We determined the crystal structure of human hematopoietic prostaglandin (PG) D synthase (H-PGDS) as the quaternary complex with glutathione (GSH), Mg$^{2+}$, and an inhibitor, HQL-79, having anti-inflammatory activities in vivo, at a 1.45-Å resolution. In the quaternary complex, HQL-79 was found to reside within the catalytic cleft between Trp$^{104}$ and GSH. HQL-79 was stabilized by interaction of a phenyl ring of its diphenyl group with Trp$^{104}$ and by its piperidine group with GSH and Arg$^{14}$ through water molecules, which form a network with hydrogen bonding and salt bridges linked to Mg$^{2+}$. HQL-79 inhibited human H-PGDS competitively against the substrate PGH$_1$ and non-competitively against GSH with $K_i$ of 5 and 3 μM, respectively. Surface plasmon resonance analysis revealed that HQL-79 bound to H-PGDS with an affinity that was 12-fold higher in the presence of GSH and Mg$^{2+}$ ($K_i$ 0.8 μM) than in their absence. Mutational studies revealed that Arg$^{14}$ was important for the Mg$^{2+}$-mediated increase in the binding affinity of H-PGDS for HQL-79, and that Trp$^{104}$, Lys$^{112}$, and Lys$^{198}$ were important for maintaining the HQL-binding pocket. HQL-79 selectively inhibited PGD$_2$ production by H-PGDS-expressing human megakaryocytes and rat mastocytes with an IC$_{50}$ value of about 100 μM but only marginally affected the production of other prostanoids, suggesting the tight functional engagement between H-PGDS and cyclooxygenase. Orally administered HQL-79 (30 mg/kg body weight) inhibited antigen-induced production of PGE$_2$ and PGF$_2$ in vivo, suggesting that the functional engagement of HQL with cyclooxygenase is important for maintaining the HQL-binding pocket. HQL-79 was stabilized by catalytic cleft between Trp$^{104}$ and GSH. HQL-79 was stabilized by a network with hydrogen bonding and salt bridges which form a network with hydrogen bonding and salt bridges linked to Mg$^{2+}$ which is important for the Mg$^{2+}$-mediated increase in the binding affinity of H-PGDS for HQL-79, and that Trp$^{104}$, Lys$^{112}$, and Lys$^{198}$ were important for maintaining the HQL-binding pocket. HQL-79 selectively inhibited PGE$_2$ production by H-PGDS-expressing human megakaryocytes and rat mastocytes with an IC$_{50}$ value of about 100 μM but only marginally affected the production of other prostanoids, suggesting the tight functional engagement between H-PGDS and cyclooxygenase. Orally administered HQL-79 (30 mg/kg body weight) inhibited antigen-induced production of PGE$_2$ and PGF$_2$, without affecting the production of PGD$_2$ and PGE$_2$ and ameliorated airway inflammation in wild-type and human H-PGDS overexpressing mice. Knowledge about this structure of quaternary complex is useful for understanding the inhibitory mechanism of HQL-79 and should accelerate the structure-based development of novel anti-inflammatory drugs that inhibit PGD$_2$ production specifically.

Prostaglandin (PG) D$_2$ is an allergic and inflammatory mediator produced by mast cells (1) and Th2 cells (2). PGD$_2$ activates 2 distinct types of receptor, i.e. DP (DP$_1$) and CRTH2 (DP$_2$). PGD$_2$ causes contraction of airway smooth muscle via DP receptors (3) and mediates the chemotaxis of eosinophils and basophils into the lungs via CRTH2 receptors (4). Thus, PGD$_2$ coordinates regulates allergic reactions, especially airway inflammation, via these 2 receptors (5).

PGD$_2$ is formed from arachidonic acid by successive enzyme reactions mediated by PG endoperoxide synthase (cyclooxygenase, COX) and PGD synthase (PGDS). The former catalyzes 2 consecutive reactions, dioxygenation of arachidonic acid to PGG$2$ and peroxidation of PGG$_2$ to PGH$_2$, the latter being a common precursor of PGs and thromboxane. PGDS then catalyzes the isomerization of PGH$_2$ to PGD$_2$, in the presence of glutathione (GSH). Two distinct types of PGDS are known: one is lipocalin-type PGDS (L-PGDS); and the other, hematopoietic PGDS (H-PGDS, (6). L-PGDS is localized in the central nervous system, male genital organs, and heart, and is involved in the regulation of sleep (7) and pain (8). On the other hand, H-PGDS is localized in mast cells, Th2 cells, microglia, necrotic muscle fibers, and apoptotic smooth muscle cells and participates in allergic and inflammatory reactions (9). Selective inhibitors of H-PGDS are considered to be more useful to suppress allergic and inflammatory reactions than COX-1 and COX-2 inhibitors, such as aspirin, indomethacin, and coxibs (10); because these COX inhibitors suppress the production of all PGs including the cytokinoprotective and anti-inflammatory PGs (11, 12). Selective inhibitors of H-PGDS may block the inflammatory signal mediated by both DP and CRTH2 receptors. Therefore, H-PGDS is a good target for anti-allergic and anti-inflammatory drugs (13).

We have already cloned the cDNA for human H-PGDS (14) and determined its x-ray crystallographic structure (15). Furthermore, in a previous study (16), we determined the x-ray crystallographic structure of human H-PGDS in a complex with a prototype of the H-PGDS inhibitor, 2-((2'-benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium chloride (BSPT). However, BSPT inhibits H-PGDS only in vitro and is not effective in vivo or even in cultured cells. On the other hand, the novel orally active anti-allergic drug 4-benzhydryloxy-1-[3-(1H-tetrazol-5-yl)-propyl]piperidine (HQL-79) was reported a few years ago (17). Although HQL-79

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The atomic coordinates and structure factors (code 2CVD) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The abbreviations used are: PG, prostaglandin; COX, cyclooxygenase; PGDS, prostaglandin D synthase; L-PGDS, lipocalin-type prostaglandin D synthase; H-PGDS, hematopoietic prostaglandin D synthase; BSPT, 2-((2'-benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium chloride (BSPT). However, BSPT inhibits H-PGDS only in vitro and is not effective in vivo or even in cultured cells. On the other hand, the novel orally active anti-allergic drug 4-benzhydryloxy-1-[3-(1H-tetrazol-5-yl)-propyl]piperidine (HQL-79) was reported a few years ago (17). Although HQL-79
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was originally developed as an antagonist for histamine H1 receptors (H1R), a part of the anti-allergic and anti-asthmatic effects of HQL-79 was proposed to be mediated by the inhibition of PGD2 production, because HQL-79 inhibited the conversion of PGH2 to PGD2 in crude extracts of mouse spleen (18). However, the biochemical characterization of HQL-79 as an inhibitor of either of the 2 types of PGDS has remained to be made.

In the present study, we show that HQL-79 is a specific inhibitor of human H-PGDS with higher potency and biological availability than those of BSPT. Kinetic analyses revealed HQL-79 to be a competitive inhibitor against PGH2 and a non-competitive one against GSH. HQL-79 highly selectively inhibited the production of PGD2 catalyzed by H-PGDS with little effect on the production of other PGs in cultured human and rat cell lines or in the lungs of ovalbumin (OVA)-immunized mice transgenic for human H-PGDS. The lack of shunting of PGH2 to downstream prostanoids other than PGD2 after H-PGDS inhibition by HQL-79 suggests a tight functional engagement between H-PGDS and COX. Both in vivo and in vitro pharmacological and biochemical experiments indicate that HQL-79 is a promising lead compound for the development of new H-PGDS inhibitors to be used as anti-allergic and anti-inflammatory drugs. Finally we determined the x-ray crystallographic structure of human H-PGDS as a quaternary complex with HQL-79 and 2 cofactors, GSH and Mg2+, whose determination is useful for understanding the inhibitory mechanism of HQL-79 and for further drug design.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—HQL-79 was purchased from Cayman–Chemical.

**Animals**—Human H-PGDS-overexpressing transgenic (TG) mice (FVB strain) were generated according to the method described previously (7), although human H-PGDS cDNA was used as the transgene instead of human L-PGDS cDNA. H1R gene knock-out (KO) mice (C57BL/6 strain) were provided by Dr. Takeshi Watanabe, RIKEN (19).

**Allergic Airway Inflammation**—Human H-PGDS-TG mice, H1R-KO mice, and wild-type (WT) mice (male, 8–9 weeks old) were actively sensitized by intraperitoneal injections of 105 ml of aluminum hydroxide gel given on days 0 and 14. On day 21, the mice were exposed to OVA (2.5% w/v in saline) for 20 min. Bronchoalveolar lavage fluid (BALF) was collected 10 min after the end of the OVA exposure. PGD2, PGE2, and PGF2α in the BALF were extracted, partially purified by HPLC, and quantified by ELISA (Cayman Chemicals) as described previously (7). The total and differential cell counts of BALF were determined 48 h after the antigen challenge. The protocols used for all animal experiments in this study were approved by the Animal Research Committee of the Osaka Bioscience Institute.

**Expression and Purification of Recombinant H-PGDS**—Human H-PGDS (15), rat H-PGDS, and its mutants of Y8F, R14E, W104I, K121E, C156L, or K198E were expressed and purified as described previously (20). In brief, Escherichia coli BL21 (DE3) cells were transformed with the prepared plasmids. The cells were collected and disrupted by sonication in phosphate-buffered saline. After removal of cell debris by centrifugation, the supernatant was applied to the GSH-Sepharose 4B column. After the column had been washed with PBS, the protein bound to the GSH-Sepharose 4B was eluted with 50 mM Tris-HCl, pH 9.0 containing 10 mM GSH. Protein concentrations were determined by the BCA method with bovine serum albumin used as a standard.

**Enzyme Assay**—The activities of H-PGDS (15), L-PGDS (21), and microsomal PG synthase (m-PGES, (22) were measured with 40 μM [1-14C]PGH2 as substrate in 100 mM Tris-HCl, pH 8.0 in the presence of 2 mM GSH, 2 mM MgCl2, and 0.1 mg/ml IgG, unless otherwise stated. The activities of COX-1 and COX-2 were measured as described (23) with 50 μM [1-14C]arachidonic acid (PerkinElmer Life Sciences) used as substrate in 100 mM Tris-HCl, pH 8.0 containing 2 μM hematin, 5 mM l-tryptophan, 1 mM GSH, and 0.1 mg/ml IgG. The kinetic constants were determined by Lineweaver-Burk plots prepared by using GraphFit software (version 3.0.8. for Windows, Erithacus Software Ltd., Horley, UK).

**Cell Culture**—Human megakaryoblastic cells (MEG-01S), human medulloblastoma cells (TE-671), and rat mastocytoma cells (RBL-2H3) were purchased from American Type Culture Collection. Human embryonic kidney (HEK)-293 cells stably transfected with human L-PGDS and COX-1 cDNAs were kindly provided by Dr. M. Murakami, the Tokyo Metropolitan Institute of Medical Science. MEG-01S cells were induced to differentiate by treatment with 12-O-tetradecanoylphorbol-13-acetate to express H-PGDS and COX-1, as described previously (24). MEG-01S, TE-671, and HEK-293 cells (5 × 105/well) were seeded into multiplates and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 4 mM l-glutamine, 4.5 g/liter glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate. After having been cultured for 1 day, the cells were stimulated with 5 μl calcium ionophore A23187 (Sigma) for 15 min in the absence or presence of HQL-79 (3–300 μM). RBL-2H3 cells (2.5 × 105/well) were seeded into multiplates and cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate. After the sensitization of these cells with 50 ng/ml monoclonal anti-dinitrophenyl IgE, the cells were stimulated with 20 ng/ml dinitrophenyl-bovine serum albumin or 5 μM A23187 for 15 min in the absence or presence of HQL-79 (1–100 μM). In some experiments, cells were pre-labeled with [1-14C]arachidonic acid (3.7 kBq/well) for 12 h before the assay. PGE2, PGE2, and PGF2α in the culture medium were quantified as described above.

**Surface Plasmon Resonance (SPR) Binding Analysis**—SPR measurements were carried out with BIAcore 2000 (BIAcore AB). Recombinant human H-PGDS, rat H-PGDS, or mutants of the latter were coupled to a CM-5 sensor chip by the amine coupling method. An empty flow cell was used as a negative control. The binding of HQL-79 to the immobilized H-PGDS (12 ng of protein giving 10,000 response units per flow cell) was measured by using the co-injection mode at a flow rate of 30 μl/min. Before the loading of HQL-79, the chip was equilibrated for 90 s with 50 mM Tris-HCl, pH 7.4, containing 2 mM MgCl2, 150 mM NaCl, and various concentrations of GSH (0–2 mM). HQL-79 at various concentrations in the same buffer was injected for 90 s. The sensogram for the empty surface was subtracted from that for the H-PGDS-immobilized surface with control software to obtain the sensogram for the specific interaction. The Kd values were derived from the sensograms after subtraction of linear, non-saturated baseline responses by steady-state analysis with BIA evaluation 3.1 software (BIAcore AB).

**Crystallization and Structure Determination**—The crystallization of the quaternary complex comprising human H-PGDS, HQL-79, GSH, and Mg2+ was achieved by a soaking method. The crystals of the ternary complex of the enzyme with GSH and Mg2+ were first obtained by the hanging drop vapor diffusion method (15), and then HQL-79 powder was added to the hanging-drop. In a few days the powder disappeared from the mother liquor, which consisted of 17% (w/v) PEG 6000, 50 mM Tris-HCl, pH 7.4, containing 2 mM MgCl2, 5 mM GSH, 5 mM diethiothreitol, and 1% (v/v) dioxane. The crystals were then soaked in a cryoprotectant solution containing 23% glycerol and flash-frozen in a stream of nitrogen gas at 100 K prior to data collection. Diffraction data beyond 1.45-Å resolution were collected at beamline 41XU at SPring-8, Japan. The complex structure was determined by the molecular replacement method with AMoRe (25) by...
using the native structure as the search model (PDB code: 1IYH; Ref. 15). The model rebuilding and the refinement of the structure were performed with O (26) and CNS (27) giving the final R-factor and R\(_{free}\)-factor of 0.192 and 0.207, respectively. The results on data collection and refinement of the complex with HQL-79 are summarized in Table 1.

Statistical Analysis—Differences arising from comparisons were analyzed for statistical significance by Student’s t test or Dunnett’s multicomparsion test. \(p < 0.05\) was considered significant.

RESULTS

Inhibition of Allergic Lung Inflammation and PGD\(_2\) Production by HQL-79 in Vivo—We applied HQL-79 (Fig. 1) to the allergic-airway inflammation model of human H-PGDS overexpressing TG mice and WT mice (FVB strain) sensitized to OVA, and determined the contents of PGD\(_2\), PGE\(_2\), and PGF\(_2\alpha\) in the BALF obtained from these mice (Fig. 2A). The inhalation of antigen significantly increased the PGD\(_2\) content in the BALF by 1.6-fold in WT mice (from 52 and 50 pg/lung) and by 2.4- and 1.5-fold in TG mice (from 53 and 75 to 131 and 110 pg/lung, respectively). Orally administered HQL-79 at doses of 10 and 30 mg/kg decreased the PGD\(_2\) content in both H-PGDS-TG and WT mice (FVB strain) sensitized to OVA, and determined the contents in the BALF were not decreased by the inhibitor.

Orally administered HQL-79 (30 mg/kg) decreased the total cell number and mononuclear cell number in BALF to 62 and 40%, respectively, in WT and to 62 and 66%, respectively, in H-PGDS-TG mice. HQL-79 also inhibited the infiltration of eosinophils to 31% in WT but had no effect in H-PGDS-TG mice (Fig. 2B). The suppressive effect of HQL-79 on the infiltration of the total cells and eosinophils in BALF was also observed in H1R-KO and WT (C57BL/6 strain) mice. Orally administered HQL-79 (30 mg/kg) decreased the numbers of total cells and eosinophils in BALF to 62 and 54%, respectively, in WT and to 60 and 52%, respectively, in H1R-KO mice (Fig. 2B). The administration of HQL-79 also decreased the PGD\(_2\) content in both H1R-KO and WT (C57BL/6 strain) mice, similarly as in the WT (FVB strain) mice (data not shown).

These results indicate that HQL-79 is an orally active H-PGDS inhibitor and a new candidate of anti-asthmatic drugs that suppress PGD\(_2\) production. These results also indicate that the anti-asthmatic effect of HQL-79 was not attributed to its antagonist activity toward H1R, although HQL-79 had been developed as an H1R antagonist (17).
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**FIGURE 3. Inhibition of H-PGDS activity by HQL-79 in vitro.** A, selective inhibition by HQL-79 of PGD₂ accumulated in the culture medium of rat mastocytoma RBL-2H3 cells. The cells were sensitized with monoclonal anti-dinitrophenyl IgE, then stimulated with 20 ng/ml dinitrophenyl-bovine serum albumin or 5 μM A23187 for 15 min in the presence of various concentrations of HQL-79 (1–100 μM). The amounts of PGD₂, PGE₂, and PGF₂α were measured by EIA. Data represent the mean ± S.E. (n = 4). *, p < 0.05; **, p < 0.01 compared with the value in the absence of HQL-79. ††, p < 0.01 as compared with the value in the presence of 100 μM HQL-79 (Dunnett’s test).

B, inhibition of [14C]PGD₂ production in H-PGDS-expressing MEG-01S cells and in L-PGDS-overexpressing HEK-293 cells. MEG-01S and HEK-293 cells were prelabelled with [1-14C]arachidonic acid and stimulated with 5 μM A23187 for 15 min in the presence of various concentrations of HQL-79 (3–300 μM). Radiolabeled arachidonic acid and its metabolites were extracted from the culture medium, separated by TLC, and analyzed by autoradiography. Positions of arachidonic acid (AA), 12-hydroxyheptadecatrienoic acid (HHT), PGD₂, PGE₂, and PGF₂α are shown by arrows on the left. C, selective inhibition by HQL-79 of PGD₂ accumulated in the culture medium of MEG-01S cells. The amounts of PGD₂, PGE₂, and PGF₂α were measured by EIA. Data represent the mean ± S.E. (n = 4). *, p < 0.05; **, p < 0.01 as compared with the value in the absence of HQL-79. †, p < 0.01 as compared with the value in the presence of 300 μM HQL-79 (Dunnett’s test).

Specific Inhibition of H-PGDS in Cell Lines by HQL-79—Quantification by EIA of the amounts of PGD₂, PGE₂, and PGF₂α released into the culture medium revealed that HQL-79 selectively inhibited the PGD₂ production catalyzed by H-PGDS and only marginally affected the production of other prostanoids (Fig. 3). HQL-79 (3–100 μM) dose-dependently inhibited either antigen- or Ca²⁺-ionophore-induced production of PGD₂ in rat mastocytoma RBL-2H3 cells (Fig. 3A), which express predominantly H-PGDS (28). HQL-79 at a concentration of 100 μM statistically significantly decreased antigen-induced PGD₂ production to 7.6 ng/10⁶ cells from the 12.2 ng/10⁶ vehicle-treated cells, whereas it slightly increased PGE₂ production to 0.33 ng/10⁶ cells from the 0.05 ng/10⁶ vehicle-treated cells and decreased PGF₂α production to 1.4 ng/10⁶ cells from the 1.5 ng/10⁶ vehicle-treated cells. HQL-79 (100 μM) also significantly decreased Ca²⁺-ionophore-induced PGD₂ production to 23 ng/10⁶ cells from the 48 ng/10⁶ vehicle-treated cells, whereas it increased PGE₂ production to 0.76 ng/10⁶ cells from the 0.19 ng/10⁶ vehicle-treated cells and decreased PGF₂α production to 4.5 ng/10⁶ cells from the 5.4 ng/10⁶ vehicle-treated cells.

HQL-79 (3–300 μM) dose-dependently inhibited Ca²⁺-ionophore (A23187)-induced production of PGD₂ from [1-14C]arachidonic acid in human megakaryocytes, MEG-01S cells (Fig. 3B), which also express predominantly H-PGDS (29). However, the production of other 14C-labeled metabolites was not inhibited by HQL-79 used up to 300 μM. Moreover, HQL-79 had no effect on the production of PGD₂ by L-PGDS-overexpressing HEK-293 cells (Fig. 3B) or human TE-671 cells (data not shown), both of which predominantly express L-PGDS (30). The IC₅₀ value of HQL-79 for inhibition of PGD₂ production in megakaryocytes was calculated by EIA to be 102 μM. HQL-79 at a concentration of 300 μM decreased PGD₂ production to 3.1 ng/10⁶ cells from the 10.1 ng/10⁶ vehicle-treated cells; whereas it increased PGE₂ production to 0.32 ng/10⁶ cells from the 0.17 ng/10⁶ vehicle-treated cells and decreased PGF₂α production to 0.23 ng/10⁶ cells from the 0.34 ng/10⁶ vehicle-treated cells. HQL-79 tested up to 300 μM did not affect at all the production of PGD₂, PGE₂ or PGF₂α in the L-PGDS-overexpressing HEK-293 cells (Fig. 3C).

These results, taken together, indicate that the inhibition of H-PGDS decreased PGD₂ production selectively without significantly affecting the biosynthesis of other PGs. Once the downstream H-PGDS was inhibited, the upstream COX was also inhibited, suggesting that H-PGDS and COX were functionally tightly engaged with each other.

Kinetic Analysis of H-PGDS Inhibition by HQL-79—The selective inhibition of H-PGDS by HQL-79 was confirmed by assays conducted on various types of the purified enzymes in the arachidonate cascade. HQL-79 inhibited the activity of purified recombinant human H-PGDS with an IC₅₀ of 6 μM, but had almost no effect on the activities of the purified COX-1, COX-2, m-PGES, or L-PGDS used up to 300 μM (Fig. 4A). As we previously reported (15), Mg²⁺ activates human H-PGDS about 2-fold and increases its affinity for GSH about 4-fold. When we determined the inhibition of H-PGDS by HQL-79 in the absence of Mg²⁺, the IC₅₀ value was increased about 3-fold from 6 μM to 16 μM (Fig. 4A). This is quite different from the manner of inhibition of H-PGDS by BSPT (16), because the IC₅₀ value of BSPT was increased from 36 μM in the absence of Mg²⁺ to 98 μM in its presence.

Kinetic analysis using the purified human H-PGDS revealed that HQL-79 inhibited the H-PGDS activity in a competitive manner against GSH (Fig. 4B, left panel), giving a Ki of 5 μM, and in a non-competitive one against GSH (Fig. 4B, right panel) with a Ki of 3 μM in the presence of 1 mM MgCl₂. In the absence of Mg²⁺, HQL-79 showed the same kinetic profile of the H-PGDS inhibition as that in the presence of Mg²⁺; however, the Ki value was increased to 55 μM for PGH₂ and to 40 μM for GSH (data not shown). These results indicate that HQL-79 bound to the PGH₂ binding site but not to the GSH-binding site and also suggest that the binding of HQL-79 to H-PGDS was enhanced in the presence of Mg²⁺ by increasing the affinity of H-PGDS for GSH.
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FIGURE 4. Kinetic analysis of H-PGDS inhibition by HQL-79. A, inhibition of H-PGDS, but not of L-PGDS, m-PGES, COX-1, or COX-2, by HQL-79. The enzyme activities were determined in the presence of various concentrations of HQL-79. The H-PGDS activity was measured in the absence or presence of 2 mM MgCl₂ and 2 mM GSH. Equilibrium response units (RU) were obtained following injection of HQL-79 (0–100 μM) over the sensor chip. B, dose-response curves of HQL-79 binding to rat H-PGDS in the absence or the presence of various concentrations of GSH and 2 mM MgCl₂. C, dose-response curves of HQL-79 binding to immobilized rat recombinant H-PGDS (○) and its mutants Y8F, R14E, W104I, K112E, C156L, and K198E in the presence of 2 mM MgCl₂ and 2 mM GSH. D, dose-response curves of HQL-79 binding to immobilized rat recombinant H-PGDS (○), the R14E mutant (■), and the K198E mutant (□) in the absence or the presence of 2 mM MgCl₂ and 2 mM GSH. Open circles, 2 mM MgCl₂ and 2 mM GSH; closed circles, (—) MgCl₂ and 2 mM GSH; open triangles, 2 mM MgCl₂ and (—) GSH; closed triangles, (—) MgCl₂ and (—) GSH.

FIGURE 5. SPR analysis of HQL-79 binding to H-PGDS. A, response curves of SPR signals for HQL-79 binding to immobilized human H-PGDS in the presence of 2 mM MgCl₂ with (right) and without (left) 2 mM GSH. Concentrations of HQL-79 are also indicated. B, dose response curves of HQL-79 binding to immobilized human H-PGDS in the absence or the presence of 2 mM MgCl₂ and 2 mM GSH. Equilibrium response units (RU) were obtained following injection of HQL-79 (0–100 μM) over the sensor chip. C, dose-response curves of HQL-79 binding to immobilized human H-PGDS in the absence or the presence of various concentrations of GSH and 2 mM MgCl₂. D, dose-response curves showing a decrease in the Kₐ of H-PGDS for HQL-79 caused by GSH in the presence of 2 mM MgCl₂. E, dose-response curves of HQL-79 binding to immobilized rat H-PGDS and its mutants Y8F, R14E, W104I, K112E, C156L, and K198E in the presence of 2 mM MgCl₂ and 2 mM GSH. F–H, dose-response curves of HQL-79 binding to immobilized rat recombinant H-PGDS (○), the R14E mutant (■), and the K198E mutant (□) in the absence or the presence of 2 mM MgCl₂ and 2 mM GSH. Open circles, 2 mM MgCl₂ and 2 mM GSH; closed circles, (—) MgCl₂ and 2 mM GSH; open triangles, 2 mM MgCl₂ and (—) GSH; closed triangles, (—) MgCl₂ and (—) GSH.

SPR Analysis of Binding of HQL-79 to H-PGDS—SPR analysis showed that HQL-79 bound to H-PGDS in a concentration-dependent, saturable, and Mg²⁺- and GSH-accelerated manner and dissociated from the enzyme-inhibitor complex immediately when washed (Fig. 5A). In the presence of 2 mM MgCl₂ and 2 mM GSH, HQL-79 bound to human H-PGDS in a concentration-dependent manner, with almost complete saturation up to 25 μM (Fig. 5B). From the association and dissociation curves, the Kₐ for HQL-79 was calculated to be 0.8 μM. In the absence of MgCl₂ and in the presence of 2 mM GSH, the HQL-79 binding to human H-PGDS significantly decreased, showing saturation at 50 μM and a Kₐ of 5 μM, indicating that the affinity of H-PGDS for HQL-79 decreased 6-fold in the absence of MgCl₂. In the absence of GSH, the HQL-79 binding decreased the total capacity to about 50% and increased the Kₐ to 11 μM in the presence of MgCl₂ and to 10 μM in its absence.

When we determined the GSH dependence on the HQL-79 binding to human H-PGDS in the presence of MgCl₂, the binding affinity increased in a GSH concentration-dependent manner (Fig. 5C). The half-effective concentration of GSH for an increase in the affinity for HQL-79 and a decrease in the Kₐ was calculated to be 0.09 mM (Fig. 5D), which is similar to the Kₐ of the H-PGDS activity for GSH (0.14 mM, Ref. 15), suggesting that GSH binding to the catalytic site of H-PGDS was involved in the increase in the binding affinity for HQL-79.

We then analyzed the binding of HQL-79 to rat H-PGDS and its mutants, i.e., Y8F, R14E, W104I, K112E, C156L, and K198E, by SPR analysis (Fig. 5E). Our previous study (20) with these mutants indicated that Lys¹¹², Cys¹⁵⁶, and Lys¹⁷⁸ are involved in the binding of PGH₂, that Trp₁⁰⁴ is critical for structural integrity of the catalytic center for GSH transferase and H-PGDS activities, and that Tyr⁸ and Arg¹⁴ are essential for activation of the thiol group of GSH. The three-dimensional geometry of these amino acid residues within the catalytic cleft is well conserved between rat H-PGDS (31) and human H-PGDS (15).

As shown in Fig. 5E, in the presence of 2 mM MgCl₂ and 2 mM GSH, rat H-PGDS and its Y8F mutant showed almost identical HQL-79 binding curves with a Kₐ of 0.7 μM. Similar to the human H-PGDS, rat H-PGDS, and the Y8F mutant showed a 5-fold decrease in their binding affinity for HQL-79, being 3.4 μM in the absence of MgCl₂, without a change in the maximum binding capacity. In the absence of GSH, both enzymes showed a decrease in their total binding capacities to about
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50% and an increase in their $K_d$ value to 22 $\mu M$ in the presence of MgCl$_2$ and to 21 $\mu M$ in its absence (Fig. 5F). These results indicate that the Tyr$^8$ residue was not essential for the HQL-79-binding.

The R14E mutant gave a binding curve quite different from those curves of the WT and other mutant enzymes, showing a remarkably decreased affinity for HQL-79 with a $K_d$ of 20 $\mu M$ in the presence of 2 mM MgCl$_2$ and 2 mM GSH (Fig. 5G). Although the HQL-79-binding was not saturated up to 100 $\mu M$, the maximum soluble concentration of HQL-79 in the assay buffer, the calculated maximum binding capacity was almost the same as those capacities of the WT and Y8F mutant enzymes. In the absence of MgCl$_2$ and in the presence of 2 mM GSH, the $K_d$ was slightly decreased to be 22 $\mu M$, but the calculated maximum binding capacity remained unchanged (Fig. 5G). In the absence of GSH, the HQL-79 binding was decreased to half of that in the presence of GSH, and the $K_d$ value was calculated to be 48 and 47 $\mu M$ with and without 2 mM MgCl$_2$, respectively. These results suggest that Arg$^{14}$ was important for the Mg$^{2+}$-mediated increase in the binding affinity of H-PGDS for HQL-79 by increasing the affinity for GSH, as previously suggested from kinetic analysis (15).

The W104I, K112E, and K198E mutants showed HQL-79-binding curves similar to each other, with a decrease in the maximum binding capacity to 26, 48, and 64%, respectively, of that of the WT enzyme and a 3–5-fold increase in the $K_d$ value (3.6, 2.3, and 3.1 $\mu M$, respectively) in the presence of 2 mM MgCl$_2$ and 2 mM GSH (Fig. 5E). In the absence of MgCl$_2$, these mutants showed an approx. 5-fold increase in their $K_d$ values for HQL-79 without a change in their maximum binding capacities. In the absence of GSH, their maximum binding capacities decreased to half of those in the presence GSH; and their $K_d$ values for HQL-79 decreased to about 16–19 $\mu M$. Typical results obtained with the K198E mutant are shown in Fig. 5H. These results suggest that the Trp$^{104}$, Lys$^{112}$, and Lys$^{198}$ residues are important for maintaining the HQL binding pocket.

The C156L mutant lost almost completely its HQL-79 binding activity (Fig. 5E), indicating that the HQL-79 binding pocket is fatally damaged by this mutation, although this mutant shows the about 50% of the GSH transferase activity of the WT enzyme (20).

Crystallographic Structure of H-PGDS-HQL-79 Complex—To elucidate the structural basis of the H-PGDS inhibition by HQL-79, we determined the crystal structure of human H-PGDS as a quaternary complex with Mg$^{2+}$, GSH, and HQL-79. The crystal of the complex was obtained with a space group of P1, in which 2-dimer molecules of H-PGDS (Mol-A and D, Mol-B and C) were located in an asymmetric unit (Fig. 6A), similar to the crystal of the enzyme complex with BSPT (16). The 2 dimer packing in the HQL-79 complex of human H-PGDS was essentially the same as that of Ca$^{2+}$- or Mg$^{2+}$-bound native form without inhibitors (15) or as that of the complex with BSPT (16).

The high resolution structure gave a clear electron density map for the HQL-79 molecule at 1.45 Å (Fig. 6B). Four independent molecules of HQL-79 and 4 of GSH were well superimposed within Mol-A to D of human H-PGDS, in which the tetrazole tail of HQL-79 showed slightly different conformations among the 4 molecules (Fig. 6C). This finding is consistent with the fact that the averaged temperature factor for the tetrazole ring of HQL-79 was the highest, 27.0 Å$^2$, and that for the glutamate residue of GSH was, the lowest, 8.4 Å$^2$ (Table 2). Among the 4 molecules, the tetrazole tail of Mol-A and -D on the outside in the packing unit (Fig. 6A) had more deviation than that of Mol-B and -C on the inside, probably because of the interaction with molecules in the neighboring packing units.

We further describe the structural characteristics of the quaternary complex in Mol-C, which had the lowest temperature factors for both GSH and HQL-79 (Fig. 6D). The HQL-79 molecule was inserted in the catalytic cleft between Trp$^{104}$ and GSH (Fig. 6E, top). No steric hindrance was detected between HQL-79 and the GSH molecule. H-PGDS has 3 pockets (pockets 1, 2, and 3) in its catalytic cleft (20, 31). Phenyl-1 and phenyl-2 of the diphenyl of HQL-79 penetrated into pocket 1 and pocket 2, respectively (Fig. 6E). The HQL-79 molecule was stabilized by weak hydrogen bonding (32) with Met$^{99}$, Phe$^{102}$, Trp$^{104}$, and Tyr$^{152}$ located within a distance of 3.5 Å (indicated by the blue-dotted lines in the bottom panel of Fig. 6E), and by Arg$^{14}$, Thr$^{159}$, and Leu$^{199}$, including GSH (by non-bonding interactions including salt bridges and hydrogen bonding) through water molecules (colored yellow). The tetrazole ring of HQL-79 was located at the entrance of pocket-3 and did not directly interact with the positively charged amino acid cluster of Lys$^{112}$ and Lys$^{198}$ in pocket-3. No direct interaction was detected around the tetrazole ring, suggesting that the tetrazone group of HQL-79 interacted with Lys$^{112}$ and Lys$^{198}$ via diffusible water molecules in pocket 3.

In the catalytic cleft, a phenyl ring of the diphenyl of HQL-79 exhibited van der Waals interaction with the indole ring of Trp$^{104}$ including weak hydrogen bonding with the ring nitrogen. In comparison with the native structure of the enzyme, the HQL-79 molecule penetrated into the ceiling of the catalytic cleft and pushed out the indole ring of Trp$^{104}$, resulting in the rotation of the indole ring by 48 degrees with a 4.3-Å shift (Fig. 6F). The movement of Trp$^{104}$ induced twisting of loop7, which linked to a long kinked α5-helix. The Cn carbon of Lys$^{107}$ located at the top of the α5-helix moved 4.4 Å, the number of which was extremely larger than the r.m.s. deviation of 0.42 Å for the Cn atoms between the complex and the native form.

DISCUSSION

Crystallographic and Biochemical Characterization of HQL-79 as a Competitive Inhibitor for Human H-PGDS—The crystallographic structure of the quaternary complex revealed that HQL-79 was inserted between Trp$^{104}$ and GSH in the catalytic cleft of H-PGDS, where the substrate PGH$_2$ is predicted to be captured (15, 20). The binding of HQL-79 to the catalytic site did not cause steric hindrance of GSH due to indirect interaction via bound water molecules (Fig. 6E). These results are completely consistent with the kinetic analyses showing that HQL-79 was a competitive inhibitor against the substrate, PGH$_2$, and a non-competitive inhibitor for GSH (Fig. 4B). The identification of the binding mode of PGH$_2$ should reveal the reaction mechanism of H-PGDS. Structural analysis of the complex with PGH$_2$ is difficult because of the instability of PGH$_2$. Therefore, more stable analogues of PGH$_2$ would be useful to understand the exact binding mode of PGH$_2$. Because HQL-79 was crystallographically shown to be bound to the PGH$_2$ binding site of H-PGDS, the competitive binding assay using H-PGDS and HQL-79, as a chemically stable decoy instead of the very labile substrate PGH$_2$, will be useful for screening for novel H-PGDS inhibitors.

The crystallographic structure also showed good agreement with the results of the SPR analyses (Fig. 5). Although our previous study with the Y8F mutant demonstrated that Tyr$^8$ residue is essential for activation of the thiol group of GSH (20), this mutant showed HQL-79 binding curves almost identical to those of the WT enzyme, indicating that Tyr$^8$ residue was not essential for the HQL-79-binding. In fact, no interaction was detected between Tyr$^8$ and HQL-79 in the crystallographic structure of H-PGDS-HQL-79 complex. On the other hand, 1 phenyl group of the diphenyl of HQL-79 penetrated into the ceiling of pocket 1 within the catalytic cleft and interacted with Trp$^{104}$ through a weak hydrogen bonding with the ring nitrogen. This binding mode is consistent with the fact that the W104H mutant showed a decrease in its maximum
Figure 6. Structure of HQL-79-bound complex of human H-PGDS. A, crystal packing of the HQL-79 complex. Two dimer molecules (Mol-A (green) and Mol-D (yellow), Mol-B (light pink), and Mol-C (cyan), respectively) located in an asymmetric unit of the crystal belonging to a space group of P1. Mol-A and Mol-B are on the outside; and Mol-C and Mol-D, on the inside. B, structure of HQL-79 in the active site of H-PGDS with Fo-Fc omit map contoured at 1.2σ. C, superimposed structures of 4 HQL-79 and 4 GSH molecules in an asymmetric unit. The flexible part of the molecule with the highest crystallographic temperature factors is shown in red, and the rigid structures with the lowest crystallographic temperature factors are depicted in blue. D, monomer structure as a surface model. Mg2⁺ (magenta), HQL-79 (pink), and GSH (green) are also shown. HQL-79 is bound to the active site. E, stereo view of the catalytic cleft of the quaternary complex of H-PGDS. Salt bridge, hydrogen bonding, and non-conventional hydrogen bonding are shown by gray-dotted lines. Each water molecule is displayed by a black sphere. F, close-up view of the superimposed structures around the active site of H-PGDS in the presence (sky blue) and in the absence (gray) of HQL-79 shown as a space-filling model. GSH molecules in the presence (green carbon atoms) and absence (gray carbon atoms) of HQL-79 are also shown. αSc and Lys107c represent the α5-helix and Lys107, respectively, of H-PGDS in the HQL-79 complex structure; and α5n and Lys107n, those of the native structure (PDB: 1IYH) (15). A, B, D, and E were drawn by MolScript (45) and Raster3D (46). C and F were prepared by using PyMOL (DeLano Scientific LLC).

Table 2

|            | H-PGDS | HQL-79 | GSH |
|------------|--------|--------|-----|
| Average    | 11.5 (23.1) | 11.5 (23.1) | 11.5 (23.1) |
| Mol-A      | 13.5 (21.0)  | 18.8     | 18.9 |
| Mol-B      | 14.2 (19.8)  | 18.9     | 18.8 |
| Mol-C      | 9.1 (24.1)   | 14.2     | 13.3 |
| Mol-D      | 9.1 (27.7)   | 14.8     | 14.4 |
| Phenyl-1   | 16.7     | 16.3    | 20.9 |
| Phenyl-2   | 23.3     | 29.3    | 22.9 |
| Piperidine  | 28.3     | 28.5    | 22.3 |
| Tetrazole  | 24.4     | 24.4    | 17.5 |
| Average    | 13.8 (25.6) | 13.8 (25.6) | 13.8 (25.6) |
| Phenyl-1   | 18.9     | 22.3    | 13.9 |
| Phenyl-2   | 28.5     | 28.5    | 13.9 |
| Piperidine  | 24.4     | 24.4    | 13.9 |
| Tetrazole  | 28.5     | 28.5    | 13.9 |
| Average    | 18.1 (28.6) | 18.1 (28.6) | 18.1 (28.6) |
| Glutamine  | 7.2 (29.8) | 7.2 (29.8) | 7.2 (29.8) |
| Cysteine   | 9.8 (29.6) | 9.8 (29.6) | 9.8 (29.6) |
| Glycine    | 12.6 (30.4) | 12.6 (30.4) | 12.6 (30.4) |
| Average    | 13.9 (22.5) | 13.9 (22.5) | 13.9 (22.5) |

*Phenyl-1 residue of HQL-79 was inserted into the ceiling of pocket 1 within the catalytic cleft.

**The values in parentheses represent B-factors of the native structure without HQL-79 in the presence of Mg²⁺ (PDB: 1IYH) (15).
capacity for HQL-79-binding to 26% and a 5-fold increase in its $K_m$ value (Fig. 5E). The other phenyl group of the diphenyl of HQL-79 reached to the bottom of pocket 2, which contains Cys$^{196}$, so that the substitution of this residue by Leu with its bulky side chain resulted in the complete loss of the HQL-79 binding activity.

In the crystal of the H-PGDS-HQL-79-GSH-Mg$^{2+}$ complex, the HQL-79 molecule was stabilized by the interaction of its piperidine group with GSH and Arg$^{14}$ through water molecules (Fig. 6E). The interaction was linked to Mg$^{2+}$ ion through a network of non-bonding interactions including salt bridges and hydrogen bonding among the thiol group of the cysteine residue, the carboxyl group of the $\gamma$-glutamyl residue of GSH, the guanido group of Arg$^{14}$, and the carboxyl group of Asp$^{96}$. These results indicate that the GSH molecule stabilized the binding pocket for HQL-79 and increased the affinity for the inhibitor in the presence of Mg$^{2+}$ ion. This interpretation is consistent with the fact that the crystallographic temperature factor of the glycine residue of GSH at the entrance of the catalytic cleft was 1.7–2.0-fold higher than that of the $\gamma$-glutamyl residue of GSH at the bottom of the cleft (Fig. 6C), whereas those values in both parts were unchanged in the native structure without inhibitors (Table 2). These results are also in agreement with the following facts: crystals of the complex with HQL-79 have never been obtained in the absence of GSH, the maximum binding capacity and the binding affinity of HQL-79 was about 2- and 14-fold, respectively, higher in the presence of GSH and MgCl$_2$ than those in their absence (Fig. 5B), and the $IC_{50}$ value of HQL-79 was improved from 16 $\mu$M in the absence of Mg$^{2+}$ to 6 $\mu$M in the presence of Mg$^{2+}$ (Fig. 4A).

In the catalytic cleft of H-PGDS, Arg$^{14}$ residue was identified to be located at a pivotal position in the salt bridge and hydrogen bonding network among HQL-79, GSH, and Mg$^{2+}$ ion (Fig. 6E). Arg$^{14}$ is important for the Mg$^{2+}$-mediated increase in the binding affinity of H-PGDS for HQL-79, by increasing the affinity for GSH, as previously suggested from kinetic analysis (15). The SPR binding assay (Fig. 5, C and D) showed that the higher the concentration of GSH, the more specifically and tightly HQL-79 bound to the active site; i.e. a better binding pocket for HQL-79 was formed in the presence of GSH and Mg$^{2+}$. The R14E mutant did not show the Mg$^{2+}$-mediated increase in the binding affinity of H-PGDS for HQL-79 (Fig. 5G). We previously demonstrated that the $K_m$ value of human H-PGDS for GSH is decreased from 0.6 ms$^{-1}$ in the presence of EDTA to 0.14 ms$^{-1}$ in the presence of Mg$^{2+}$ (15) and that Arg$^{14}$ is essential for activation of the thiol group of GSH (20). Taken together, these results indicate that the Arg$^{14}$ residue is the key residue for both the catalytic reaction and the Mg$^{2+}$-mediated increase in HQL-79 binding.

The binding site of HQL-79 in the catalytic cleft of H-PGDS was similar to that of another H-PGDS inhibitor, BSPT (16), but differed in a sense that BSPT shows steric hindrance with GSH and does not interact with bound Mg$^{2+}$, GSH, and water molecules in the crystal structure. BSPT is a competitive inhibitor for GSH, and the binding efficiency of BSPT is decreased in the presence of MgCl$_2$ and GSH, increasing the $IC_{50}$ value of BSPT from 36 $\mu$M to 98 $\mu$M upon binding of Mg$^{2+}$. In contrast, HQL-79 increased the binding affinity and the inhibition potency against H-PGDS in the presence of MgCl$_2$ and GSH as described above. Moreover, the intracellular concentrations of GSH and MgCl$_2$ are considered to be several ms$^{-1}$ (33). Thus, HQL-79 may be considered to be a better lead compound than BSPT to design novel inhibitors for H-PGDS.

The crystal structure of the H-PGDS-HQL-79 complex, together with the results of the kinetic and SPR analyses, suggests the possible strategy for drug designing. The tetrazol ring of HQL-79 was located at the entrance of pocket-3 and did not directly interact with the positively charged amino acid cluster in pocket-3, although the SPR analysis with the K112E and K198E mutants showed that Lys$^{112}$ and Lys$^{198}$ residues were important for HQL binding (Fig. 5, E and H). Thus, one of promising modifications of HQL-79 would be the elongation of the side chain of the tetrazol group to provide a better interaction with the positively charged residues. The HQL-79 binding activity was almost completely lost by substitution of Cys$^{196}$ by Leu in pocket 2, indicating that this pocket is almost occupied by the phenyl group of the diphenyl group of HQL-79 and suggesting that only minor modification with a small-sized group would be possible for this phenyl group. On the other hand, the other phenyl group of HQL-79 penetrated into the ceiling of pocket 1, resulting in the rotation of the indole ring of Trp$^{104}$ and the twisting of loop7 (Fig. 6F). Moreover, the substitution of Trp$^{104}$ in pocket 1 by Ile resulted in 26% of the HQL-79 binding (Fig. 5E). Therefore, derivatization is more acceptable for the phenyl group of HQL-79 within pocket-1 than that in pocket-2. The development of novel H-PGDS inhibitors with increased selectivity and inhibitory potency is now being extensively pursued by our group and others, based on the crystal structure of the H-PGDS-HQL-79 complex.

**Tight Functional Coupling between H-PGDS and COX**—The most interesting and unexpected our finding was that HQL-79 inhibited the H-PGDS-catalyzed PGD$_2$ production without shunting PGH$_2$ toward the production of other PGs either in vivo (Fig. 2) or in cultured cells (Fig. 3). This situation is quite different from the previous prediction about the utility of inhibitors for the terminal PG synthase including PGDSs; i.e. those inhibitors may alter the metabolic flow within the PG cascade without changing the total amount of PGs. However, as shown in Fig. 3, HQL-79 inhibited highly selectively PGD$_2$ production while causing only a marginal change in other PGs in human megakaryocyte MEG-01S cells and rat mastocytoma RBL-2H3 cells. For example, in MEG-01S cells, 300 $\mu$M HQL-79 decreased the Ca$^{2+}$-ionophore-induced PGD$_2$ production from 10.1 to 3.1 ng/10$^6$ cells but increased the PGE$_2$ production from 0.17 to 0.32 ng/10$^6$ cells and decreased the PGF$_{2\alpha}$ production from 0.34 to 0.23 ng/10$^6$ cells. In RBL-2H3 cells, 100 $\mu$M HQL-79 decreased antigen- and Ca$^{2+}$-ionophore-induced PGD$_2$ production from 12.2 to 7.6 ng/10$^6$ cells and from 48 to 23 ng/10$^6$ cells, respectively, but increased PGE$_2$ production from 0.05 to 0.33 ng/10$^6$ cells and from 0.19 to 0.76 ng/10$^6$ cells, respectively, and decreased the PGF$_{2\alpha}$ production from 1.5 to 1.4 ng/10$^6$ cells and from 5.4 to 4.5 ng/10$^6$ cells, respectively. These results clearly indicate that, once the downstream H-PGDS is inhibited, the upstream COX, probably COX-1 in these cells (24), is also inhibited. However, in the *in vitro* experiment using the purified enzymes (Fig. 4) and in cultured L-PGDS/COX-1-containing HEK-293 cells (Fig. 3), HQL-79 did not inhibit COX activities and total PG production, respectively. These results, taken together, suggest that H-PGDS and COX are tightly engaged functionally with each other.

The functional coupling between H-PGDS and COX may arise from the complex formation of these 2 enzymes within the membrane. We previously found that H-PGDS was translocated from the cytoplasm to the perinuclear region or to a membranous structure in the cytoplasm of H-PGDS-overexpressing HEK-293 cells, where COX-1 or COX-2 exists, after stimulation with A23187 or IL-1 (34). Similar translocation of the complex formation of these 2 enzymes within the membrane. We previously found that H-PGDS was translocated from the cytoplasm to the perinuclear region or to a membranous structure in the cytoplasm of H-PGDS-overexpressing HEK-293 cells, where COX-1 or COX-2 exists, after stimulation with A23187 or IL-1 (34). Similar translocation of the complex formation of these 2 enzymes within the membrane. We previously found that H-PGDS was translocated from the cytoplasm to the perinuclear region or to a membranous structure in the cytoplasm of H-PGDS-overexpressing HEK-293 cells, where COX-1 or COX-2 exists, after stimulation with A23187 or IL-1 (34). Similar translocation of the complex formation of these 2 enzymes within the membrane.
and structural studies of the interaction between H-PGDS and COX are needed to confirm the functional and topological coupling between these 2 enzymes that we have proposed here.

Pharmacological Characterization of HQL-79 as an Excellent Lead Compound for Development of Orally Effective Inhibitors for Human H-PGDS—Here, we demonstrated pharmacologically and biochemically that HQL-79 is an orally effective inhibitor selective for H-PGDS. Especially, it should be noted that HQL-79 specifically inhibited the production of PGD2 catalyzed by H-PGDS but only marginally affected the production of other prostanooids. In this sense, HQL-79 is an even better PG-blocking compound than those available today (13). Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used as anti-inflammatory drugs that ameliorate pain, fever, and inflammation by blocking PG production. However, NSAIDs accelerate asthmatic reactions by leading to a shunting of arachidonic acid metabolism toward the production of lipoxins and leukotrienes. Moreover, NSAIDs inhibit the production of all prostanooids, including the cytoprotective and anti-inflammatory PGs. For example, aspirin and indomethacin induce gastrointestinal toxicity by blocking PGE production (11, 12). The anti-inflammatory action of PGE mediated by EP3 receptors was also very recently reported (38). We have previously demonstrated that PGD2 produced by L-PGDS prevents neuronal and oligodendroglial apoptosis during neuroinflammation in a genetic demyelination mouse model, i.e. twitcher (39). Thus, HQL-79 may be predicted to selectively suppress the inflammatory reaction mediated by H-PGDS-catalyzed PGD2 without various side effects caused by the suppression of cytoprotective and anti-inflammatory PGs.

HQL-79 suppressed OVA-induced allergic airway inflammation in WT, human H-PGDS-TG, and H1R-KO mice (Fig. 2). The anti-asthmatic and anti-allergic properties of HQL-79 were originally explained by the antagonistic activity of the drug against H1R (17, 18). However, HQL-79 exhibited a 10-fold more potent anti-allergic effect than other anti-H1R drugs, such as epinastine and ketotifen; although the anti-histaminic effect of HQL-79 was 10-fold less potent than that of the latter drugs (17, 18). Because HQL-79 inhibited allergic reaction even in H1R-KO mice, the anti-allergic effect was not caused by the anti-H1R antagonistic activity but to the anti-H-PGDS activity. In other word, HQL-79 is a unique dual functional drug associated with both anti-H-PGDS and anti-H1R activities.

In the OVA-induced asthma model, antigen provocation increased the PGD2 content in the BALF (Fig. 2) and induced a variety of PGD2-mediated biological actions, including vasodilation and bronchoconstriction (1). The asthmatic reaction is reduced in DP1 receptor-KO mice (3) and suppressed by ramatroban, an antagonist against DP2 (CGRTH2) receptor (40), indicating that PGD2 coordinates regulates allergic reactions, especially airway inflammation, via these 2 receptors (5). Therefore, H-PGDS inhibitors such as HQL-79 may be considered to more effectively suppress the PGD2-mediated asthmatic and inflammatory reactions than the antagonist of each DP receptor and to function as a non-selective antagonist against DP1 and DP2 receptors.

Recently, we and many other research groups reported that PGD2 produced by H-PGDS is involved in a variety of allergic and non-allergic disorders (9). For example, H-PGDS is expressed in mast cells that accumulate in the nasal mucosa of patients with polyposis (41); in infiltrates of mast cells, eosinophils, macrophages, and lymphocytes in the nasal mucosa of patients with allergic rhinitis (42); in necrotic muscle fibers of patients with Duchenne’s muscular dystrophy or polymyositis (43); in microglial cells around the demyelinating region of twitcher mice (44), an animal model of human Krabbe’s disease; and in rat and mouse brains after stab-wounding or traumatic brain injury.4 Therefore, H-PGDS inhibitors would also be predicted to suppress the progression of those diseases. In fact, we have already confirmed that HQL-79 administration suppressed the muscular necrosis of mdx mice, an animal model of Duchenne’s muscular dystrophy, and the astrogliosis found in the twitcher brain (44) or after stab-wounding brain injury.4 Therefore, HQL-79 is an excellent lead compound for the development of novel H-PGDS inhibitors that promise to be new concept drugs against a variety of allergic and non-allergic diseases.

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