Mast Cells Contain Spleen-type Prostaglandin D Synthetase*

Yoshihiro Urade‡, Mayumi Ujihara‡, Yuji Horiguchi‡, Makoto Igarashi‡, Akihisa Nagata‡, Kouichi Ika‡, and Osamu Hayashi**

From the ‡Department of Enzymes and Metabolism, Osaka Bioscience Institute, Furuedai, Suita, Osaka 565, Japan, the  †Department of Dermatology, Kyoto University Faculty of Medicine, Shogoin, Sakyoku-ku, Kyoto 606, Japan, and the ‡Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565, Japan

Prostaglandin D synthetase activity in the cytosol (100,000 x g, 1-h supernatant) fraction of peritoneal mast cells of adult rats (105.0 nmol/min/mg protein) was the highest among such activities in various rat tissues and cells. As judged by the absolute requirement for glutathione for the reaction (Km = 300 μM), the Km value for prostaglandin H2 (200 μM), and insensitivity of the activity to 1 mM 1-chloro-2,4-dinitrobenzene, the enzyme in mast cells was similar to rat spleen prostaglandin D synthetase and differed from rat brain prostaglandin D synthetase or glutathione S-transferase, all of which catalyze the isomerase reaction from prostaglandin H2 to prostaglandin D2. In immunotitration analysis, the activity in mast cells showed a titration curve exactly identical with that of the purified spleen-type enzyme and almost completely absorbed by an excess amount of antibody against this enzyme, but it remained unchanged after incubation with antibodies against the brain-type enzyme and glutathione S-transferase isozymes thus far purified. In Western blot after two-dimensional electrophoresis of crude extracts of mast cells, a single immunoreactive spot was observed with antibody against the spleen-type enzyme at the same position as that of the purified enzyme (M, = 26,000, pI = 5.2). Furthermore, the immunoreactive protein obtained from mast cells showed the same peptide fingerprints as those of the purified spleen-type enzyme, after partial digestion with Staphylococcus aureus V8 protease or trypsin. In immunoperoxidase staining, the immunoreactivity of the spleen-type enzyme was found in the cytosol of tissue mast cells in various organs such as thymus, intestine, stomach, and skin of adult rats. These findings indicate that prostaglandin D2 is produced by the spleen-type synthetase in mast cells of various tissues.

Prostaglandin (PG) synthetase is a major cyclooxygenase product formed in mast cells obtained from rats and humans (1-3). It shows various pharmacological activities such as inhibition of human platelet aggregation (4), peripheral vasodilation and pulmonary vasoconstriction in dogs (5), and bronchoconstriction in dogs (5) and humans (6). PGD2 is produced in mast cells during pharmacological or physiological stimulation with calcium ionophore or anti-IgE antibody (3, 7-9). In addition to these in vitro findings, Roberts et al. (10) observed overproduction of PGD2 in several patients with systemic mastocytosis as evidenced by their elevated levels of urinary metabolites of PGD2. They also showed that chronic aspirin therapy reduced overproduction of PGD2 in those patients as well as suppressed their life-threatening hypotensive episodes (10). Therefore, PGD2 produced in mast cells is thought to be significantly involved in the anaphylactic process.

The enzymic conversion of PGH2 to PGD2 is observed in the soluble fraction of rat peritoneal mast cells (11), but quantitative measurement of the activity and identification of the enzyme remain to be elucidated. Two different types of PGD synthetase (prostaglandin H2 n-isomerase, EC 5.3.99.2) have been purified from rats; one is glutathione (GSH)-independent enzyme from brain (12) and the other is GSH-requiring enzyme from spleen (13, 14). These two PGD synthetases are similar acidic proteins with the same M, of 26,000 but differ from one another in terms of their amino acid compositions, catalytic properties, antigenic determinants (14), and mRNA sequences (15). Furthermore, we also showed that several isoforms of GSH S-transferase (EC 2.5.1.18) purified from various rat tissues catalyze the conversion of PGH2 to produce PGD2 (16).

In this study, we report that PGD synthetase activity in rat peritoneal mast cells is the highest among such activities in various rat tissues so far determined and demonstrate that the enzyme in these cells is biochemically and immunologically the same enzyme as that purified from rat spleen. We also examine the immunocytochemical localization of the spleen-type enzyme in tissue mast cells in various organs, indicating that the enzyme is responsible for biosynthesis of PGD2 in this type of cells.

EXPERIMENTAL PROCEDURES

Materials—[1-14C]Arachidonic acid (52.0 mCi/mmol) was purchased from Du Pont-New England Nuclear. [1-14C]PGH2 was prepared as described previously (17). PGD2, PGE2, and PGE3, were generous gifts from Ono Central Research Institute, Osaka, Japan. Dithiothreitol and 5-mercaptoethanol were obtained from Macalai Tesque, Kyoto, Japan. GSH was from Sigma. 1-Chloro-2,4-dinitrobenzene was from Tokyo Kasei, Tokyo, Japan. Staphylococcus V8 protease, bovine pancreatic trypsin, and d-biotin-N-hydrosuccinimide ester were obtained from Boehringer Mannheim. All other chemicals were of analytical grade.

Enzyme Purification—Rat brain PGD synthetase (12) and rat spleen PGD synthetase (14) were purified by our previously reported methods.

Antibodies—Preparation procedures and characterization of polyclonal antibodies specific for rat brain and spleen PGD synthetase

(Received for publication, July 14, 1989)
M. embedded in OCT compound (Tissue-Tek II; Miles). Cryosections were washed overnight with PBS containing 10-20% sucrose and incubated with various amounts of antibody at 4 °C overnight. The mixture was then centrifuged at 10,000 x g for 1 h and the supernatant was used for the assay of PGD synthetase activity. The enzyme reaction was performed in 50 μl of 0.1 M Tris-HCl (pH 8.0) containing 1 mM GSH at 25 °C for 1 min after addition of [1-14C]PGH₂ (final concentration 40 μM), unless otherwise stated. When the requirement of sulfhydryl compounds for the reaction was examined, the enzyme solution was applied to a PD-10 column (Pharmacia LKB Biotechnology Inc.) equilibrated with the homogenization buffer to remove intrinsic GSH, and the eluate was used for the assay. Termination of reaction, extraction, and quantification of products were performed as described previously (12, 14).

Enzyme Assays—About 5 x 10⁵ cells were homogenized in 500 μl of 10 mM potassium phosphate (pH 7.4) and 150 mM NaCl (PBS) by sonicating (4 x 15 s, in an ice bath) with a Heat Systems-Ultrasonics model 220 Sonifier. The homogenate was centrifuged at 100,000 x g for 1 h and the supernatant was used for the assay of PGD synthetase activity. The enzyme reaction was performed in 50 μl of 0.1 M Tris-HCl (pH 8.0) containing 1 mM GSH at 25 °C for 1 min after addition of [1-14C]PGH₂ (final concentration 40 μM), unless otherwise stated. When the requirement of sulfhydryl compounds for the reaction was examined, the enzyme solution was applied to a PD-10 column (Pharmacia LKB Biotechnology Inc.) equilibrated with the homogenization buffer to remove intrinsic GSH, and the eluate was used for the assay. Termination of reaction, extraction, and quantification of products were performed as described previously (12, 14). Protein concentration was determined by the method of Lowry et al. (22) using bovine serum albumin as standard.

Immunotitration—The cells were homogenized in PBS containing 0.5 μM dithiotreitol to prevent inactivation of the enzyme during incubation. A given volume of the 100,000 x g supernatant was incubated with various amounts of antibody at 4 °C overnight. The mixture was then centrifuged at 10,000 x g for 10 min to remove the immunoprecipitated and the residual PGD synthetase activity was determined in the resulting supernatant.

Western Blot—The cytosol fraction (100,000 x g supernatant) of peritoneal mast cells or the purified rat spleen PGD synthetase was applied to two-dimensional polyacrylamide gel electrophoresis (PAGE) by the method of O’Farrell (25). The gels after PAGE were electrophoretically transferred to a Zeta-Probe nylon membrane (Bio-Rad). The two-dimensional gel electrophoresis solution (pH 3.5-9.5) containing 3% gelatin and 0.05% Tween 20 in PBS at 45 °C for 1 h and then incubated with anti-rat spleen PGD synthetase IgG (100 μg/ml) at 40 °C overnight. After incubation, the membrane blot was immuno-stained by the method of Hau et al. (24) with biotinylated anti-rabbit IgG antibody and Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA).

Peptide Mapping—The 100,000 x g supernatant of mast cells was incubated at 4 °C overnight with an excess amount of anti-rat spleen PGD synthetase IgG. The immunoprecipitate was recovered by centrifugation at 100,000 x g for 1 h and the supernatant was used for the assay of PGD synthetase activity. The enzyme reaction was performed in 50 μl of 0.1 M Tris-HCl (pH 8.0) containing 1 mM GSH at 25 °C for 1 min after addition of [1-14C]PGH₂ (final concentration 40 μM), unless otherwise stated. When the requirement of sulfhydryl compounds for the reaction was examined, the enzyme solution was applied to a PD-10 column (Pharmacia LKB Biotechnology Inc.) equilibrated with the homogenization buffer to remove intrinsic GSH, and the eluate was used for the assay. Termination of reaction, extraction, and quantification of products were performed as described previously (12, 14). Protein concentration was determined by the method of Lowry et al. (22) using bovine serum albumin as standard.

RESULTS

Biochemical Characterization of PGD Synthetase Activity in Peritoneal Mast Cells—When the mast cell homogenate was incubated with 40 μM PGH₂ in the presence of 1 mM GSH, concentration-dependent conversion of PGH₂ to PGD₂ was observed (29.6 ± 3.1 nmol/min/10⁶ cells; mean ± S.E. of three determinations). No enzymic conversion of PGH₂ to PGE₂ or PGF₂α was found during the incubation under our standard assay conditions. The concentration-dependent formation of PGD₂ was abolished after a heat treatment of the homogenate at 100 °C for 5 min. On the other hand, no significant PGD synthetase activity was detected in homogenates of macrophages, lymphocytes, or polymorphonuclear leukocytes (<0.3 nmol/min/10⁶ cells). When the homogenate of peritoneal mast cells was mixed with those of the other types of cells, neither inhibition nor activation of the PGD synthetase activity was observed.

About 98% of PGD synthetase activity in homogenates of peritoneal mast cells was recovered in the 100,000 x g supernatant fraction. The specific activity (105.0 ± 10.0 nmol/min/mg protein) was about 2-fold higher than that of rat spleen (57.1 nmol/min/mg protein), making it the highest activity among various rat tissues (26). The PGD synthetase from mast cells absolutely required GSH for the reaction and was virtually inactive with other sulfhydryl compounds such as 1 mM β-mercaptoethanol or 0.5 mM dithiobitol. The KM value for GSH was calculated to be about 300 μM. In the presence of 1 mM GSH, the synthetase activity increased almost linearly with the concentration of PGH₂ up to 100 μM, and the KM value for PGH₂ was calculated to be around 200 μM. These catalytic properties are similar to those of rat spleen PGD synthetase and clearly different from those of rat brain PGD synthetase (14).

Furthermore, the synthetase from mast cells showed almost full activity in the presence of 1 mM 1-chloro-2,4-dinitrobenzene, a reagent which inhibits rat brain PGD synthetase completely and GSH S-transferase-associated PGD synthetase activity partially (26), but not rat spleen PGD synthetase activity (14).

Immunological and Molecular Properties of PGD Synthetase in Peritoneal Mast Cells—In an immunoelectron microscopy analysis with antibody against rat spleen PGD synthetase (Fig. 1), the activity from peritoneal mast cells decreased dose-dependently and showed a titration curve identical with that of the purified spleen-type PGD synthetase, indicating that the enzyme in these cells is immunologically the same as that purified from rat spleen. After incubation with an excess amount of the antibody, the synthetase activity from these cells decreased to less than 5% of the initial activity before incubation. On the other hand, the activity remained unchanged after incubation with excess amounts of antibodies against rat brain PGD synthetase and GSH S-transferase
isozymes 1-2, 3-4, 6-6, and 7-7, or of non-immunized rabbit IgG.

In a Western blot after two-dimensional PAGE of crude extracts of peritoneal mast cells (Fig. 2), an immunoreactive spot was intensively stained with antibody against the spleen-type PGD synthetase at the same position as that of the purified enzyme ($M_r = 26,000, pI = 5.2$) (12). Although weakly stained proteins were observed at regions of more acidic $pI$ and smaller $M_r$ than those of the major immunoreactive protein, all of the positions were clearly different from that of rat brain PGD synthetase (27) or of various rat GSH S-transferase isozymes characterized thus far. When antibody against the brain-type PGD synthetase was used for immunostain, no positive spot was detected in crude extracts of mast cells. After partial digestions with S. aureus V8 protease or trypsin, the spleen-type PGD synthetase-like immunoreactive protein recovered from peritoneal mast cells showed peptide fingerprint identical to those of the purified spleen-type enzyme (Fig. 3). The immunoreactive proteins of smaller $M_r$ ($-12,000$ and $14,000$) in mast cells do not change in size upon digestion with $S$. aureus V8 protease and were degraded by trypsin. Note that a major proteolytic product of PGD synthetase by V8 protease migrates to the same position as that of the immunoreactive protein of $M_r = 14,000$. These immunoreactive peptides were not cross-reactive with antibody against the brain-type PGD synthetase. Furthermore, both fragmentation profiles and the proteolytic sensitivity of the brain-type enzyme were quite different from those of PGD synthetase in spleen and mast cells.

These results both confirmed the specificity of the antibody and showed that PGD synthetase of the spleen-type, but not the brain-type enzyme nor GSH S-transferase, is responsible for biosynthesis of PGD$_2$ in peritoneal mast cells.

**Immunocytochemical Localization of Spleen-type PGD Synthetase in Tissue Mast Cells of Various Rat Organs**—When immunoperoxidase staining was carried out with antibody against spleen-type PGD synthetase on sections of various tissues of adult rats, significant positive staining was observed in several mast cells located in the connective tissues, such as the septum of thymus (Fig. 4a), the submucosal layer of stomach (Fig. 4c) and intestine, dermis (Fig. 4e), and subcutaneous tissue. The positive staining was inhibited by preabsorption of the antibody with the purified rat spleen PGD synthetase (Fig. 4, b, d, and f) and was not detected with nonimmunized IgG or anti-rat brain PGD synthetase IgG. The immunoreactivity of spleen-type PGD synthetase was undetectable in brain, spinal cord, kidney, and testis, where either brain-type PGD synthetase or GSH S-transferase is responsible for biosynthesis of PGD$_2$ in homogenates of such tissues (16, 26).

Immunoelectron microscopy confirmed the localization of the immunoreactivity of this enzyme in mast cells (Fig. 5). Fine granular reaction products were widely distributed in the cytoplasm of mast cells, in agreement with the observation that the PGD synthetase activity in these cells was nearly completely recovered in the cytosolic (100,000 $\times g$ supernatant) fraction.

**DISCUSSION**

In this study, we show that the spleen-type PGD synthetase, and not the brain-type enzyme or the known isozymes of GSH S-transferase, is the major enzyme responsible for the biosynthesis of PGD$_2$ in peritoneal mast cells, as judged by the kinetic ($K_m$ (GSH) = 300 $\mu$M, $K_m$ (PGH$_2$) = 200 $\mu$M), immunological (Figs. 1 and 2), and molecular ($M_r = 26,000$, $pI = 5.2$, Fig. 3) properties of the enzyme. By using immunoperoxidase staining with antibody specific for the spleen-type enzyme (Figs. 4 and 5), we also demonstrate that this enzyme

---

**FIG. 1.** Immunotitration test of the PGD synthetase activity in the 100,000 $\times g$ supernatant of peritoneal mast cells obtained from adult rats. Symbols represent antibodies specific for each rat spleen PGD synthetase (O), rat brain PGD synthetase (A), GSH S-transferase isozymes 1-2 (A.), 3-4 (A.), 6-6 (O.), and nonimmunized rabbit IgG (M). The residual enzyme activities are expressed as percentages of those before incubation (25.2 nmol/min). The titration curve of the purified rat spleen PGD synthetase with antibody against this enzyme (O) is also shown.

**FIG. 2.** Western blot with anti-rat spleen PGD synthetase IgG after two-dimensional PAGE of crude extracts of peritoneal mast cells. First dimension, isoelectric focusing (IEF) in 5% polyacrylamide gel containing 2% Ampholine, pH 5.5-10; second dimension, SDS-PAGE (10.6% gel). Proteins (10 $\mu$g) separated in the gel were transferred to a nylon membrane and then immunostained as described under "Experimental Procedures." Arrowheads indicate the positions of purified spleen-type PGD synthetase in IEF (bottom) and SDS-PAGE (right). The positions of biotinylated $M_r$ marker proteins (Bio-Rad) (arrow) and their $M_r$, values in thousands are indicated on the right: phosphorylase b (97,400), bovine serum albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).
Prostaglandin D Synthetase in Mast Cells

S. aureus V8 protease (pg)

B C

31.0 -em-

21.5

14.4

1 FKVll

mwsin (w)

--- --

C

5 10 20

l. c

FIG. 4. Immunoperoxidase staining of rat spleen PGD synthetase in tissue mast cells of rat thymus (a and b), stomach (c and d), and skin (e and f). Serial sections of each tissue were stained with anti-rat spleen PGD synthetase antibody (a, c, and e) or the antibody preabsorbed with the purified enzyme as the control (b, d, and f). Immunoreactive mast cells are indicated by arrowheads and also by circles in the control sections. Interlobular space (* in a and b), blood vessels (small arrows in c and d), and the collagenous bundles (dotted line in e and f) are also marked to indicate the position of each serial section. Bar = 50 μm.

FIG. 3. Immunoreactive peptide fingerprints of PGD synthetases purified from rat spleen (A), and brain (C) and recovered from peritoneal mast cells (B) after partial digestion with S. aureus V8 protease (upper panels) or trypsin (lower panels). Each sample (1.0 μg protein) was digested at 37 °C for 30 min with various amounts of each protease as described under “Experimental Procedures.” The digest was subjected to SDS-PAGE (15.0% gel) and transferred to a nylon membrane. The nylon blots (A) and (B) were immunostained with antibody against spleen-type PGD synthetase, and the blot (C) was with antibody against brain-type PGD synthetase. When the heterologous antibody was used for immunostain of these blots, no positive stain was detected. The positions of biotinylated M, marker proteins (arrow) and their M, values in thousands are indicated on the left.

is widely distributed in tissue mast cells of various organs of adult rats.

Although Sun et al. (28) reported PGD synthetase activity to be present in homogenates of rat polymorphonuclear leukocytes, we could not observe any significant activity of the enzyme in the homogenate of this type of cell (<0.3 nmol/min/10⁶ cells or <1.6 nmol/min/mg protein in the 100,000 × g supernatant). Neither did we detect PGD synthetase activity in the homogenate of peritoneal and alveolar macrophages or of blood monocytes (<1.0 nmol/min/mg protein), which is consistent with previous studies (29–31) showing that this type of cell is inactive in terms of PGD2 synthesis both before and after stimulation with various activators of macrophages. Furthermore, in Western blots of crude extracts of leukocytes and macrophages, no immunoreactive protein was detected with either of antibodies against the brain- and spleen-type PGD synthetases. Recently, we isolated cDNA clones specific for rat brain PGD synthetase (18). In Northern blot analyses with cDNA for the brain-type enzyme, no positive transcript was detected in total RNA obtained from peritoneal macrophages and mast cells² which is in agreement with the present study. However, identification of spleen-type PGD synthetase mRNA in mast cells remained to be elucidated, since the cDNA coding for the spleen-type enzyme has not yet been isolated.

²Y. Urade, M. Igarashi, and A. Nagata, unpublished results.
The immunoreactivity of spleen-type PGD synthetase was observed in many, but not all, mast cells of the connective tissues of various organs (Figs. 4 and 5), but it was not significantly detected in mast cells of the parenchymal portions of spleen, thymus, and digestive tract. In those areas, so-called “mucosal mast cells,” but not “tissue mast cells,” are known to be distributed. Such heterogeneity of the distribution of the immunoreactivity in mast cells may reflect the functional heterogeneity of both types of mast cells (32).

The specific activity of PGD synthetase in mast cells is also suggested by the study using w/"w" mutant mice deficient in tissue mast cells (37), in which PGD content and the PGD synthetase activity in various tissues of these mice were shown to be almost the same as those of congenic normal mice. On the other hand, PGD is also produced in the central nervous system of rats by brain-type PGD synthetase (12). Previously, we have shown that the brain-type enzyme is localized in neurons and oligodendrocytes of rat brain (27), ganglion cells in the retina (38, 39), and hair cells in the cochlea (40). Therefore, although mast cells are likely to be the active source, they are not the only type of cell that produces PGD.

Since the physiological significance of PGD in mast cells and the mechanism(s) of action of PGD on modification of the anaphylactic process are still a matter of speculation, characterization of catalytic properties of spleen-type PGD synthetase (14) and screening of its inhibitors (41, 42) should prove useful in addressing these problems.

Acknowledgments—We are grateful to Drs. H. Kawabe and H. Hayashi, N. Eguchi, N. Fujimoto, Y. Nakayama, and S. Hitomi of Hayashi Bioformation Transfer Project for their technical and secretarial assistance. We also thank Prof. K. Sato, Department of Biochemistry, Hiroaki University School of Medicine, for providing antibodies against GSH S-transferases and Prof. S. Imamura of the Department of Dermatology, Kyoto University, and Prof. S. S. Tate of Cornell University for critical reading of the manuscript.

REFERENCES

1. Needleman, P., Turk, J., Jakshik, B. A., Morrison, A. R., and Leffkovitch, J. B. (1980) Ann. Rev. Biochem. 55, 69-102
2. Lewis, R. A., and Austen, K. F. (1981) Nature 293, 103-108
3. Lewis, R. A., Soter, N. A., Diamond, P. T., Austen, K. F., Oates, J. A., and Roberts, L. J., II (1982) J. Immunol. 129, 1627-1631
4. Watanabe, T., Shimizu, T., Narumiva, S., and Havaishi, O. (1982) J. Allergy Clin. Immunol. 68, 383-391
5. Wescott, S., and Kaliner, M. (1981) J. Allergy Clin. Immunol. 68, 383-391
Mast cells contain spleen-type prostaglandin D synthetase.
Y Urade, M Ujihara, Y Horiguchi, M Igarashi, A Nagata, K Ikai and O Hayaishi

J. Biol. Chem. 1990, 265:371-375.

Access the most updated version of this article at http://www.jbc.org/content/265/1/371

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/1/371.full.html#ref-list-1