A Mechanistic Study of Self-inactivation of the Peroxidase Activity in Prostaglandin H Synthase-1*

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Prostaglandin H synthase (PGHS) is a self-activating and self-inactivating enzyme. Both the peroxidase and cyclooxygenase activities have a limited number of catalytic turnovers. Sequential stopped-flow measurements were used to analyze the kinetics of PGHS-1 peroxidase self-inactivation during reaction with several different hydroperoxides. The inactivation followed single exponential kinetics, with a first-order rate constant of 0.2–0.5 s⁻¹ at 24 °C. This rate was independent of the peroxide species and concentration used, strongly suggesting that the self-inactivation process originates after formation of Compound I and probably with Intermediate II, which contains an oxyferryl heme and a tyrosyl radical. Kinetic scan and rapid scan experiments were used to monitor the heme changes during the inactivation process. The results from both experiments converged to a simple, linear, two-step mechanism in which Intermediate II is first converted in a faster step (0.5–2 s⁻¹) to a new compound, Intermediate III, which undergoes a subsequent slower (0.01–0.05 s⁻¹) transition to a terminal species. Rapid-quench and high pressure liquid chromatography analysis indicated that Intermediate III likely retains an intact heme group that is not covalently linked with the PGHS-1 protein.

Prostaglandin H synthase (PGHS)¹ (1) catalyzes the first committed step in the biosynthesis of many important prostanoids. PGHS exhibits two enzymatic activities as follows: a cyclooxygenase activity that converts arachidonic acid to PGG₂ and a peroxidase activity that transforms PGG₂ to PGH₂. Several oxidized reaction intermediates have been identified (1–4). PGHS first interacts with a peroxide substrate to generate Intermediate I, equivalent to Compound I of horseradish peroxidase. Intermediate I then converts to Intermediate II, equivalent to Complex ES of cytochrome c peroxidase, through an intramolecular electron transfer from a tyrosine residue to the oxidized porphyrin. The transiently formed tyrosyl radical present in Intermediate II has been demonstrated to be capable of oxidizing arachidonic acid to generate an arachidonic acid radical and beginning cyclooxygenase catalysis in both PGHS isomers (5, 6). X-ray crystallographic data revealed that the presumed site of the tyrosyl radical, Tyr-385 in PGHS-1 (Tyr-371 in PGHS-2), is located between the heme- and arachidonate-binding sites, well positioned to serve a role in coupling the two enzyme activities (7–9). These recent crystallographic findings substantiate the original branched-chain mechanism (2, 3) which proposed that a tyrosyl radical generated in the peroxidase cycle leads to production of PGG₂ as long as arachidonate is present.

One important fundamental limit on prostaglandin synthesis is the characteristic self-inactivation of PGHS (10). A typical reaction kinetics profile for PGHS with arachidonate, monitored by oxygen uptake, shows an initial burst, reaches a maximum velocity, and gradually decreases to zero (10). This fall in cyclooxygenase activity is not due to exhaustion of substrate or product inhibition but is rather a consequence of suicide inactivation (10–13). Each PGHS molecule thus exhibits only a limited number of turnovers before the cyclooxygenase activity disappears. With purified PGHS, this number of turnovers can be as high as 1300 and as low as 10 depending on the concentration of reductants and other factors (14, 15).

The detailed mechanism of the self-inactivation process has not been thoroughly characterized. Degradation of both peroxidase and cyclooxygenase activities have been observed during the self-inactivation process, as evidenced by decay of enzyme activities (15–17) and heme spectral changes (18). Based on the protective effect of various reducing cosubstrates against self-inactivation in both enzyme activities (15, 19–22), a consensus has emerged that certain active radical intermediates generated in peroxidase or cyclooxygenase catalysis lead to irreversible loss of enzyme activity. However, a direct linkage between a particular radical species and self-inactivation has yet to be established. The present study concentrates on peroxidase self-inactivation in the absence of exogenous reducing substrate to simplify the interpretations. The results provide convincing evidence that the self-inactivation step occurs subsequent to formation of Intermediate II and is thus independent of peroxidase structure and concentration.

**Experimental Procedures**

Hydrogen peroxide, guaiacol, and hemin were purchased from Sigma. Arachidonic acid was from Nu Chek Prep (Elysian, MN). ET-OOH was purchased as a 5% aqueous solution from Polysciences Inc. (Warrington, PA) or from Accurate Chemical and Scientific Corp. (Westbury, NY) as a 10% solution. PPHP was the product of Cayman Chemical Co. (Ann Arbor, MI). Chloroperoxidase acid was from Aldrich and was recrystallized in water. 15-HPETE was prepared according to Graff et al. (23) using arachidonate and soybean lipoxygenase. The purity of 15-HPETE was assessed chromatographically and its concentration quantified from the oxidation of TMPD catalyzed by excess PGHS-1 as described previously (17). Octyl-β-D-glucopyranoside was...
Examination of Heme Modification by Chemical Quench and HPLC Analysis—Covalent changes in the heme during the peroxide-induced self-inactivation were examined by a combination of rapid quench and HPLC analysis. PGHS (5–10 μM) was reacted with 10 eq of EtOOH for a defined period and then quenched with solvent (60% acetonitrile and 1.2% trifluoroacetic acid) at 24 °C in an Update Instrument System 1000 Chemical/Freeze Quench Apparatus (Madison, WI). The final concentrations of the reactants and quenching solvent after the two-stage mixing procedure were one-third the original. Chemical-quenched samples were stored on Dry Ice until HPLC analysis. HPLC was performed with a Waters Model 600S controller and model 996 photodiode array detector and a Vydac C4 column (5 μm; 0.21 × 15 cm) equilibrated with Solvent A (0.1% trifluoroacetic acid) at a flow rate of 0.3 ml/min. A gradient was run to 75% Solvent B (0.1% trifluoroacetic acid in acetonitrile) over 30 min and then to 100% Solvent B over the next 5 min. The absorbance was monitored at 220 and 400 nm to detect protein and heme species, respectively. Control experiments with sperm whale myoglobin and hydrogen peroxide confirmed efficient trapping of the reaction with this solvent quenching system (data not shown).

RESULTS

Kinetics of PGHS Peroxidase Inactivation during Reaction with EtOOH—PGHS was reacted with 250 μM EtOOH for various times, and the surviving peroxidase activity was monitored by H2O2-dependent oxidation of guaiacol in a secondary reaction, as described under “Experimental Procedures” (Fig. 1A). The initial velocity in the second stage reaction (with H2O2/guaiacol) is plotted as a function of the duration of the first stage reaction (with EtOOH) in Fig. 1B, along with corresponding data involving first stage reactions with other EtOOH levels. The surviving peroxidase activity (measured in the second stage reaction) followed essentially the same single exponential decay kinetics for all EtOOH levels between 7.8 to 250 μM (Fig. 2). In each instance, the surviving peroxidase activity was found to follow first-order kinetics as the first stage reaction proceeded (Fig. 1B). The rates of the reduction steps (k4, k5, k6, and k7) fall in the range commonly observed with PGHS peroxidase (19–22). No spectral distinction was made between Fe(IV) and Fe(IV)Tyr’ intermediates or between Fe(III)/Tyr’ and Fe(III) species. The rate of reduction of tyrosine radical in Fe(IV)/Tyr’ was assumed to be the same as that in Fe(III)/Tyr’.

Kinetics of PGHS Peroxidase Inactivation during Reaction with EtOOH

Substrate Specificity—Kinetic measurements of the peroxidase self-inactivation were extended to other peroxide substrates, including H2O2, 15-HPETE, and PPHP, at PGHS levels of 0.2–1.3 μM (Fig. 2). In each instance, the surviving peroxidase activity was determined by a second stage reaction with H2O2 and guaiacol, as described above for the experiments in Fig. 1A. In each case, the decay in peroxidase activity was found to follow first-order kinetics as the first stage reaction proceeded (Fig. 2).
time was increased (data not shown). The rates of self-inactivation were calculated by fitting the data to single-exponential equations, as in Fig. 1B. From the data in Fig. 2 it is clear that the rate of peroxidase self-inactivation was essentially independent of peroxide structure and peroxide concentration for the four peroxides examined, with rates of 0.20–0.44 s\(^{-1}\) for H\(_2\)O\(_2\), 0.22–0.54 s\(^{-1}\) for EtOOH, 0.22–0.46 s\(^{-1}\) for PPHP, and 0.1–0.5 s\(^{-1}\) for 15-HPETE. The overall average self-inactivation rate for the data in Fig. 2 was 0.40 s\(^{-1}\). It is also apparent from the data in Fig. 2 that the rate of peroxidase self-inactivation was insensitive to the PGHS level over the 0.15–1.5 \(\mu\)M range.

PGHS Heme Spectral Intermediates Generated during Peroxidase Self-inactivation—Optical spectral changes in the PGHS heme center were monitored during self-inactivation (Fig. 3). Reconstructed spectra after SVD analysis from 390 to 430 nm for reaction between 1 \(\mu\)M PGHS-1 and 30 \(\mu\)M EtOOH are shown in Fig. 3A. Resting enzyme had a Soret peak near 410 nm (spectrum a). Early in the reaction with EtOOH (0–0.5 s), the intensity of the Soret peak decreased and shifted to longer wavelengths, consistent with rapid sequential formation of Intermediates I and II. Subsequent spectra obtained after 0.5 s of reaction (\textit{thin lines} in Fig. 3A) were consistent with a single transition. Spectral changes in this reaction period were highlighted by a maximal increase near 392 nm and a maximal decrease near 420 nm, with an isosbestic point at 405–406 nm. An additional, slower transition (\textit{dashed lines} in Fig. 3A) followed, finally yielding a spectrum characterized by a peak at 405 nm with a low absorption coefficient (\textit{lower heavy line} in Fig. 3A). These spectral data after the first 50 ms of reaction were further analyzed by the global fitting package, Glint, to obtain the spectra of the major components present in this part of the reaction. A simple mechanism, represented by Reaction 1, was used for the fitting, because the SVD analysis indicated three dominant species were present in that time range.

\[
A \rightarrow B \rightarrow C \quad (\text{Reaction 1})
\]

The three prominent spectral intermediates resulting from this fit are shown in Fig. 3B, together with the spectrum of the resting enzyme. The first intermediate considered here (A in Reaction 1), with a peak at 413–414 nm (\textit{spectrum b}), is very similar to published spectra of PGHS-I Intermediate II, containing an oxyferryl heme and a tyrosyl radical (1–4). The second intermediate observed (B in Reaction 1), called Intermediate III, exhibited a peak at 403–404 nm (\textit{spectrum c} in Fig. 3B). The last spectroscopic intermediate observed (C in Reaction 1) had a peak at 412–413 nm (\textit{spectrum d} in Fig. 3B) and appears similar to the overall spectrum at the terminal stage of the reaction (\textit{spectrum b} in Fig. 3A). Accordingly, the mechanism in Reaction 1 can be refined as shown in Reaction 2.

Intermediate II \(\rightarrow\) Intermediate III \(\rightarrow\) terminal species \quad (\text{Reaction 2})

The rates of the two steps in Reaction 2 determined by global fit were 1.98 and 0.02 s\(^{-1}\), respectively. The inactivation rate determined for peroxidase activity, 0.4 s\(^{-1}\) (Figs. 1 and 2), was much closer to that for the first spectroscopic transition, implying that irreversible loss of peroxidase activity accompanies formation of Intermediate III.

The spectral changes observed during reaction of PGHS with PPHP and 15-HPETE were very similar to those shown in Fig. 3A for EtOOH (data not shown). As was described for the EtOOH reactions, spectra for two intermediates were resolved by fitting the data to a two-step process (Reaction 2). The spectrum resolved for the earlier intermediate (Intermediate III in Reaction 2) had its peak at 415–416 nm for the reactions with PPHP and 15-HPETE, slightly longer than 413–414 nm found for the same intermediate during reaction with EtOOH. There were no obvious differences observed for the terminal species with different hydroperoxides.

The double-monochromator Olis rapid scan instrument was used to obtain spectral data extending to the visible region for inactivation intermediates. Due to the weak absorbance of the intermediates in the visible region, a higher enzyme concentration (2.5 \(\mu\)M) was employed. Rapid scan spectral data obtained during reaction of PGHS with EtOOH were consistent with the two-step inactivation process in Reaction 2 and were fitted to the same mechanism (Fig. 4). The spectral changes observed in the Soret region with the rapid scan instrument were very similar to those obtained by kinetic scan at incremental wavelengths using a lower level of PGHS (0.5 \(\mu\)M), thus confirming that the increased enzyme concentration did not change the self-inactivation mechanism. The resolved spectrum for Intermediate III obtained by SVD analysis and global-fitting of the rapid scan data to Reaction 2 (\textit{spectrum b} in Fig. 4) was very similar to that obtained by the kinetic scan approach (\textit{spectrum c} in Fig. 3B) both in its absorption peak position and its intensity. In the visible region, Intermediate III displayed a single broad peak centered at 560 nm, with an extinction coefficient of 22.7 mM\(^{-1}\) cm\(^{-1}\). This feature is absent from the spectra of resting enzyme and the terminal species (Fig. 4), indicating that the heme electronic configuration in Intermediate III is distinct from those of other recognized peroxidase intermediates. The rates obtained for the two steps in Reaction 2 from the rapid scan data were 0.46 and 0.032 s\(^{-1}\), respectively. The value for the fast spectral change here is essentially the same as that for peroxidase inactivation (Fig. 2), supporting the contention that peroxidase inactivation accompanies Intermediate III formation.

Analysis of Heme Structural Changes during Peroxidase Self-inactivation—Rapid-quench experiments were conducted to look for covalent changes of the PGHS heme group. For this, PGHS was reacted with 10 eq of EtOOH, and aliquots were removed at various times for solvent quenching and HPLC analysis. As shown in Fig. 5A, the chromatographic profiles for the control sample (EtOOH was replaced by buffer) and the sample obtained at 5 s of reaction were very similar. The main heme peak, monitored by absorbance at 400 nm, eluted at 20.5 min and was clearly separated from the PGHS apoprotein peak registered by 220 nm absorbance, which elutes near 26 min. A time-dependent increase of a 400-nm absorbing species with a 21.7-min retention time was noticed at expanded scales, but it accounted for only a very small percentage the total heme. As shown in Fig. 5B, the integrated area of the main A\textsubscript{400} peak changed little with reaction time. Thus, most of the PGHS heme remained chemically intact during the time the peroxidase was inactivated.

Mathematical Fitting to the Mechanistic Model of Peroxidase Catalysis in PGHS—Early kinetic events in the reaction of PGHS with peroxide, including formation and degradation of Intermediate I, were not considered in the SVD and global
fitting to Reaction 2. In addition, minor spectral species and reactions involving recycling of higher oxidation states of heme were ignored to simplify data analysis. To validate these data manipulations, further analysis of PGHS self-inactivation by peroxide was conducted by simulation and fitting to the more complete mechanism shown in Scheme 1 (Steps 1–8 listed in Table I) using the SCoP numerical integration program. In this mechanism, the reaction pathway divides at Intermediate II, with one route leading to Intermediate III and irreversible inactivation (via \( k_7 \)) and the other route recycling the enzyme sequentially back to Compound II (or Fe(III) Tyr') and resting PGHS (via \( k_3 \) or \( k_5 \)). The rate constants for Steps 1 and 2 were taken from published data (4). The rate constants for Steps 3–6, were set at 10^3 to 10^5 M^-1 s^-1, in line with literature values for various PGHS peroxidase substrates (19–22). Five equivalents of endogenous reductant were assumed in the simulations based on earlier estimates (25).

The simulations indicated that the flux through the recycling steps is minimal unless exogenous cosubstrate is added. The main determining factors for the speed of transitions in the 0.5–5-s period were the values of \( k_7 \) and \( k_8 \). To fit the simulations to the observed absorbance change kinetics, molar absorbance coefficients for individual wavelengths were based on the resolved spectra for Intermediates II and III and terminal species obtained in this study, and on previously published spectra corresponding to resting PGHS and Intermediate I (4). The floating parameters in the computer fitting included \( k_7 \) and \( k_8 \). Fittings that floated the \( k_3 \) to \( k_6 \) parameters always converged with rate constant values of less than 10^5 M^-1 s^-1. Floating the amount of endogenous reductant usually converged to values close to 0. These results indicate a minimal contribution of the reductive recycling steps (\( k_3 \) to \( k_6 \)) to the overall kinetics. Typical kinetic data and fittings for three different wavelengths are shown in Fig. 6. The initial rapid
changes before 0.5 s reflect the formation of Intermediates I and II. The increase in $A_{392}$ and decrease in $A_{428}$, accompanied by a relatively static $A_{404}$, in the 0.1–5-s period coincide with the conversion of Intermediate II to Intermediate III. The optimal values for $k_7$ and $k_8$ obtained in this fitting were 1.87 and 0.01 s$^{-1}$, respectively. These values match nicely with those obtained by global fitting to the simple sequential model represented by Reaction 2, indicating that the fitting results with the complete mechanism in Table I are essentially the same as those obtained with the simplified mechanism in Reaction 2.

DISCUSSION

The basic kinetics of formation of Intermediate I (Compound I) and Intermediate II during reaction of PGHS-1 with peroxides are well known (1–4). Formation of Intermediate I is very rapid, with rate constants ranging from $10^8$ M$^{-1}$ s$^{-1}$ for lipid hydroperoxides to $10^5$ M$^{-1}$ s$^{-1}$ for H$_2$O$_2$ (1–4). Formation of Intermediate II occurs as a largely unimolecular process, with a rate constant of 50–500 s$^{-1}$ (3, 4). It is clear from the present results that both of these processes are much more rapid than the loss of peroxidase activity, which averaged about 0.4 s$^{-1}$ (Fig. 2). A second striking aspect of the present results is that inactivation of the peroxidase is a first-order process, independent of peroxide concentration and structure. This behavior strongly indicates that the inactivation reaction step occurs after formation of Intermediate I or II. Two lines of evidence indicate that the starting point for inactivation is Intermediate II. First, little Intermediate I is present during the period when the bulk of self-inactivation occurs under our experimental conditions. Second, kinetic scan (Fig. 3) and rapid scan (Fig. 4) data show no evidence of an additional reaction step before the conversion of Intermediate II to Intermediate III, and the observed rate constant of this conversion is in the range of that observed for loss of peroxidase activity (Figs. 1 and 2).

Previous examinations of PGHS peroxidase self-inactivation...
found the process to be sensitive to peroxide structure and concentration (16, 17), but these studies were done under "steady-state" conditions with exogenous reductant present. The cosubstrate facilitates recycling of Intermediate II back to resting enzyme, making the steady-state level of Intermediate II, and thus the inactivation rate, dependent on the rate of Intermediate II regeneration by additional peroxide (via Intermediate I). As a result, the steady-state level of Intermediate II under such conditions is far from stoichiometric, and the earlier results are not directly comparable with those from this study.

The proposed inactivation of PGHS peroxidase from Intermediate II contrasts with the inactivation pathway involving Compound I reported for the reaction of horseradish peroxidase with excess \( m \)-chloroperbenzoic acid (26). Horseradish peroxidase Compound I is rather stable, whereas PGHS Compound I quickly converts to Intermediate II. It may well be that this large difference in the lifetime of Compound I leads to a different inactivation processes in PGHS and horseradish peroxidase. For horseradish peroxidase, as few as 2 eq of \( m \)-chloroperbenzoic acid are sufficient to induce inactivation, permitting calculation of the partition ratio between the inactivation and recycling processes (26). For PGHS-1, the presence of endogenous reductant complicates a quantitative analysis because the endogenous reductant is not titratable by oxidants such as ferricyanide, and its quantity varies from preparation to preparation. To simplify the analysis, we did not include exogenous reducing cosubstrate in the present experiments. This simplified reaction system led to minimal recycling of the enzyme to resting state via reductive steps. Bakovic and Dunford (27) observed a rate of 0.08–0.11 s\(^{-1}\) for the decay of Intermediate II in the reaction of PGHS-1 and \( m \)-chloroperbenzoic acid. This rate was zero-order with respect to the peroxide concentration, a finding very similar to our results, and their rate constant

\[ k = \text{rate constant} \]

FG. 5. HPLC analysis of PGHS heme structural changes during peroxidase self-inactivation. Equal volumes of PGHS-1 (5.8 \( \mu \)M in 0.1 M potassium phosphate, pH 7.2, containing 0.1% Tween 20, 10% glycerol, and 0.1% octyl-\( \beta \)-D-glucopyranoside) and 60 \( \mu \)M EtOOH were reacted with at 24 °C for 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, and 60 s and quenched by an aqueous solution of 60% acetonitrile and 1.2% trifluoroacetic acid. The final concentrations of protein, peroxide, and the quenching solvents in the quenched sample were 1/3 those of the originals. A, HPLC analysis were performed as detailed under "Experimental Procedures." As the profiles for every sample were very similar, only the results from the samples quenched at 0 and 5 s are shown. B, integrated areas for the main heme chromatographic peak (monitored by \( A_{400} \)) are plotted against reaction time.

FG. 6. Computer fitting of single-wavelength kinetic data to mechanistic model. Kinetic data from the experiment shown in Fig. 3B are compared with predicted kinetics at the same wavelengths generated by computer fitting to the mechanism represented by Steps 1–8 in Table I. The values of rate constants \( k_1 \) and \( k_2 \) were fixed at 1.4 \( \times \) \( 10^7 \) M\(^{-1}\) s\(^{-1}\) and 320 s\(^{-1}\) (4), respectively; \( k_3 \) to \( k_6 \) were fixed at \( 10^2 \) M\(^{-1}\) s\(^{-1}\); and endogenous reductant was set to 3 \( \mu \)M (25). Absorbance coefficients at 404, 392, and 428 nm for each enzyme species were obtained from the resolved spectra shown in Fig. 3B. The optimal values obtained for \( k_7 \) and \( k_8 \) were 1.87 and 0.01, respectively.
value falls between the rate constants we obtained for steps 1 and 2 in Reaction 2. The differences between the present results and their studies (27) may partly be due to the unresolved two reaction steps (k₁ and k₂ in Scheme 1) in their data analysis of the inactivation measurements and the presence of added reductant, diethyldithiocarbamate, in their reaction mixture.

The present studies have isolated a new spectral intermediate in the self-inactivation process, i.e. Intermediate III, using both kinetic scan and rapid scan approaches (Figs. 3 and 4). Intermediate III appears to be produced directly from Intermediate II, which contains Fe(IV)=O and a tyrosyl radical. The spectral changes in the Soret region during the transition from Intermediate II to III showed an isosbestic point at 406 nm (Fig. 3A), near that for the transformation of Intermediate I to Intermediate II (4). However, the spectral changes in these two transitions are quite distinct, in that the absorbance changes on the two sides of the isosbestic point occurred in opposite directions. The overall spectral line shape of Intermediate III roughly resembles that reported for covalently bound heme in myoglobin pretreated with hydrogen peroxide (28). However, heme analysis during the peroxidase inactivation rules out the possibility of significant covalent linkage between the heme group and the PGHS-1 protein, because no metalloporphyrin chromophore was associated with protein peak in the HPLC profile (Fig. 5A). Furthermore, the PGHS-1 heme structure was not changed by peroxidase self-inactivation, as evidenced by the unchanged chromatographic behavior of the heme isolated from samples taken at different times during reaction with EtOOH (Fig. 5). Taken with the observed spectral changes, the chromatographic results make it likely that the heme group found in Intermediate III is not properly coordinated to the histidine ligand(s) but still remains non-covalently associated with the protein. Irreversible heme destruction can be observed during cyclooxygenase catalysis (12), but this appears to be a much slower process than conversion from Intermediate II to III.

Several observations indicate that the process of PGHS peroxidase self-inactivation begins with Intermediate II. First of all, the rate of conversion from Intermediate II to Intermediate III, 0.5–2 s⁻¹, is only slightly faster than the decay rate for peroxidase activity (∼0.4 s⁻¹). In fact, measurements using m-chloroperbenzoic acid instead of H₂O₂ in the peroxidase assay gave decay rates around 1–2 s⁻¹, very similar to the rate for the Intermediate II to Intermediate III transition (data not shown). The similar rates for conversion from Intermediate II to Intermediate III measured by spectral changes and for the decay of peroxidase indicate that they reflect the same kinetic event. Second, the observed rate for Intermediate III transformation to the terminal spectral species (0.01–0.05 s⁻¹) was kinetically quite distinct from the reaction of Intermediate II to Intermediate III. It is thus concluded that Intermediate III is itself catalytically inactive.

Reduction of the peroxide-generated radical in crude PGHS by added cosubstrate was found to decrease the amount of self-inactivation (29). Various reducing cosubstrates have been reported to extend the peroxidase activity (15, 19–22), and there was an inverse correlation between the residual peroxidase activity and the tyrosyl radical intensity when purified PGHS-1 was reacted with peroxide (18, 30). These observations indicate the linkage between tyrosyl radical and self-inactivation damage in PGHS. However, the present analyses of heme structure (Fig. 5) show clearly that a reaction covalently linking a tyrosyl radical to the heme moiety is not the cause for peroxidase inactivation.

The relationship of PGHS peroxidase inactivation to cyclooxygenase inactivation is of considerable interest. Previous measurements of the cyclooxygenase inactivation rate during reaction with fatty acid (31) produced a value that is roughly comparable to those obtained for peroxidase inactivation in this study. Much slower rates for cyclooxygenase inactivation (1–5 min⁻¹) were reported earlier by Lands and co-workers (10, 12). The present results for cyclooxygenase and peroxidase inactivation are considerably complicated by the reaction conditions used for the cyclooxygenase measurements. The peroxide levels in the cyclooxygenase reactions vary widely with time and are sensitive to the level of reducing cosubstrate present (31). In the branched-chain mechanism (3), cyclooxygenase catalysis requires reaction of the enzyme with peroxide to generate the tyrosyl radical. This predicts that inactivation of the cyclooxygenase by peroxide should be at least as fast as peroxidase inactivation. Further quantitation of the cyclooxygenase self-inactivation will be needed to test this hypothesis.

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REFERENCES

1. Lambeir, A. M., Markay, C. M., Dunford, H. B., and Marnett, L. J. (1985) J. Biol. Chem. 260, 14894–14896
2. Karthikeyan, R., Dietz, R., Nastainczyk, W., and Ruf, H. H. (1988) Eur. J. Biochem. 171, 313–320
3. Dietz, R., Nastainczyk, W., and Ruf, H. H. (1988) Eur. J. Biochem. 171, 321–328
4. Tsai, A., Wei, C., Baek, H. K., Kulmacz, R. J., and Van Wart, H. E. (1997) J. Biol. Chem. 272, 8885–8894
5. Tsai, A., Kulmacz, R. J., and Palmer, G. (1995) J. Biol. Chem. 270, 10505–10508
6. Tsai, A., Palmer, G., Xiao, G., Swinney, D. C., and Kulmacz, R. J. (1998) J. Biol. Chem. 273, 3888–3894
7. Picot, D., Loll, P. J., and Garavito, R. M. (1994) Nature 367, 243–249
8. Luong, C., Miller, A., Barnett, J., Chow, J., Ramesha, C., and Browner, M. F. (1996) Nat. Struct. Biol. 3, 927–933
9. Kambhaili, R. G., Stevens, M., Giese, J. K., McDonald, J. J., Stegeman, R. A., Pak, Y. Y., Gileshaus, D., Miyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C., and Stallings, W. C. (1996) Nature 384, 644–648
10. Smith, W. L., and Lands, W. E. M. (1972) Biochemistry 11, 3276–3285
11. Sato, S., Ogino, N., Yamamoto, S., and Hayashi, O. (1979) J. Biol. Chem. 254, 829–836
12. Hemler, M. E., and Lands, W. E. M. (1980) J. Biol. Chem. 255, 6253–6261
13. Marnett, L. J., and Maddipati, K. R. (1991) in Peroxidases in Chemistry and Biology (Everse, J., Everse, K. E., and Grisham, M. B., eds) Vol. 1, pp. 14894–14896
14. Marshall, P. J., Kulmacz, R. J., and Lands, W. E. M. (1987) J. Biol. Chem. 262, 3510–3517
15. Morrey, C. M., Alward, A., Weller, P. E., and Marnett, L. J. (1987) J. Biol. Chem. 262, 6266–6279
16. Marshall, P. J., and Kulmacz, R. J. (1988) Arch. Biochem. Biophys. 266, 162–170
17. Kulmacz, R. J. (1986) Arch. Biochem. Biophys. 249, 273–285
18. Tsai, A., Palmer, G., and Kulmacz, R. J. (1992) J. Biol. Chem. 267, 17665–17659
19. Hsuanuy, Y., and Dunford, H. B. (1990) Biochim. Biophys. Acta 10503–10508
20. MacDonald, I. D., Graft, G., Anderson, L. A., and Dunford, H. B. (1989) Arch. Biochem. Biophys. 272, 194–202
21. Hsuanuy, Y., and Dunford, H. B. (1992) Arch. Biochem. Biophys. 292, 213–220
22. Hsuanuy, Y., and Dunford, H. B. (1992) J. Biol. Chem. 267, 17649–17657
23. Graft, G., Anderson, L. A., and Dunford, H. B. (1990) Annu. Rev. Biochem. 59, 47–71
24. Kulmacz, R. J., and Lands, W. E. M. (1987) in Proteindases and Related Substances: A Practical Approach (Benedetto, C., McDonald-Gibson, B. G., Nigam, S., and Slater, T. F., eds) pp. 209–227, IRL Press, Washington, D. C.
25. Okai, S., Wu, G., and Kulmacz, R. J. (1997) Biochemistry 36, 13083–13084
26. Rodriguez-Lopez, J. N., Hernandez-Ruiz, J., Garcia-Carnova, F., Thorneley, R. N. F., Acosta, M., and Arnao, M. B. (1997) J. Biol. Chem. 272, 5469–5476
27. Bakovic, M., and Dunford, H. B. (1990) J. Biol. Chem. 265, 2048–2056
28. Osawa, Y., and Korzkev, K. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 7081–7085
29. Egan, R. W., Gale, P. H., and Knebel, F. A. (1979) J. Biol. Chem. 254, 3285–3302
30. Lassmann, G., Odenwallner, R., Curtis, J. F., DeGray, J. A., Mason, R. P., Marnett, L. J., and Elzing, T. E. (1991) J. Biol. Chem. 266, 20045–20055
31. Wei, C., Kulmacz, R. J., and Tsai, A. L. (1995) Biochemistry 34, 8499–8512