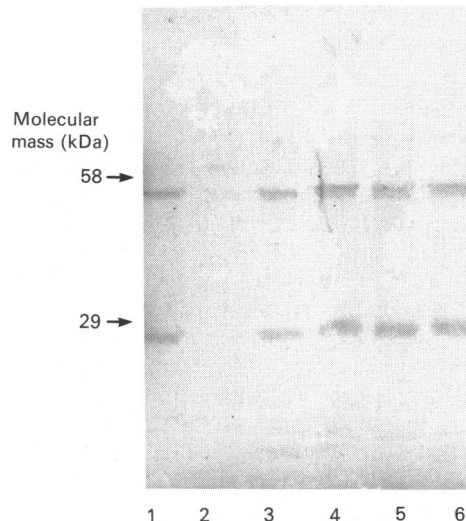
HL-60 cells ( $5 \times 10^5$ /ml) were untreated (○), or treated with 1.25% DMSO (◆), 1.25% DMSO plus 10 nM-staurosporine (●), or 1.25% DMSO plus 50 μM-H-7 (◇) for the indicated lengths of time. Cells were washed and sonicated for determination of 15-PGDH activity as described in the Experimental section. Each point is the average of two determinations, and the figure is representative of two separate experiments with qualitatively similar results.



**Fig. 5.** Western blot analysis of 15-PGDH from HL-60 cells treated with PMA or PMA plus transcriptional or translational inhibitors

HL-60 cells were treated with PMA alone (10 nM) (lane 2), PMA plus actinomycin D (1 μM) (lane 3), PMA plus cycloheximide (1 μM) (lane 4) or not treated (lane 1) for 24 h. Cells were then lysed and an equivalent of  $1 \times 10^6$  cells was applied to each lane for SDS/PAGE and immunoblot analysis. Purified human placental 15-PGDH (lane 5) and biotinylated protein standards (lane 6) were run at the same time. Membranes were treated with monoclonal antibody to 15-PGDH, and other biotinylated rabbit anti-mouse IgG and streptavidin labelled with alkaline phosphatase were used to localize antigenic proteins as described in the Experimental section.

treatment (lane 1). These two immunoreactive bands were also observed with purified human placental 15-PGDH (lane 5). Simultaneous treatment of the cells with PMA and cycloheximide (lane 4) or actinomycin D (lane 3) abolished the PMA-induced enhancement of both immunoreactive bands. Actinomycin D or cycloheximide added to HL-60 cells which had been treated with PMA for 24 h also totally inhibited expression of 15-PGDH activity assayed at 40 h (results not shown). The disappearance of immunoreactive 15-PGDH in the presence of actinomycin D or cycloheximide was not due to cell death, since viability of these cells was greater than 95% during the course of the experiments.



**Fig. 6.** Western blot analysis of 15-PGDH from HL-60 cells treated with different concentrations of PMA

HL-60 cells were treated for 24 h with different concentrations of PMA as described in the legend to Fig. 2(b). Cells were then lysed and an equivalent of  $1 \times 10^6$  cells was applied to each lane for SDS/PAGE and immunoblot analysis as described in the Experimental section. Purified human placental 15-PGDH (lane 1) was run with HL-60 cells without treatment (lane 2) or treated with 5 nM-PMA (lane 3), 10 nM-PMA (lane 4), 20 nM-PMA (lane 5) or 50 nM-PMA (lane 6).

The effect of PMA on methionine incorporation into newly synthesized 15-PGDH was then examined by immunoprecipitation and SDS/PAGE analysis. HL-60 cells were first incubated in the presence or the absence of PMA for 24 h, and then changed to methionine-free medium containing L-[<sup>35</sup>S]methionine for 4 h. The cells were then lysed and the supernatant was incubated with monoclonal antibodies to 15-PGDH. The antibody-15-PGDH complex was precipitated by *S. aureus* cells and then subjected to SDS/PAGE analysis. PMA-treated cells exhibited an enhanced 28 kDa band compared with non-treated cells, similar to the immunoblot studies above (results not shown). The enhancement was not due to increased uptake of L-[<sup>35</sup>S]methionine, since the uptake was not affected by PMA.

It was shown in Fig. 2 that 15-PGDH activity was optimally stimulated by PMA at 10 nM, but poorly enhanced at 50 nM. It is not clear if higher concentrations of PMA showed poor induction of the enzyme or caused its inactivation. We examined this point by carrying out immunoblot studies on induced enzyme at various concentrations of PMA. Fig. 6 indicated that PMA at 50 nM induced as much, if not more, immunoreactive enzyme as did PMA at 10 nM, as revealed by densitometric measurement. It thus appears that high concentrations of PMA induced inactivation of the enzyme. In order to clarify this point, 15-PGDH activity in HL-60 cells was induced first by DMSO, and the cells were then treated with 50 nM-PMA for different lengths of time before enzyme activity was determined. It was found that approx. 70% of 15-PGDH activity was lost during the first 40 min of exposure (Fig. 7). Concurrent addition of staurosporine at 10 nM or H-7 at 50 μM partially protected the enzyme from inactivation.

Further experiments were carried out to determine the half-life of 15-PGDH. HL-60 cells were incubated in the presence or absence of 10 nM-PMA for 24 h. The cells were washed and then incubated in the methionine-free medium containing L-[<sup>35</sup>S]methionine for another 4 h. The labelled cells were washed and incubated in regular medium containing methionine in the presence or the absence of PMA for 60 min. At designated times,

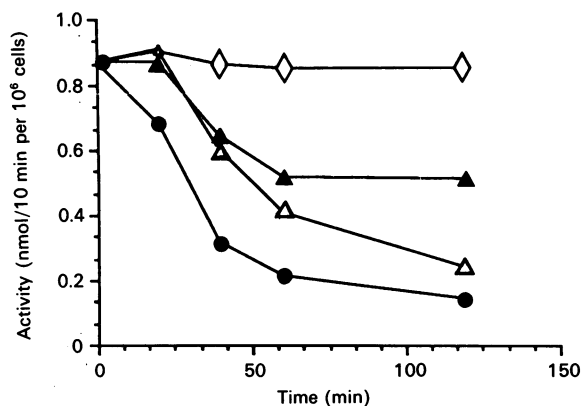


Fig. 7. Inactivation of DMSO-induced 15-PGDH activity by short-term incubation with a high concentration of PMA

HL-60 cells were treated with 1.25% DMSO for 3 days and then divided into four groups. ●, treated with 50 nM-PMA; △, treated with PMA plus 50  $\mu$ M-H-7; ▲, treated with PMA plus 10 nM-staurosporine; ◇, no further treatment. At the indicated times, cells in each group were immediately spun down, washed and kept on ice. Cell homogenates were then made and 15-PGDH activity was assayed as described in the Experimental section. Each point is the average of two determinations.

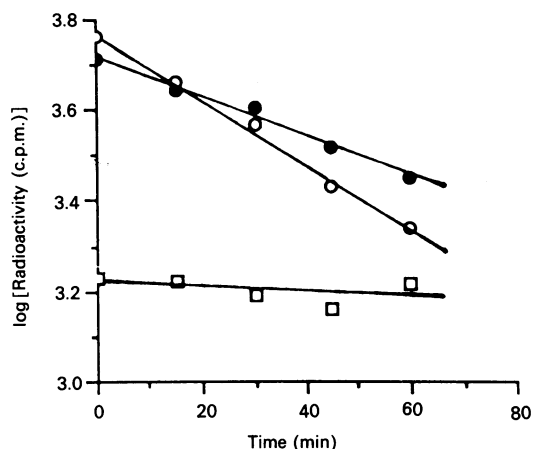


Fig. 8. Half-life of 15-PGDH in HL-60 cells treated with and without PMA

HL-60 cells were treated with or without PMA (10 nM) for 24 h to induce 15-PGDH. The cells were washed and labelled with L-[ $^{35}$ S]methionine for 4 h, and then washed again and suspended in regular medium containing methionine. Cells treated with PMA were divided into two groups. One group was treated again with PMA (10 nM) (○), and the other group received no further treatment (●). At the indicated time, cells were removed, washed and lysed. The lysate was incubated with microwell strip precoated with monoclonal antibody to 15-PGDH. After extensive washing each well was counted for radioactivity in a liquid scintillation counter as described in the Experimental section. The cells that were not treated with PMA at the beginning were used to monitor background radioactivity in the wells (□). Each point is the average of two determinations.

cells were washed, lysed and the L-[ $^{35}$ S]methionine incorporated into 15-PGDH was determined by reverse immunoblotting. Plots of log (labelled enzyme) versus time in the presence and the absence of PMA during unlabelled methionine chase gave two statistically different slopes (Fig. 8). Estimation from these slopes gave half-lives of labelled enzyme of 33 min and 47 min in the presence and absence respectively of PMA.

## DISCUSSION

Human promyelocytic leukaemia (HL-60) cells have been shown to exhibit prostaglandin and thromboxane biosynthetic activity following exposure to phorbol ester [22], 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> [23], retinoic acid [23] and DMSO [24]. The increase in biosynthetic activity is thought to be due to induction of cyclo-oxygenase and prostaglandin endoperoxide isomerases synthesis, since actinomycin D and cycloheximide block the biosynthetic activity induced by these agents. Whether these agents also increase the catabolism of prostaglandins in HL-60 cells has not been extensively explored, except in one instance. Agins *et al.* [25] reported that dimethyl formamide-treated HL-60 cells produced a cyclo-oxygenase-derived u.v.-absorbing metabolite which was not found in untreated cells. This compound was later identified to be 12-oxo-5,8,10-heptadecatrienoic acid (12-oxo-HHT) [26]. This finding is consistent with our report that 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), an alternative product of thromboxane synthase, is an excellent substrate for 15-PGDH [27], and further suggests that dimethyl formamide stimulates 15-PGDH activity in HL-60 cells, which accounts for the production of 12-oxo-HHT. In the present investigation we have examined the effects of PMA and DMSO in stimulating 15-PGDH activity in HL-60 cells. We found that both PMA and DMSO induced 15-PGDH activity. The time course of induction of 15-PGDH activity was found to be relatively short for PMA. PMA-induced 15-PGDH activity reached a maximum within 24 h of exposure, whereas DMSO required 3 days for maximal induction. In view of the rapid induction and the potential role of protein kinase C in regulating 15-PGDH activity, we have systematically examined the induction of 15-PGDH by PMA.

Stimulation of 15-PGDH activity by PMA is apparently due to the induction of synthesis *de novo* of the enzyme protein. This is evident from three lines of evidence. First, up-regulation of 15-PGDH activity is blocked by actinomycin D and cycloheximide. Secondly, immunoreactive 15-PGDH was found to appear following PMA exposure and to disappear completely in the presence of actinomycin D or cycloheximide. Thirdly, [ $^{35}$ S]methionine-labelled 15-PGDH was shown to increase significantly following stimulation by PMA. Synthesis of new 15-PGDH appears to be limited to a single and regular form of the enzyme, since no other form was detected immunologically. The presence of an immunoreactive band of 56 kDa in the Western blot is probably due to incomplete dissociation into subunits of 28 kDa during preparation of the sample for SDS/PAGE analysis, since purified placental 15-PGDH also exhibits two immunoreactive bands of similar sizes.

The induction of 15-PGDH appears to be mediated by the activation of protein kinase C. This is supported by several lines of evidence. PMA, a potent activator of protein kinase C [28], produced time- and concentration-dependent induction of 15-PGDH enzyme protein and activity, whereas PPD, an inactive analogue, did not exhibit any effect. Such an induction was found to be inhibited by protein kinase C inhibitors such as staurosporine and H-7. The concentrations of H-7 used have previously been shown to inhibit protein phosphorylation in HL-60 cells [29]. DMSO, which has been shown to induce protein kinase C in HL-60 cells [30,31], stimulated 15-PGDH activity, and this stimulation was again blocked by staurosporine or H-7. The induction of 15-PGDH by DMSO is very likely to be mediated by DMSO-induced protein kinase C activation. 15-PGDH appears to be the second enzyme that is regulated by protein kinase C [22,32] in the eicosanoid metabolic pathway, the first being cyclo-oxygenase.

The induction of 15-PGDH activity was found to occur with

a relatively narrow range of PMA concentrations. A concentration-dependent increase in 15-PGDH activity was observed between 1 and 10 nM-PMA. The lower 15-PGDH activity with higher PMA concentrations is not due to decreased induction of enzyme synthesis, since the induced enzyme protein was found to be present in equal, if not greater, quantities at 50 nM-PMA as at 10 nM-PMA, as revealed by the Western blot studies. It appears that low concentrations of PMA (< 10 nM) stimulate primarily enzyme induction whereas high concentrations of PMA (> 10 nM) have an additional effect of enzyme inactivation, presumably via protein kinase C-mediated phosphorylation. Similarly, a decrease in 15-PGDH activity at 36 h during the time course study did not reflect less enzyme synthesis, as revealed by the Western blot studies (results not shown). It is very likely that 15-PGDH activity is subjected to regulation by phosphorylation/dephosphorylation, as with other enzymes showing diurnal variation and regulation by phosphorylation [33]. In order to provide evidence for regulation by protein kinase C-mediated phosphorylation, 15-PGDH was induced by DMSO and the cells were then subjected to treatment with 50 nM-PMA in the absence and the presence of protein kinase C inhibitors such as staurosporine and H-7. Indeed, PMA caused a time-dependent inactivation of the enzyme during the 2 h treatment. Concentrations of PMA lower than 50 nM also induced inactivation of the enzyme, although the time required to reach 50% inactivation was longer. For rapid demonstration of a PMA effect, a concentration of 50 nM was used. Inactivation was significantly blocked by the presence of staurosporine or H-7, suggesting that inactivation of the enzyme is mediated by the activation of protein kinase C. However, definitive proof of enzyme inactivation by protein kinase C-mediated phosphorylation requires a purified enzyme preparation in order to demonstrate its inactivation by phosphorylation.

Blackwell *et al.* [34] reported that rat kidney and lung pre-treated with cycloheximide rapidly lost their ability to metabolize prostaglandins. These workers estimated the half-life of 15-PGDH to be 45–75 min. By directly measuring the disappearance of L-[<sup>35</sup>S]methionine-labelled 15-PGDH we found that the PMA-induced enzyme in HL-60 cells incubated with medium alone undergoes rapid degradation, with a half-life of approx. 47 min. Addition of PMA during the chase period shortens the half-life to 33 min, suggesting that PMA might have an additional effect on 15-PGDH degradation. The kinetics of degradation shows first-order kinetics, which is different from the case with the turnover of cyclo-oxygenase [31]. In view of the rapid turnover of 15-PGDH, enzyme activity will depend on continued enzyme synthesis, and this could be very susceptible to hormonal and drug control mechanisms. Several reports have demonstrated alterations in 15-PGDH activity caused by hormonal changes [8], steroid administration [11], endotoxin shock [35] or cigarette smoking [36] and in tissues from spontaneously hypertensive rats [37]. Although alteration of these enzyme activities may be the consequence of post-translational modifications of 15-PGDH, such as the phosphorylation suggested above, changes in enzyme synthesis and content are also very likely possibilities. Recently a full-length cDNA for human placental 15-PGDH has been cloned and sequenced [38]. The availability of this cDNA should make it possible to differentiate between the above possibilities and will facilitate studies on the regulation of this important eicosanoid metabolic enzyme.

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