Hydroperoxide Dependence and Cooperative Cyclooxygenase Kinetics in Prostaglandin H Synthase-1 and -2*

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Prostaglandin H synthase isofom-1 (PGHS-1) cyclooxygenase activity has a cooperative response to arachidonate concentration, whereas the second isoform, PGHS-2, exhibits saturable kinetics. The basis for the cooperative PGHS-1 behavior and for the difference in cooperativity between the isoforms was unclear. The two cyclooxygenase activities have different efficiencies of feedback activation by hydroperoxide. To determine whether the cooperative kinetics were governed by the feedback activation characteristics, we examined the cyclooxygenase activities under conditions where feedback activation was either assisted (by exogenous peroxide) or impaired (by replacement of heme with manganese protoporphyrin IX to form MnPGHS-1 and -2). Heme replacement increased PGHS-1 cyclooxygenase cooperativity and changed PGHS-2 cyclooxygenase kinetics from saturable to cooperative. Peroxide addition decreased or abolished cyclooxygenase cooperativity in PGHS-1, MnPGHS-1, and MnPGHS-2. Kinetic simulations predicted that cyclooxygenase cooperativity depends on the hydroperoxide activator requirement and initial peroxide concentration, consistent with observed behavior. The results indicate that PGHS-1 cyclooxygenase cooperativity originates in the feedback activation kinetics and that the cooperativity difference between the isoforms can be explained by the difference in feedback activation loop efficiency. This linkage between activation efficiency and cyclooxygenase cooperativity indicates an interdependence between fatty acid and hydroperoxide levels in controlling the synthesis of potent prostanoid mediators.

The cyclooxygenase activity of prostaglandin H synthase (PGHS) catalyzes the oxygenation of arachidonic acid to PGG2, a key control step in the biosynthesis of all prostanoids (1). Two isoforms of PGHS are known: PGHS-1 is present at relatively stable levels in many cells, and is considered a housekeeping enzyme, whereas PGHS-2 is absent from most quiescent human PGHS-2 indicates that the two isoforms have similar peroxidase and cyclooxygenase specific activities (3). Studies with recombinant human PGHS-1 and -2 indicate that the two isoforms have similar peroxidase and cyclooxygenase activities (3). Studies with recombinant human PGHS-1 and -2 indicate that the two isoforms have similar peroxidase and cyclooxygenase specific activities (4).

Cyclooxygenase synthetic capacity tends to be much greater than the actual prostaglandin synthesis from endogenous fatty acid in vivo, demonstrating that regulation of cyclooxygenase catalysis plays a major role in controlling cellular prostanoid synthesis (5). In this regard, it is particularly interesting that the two isoforms’ cyclooxygenase catalytic activities are differentially controlled in several cells where both PGHS-1 and -2 are present (2). In these cells, the large pulse of prostaglandin synthesis that follows cytokine treatment comes from cyclooxygenase catalysis by freshly induced PGHS-2; the considerable PGHS-1 cyclooxygenase capacity in the same cells remains latent unless high levels of arachidonate are added.

One possible explanation for this differential control of cyclooxygenase catalysis in the two PGHS isoforms has been suggested by a recent study of their responses to changes in arachidonate concentration (6). It was found that PGHS-1 exhibits cooperative changes in cyclooxygenase activity in response to the arachidonate level, whereas PGHS-2 shows simple saturable cyclooxygenase kinetics. As a result, cyclooxygenase catalysis by PGHS-2 was severalfold greater than that by PGHS-1 at the submicromolar arachidonate levels expected in the cellular environment (6).

The physical basis for the cooperative response of the PGHS-1 cyclooxygenase remained unresolved. Both detergent-solubilized PGHS isoforms are homodimers with one specific binding site for arachidonate recognized in each subunit (7–9). This led to a suspicion that the observed cooperativity in PGHS-1 was due to conventional allosteric interactions between arachidonate sites on the two PGHS-1 subunits. On the other hand, the grossly similar dimeric interfaces found in the two PGHS isoforms (7–9) offer few obvious clues as to why cooperativity is present only in one isoform. Further, cooperativity was prominent at submicromolar arachidonate levels, whereas the $K_m$ value for the fatty acid was approximately 10 $\mu$M (6). In addition, cooperative behavior in PGHS-1 required the presence of phenol, a peroxidase cosubstrate (6), an observation that is difficult to reconcile with a conventional allosteric effect. However, phenol is known to alter PGHS-1 cyclooxygenase kinetics, in particular the feedback activation of the cyclooxygenase by its product hydroperoxide, PGG2 (10).

This led to a suspicion that the PGHS-1 cooperativity might be a consequence of the complex cyclooxygenase kinetics. To test this possibility, the cyclooxygenase kinetics of PGHS-1 and -2 were examined for cooperativity under conditions where the cyclooxygenase feedback activation was perturbed.

**EXPERIMENTAL PROCEDURES**

Materials—Arachidonic acid was purchased from NuChek Prep (Elysian, MN), and [5,6,8,9,11,12,14,15-2H]arachidonic acid (91.8 Ci/mmol,
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The reaction products were analyzed by high pressure liquid chromatography on a Rainin/Varian (Walnut Creek, CA) Microsorb C18 column (0.46 x 30 cm) eluted at 1.0 ml/min with a gradient of methanol in 0.1% acetic acid (adjusted to pH 6.1 with ammonium hydroxide). The methanol concentration was 20% for the first two min, increased to 60% methanol at 6 min and to 100% methanol at 20 min, and then declined from 100 to 20% between 26 and 30 min. Eluting lipids were quantitated using a Beta-RAM Model 2B radioisotope flow detector (INUS Systems, Tampa, FL) with a 1,000 µl flow cell and a scintillant:elu rate ratio of 3.1. Arachidonate eluted near 22 min, and the major prostanoic products eluted near 12 min.

**RESULTS**

**Measurement of PGHS-1 Cyclooxygenase Kinetics—PGHS-1 cyclooxygenase kinetics were determined for reactions with 0.5–20 µM arachidonate using both radioisotope and oxygen electrode measurements (Fig. 1). The independent measurements of cyclooxygenase activity agreed well, with the individual time points from the isotope assays falling close to the traces determined by continuous measurements of oxygen consumption. Two trends are readily apparent from the data. First, the cyclooxygenase velocity, determined by the maximal slope of the individual traces, increased with the arachidonate level, much as expected. Second, the maximum velocity was reached earlier in reactions with higher arachidonate levels than in reactions with less substrate. As a result of this variation in lag time with substrate level, it was not possible to use a single time point for accurate determination of cyclooxygenase velocity using radioisotope analysis. Fortunately, the continuous oxygen electrode measurements permit determination of cyclooxygenase velocity regardless of the lag time. Accordingly, oxygen electrode measurements were used to monitor cyclooxygenase velocity in the remaining experiments in this study.**

The rather slow acceleration observed in reactions with low micromolar arachidonate levels (Fig. 1) permitted an additional simplification in data analysis. Dampening of the electrode signal because of the protecting Teflon membrane is severe and needs correction when fast acceleration is encoun-

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0.10 mM (in ethanol) was from NEN Life Science Products. Heme, butylated hydroxytoluene, and hydrogen peroxide were obtained from Sigma, and manganese porphyrin IX (MnPPIX) from Porphyrin Products (Logan, UT). Tween 20 (10% solution) was purchased from Pierce. Monolux 2 scintillation fluid was from National Diagnostics (Atlanta, GA). Purification of ovine PGHS-1 from seminal vesicles and of recombinant human PGHS-2 from a baculovirus expression system have been described previously (11, 12); both are isolated in apoenzyme form.

**Preparation of Arachidonic Acid Stock Solutions—**Highly purified arachidonic acid (>99% pure by gas chromatography) was supplied in sealed ampules. Upon opening an ampule, the content was immediately weighed into a small glass screw cap vial, dissolved in ice-cold ethanol containing 0.125% acetic acid and chilled on ice for at least 30 min. The solvent was removed under vacuum in a Speed-Vac centrifuge (Savant, Bathesda, MD) and the lipid residue was dissolved in 0.40 ml of methanol/water/acetic acid (50:50:1).

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**Computer Simulation of Cyclooxygenase Kinetics—**Numerical integration was performed with the ScOp program (Simulation Resources, Redlands, CA) using a simple model based on the mechanism used for simulation of the effects of peroxide scavengers on cyclooxygenase kinetics (12, 17). In the mechanism used here, the peroxidase activity was assumed to depend on the level of substrate PGG2 (governed by the constant K_{p(POX)} (Eq. 1). The cyclooxygenase velocity was assumed to respond to both the level of hydroperoxide (as initiator, governed by the constant K_{h} and the level of arachidonate (as substrate, governed by the constant K_{a}) (Eq. 2).

Peroxidase velocity = \( EV_{\text{max,POX}}(1 + K_{p(POX)}[\text{PGG}]) \) (Eq. 1)

Cyclooxygenase velocity = \( EV_{\text{max,COX}}(1 + K_{a}[\text{AA}](1 + K_{h}[\text{PGG}]]) \) (Eq. 2)

E is the level of PGHS. The derivative equations for hydroperoxide (PGG2), arachidonate, and PGHS were as follows.

\[
\frac{d[\text{PGG}]}{dt} = EV_{\text{max,POX}}(1 + K_{a}[\text{AA}](1 + K_{h}[\text{PGG}]]) - EV_{\text{max,POX}}(1 + K_{p(POX)}[\text{PGG}])
\]

\[
\frac{d[\text{AA}]}{dt} = -EV_{\text{max,COX}}(1 + K_{a}[\text{AA}](1 + K_{h}[\text{PGG}]])
\]

\[
\frac{dE}{dt} = -(0.001)EV_{\text{max,COX}}(1 + K_{a}[\text{AA}](1 + K_{h}[\text{PGG}]])
\]

The decline in PGHS specified by Eq. 5 simulates the self-inactivation process, which is typically complete within about 1,000 catalytic turnovers of the PGHS-1 cyclooxygenase (5).

The values of the fixed parameters were: \( V_{\text{max,POX}} \) 165 s\(^{-1}\); \( K_{p(POX)} \) 2.5 µM; \( V_{\text{max,COX}} \) 15 s\(^{-1}\); \( K_{a} \) 3 µM; \( K_{h} \) 21 nm (for PGHS-1) or 2.3 nm (for PGHS-2). These values are based on experimental observations (6, 12, 17). The \( V_{\text{max}} \) values were scaled to give comparable activities for PGHS-1 and -2, based on the reported cyclooxygenase activities of the recombinant human isoforms (6). The initial conditions were: E, 10 nM; AA, 0.4–100 µM, and PGG2 (or equivalent hydroperoxide), 1 nM.

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**Polargraphic Assay of Cyclooxygenase Activity—**Quantitation of oxygen uptake during cyclooxygenase catalysis was done with a Model 53 oxygen electrode and monitor from YSI, Inc. (Yellow Springs, OH), following general procedures described previously (11). Unless otherwise noted, experiments were run in 3 ml of 0.1 mM potassium phosphate, pH 7.2, containing 1 mM phenol, 0.02% Tween 20, and 1 µM heme at 23 °C. Inclusion of the detergent was crucial to obtaining reproducible results. Reactions were started by injection of a small volume (<6 µl) of working fatty acid stock solution. The oxygen electrode monitor was modified with electronic offset circuitry (14) to produce a 10 mV output signal representing a 10% decrement in oxygen content. The 10 mV signal was displayed by a chart recorder set at 10 mV/full scale, so that an excursion of 0.1 full scale on the recorder corresponded to consumption of 2.60 µM O\(_{2}\) (at 23 °C). When care was taken to condition the electrode by operating it for several hours after changing the Teflon membrane, the noise amplitude was below 0.005 full scale and the noise frequency was relatively fast (~1 s\(^{-1}\)), permitting determination of cyclooxygenase velocities in reactions with as little as 0.5 µM arachidonate.

For digital analysis, the 10 mV electrode output signal was sampled (10 s\(^{-1}\)) by a model ADC-1 A/D converter (Remote Measurement Systems, Seattle, WA) connected to a Macintosh SE computer (Apple Computer, Cupertino, CA) running a program based on control software modules supplied by Remote Measurement Systems. The program, written in QuickBasic (Microsoft, Redmond, WA), averaged groups of five sequential digital readings to produce a tab-delimited data file with two [O\(_{2}\)] values/s. When oxygen electrode data were to be compared with radiochemical cyclooxygenase data, it was necessary to correct for the damping caused by the Teflon membrane protecting the electrode, damping that is prominent for rapidly accelerating reactions (15). The mathematical formula used to correct the digital electrode data for membrane effects (16) has been described previously (16); the calculations were implemented using Excel software (Microsoft), with a membrane diffusion constant of 0.22 s\(^{-1}\). Corrected electrode data were divided by 2 to account for the stoichiometry of oxygen incorporation into arachidonate during cyclooxygenase catalysis and smoothed using a Kaleidagraph (Synergy Software, Reading, PA) smoothing algorithm.

One unit of cyclooxygenase produces a maximal velocity of 1 nmol O\(_{2}\)/min in 0.1 M potassium phosphate, pH 7.2, containing 1 mM phenol and 1 µM heme in a total volume of 195 µl in a glass aggregeomere cuvette (Chrono-Log Corp., Havertown, PA) stirred with a Teflon-coated magnet at room temperature (22–23 °C). Reactions were started by addition of 5 µl of 4–800 µM [\(^{3}H\)]arachidonate to give final fatty acid levels of 0.1–20 µM and a final Tween 20 concentration of 0.02%. The arachidonate stocks were prepared by adding one volume of the original [\(^{3}H\)]arachidonate solution in ethanolic volumes of 4–90 min and sealed glass ampules. Upon opening an ampule, the content was chilled on ice for at least 30 min. The solvent was removed under vacuum in a Speed-Vac centrifuge (Savant, Bathesda, MD) and the lipid residue was dissolved in 0.40 ml of methanol/water/acetic acid (50:50:1).
Addition of exogenous hydroperoxide considerably de-

A

B

Fig. 1. Effect of arachidonate level on PGHS-1 cyclooxygenase kinetics. Arachidonate consumption was monitored as a function of time using either radioisotope (symbols) or oxygen electrode (lines) assays in reactions of 5 nM PGHS-1 in 25 mM potassium phosphate, pH 7.2, containing 1.0 mM phenol, 1.0 mM heme, and 0.02% Tween 20. The arachidonate concentration was 0.5 (squares and dashed/dotted line), 1.0 (diamonds and dotted line), 2.0 (circles and dashed line), or 20 μM (triangles and solid line). Reactions with radioisotope were at room temperature (22–23 °C); reactions monitored by oxygen electrode were at 22.5 °C. The radioisotope data represent the averages from two separate reactions, such as for cyclooxygenase activity with saturating levels of fatty acid (15). On the other hand, electrode dampening is negligible and correction is unnecessary for slowly accelerating oxygenase reactions, such as that with soybean lipoxygenase (15). For the critical range of arachidonate levels below 2 μM where cooperativity is observed for PGHS-1 (see below), the slowly accelerating cyclooxygenase kinetics resemble those of soybean lipoxygenase (Fig. 1 and Ref. 15). This allowed estimation of cyclooxygenase velocity by direct analysis of the electrode signals in the subsequent experiments.

Dependence of PGHS-1 Cyclooxygenase Kinetics on Arachi-
donate Concentration—The cyclooxygenase velocity of PGHS-1 was measured at various arachidonate levels (Fig. 2A). A slightly sigmoidal curve was seen for the data from low arachidonate levels; this positive cooperativity is clearly evident in the convex shape of the diagnostic v/S versus v plot (Fig. 2A, inset) (18). The major part of the cooperativity occurred below 2 μM arachidonate, where contaminating hydroperoxide levels in the substrate were only about 3 nM (see “Experimental Procedures”). This peroxide level is too low to contribute much to direct activation of the cyclooxygenase, which is half-maximal only at 20 nM (17). A Hill plot of the data (not shown) produced a biphasic curve, with an initial linear portion having a slope of 2.3 (up to about 1 μM arachidonate) and a subsequent linear portion at higher arachidonate levels having a slope of 0.9. The slope of a single line fitted to all the data suggests a Hill number of 1.2 ± 0.1, although the downward break in the data at a point well below the apparent K_p (3.7 ± 0.5 μM for this data) is difficult to interpret. The results here confirm the earlier observations of modestly cooperative behavior for PGHS-1 cyclooxygenase activity (Hill number = 1.29 ± 0.06; Ref. 6). Addition of exogenous hydroperoxide considerably de-

Fig. 2. Effects of exogenous hydroperoxide on the cooperativ-
ity of PGHS-1 cyclooxygenase. Panel A, the cyclooxygenase activity (V_{opt}) of 5 nM PGHS-1 was determined by oxygen electrode measure-
ments for the indicated arachidonate levels. Inset, the data are plotted in v/S versus v format. Panel B, the cyclooxygenase activity of PGHS-1 was determined in the presence of 1 μM MCPBA. The other conditions were as in panel A. Inset, the data are plotted in v/S versus v format. Increased the convex curvature of the v/S versus v plot for PGHS-1 (Fig. 2B, inset), indicating that promoting cyclooxygenase activation decreased the cooperative response to the fatty

Dependence of MnPGHS-1 Cyclooxygenase Kinetics on Arachi-
donate Concentration—The cyclooxygenase velocity was measured at various arachidonate levels for PGHS-1 supple-
mented with MnPPIX instead of heme (Fig. 3A). The sigmoidal shape of the activity versus substrate curve is quite pronounced, and this cooperative behavior is confirmed by the convex shape of the v/S versus v format (Fig. 3A, inset). Compar-
ison of the data for MnPGHS-1 (Fig. 3A) with that for PGHS-1 itself (Fig. 2A) shows that the cooperativity of the PGHS-1 cyclooxygenase is considerably increased by the substitution of MnPPIX for heme. MnPGHS-1 has a higher activator hy-
droperoxide requirement (K_p value of 100 nM), and so a weaker hydroperoxide feedback activation, than PGHS-1 (19). A weak-
ened feedback activation was thus associated with increased cooperativity. The linkage between cooperativity and feedback activation in MnPGHS-1 was dramatically demonstrated by the ability of added hydroperoxide to essentially abolish the cooperative response (Fig. 3B).

Dependence of PGHS-2 and MnPGHS-2 Cyclooxygenase Ki-


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PGHS-2 cyclooxygenase velocity was measured at various arachidonate levels from 0.33 to 20 μM (Fig. 4). Very little indication of cooperative behavior for the PGHS-2 cyclooxygenase was seen in the activity versus substrate or v/S versus v plots, confirming previous observations with this isoform (6).

When the cyclooxygenase velocity was measured for PGHS-2 supplemented with MnPPIX, the response to fatty acid level was slightly sigmoidal (Fig. 5A). The cooperative behavior is clearly evident in the convex curvature of the corresponding v/S versus v plot (Fig. 5A, inset). Thus, although the PGHS-2 cyclooxygenase did not have a cooperative response to the arachidonate level when heme is the prosthetic group, the enzyme did show cooperative behavior with MnPPIX present. Addition of exogenous hydroperoxide abolished the cooperative behavior of MnPGHS-2 (Fig. 5B), indicating that the cooperativity was because of an impaired feedback activation of the cyclooxygenase.

Computer Simulation of the Effect of Hydroperoxide Activation Efficiency on the Response of Cyclooxygenase Velocity to Arachidonate Level—Computer simulations were used to predict the response of cyclooxygenase kinetics to arachidonate level for a relatively simple PGHS mechanism with feedback activation of the cyclooxygenase (Fig. 6). With a higher efficiency feedback activation (Kp = 2.3 nM) corresponding to PGHS-2 (12), the cyclooxygenase activity was predicted to be essentially a simple saturable function of the arachidonate concentration (Fig. 6), as shown more clearly by the nearly linear curve in the v/S versus v plot in Fig. 6. With a lower efficiency feedback activation (Kp = 21 nM) corresponding to PGHS-1 (12, 17), the cyclooxygenase activity was predicted to have a sigmoidal response to arachidonate, particularly at substrate levels below 1 μM (Fig. 6). This predicted cooperative behavior is more clearly apparent in the convex curvature of the v/S versus v plot in the Fig. 6 inset. Setting the hydroperoxide level to 1 μM at the start of simulated reactions with an assumed Kp of 21 nM increased the cyclooxygenase velocity slightly and removed the cooperativity (Fig. 6). When the Kp value in the simulations was increased to 100 nM to mimic MnPGHS-1 (19), the predicted cyclooxygenase velocity was decreased and the cooperativity greatly accentuated (Fig. 7A). With 1 μM hydroperoxide present at the start of simulations with a Kp value of 100 nM, the cooperativity was abolished, as evident from the linear v/S versus v plot in Fig. 7B. Thus, the mechanistic model predicts that decreasing the activator efficiency increases the positive cooperativity, and addition of hydroperoxide abolishes the cooperativity.

**DISCUSSION**

The cyclooxygenase activity of PGHS-1 was recently shown to exhibit a positive cooperative response to substrate fatty acid, whereas PGHS-2 displayed a simple saturable response over the same submicromolar substrate range (6). The present results have confirmed these observations using a continuous assay for cyclooxygenase activity, which is not affected by the markedly weaker accelerative phase of the reaction encountered at low substrate levels (Figs. 1, 2, and 4). A key issue in interpreting the cooperative behavior observed with PGHS-1 cyclooxygenase is in understanding the origin of the cooperativity. Allosteric transitions within individual PGHS-1 subunits have been proposed as one explanation for the cooperativity (6, 20), and the dimeric structure of the protein does present an opportunity for allosteric interactions between subunits. The present studies have focused on the possibility of a kinetic, rather than allosteric, explanation for cooperativity in PGHS-1.
The cyclooxygenase activity of PGHS-1 has long been recognized to require initiation by hydroperoxide (10); more recently the same was demonstrated for PGHS-2 (12, 21). The initiation process is believed to entail reaction of the hydroperoxide with the heme in the peroxidase site to form an oxidized intermediate, Compound I, which then undergoes an intramolecular redox step to generate a tyrosyl radical in the cyclooxygenase pocket (22). Tyrosyl radicals have been shown to be capable of serving as the initial oxidant in the cyclooxygenase catalytic cycle for both PGHS isoforms (23, 24). Because the product of cyclooxygenase catalysis, PGG2, is itself a hydroperoxide, cyclooxygenase catalysis in bulk solution is a branched-chain process with feedback acceleration as PGG2 produced by enzyme activated early on diffuses away to initiate cyclooxygenase catalysis in previously latent enzyme (16). The feedback loop has been shown to be much stronger in PGHS-2, where half-maximal cyclooxygenase activation requires about 2 nM PGG2, than in PGHS-1, where about 20 nM PGG2 was required (12).

Substitution of MnPPIX for heme is known to greatly decrease the efficiency of feedback activation by hydroperoxide (19), presumably via the large decrease in peroxidase activity with the unnatural metalloporphyrin (25–27). Given the basic mechanistic similarities between the two isoforms, it seems likely that MnPPIX substitution has similar effects on PGHS-2 peroxidase activity and cyclooxygenase feedback activation. MnPPIX substitution dramatically accentuated the cooperative behavior in PGHS-1 (Fig. 3) and even elicited cooperative behavior in PGHS-2 (Fig. 5). Impairment of the feedback activation cycle thus increased the cooperative cyclooxygenase response in both PGHS isoforms. In contrast, addition of exogenous hydroperoxide, which promotes the feedback activation process (28), abolished or decreased the cooperative cyclooxygenase behavior in PGHS-1, MnPGHS-1, and Mn-PGHS-2 (Figs. 2, 3, and 5). An effect of exogenous peroxide on cyclooxygenase cooperativity is difficult to reconcile with the proposed allosteric mechanism (6, 20).

Although the redox behavior of heme and MnPPIX are very different, the two metalloporphyrins have similar dimensions, and switching the metalloporphyrin was found to have very little effect on the overall structure of another hemoprotein, hemoglobin (29). Thus, substitution of MnPPIX for heme in PGHS-1 and -2 should be conservative in terms of the holoenzyme structures, making it unlikely that the increased cooperativity seen in MnPGHS-1 and -2 was because of perturbed allosteric transitions in the two proteins. On the other hand, the large effects of the change in metalloporphyrin on PGHS peroxidase kinetics are well established, and the concomitant effects on cyclooxygenase activation efficiency can be readily rationalized in terms of a consequent impaired generation of the active site tyrosyl radical (25–27, 30).

The kinetic simulations in the present study strongly corroborated the experimental observations. In particular, the simulations predicted that a positive cooperative response to arachidonate is a consequence of the complex feedback activation loop, which is a hallmark of cyclooxygenase kinetics, and that a
lower feedback loop efficiency (i.e. higher $K_p$ value) gives a greater degree of cooperativity (Figs. 6 and 7). The observation of cooperativity in PGHS-1 and not PGHS-2 (Figs. 2 and 4; and Ref. 6) can thus be theoretically accounted for by the difference in $K_p$ value between the isoforms (Fig. 6). The observed increase in cooperativity in PGHS-1 upon MnPPIX substitution (Fig. 3) is also predicted by the kinetic simulations (Fig. 7). Further, the simulations predict that added hydroperoxide decreases the cyclooxygenase cooperativity (Figs. 6 and 7), as was experimentally observed (Figs. 2, 3, and 5).

Overall, the present evidence indicates that the cooperativity in the response of PGHS-1 cyclooxygenase to the arachidonate level originates in the complex feedback activation kinetics of the enzyme. In this light, the difference between the two PGHS isoforms in the degree of cyclooxygenase cooperativity can be simply explained by the difference in the efficiency of the hydroperoxide feedback loops in PGHS-1 and PGHS-2. This linkage between hydroperoxide activator efficiency and cooperative cyclooxygenase responses at submicromolar levels of arachidonate brings an important new perspective to understanding the cellular control of prostaglandin biosynthesis. One should consider not just the arachidonate or hydroperoxide levels by themselves, but how the two levels act together to determine what fraction of PGHS-1 or -2 cyclooxygenase catalytic capacity is utilized in a particular cellular situation. As the results of computer simulations (Figs. 6 and 7) suggest, this concept of an interdependence between fatty acid substrate and hydroperoxide activator provides a useful way to grasp and predict some complex and unintuitive aspects of catalytic control in these physiologically important enzymes. 

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Fig. 7. Computer simulation of cyclooxygenase substrate dependence for MnPGHS-1. Numerical integration based on the mechanistic model was used to predict the cyclooxygenase velocity ($V_o$) for a $K_p$ value of 100 nM (corresponding to MnPGHS-1) with an initial hydroperoxide level of either 1 nM (panel A) or 1 μM (panel B) and the indicated arachidonate levels. Insets: the indicated arachidonate levels.

Inset: $v/S$ versus $v$ format. Details are described under “Experimental Procedures.”