Differential Allosteric Regulation of Prostaglandin H Synthase 1 and 2 by Arachidonic Acid*

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Prostaglandins are synthesized by prostaglandin H synthase (PGHS) 1 and 2. PGHS2 is regulated through inductive expression. We report here the regulation of PGHS1 activity by substrate-dependent cooperative activation. The cooperativity is characterized by a Hill coefficient of 1.29 ± 0.06, a curved Eadie-Scatchard plot, and activation by low concentrations of competitive inhibitors. The activation also appears to induce a conformational change in the cyclooxygenase site. The cooperativity produces a 2–4-fold greater rate of PGHS2-dependent prostaglandin formation compared with PGHS1-dependent prostaglandin formation at arachidonic acid concentrations below 0.5 μM. A consequence of the PGHS1 cooperativity is that the affinity of many cyclooxygenase inhibitors for PGHS1 decreases in parallel to the activation by arachidonic acid. In contrast, the affinity of these inhibitors for PGHS2 is unaffected by the changes in arachidonic acid concentration. This results in a dramatic difference in PGHS2/PGHS1 selectivity at different arachidonic acid concentrations.

Prostaglandin H synthase (PGHS)1 oxidizes arachidonic acid to prostaglandin H₂, which is the precursor of all other prostaglandins. Two forms of PGHS are known: constitutively expressed PGHS1 and inducible PGHS2. PGHS1 is considered to be the housekeeping enzyme, whereas PGHS2 is induced in response to cellular stress and is thought to play a role in inflammation (1, 2). Aspirin and other nonsteroidal anti-inflammatory drugs inhibit both enzymes (3). Chronic use of these drugs causes stomach bleeding and kidney failure. These dangerous side effects are thought to be due to inhibition of PGHS1-dependent prostaglandin synthesis and not inhibition of PGHS2 (4). Recently, PGHS2 expression has been shown to be associated with colon cancer (5, 6) and Alzheimer’s disease (7, 8). Accordingly, extensive biological and pharmaceutical research has focused on factors that differentiate between these two routes of prostaglandin biosynthesis.

Accumulating biological evidence points to a functional differentiation in the formation of prostaglandins by PGHS1 and PGHS2. Both enzymes are located in the endoplasmic reticulum and the nuclear envelope with PGHS2 somewhat more concentrated in the nuclear envelope (9). Murakami et al. (10, 11) showed that both enzymes function independently by coupling different stimulus-initiated pathways to prostaglandin D₂ generation from endogenous arachidonic acid. Reddy and Herschman (12, 13) have proposed at least two independent pathways for prostaglandin synthesis: a secretory phospholipase A₂-mediated, PGHS1-dependent transcellular pathway and an intracellular cytosolic phospholipase A₂-mediated, PGHS2-dependent pathway. This is consistent with the hypothesis that PGHS1 prefers exogenous arachidonic acid, and PGHS2 prefers endogenous arachidonic acid (12). Chulada and co-workers (14) quantitated the differences in the rate of prostaglandin formation by PGHS1 and PGHS2 in mouse embryonic fibroblasts and Chinese hamster ovary cells with stably expressed enzymes. They observed that the levels of PGHS1 activities were 3–5-fold higher than those of PGHS2 when 10 μM arachidonic acid was used as substrate. In contrast, PGHS2 activity was up to 5-fold higher when endogenous arachidonic acid was the substrate (14). These observations support the existence of discrete PGHS1 and PGHS2 biosynthetic pathways but give little insight to why PGHS1 has less activity than PGHS2 when utilizing endogenous arachidonic acid.

Pharmaceutical research has focused on the selective intervention of PGHS2-dependent prostaglandin formation. Selective PGHS2 inhibition is hypothesized to alleviate the side effects associated with the use of nonselective nonsteroidal anti-inflammatory drugs and to be useful in treating inflammatory diseases as well as colon cancer and Alzheimer’s disease. A number of PGHS2 selective inhibitors have been reported recently including NS-398 and SC-58125 (15, 16). However, the degree of selectivity with these inhibitors and others changes dramatically with the assay conditions (17).

We report here the contribution of an inhibitor-sensitive substrate-dependent cooperative activation of PGHS1 in the functional segregation of PGHS1 and PGHS2 biosynthetic pathways. The allosteric activation appears to induce a conformation change in the PGHS1 cyclooxygenase binding site which causes a loss in PGHS1 affinity for PGHS2-selective inhibitors. Nonselective inhibitors are unaffected. These findings allow us to conclude that the production of prostaglandins is regulated coordinately through transcriptional regulation of PGHS2 activity and allosteric regulation of PGHS1 activity.

EXPERIMENTAL PROCEDURES

Materials—[3H]Arachidonic acid and [3H]prostaglandin E₁ were purchased from DuPont NEN and unlabeled arachidonic acid from Nu-Chek-Prep, Inc. (Elysian, MN). Hemin, tyloxapol, and dilauroylphosphatidylcholine were obtained from Sigma. 15(S)-HPETE and 15-HETE were purchased from Biomol (Plymouth Meeting, PA). All other chemicals were of the highest grade available. Human PGHS1 and PGHS2 were expressed and purified from a baculovirus/insect cell culture system as described previously (18). Enzyme concentrations were determined from the absorbance at 411 nm using an extinction coefficient of 123 mM⁻¹ cm⁻¹ (19).

Incubation Conditions—Reactions contained [3H]arachidonic acid (50 nM–20 μM; 0.5 μCi), tyloxapol (0.05%), phenol (1.2 mM), hemin (0.6 μM), and potassium phosphate buffer (100 mM, pH 8.0) in a total volume of 1 cm³.
of 0.1 ml unless otherwise stated. Reactions were incubated at 30 °C for 30 s and stopped with 0.4 ml of ice-cold ethanol and placed on ice. After approximately 30 min on ice the reactions were evaporated to dryness, reconstituted in 0.2 ml of 50:50:1 water/methanol/acetic acid, and 0.1 ml was injected onto the HPLC for analysis. The substrate solution was prepared by combining unlabeled arachidonic acid in ethanol, [3H]arachidonic acid in ethanol, and 10 μl of a 0.5% solution of tyloxapol in acetone and evaporating to dryness. This was reconstituted in 0.05 ml of water. The reactions were initiated by adding the detergent-solubilized substrate solution to the enzyme mix containing buffer, phenol and hemin which had been preincubated at room temperature from 1–5 min.

**HPLC Conditions**—The prostaglandins were separated from arachidonic acid by reverse phase HPLC using a 25-cm, 5-μm Jones chromatography apex octadecyl column and detected with a Packard Flo-one A-500 radioflow detector with a scintillant mixture/HPLC eluant ratio of 3:1. The strong component of the mobile phase was 0.1% acetic acid, ammonium hydroxide buffer, pH 6.1, and the eluting solvent was methanol. The flow rate was 1 ml/min. The following elution profile was used: 0–5 min, 20% methanol; 6–15 min, −7 convex gradient to 60% methanol; 16–20 min, 60% methanol; 21–30 min, −7 convex gradient to 80% methanol; 31–35 min, 80% methanol; 36–45 min, 100% methanol. The prostaglandins eluted around 25 min and arachidonic acid at 44 min.

**Cell-based Prostaglandin Formation**—The activity of cell-associated PGHS1 was measured using human HFF 1491 cells expressing basal PGHS1 activity. Cells were plated in 96-well microtiter plates at a density of 8×10^4 cells/well in RPMI 1640 medium (Life Technologies, Inc.) and grown for 24 h at 37 °C. The medium was removed, and cells were washed two times with phosphate-buffered saline (Life Technologies, Inc.) and replaced with 0.225 ml/well of fresh medium containing inhibitors in dimethyl sulfoxide or dimethyl sulfoxide alone. Following incubation at 37 °C for 30 min, arachidonic acid in dimethyl sulfoxide was added to the cells and incubated for an additional 10 min. The reaction was stopped by transferring the plates to ice, and the amount of prostaglandin E₂ in the culture supernatants was quantitated using enzyme-linked immunosorbent assay kits (Cayman Chemicals, Ann Arbor, MI).

**Fluorescence Spectroscopy**—The extent of fluorescence quenching was measured using a Perkin-Elmer LS 50B spectrometer with excitation at 280 nm (slit width 2.5 nm) and emission at 327 nm (slit width 10 nm). PGHS1 or PGHS2 was mixed with 1.5 eq of hemin in 0.1 M potassium phosphate buffer, pH 8.0, for 1–2 min at 30 °C. When the fluorescence emission had stabilized, the inhibitor was added sequentially until the quench was saturated. All concentrations were volume adjusted. The concentrations of PGHS2 needed for detectable fluorescence emission were greater than the association constant of RS-57067, therefore we employed a tight binding kinetic analysis using four enzyme concentrations (0.1, 0.25, 0.5, and 0.8 μM) to determine the association constant for RS-57067 binding to PGHS2. The Kₜ was determined using a nonlinear least squares analysis from the following equation

\[ [I] / [E] = K_t (1/E_t - [I]) + 1 \quad \text{(Eq. 1)} \]

where \([I] = E_t (k_F/k_m)\).

**RESULTS AND DISCUSSION**

**Positive Cooperativity-Substrate Kinetics**—The kinetics of arachidonic acid oxidation by PGHS1 and PGHS2 were determined using baculovirus-expressed, purified human enzymes reconstituted with hemin and the cosubstrate phenol (18). The arachidonic acid was not purified to remove peroxides, and therefore no lag in product formation was detected. Reactions were typically carried out for 30 s. Substrate velocity plots associated with the oxidation of 0.05–20 μM arachidonic acid by human PGHS1 and human PGHS2 are shown in the inset of Fig. 1A. The maximum velocities determined by fitting to the Michaelis-Menten equation were 14.9 ± 1.04 s⁻¹ and 13.0 ± 0.68 s⁻¹, respectively, for PGHS1 and PGHS2. The apparent Kₚ values, determined from one-half Vₘₜₐₓ were 10.2 ± 1.5 μM and 10.0 ± 1.1 μM, respectively. A slight sigmoidal relationship was observed at low substrate concentration with PGHS1. This resulted in PGHS2 having 2–4-fold more activity than PGHS1 at substrate concentrations below 0.5 μM, whereas at substrate concentrations above 2.5 μM, PGHS1 was slightly more active than PGHS2 (Fig. 1A). Positive cooperativity for PGHS1 was evident from the shape of the Eadie-Scatchard plot, which was curved and passed through a maximum (Fig. 1B) (20). The plot was linear for PGHS2 (Fig. 1B). The apparent catalytic affinity of the enzyme for substrate must change with substrate concentration, since the slope of the Eadie-Scatchard, −1/Kₚ, changes with the substrate concentration. Hill plots confirmed the PGHS1-associated positive cooperativity. The Hill coefficient (n) for PGHS1 was 1.29 ± 0.06. The Hill coefficient was determined from three separate experiments where it ranged from 1.20 to 1.39 with all r² values greater than 0.985. Positive cooperativity was also observed with rat PGHS1 expressed and purified from a baculovirus insect cell system.

There was no evidence for detectable positive cooperativity associated with arachidonic acid turnover by PGHS2 under the conditions of these experiments. Not only was the slope of the Eadie-Scatchard plot linear, the Hill coefficient associated with PGHS2 was 0.95 ± 0.04. The r² values were all greater than 0.984. These results show that PGHS1 has a requirement for the cooperative activation and PGHS2 does not; thus, PGHS2 has more activity at lower substrate concentrations.

Since product formation was linear for 60 s at all substrate concentrations determined using a nonlinear least squares analysis from the following equation

\[ [I] / [E] = K_t (1/E_t - [I]) + 1 \quad \text{(Eq. 1)} \]

where \([I] = E_t (k_F/k_m)\).
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with low affinity binding in the presence of 20 μM arachidonic acid (IC\textsubscript{50} values of 390 and 730 μM, respectively) and relatively high affinity binding in the presence of 50 nM arachidonic acid (IC\textsubscript{50} values of 4.0 and 0.15 μM, respectively) (Table I). This results in a 98- and 4867-fold change in the IC\textsubscript{50} values associated with a 400-fold change in substrate concentration.

This much variation in substrate-dependent IC\textsubscript{50} values is inconsistent with reversible inhibition at a single enzyme site obeying Michaelis-Menten kinetics. According to theory, the intrinsic inhibition constant, \( K_I \), is independent of the substrate concentration. The \( K_f \) for a competitive inhibitor can be determined from the IC\textsubscript{50} values using the following equation.

\[
K_f = K_m [IC_{50}]/(K_m + [S])
\]

A large difference in the calculated \( K_f \) values was observed for the different arachidonic acid concentrations (based on the measured IC\textsubscript{50} values and assuming a \( K_m \) of 10 μM). The differences in calculated \( K_f \) values between 20 μM and 50 nM arachidonic acid were 33- and 1620-fold, respectively, for RS-57067 and SC-58125. These results are clearly inconsistent with normal Michaelis-Menten kinetics and show that the positive cooperativity is accompanied by a dramatic decrease in the apparent affinity of these inhibitors for PGHS1.

Are All Inhibitors Sensitive to the Positive Cooperativity?—We have observed that the affinity of the cyclooxygenase inhibitors RS-57067 and SC-58125 changes in parallel to the substrate-induced positive cooperativity of PGHS1. These compounds are PGHS2-selective when assayed with 20 μM and nonselective using 50 nM arachidonic acid (Table I). Are all compounds sensitive to the positive cooperativity or only the PGHS2-selective compounds? This question was addressed with ibuprofen and RS-101575, two compounds that are relatively nonselective when assayed with 20 μM arachidonic acid (Fig. 3). The affinities of ibuprofen and RS-101575 for both PGHS1 and PGHS2 were unaffected by the change in substrate concentrations (Table I). The calculated \( K_f \) values for PGHS1 inhibition changed only 0.2- and 1.4-fold, respectively. These results show that the activation of PGHS1 by arachidonic acid does not change the binding affinity for every cyclooxygenase inhibitor. We interpreted this to indicate that a conformational change is produced in the cyclooxygenase binding site upon the cooperative activation of PGHS1, and the affinities of only some of the cyclooxygenase inhibitors are affected by this change. Of importance is the fact that only the affinities of PGHS2-selective inhibitors are influenced by the change. We conclude that the molecular factors involved in the conformation change contribute to the selectivity of PGHS2 inhibitors.

**Tryptophan Fluorescent Quenching Equilibrium Binding Experiments Provide Evidence for PGHS2-dependent Positive Cooperativity**—Human PGHS1 and PGHS2 have nine and six tryptophans, respectively. Excitation at 280 nm results in a broad maximum fluorescence emission from 320 to 350 nm. RS-57067, when excited at 280 nm, has very little emission and a broad maximum fluorescence emission from 320 to 350 nm. RS-57067, when excited at 280 nm, has very little emission and a broad maximum fluorescence emission from 320 to 350 nm. Rs-57067. The binding constants, \( K_B \), were determined assuming tight binding kinetics by comparing the saturation curves at four protein concentrations. The \( K_B \) values associated with PGHS1 and PGHS2 were 11.2 ± 0.27 μM and 2.9 ± 0.8 nM, respectively. The \( K_f \) values represent the affinity of the inhibitors for the enzyme in the absence of substrate, conditions in

**Low Concentrations of Competitive Inhibitors Activate the Cyclooxygenase Activity of PGHS1—RS-57067, RS-101575, SC-58125, and ibuprofen are competitive inhibitors of PGHS1 and PGHS2 (data not shown).**

![Graph](image)

Fig. 2. Activation of PGHS1 activity by competitive cyclooxygenase inhibitors. ●, RS-57067; ▲, ibuprofen; ▲, RS-101575; and ■, SC-58125. For comparative purposes the data are normalized to the uninhibited velocity (\( V_v \)). Inhibitors were incubated with enzyme and 50 nM arachidonic acid as described in Fig. 1.

Concentrations examined, the observed positive cooperativity could not be attributed to different rates of catalytic activation or autoinactivation. Changing the ratio of substrate to enzyme did not significantly affect the apparent positive cooperativity. There was no difference between arachidonic acid being added to the reaction in ethanol or reconstituted with 0.6% tyloxapol, a nonionic detergent. In addition, reconstitution of the purified enzymes in dilauroylphosphatidylcholine (10 μM) did not alter the apparent positive cooperativity.

**Effect of Positive Cooperativity on the Affinity of Cyclooxygenase Inhibitors for PGHS1 and PGHS2—RS-57067 and SC-58125 are rapidly reversible competitive inhibitors of PGHS1...**
which the enzyme must be unactivated. The $K_p$ for PGHS2 was approximately 1000-fold lower than the $K_p$ value (3.8 $\mu M$) determined with 200 $\mu M$ arachidonic acid assuming slow binding inhibition (22). One interpretation of the results is that PGHS2 undergoes a conformation transition similar to PGHS1; however, it occurs at a substrate concentration below our limits of detection (50 nM arachidonic acid). We confirmed that arachidonic acid was competitive for RS-57067 in these dissociation experiments by the fact that 50 nM arachidonic acid (which does not quench the tryptophan fluorescence) completely reverses the quenching associated with 1.1 $\mu M$ RS-57067 bound to PGHS2.

Effect of Arachidonic Acid Concentration upon Inhibition of PGHS1 in Intact Cells—IC$_{50}$ values were determined in PGHS1-expressing uninduced HFF 1491 cells (Table II). HFF 1491 cells have a high basal rate of PGHS1-dependent prostaglandin formation. Insufficient quantities of prostaglandin $E_2$ were formed using 50 nM arachidonic acid in the other PGHS1-expressing cell line tested (U937). Similar to the results with the purified enzyme the IC$_{50}$ values associated with RS-57067 and SC-58125 decreased with increasing arachidonic acid concentrations, and the affinity RS-101575 was unaffected by the different concentrations. The observed 40- and 9-fold, respective, decrease in the affinity of RS-57067 and SC-58125 represents the minimum change. These compounds were insoluble in cell-based assays at concentrations greater than 100 $\mu M$. The change in IC$_{50}$ values associated with ibuprofen inhibition in the cell-based assay was not as well correlated to the change obtained with purified enzyme. We assume this is because of the large experimental error associated with the IC$_{50}$ value determined using 20 $\mu M$ arachidonic acid. These results confirm that the conformation change associated with substrate activation of PGHS1 occurs in intact cells and is not an artifact associated with enzyme isolation.

Role of Peroxidase Activity in Positive Cooperativity—The cyclooxygenase activity associated with PGHS requires catalytic activation via the peroxidase component of the enzymes. The mechanism of the peroxidase reaction has been investigated extensively and involves the binding of hydroperoxide to the heme to form an oxyferryl intermediate that is reduced back to the ferric resting enzyme by arachidonic acid and reducing cosubstrates such as phenol and TMPD (24). We used the affinity of RS-57067 as an end point for the study of the involvement of the peroxidase activity in the conformational positive cooperativity. The involvement of heme in positive cooperativity is well documented for hemoglobin (25). Potassium cyanide (5 mM), which coordinates to heme and can induce the cooperative effects in hemoglobin, had minimal effect of the cooperative effects in hemoglobin. The observed 40- and 9-fold, respective, decrease in the affinity of RS-57067 and SC-58125 represents the minimum change. These compounds were insoluble in cell-based assays at concentrations greater than 100 $\mu M$. The change in IC$_{50}$ values associated with ibuprofen inhibition in the cell-based assay was not as well correlated to the change obtained with purified enzyme. We assume this is because of the large experimental error associated with the IC$_{50}$ value determined using 20 $\mu M$ arachidonic acid. These results confirm that the conformation change associated with substrate activation of PGHS1 occurs in intact cells and is not an artifact associated with enzyme isolation.

### Table I

| Inhibitor | Enzyme | Inhibition mechanism | IC$_{50}$ 50 nM Arachidonic acid | IC$_{50}$ 20 $\mu M$ Arachidonic acid | Ratio 20 $\mu M$/50 nM |
|-----------|--------|----------------------|----------------------------------|---------------------------------------|------------------------|
| RS-57067  | PGHS1  | Reversible           | 4.0 ± 2.5                        | 390 ± 69                              | 98                     |
|           | PGHS2  | Slow binding         | 0.5 ± 0.15                       | 0.6 ± 0.4                             | 1.2                    |
| SC-58125  | PGHS1  | Reversible           | 0.15 ± 0.07                      | 730 ± 460                             | 4867                   |
|           | PGHS2  | Irreversible         | <0.03                            | 0.8 ± 0.9                             | >26                    |
| Ibuprofen | PGHS1  | Slow binding         | 2.7 ± 2.0                        | 2.0 ± 1.1                             | 0.74                   |
|           | PGHS2  | Slow binding         | 0.82 ± 0.06                      | 1.2 ± 0.9                             | 1.5                    |
| RS-101575 | PGHS1  | Reversible           | 0.30 ± 0.03                      | 1.2 ± 1.0                             | 4.2                    |
|           | PGHS2  | Slow binding         | 0.77 ± 0.11                      | 0.3 ± 0.1                             | 0.4                    |

* A reversible mechanism of inhibition indicates no time dependence and competition by substrate. Slow binding inhibition is defined by reversible time dependence and competition by substrate. The kinetic constants associated with the slow binding inhibition were determined using 200 $\mu M$ arachidonic acid as described by Callan and co-workers (22). The $K_p$ values associated with overall slow binding inhibition were determined to be 0.24 ± 0.02 and 14 ± 2.8 $\mu M$ for the respective inhibition of PGHS1 and PGHS2 by ibuprofen and 3.8 ± 2.5 and 2.5 ± 0.24 $\mu M$, respectively, for the slow binding inhibition of PGHS2 by RS-57067 and RS-101575. SC-58125 is an irreversible inhibitor of PGHS2 as described by Copeland and co-workers (23).

### Table II

| Inhibitor | IC$_{50}$ 50 nM arachidonic acid | IC$_{50}$ 20 $\mu M$ arachidonic acid | Ratio 20 $\mu M$/50 nM |
|-----------|---------------------------------|--------------------------------------|------------------------|
| RS-57067  | 2.5 ± 0.3                       | >100                                 | >40                    |
| SC-58125  | 11 ± 6.1                        | >100                                 | >9                     |
| Ibuprofen | 2.5 ± 1.1                       | 25 ± 16                              | 10                     |
| RS-101575 | 1.5 ± 0.9                       | 6.0 ± 2.1                            | 4.2                    |

### Fig. 3. Structure of inhibitors.
the heme iron does not contribute to the cooperativity.

The role of hydroperoxides that initiate the peroxidase activity was investigated using 15(S)-HPETE. There was little effect on the IC$_{50}$ values associated with 50 nM arachidonic acid and 1.5–20 M 15(S)-HPETE (Table III). These concentrations of 15(S)-HPETE rapidly activated the peroxidase activity as measured by the oxidation of TMPD. We conclude that activation of the peroxidase activity by hydroperoxide alone is not responsible for the conformation changes in the cyclooxygenase site. The product of the peroxidase reaction 15-HETE (1.5 μM) also had no effect on the kinetics.

The remaining component of the peroxidase cycle is the reducing cosubstrate that provides the electrons to reduce the hydroperoxide to the corresponding alcohol, water, and ferric enzyme. Removing the cosubstrate essentially disables the peroxidase activity. This has only a minimal effect on the cyclooxygenase activity (26). Surprisingly, PGHS1 with the peroxidase activity disabled by the absence of the reducing cosubstrate phenol showed classical Michaelis-Menten kinetics associated with the cyclooxygenase reaction (Fig. 1). The IC$_{50}$ for inhibition by RS-57067 did not change between 0.05 and 20 M arachidonic acid (29–46 μM, respectively; Table III), the Eadie-Scatchard plot was linear (Fig. 1B), and the Hill coefficient was near 1 (n = 0.96, r$^2$ = 0.994). The apparent $K_m$ was 2.9 μM, 3-fold lower than the fully activated enzyme in the presence of phenol. The $V_{\text{max}}$ was approximately 15 times less, 0.87 s$^{-1}$ compared with 14.9 s$^{-1}$ in the presence of phenol. The V/K was approximately 5-fold lower in the absence of phenol. These data indicate a role for the active peroxidase component of PGHS1 in the cooperative kinetics and imply that reducing cosubstrate concentrations can dramatically influence cyclooxygenase function.

**Is There Biological Relevance for the Selective Cooperative Regulation of PGHS1?**—The obvious biological impact of the positive cooperativity is that under conditions of limiting arachidonic acid or reducing cosubstrates there will be a limited rate of production of PGHS1-derived prostaglandins. What is the biological significance of this observation? One possibility is to enhance the selectivity of PGHS2-derived prostaglandins over PGHS1 in locations where they are coexpressed and substrate concentration is limiting. This would allow the inducible enzyme to be the primary source of prostaglandin production. Another possibility is to limit the formation of PGHS1-derived prostaglandins until mandated via a signal-induced stimulus. The positive cooperativity could have a dual role in this: first, the unactivated enzyme would not utilize arachidonic acid needed for other cellular processes; and second, it would prevent the autoinactivation of PGHS1 and thus allow it to remain quiescent and viable for long periods of time. This may be particularly important in cells such as platelets, which are incapable of synthesizing new protein but must be able to make PGHS1-derived prostaglandins upon demand.

Recently a number of reports have suggested that PGHS1 and PGHS2 utilize different sources of arachidonic acid (12, 13). PGHS2 is proposed to utilize endogenous arachidonic acid preferentially, whereas PGHS1 is proposed to utilize exogenous arachidonic acid. Additionally, Kulmacz and Wang (27) reported a large intrinsic difference between PGHS1 and PGHS2 in cyclooxygenase initiation efficiency, with PGHS2 being initiated at a much lower hydroperoxide concentrations. In this manuscript we report a greater PGHS2 activity under conditions of limiting substrate and conclude that the substrate-dependent, enzyme-selective, positive cooperativity will also contribute to differences between PGHS1 and PGHS2 activities.

The molecular mechanism underlying the cooperative effects is not completely understood. We know it is dependent upon substrate concentration, involves a conformation change in the cyclooxygenase site, requires an active peroxidase reaction, and can be activated by low concentrations of competitive inhibitors. We do not know if it involves both subunits of the homodimer or is the result of an interaction between the cyclooxygenase and peroxidase sites of the individual subunits.

The significance of these findings is: 1) the contribution of an inhibitor-sensitive substrate-dependent cooperative activation of PGHS1 in the functional segregation of PGHS1 and PGHS2 biosynthetic pathways; 2) the selectivity and potency of inhibitors will depend on the relative state of activation of PGHS1; and 3) the solved x-ray structures of inhibitor-bound, substrate-free enzymes may not accurately represent the conformations of the substrate-activated enzymes (28–30). This may influence the interpretation of specific inhibitor-enzyme interactions.

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