Characterization and Distribution of Prostaglandin D Synthetase in Rat Skin*

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The biochemical properties and immunohistochemical localization of prostaglandin D synthetase were investigated in adult rat skin. The activity of prostaglandin D synthetase, which isomerizes prostaglandin H_2 to prostaglandin D_2, was detected in the 100,000 *g* supernatant of the homogenate of adult rat skin. Whole skin showed considerable activity (1.9 nmol/min/mg protein), and prostaglandin D_2 was the major prostaglandin among those formed from prostaglandin H_2 in the presence of glutathione. The epidermis, which was separated from whole skin by heating (55°C, 30 s), exhibited about three times higher activity (3.5) than the dermis (1.0). The enzymatic properties of both layers were similar; they were absolutely glutathione-dependent, were inhibited only a few percent by 1 mM 1-chloro-2,4-dinitrobenzene, and were completely absorbed by anti-rat spleen prostaglandin D synthetase antibody. Immunohistochemical studies, using anti-rat spleen prostaglandin D synthetase antibody and the immunoperoxidase method, showed that prostaglandin D synthetase was localized in Langerhans cells (not in keratinocytes) in the epidermis, in macrophages or histiocytes, and also in mast cells in the dermis. Immunoelectron microscopy also supported these findings. These results suggest that prostaglandin D_2 is one of the most important arachidonic acid metabolites and plays a significant role in immunological function in the skin via Langerhans cells and macrophages. *J Invest Dermatol 90:448–451, 1988*

Prostaglandin (PG) D_2 is a naturally occurring prostanoid [1–3] that shows various kinds of pharmacological activity such as inhibition of platelet aggregation [4], bronchoconstriction [5], sleep induction [6], and hypothermia [7] under various physiological and pathological conditions. In the skin, however, the in vivo function of PGD_2 is not yet fully understood, although PGD_2 is the major cyclooxygenase product from arachidonic acid in the epidermis and dermis of guinea pig skin [8]. Intradermal injection of PGD_2 in the rat causes increased vascular permeability and in humans produces prolonged cutaneous dose-related erythema [9]. PGD_2 is known to be actively produced by mast cells during IgE stimulation and to modify the anaphylactic process [10,11]. Therefore, PGD_2 seems to be an important mediator in the development of acute and chronic inflammation in the skin. Furthermore, Langerhans cells have been reported to be active for PGD_2 synthesis from arachidonic acid [12,13], suggesting that PGD_2 also plays a significant role in immunological and allergic reactions in the skin.

In this paper, we report the enzymatic properties and immunohistochemical localization of PGD synthetase in adult rat skin to make clear the biological significance of PGD_2 in the skin.

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Abbreviations:
CDNB: 1-chloro-2,4-dinitrobenzene
DAB: diaminobenzidine
GSH: glutathione
PBS: phosphate-buffered saline
PG: prostaglandin

MATERIALS AND METHODS

Materials [1-^{14}C]Arachidonic acid (56.0 mCi/mmol) was purchased from New England Nuclear. [1-^{14}C]PGH_2 was prepared as described previously [14]. PGD_2, PGE_2, and $PGF_{2\alpha}$ were generous gifts from Ono Pharmaceutical, Osaka, Japan. Precoated silica gel plates (Kiesel gel 60 F254) were from Merck. 1-Chloro-2,4-dinitrobenzene (CDNB) was from Tokyo Kasei, Tokyo, Japan. Glutathione (GSH) was from Sigma. All other chemicals used were of analytical grade.

Animals and Skin Preparations Male Wistar rats weighing 250–300 g were used and the dorsal hair of each rat was shaved with surgical clippers 48 h before use. Epidermis and dermis were separated by the method of Raineri et al. [15] with minor modifications. Briefly, pieces of dorsal skin of rats killed by decapitation were placed in water at 55°C for 30 s and then in ice-cold water for 30 s. Then the skin was placed epidermis-side-up on a cold plate and the epidermis was scraped off with a razor blade. Each weighed tissue was homogenized in 3 vol of 150 mM NaCl and 10 mM potassium phosphate (PBS; pH 7.2) with an Ultra-Turrax homogenizer at top speed for 30 s. Homogenates were centrifuged at 100,000 *g* for 1 h at 4°C and the supernatant solutions were used as enzyme sources.

Assay of PGD Synthetase The reaction mixture for the assay of PGD synthetase activity contained 0.1 M Tris–HCl (pH 8.0), 1 mM GSH, and the enzyme in a total volume of 50 μl. After preincubation at 25°C for 10 min, the reaction was started by the addition of [1-^{14}C]PGH_2 (final 40 μM) dissolved in 1 μl of diethylene glycol dimethyl ether and carried out at 25°C for 1 min. Termination of the reaction and extraction and quantification of the products were performed as described previously [16]. To examine the dependence of PGD synthetase activity on GSH, the supernatant solutions were passed twice through a PD-10 column (Pharmacia) equilibrated with homogenizing buffer to exclude intrinsic GSH, and assayed in the presence of 1 mM β-mercaptoethanol instead of 1 mM GSH.

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Protein was determined by the method of Lowry et al. [17] with bovine serum albumin as a standard.

Immunochemical Study Each antibody against rat spleen PGD synthetase [16] and rat brain PGD synthetase [18] was prepared as described previously. In immunotitration analyses, the 100,000 *g* supernatant of the skin preparation was incubated at 4°C overnight with various amounts of these antibodies in the presence of 1 mM dithiothreitol to prevent inactivation of the enzyme. After incubation with each antibody, the reaction mixture was centrifuged at 10,000 *g* for 10 min, and the resulting supernatant was recovered for the determination of PGD synthetase activity.

Immunoperoxidase Staining Pieces of dorsal skin of adult rats killed by perfusion with an ice-cold Ringer solution were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.5, at 4°C overnight. After being washed overnight with PBS containing 10–20% sucrose, the tissue specimens were embedded in OCT compound (Tissue-Tek II, Miles). Sections (thickness = 10 μm) were cut on a cryostat, mounted on albumin-coated glass slides, and air-dried in preparation for immunoperoxidase staining. Sections were stained by the method of Hsu et al. [19] using anti-rat spleen PGD synthetase IgG (10 μg/ml PBS) as the first antibody, biotinylated anti-rabbit IgG antibody as the second labeled antibody, and a Vectastain ABC Kit (Vector Lab.). The control was stained with nonimmunized rabbit IgG as the first reagent. Sections were then reacted with a diaminobenzidine (DAB)–hydrogen peroxide solution for 30 min.

Immunoelectron Microscopy Some of the immunoperoxidase-stained sections were postfixed in 1% osmium tetroxide after reaction with DAB–hydrogen peroxide, dehydrated in an ethanol series, and embedded in polyethylene capsules of epoxy resin. The ultrathin sections were observed under a Hitachi-300 electron microscope without electron-optical counterstaining as described previously [20].

RESULTS

Location of PGD Synthetase Activity in the Skin of Adult Rats PGD synthetase activity was found in whole skin of adult rats (1.9 nmol/min/mg protein), when PGH_2 was incubated with 100,000 *g* supernatants of adult rat skin in the presence of 1 mM GSH (Table I). Although the PGD synthetase activity of whole skin was reduced by the heat treatment at 55°C for 30 s (1.2 nmol/min/mg protein), the skin was divided into the epidermis and dermis and the activities in these layers were compared. Enzyme activity was found in both layers, but was about three times higher in the epidermis (3.5 nmol/min/mg protein) than in the dermis (1.0 nmol/min/mg protein). Activity in both layers was absolutely GSH-dependent for the reaction (Table I). Furthermore, the activities were inhibited only a few percent by 1 mM CDNB (data not shown), which is one of the substrates of GSH *S*-transferase [21].

Table I. Location of PGD Synthetase Activity in Adult Rat Skin

Heat Treatment[a]	1 mM GSH	PGD Synthetase Activity (nmol/min/mg protein)[b] Whole skin[c]	Epidermis[c]	Dermis[c]
+	+	1.22 ± 0.13	3.49 ± 0.10	0.98 ± 0.16
	−	0.13 ± 0.03	0.19 ± 0.01	0.11 ± 0.02
−	+	1.91 ± 0.06	—	—
	−	0.26 ± 0.06	—	—

[a] Whole skin was placed in water at 55°C for 30 s and then in ice-cold water for 30 s. The epidermis was separated from the dermis as described under Materials and Methods.

[b] Supernatants (100,000 *g*, 1 h) of homogenates of rat skin preparations were incubated with 40 μM [1-^{14}C]PGH_2 at 25°C for 1 min in the presence of 1 mM GSH or 1 mM β-mercaptoethanol. Extraction, thin-layer chromatography, and quantification of PGD_2 were performed as described under Materials and Methods. Values represent means of three samples ± SE.

[c] Protein concentrations of the enzyme sources were as follows: whole skin, 5.4 mg/ml; epidermis, 7.2 mg/ml; dermis, 4.4 mg/ml.

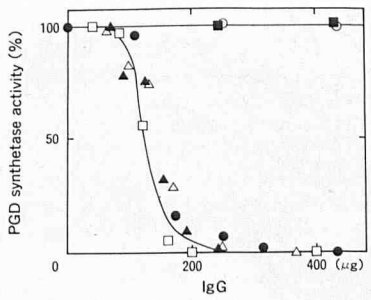

Figure 1. Immunotitration analyses of PGD synthetase activities in supernatants (100,000 *g*, 1 h) of skin preparations of adult rats, with antibodies against PGD synthetases purified from the spleen and brain. Various amounts of the antibodies were added to the supernatants containing a given activity of PGD synthetase. After incubation at 4°C overnight, the residual activity was determined as described under Materials and Methods. Activity was expressed as a percentage of that before incubation (25.2 nmol/min). Symbols represent the residual activity of whole skin (●), epidermis (△), dermis (▲), and spleen (□) after incubation with anti-rat spleen PGD synthetase IgG, and the residual activity of whole skin after incubation with anti-rat brain PGD synthetase IgG (○) or nonimmunized IgG (■).

Immunochemical Properties of PGD Synthetase of Adult Rat Skin In immunotitration analyses with the antibody against rat spleen PGD synthetase, the activities in whole skin, epidermis, dermis, and spleen of adult rats showed identical titration curves (Fig 1). Activity almost completely disappeared after incubation with excess amounts of antibody to spleen enzyme, but were unchanged after incubation with antibody to brain enzyme or nonimmunized IgG.

Immunohistochemical Observation of PGD Synthetase in Rat Skin In adult rat skin, cytoplasmic distribution of PGD synthetase was prominent in the intraepidermal dendritic cells (Fig 2*A*). The positively stained dendritic cells were rich in hair follicles (Fig 2*B*). In the dermis, infiltrating cells were immunohistochemically positive for PGD synthetase (Fig 2*A*). The intensity of positive stain in a portion of these infiltrating cells was somewhat lower than that of the other positive cells (Fig 2*B*, *arrow*). These weakly immunoreactive cells were identified as mast cells, as metachromasia was observed after staining with 0.01% toluidine blue (data not shown). Control specimens revealed no specific immunostaining (Fig 2*C*).

Immunoelectron Microscopy of PGD Synthetase in Rat Skin The positively stained cells in the epidermis ultrastructurally showed markedly folded nuclei and Birbeck granules with their characteristic rod-shaped structure. The fine granular reaction products were homogeneously distributed in the cytoplasm of the cells (Fig 3*A* and *inset*). These cells in the epidermis were considered to be Langerhans cells. In the dermis, some of the positive cells showed large nuclei and irregularly projecting narrow cytoplasm with abundant vacuoles and lysosomes (Fig 3*B*). The other positive cells contained oval nuclei and numerous granules in the cytoplasm (Fig 3*C*). The reaction products were also distributed homogenously in the cytoplasm. In the dermis, the former cells were considered macrophages or histiocytes, and the latter, mast cells.

DISCUSSION

Our experiments show that adult rat skin, not only epidermis but also dermis, has active PGD synthetase. PGD synthetase in both layers of rat skin is absolutely GSH-dependent, inhibited only a few percent by 1 mM CDNB, and completely absorbed by anti-rat spleen PGD synthetase antibody. Recently, the authors found at least three different types of PGD synthetase in adult rats: spleen type, brain type, and GSH *S*-transferase [22]. Judging from the enzymatic and immunochemical properties described earlier, rat skin PGD synthetase is of the spleen type.

The immunohistochemical results for PGD synthetase in rat skin

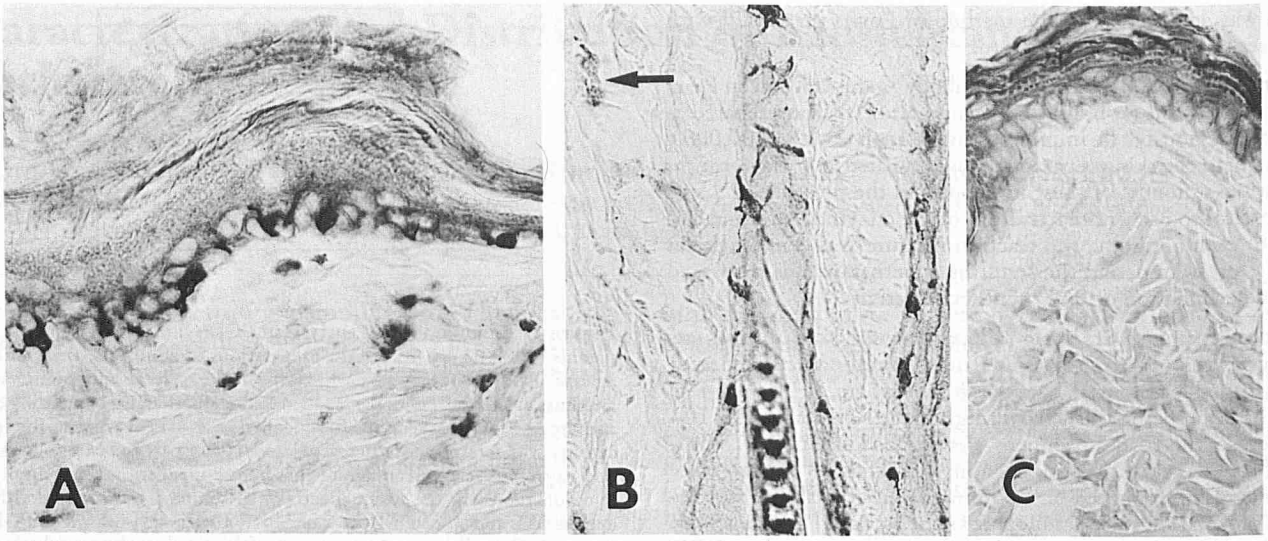

Figure 2. Immunohistochemical distribution of PGD synthetase in rat skin by anti-rat spleen PGD synthetase antibody. *(A)* Cytoplasmic distribution of PGD synthetase was prominent in dendritic cells in the epidermis. In the dermis, infiltrating cells were stained. *(B)* The positively stained dendritic cells were abundant in hair follicles. Immunoreactivity of mast cells *(arrow)* was positive but somewhat lower than that of the other positive cells. *(C)* No specific immunostaining was observed by incubation with nonimmunized rabbit IgG. *A*, ×400; *B*, ×400; *C*, ×400.

are quite compatible with biochemical findings that spleen-type PGD synthetase exists in both layers of the skin and that the activity is higher in the epidermis than in the dermis. In the epidermis, immunostaining of this enzyme is prominently and exclusively positive in Langerhans cells, not in keratinocytes. This finding agrees with reports that enriched Ia-positive Langerhans cells produce mainly PGD_2 from arachidonic acid and that Langerhans cell-depleted epidermal cells produce scarcely any PGD_2 [12,13]. A marked decrease in PGD_2 formation was reported in experimental contact dermatitis [23] and UVB-irradiated skin [24]. These results can be explained by our observation that PGD_2 in the epidermis is synthesized mainly in Langerhans cells, because the number and function of Langerhans cells are remarkably reduced under these conditions [25].

In the dermis, PGD synthetase is localized in histiocytes or macrophages and in mast cells. As mast cells actively produce PGD_2 [10] and are rich in GSH-requiring PGD synthetase [26], they are believed to be a major source of PGD_2 in various tissues; however, the intensity of the positive stain in mast cells is somewhat lower than in Langerhans cells and in histiocytes or macrophages. Furthermore, immunoreactive mast cells are fewer in number than those cells. These results are consistent with the previous observation that no

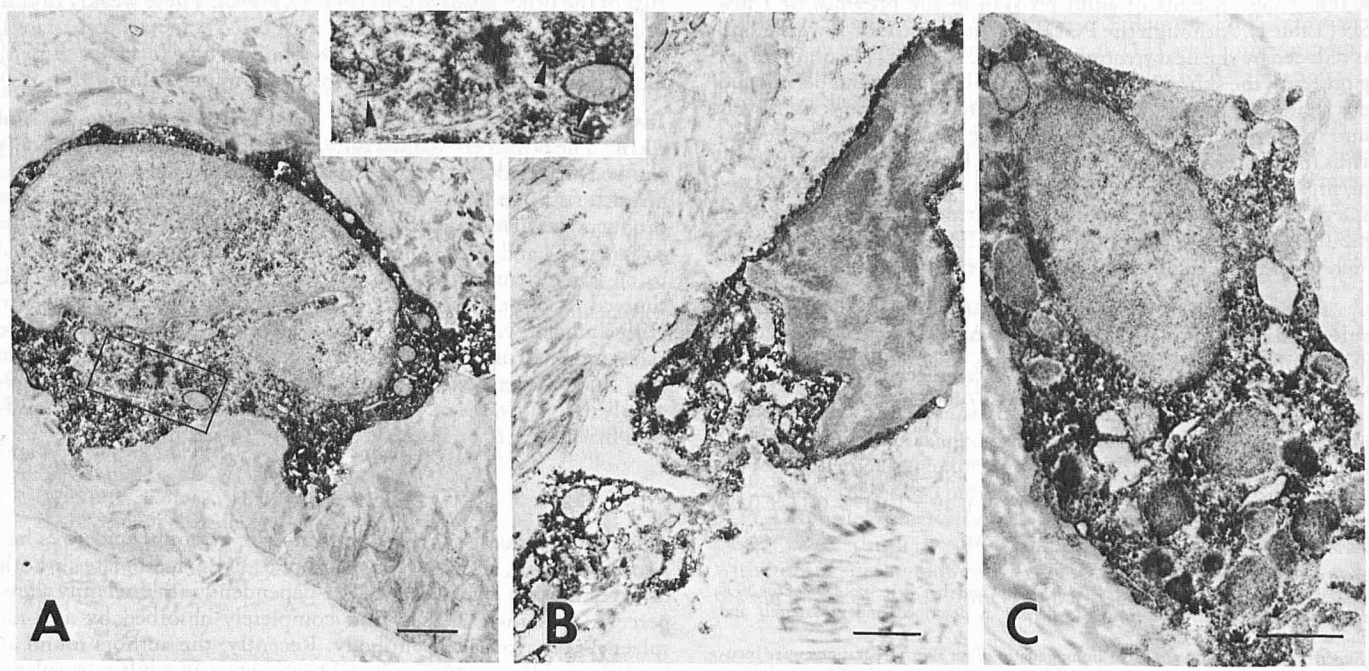

Figure 3. Immunoelectron microscopy of PGD synthetase in rat skin. *(A)* Fine granular reaction products were observed in the cytoplasm of Langerhans cells in the epidermis. *Inset: Arrows* indicate rod-shaped Birbeck granules in the Langerhans cells. *(B)* Reaction products were distributed in the cytoplasm of histiocytes or macrophages in the dermis. *(C)* Reaction products were observed in the cytoplasm of mast cells in the dermis. *A*, ×8000; *inset*, ×16,000; *B*, ×9100; *C*, ×11,800. *All bars* = 1 μm.

difference in PGD_2 synthesis in the skin is found between mast cell deficient W/W^v mice and their control littermates [27]. The contribution of mast cells to PGD_2 formation is, therefore, thought not to be so significant in adult rat skin.

Tissues rich in spleen-type PGD synthetase are spleen, thymus, bone marrow, intestine, and stomach [22]. In these tissues, the immunostaining of spleen-type PGD synthetase is localized mainly in histiocytes or macrophages [28]. In the liver, Kupffer cells, which also belong to the reticuloendothelial system, are exclusively positive for the enzyme [28]. In the skin, Langerhans cells and histiocytes or macrophages are positive for PGD synthetase of the spleen type. Langerhans cells have some surface-marker characteristics in common with macrophages: Fc and C3 receptors and Ia antigens [25]. Ia antigens are immune response gene-associated antigens shared by immunologically active cells. These results suggest that PGD_2 plays a significant role in immunological function, such as inflammatory, and immediate and delayed immune reactions in the skin via Langerhans cells and macrophages.

Besides regulating the inflammatory reaction, PGD_2 might participate in the regulation of epidermal proliferation and differentiation [2,3]. PGD_2 also shows potent cytotoxic activity on various cell lines [29–31], including transformed mouse epidermal cells [32]. A reevaluation of PGD_2 in normal and diseased skin should provide more conclusive results with regard to the pathogenesis of inflammatory, allergic, hyperproliferative, and neoplastic skin diseases.

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