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Kinetic Models of Cyclooxygenase and Peroxidase Inactivation of Prostaglandin-H-synthase during Catalysis

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Abstract—Kinetic models of inactivation of cyclooxygenase and peroxidase activities of prostaglandin-H-synthase (PGHS) during cyclooxygenase and peroxidase reactions catalyzed by the enzyme and also on preincubation with H_2O_2 have been developed; these models account for data obtained by the authors as well as data from the literature. Being rather simple, these models simultaneously describe the processes of cyclooxygenase and peroxidase inactivation of PGHS, using the minimal set of experimental parameters.

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Key words: prostaglandin-H-synthase, kinetic mechanism, cyclooxygenase activity, peroxidase activity, inactivation

Irreversible inactivation of prostaglandin-H-synthase (PGHS, EC 1.14.99.1) during the reactions catalyzed by it is a well-known fact [1-3], but the nature of this phenomenon is still not elucidated. As was established in the first detailed kinetic study of inactivation of microsomal PGHS [2], suppression of cyclooxygenase oxidation of arachidonic acid is not caused by substrate consumption or inhibition by the reaction products. The rate and degree of the reaction completeness increased in the presence of phenol or methional in the reaction mixture, and preincubation of the enzyme with prostaglandin G_2 also resulted in the loss of PGHS cyclooxygenase activity [2]. It was suggested that inactivation of the enzyme results from its oxidation by hydroxylic radicals formed on reduction of prostaglandin G_2 [2].

Kinetic mechanisms of PGHS inactivation have been suggested in several works [4-8]. A common feature of these mechanisms is a proper description of one kinetic feature but inconsistency with other experimental data. The mechanism presented in [5] suggests that all intermediate forms of enzyme containing tyrosyl radical are inactivated and that complete loss of cyclooxygenase and peroxidase activities occurs simultaneously. However, such mechanism means that the concentrations of enzyme forms containing tyrosyl radical decrease to zero, and meanwhile, the concentration of electron donor increases without limit. This should result in complete suppression of cyclooxygenase activity, but the latter is not experimentally observed.

The scheme suggested in [5] was improved in [6]: having lost cyclooxygenase activity, PGHS retained peroxidase activity, and the process of peroxidase activity loss proceeded via this intermediate form of PGHS lacking cyclooxygenase activity. However, it is not experimentally proved that retention of cyclooxygenase activity is impossible after complete peroxidase inactivation of PGHS [3].

Materials on phenomenological description of cyclooxygenase and peroxidase inactivation of PGHS during catalysis are presented in [3]. To study the mechanisms of cyclooxygenase and peroxidase inactivation, we compared the rate of PGHS cyclooxygenase activity loss caused by the proceeding of the cyclooxygenase reaction itself (that is, the rate of inactivation during reaction) with the rate of PGHS peroxidase activity loss caused by proceeding of the cyclooxygenase reaction.

Abbreviations: AA) arachidonic acid; ABTS) 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid); PGHS) prostaglandin-H-synthase; TMPD) N,N,N′,N′-tetramethyl-*p*-phenylenediamine.

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And vice versa, we compared the rate of peroxidase activity loss caused by proceeding of the peroxidase reaction itself (that is, the rate of inactivation during reaction) with the rate of cyclooxygenase activity loss caused by proceeding of the peroxidase reaction. We also studied the effect of PGHS preincubation with hydrogen peroxide on cyclooxygenase and peroxidase activities of PGHS.

In this study, we performed kinetic analysis of these data and developed a model describing these and the literature data on PGHS inactivation.

RESULTS

Preincubation with hydrogen peroxide. Kinetics of change in PGHS cyclooxygenase activity on preincubation with H_2O_2 (Fig. 1; this is Fig. 4 from [3]) can be presented as a typical curve with lag period, which cannot be described by one exponential (systematic deviations are presented in Fig. 2; this is Fig. 5 from [3]). This indicates that the mechanism of cyclooxygenase activity loss consists of at least two consecutive stages and the latter results in inactivation.

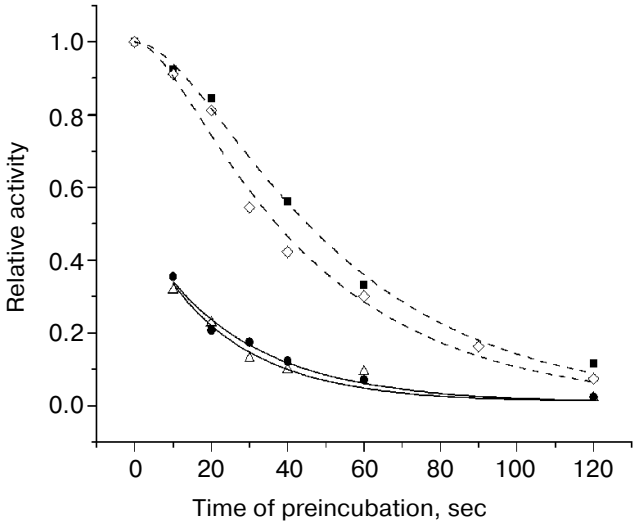


Fig. 1. Relative cyclooxygenase activity of purified (squares, rhombuses) and relative peroxidase activity of solubilized (circles, triangles) PGHS preparations (C_{rel}) versus preincubation time with hydrogen peroxide. Squares: 6.7 μg/ml PGHS, 2 μM hemin, 1.5 mM H_2O_2. Cyclooxygenase activity was determined after addition of 150 μM arachidonic acid (AA) into the reaction mixture. Rhombuses: 4.7 μg/ml PGHS, 2 μM hemin, 1.5 mM H_2O_2. Cyclooxygenase activity was determined after addition of 150 μM AA and 1 mM potassium ferrocyanide into the reaction mixture. Circles: 9 μg/ml PGHS (solubilized), 1.5 mM H_2O_2. Peroxidase activity was determined after addition of 60 μM N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) into the reaction mixture. Triangles: 9 μg/ml PGHS (solubilized), 0.15 mM H_2O_2. Peroxidase activity was determined after addition of 1.35 mM H_2O_2 and 60 μM TMPD into the reaction mixture. Solid lines are plotted by approximation of experimental data using Eq. (7), and dashed lines using Eq. (6).

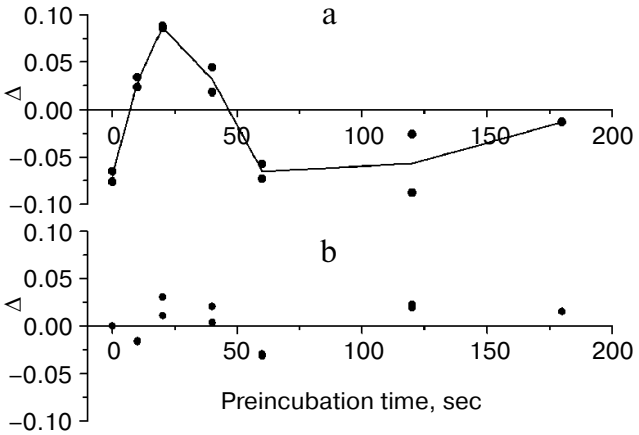


Fig. 2. Difference between experimental values of the relative rate of cyclooxygenase reaction obtained on PGHS preincubation with H_2O_2 and theoretical values obtained by approximation (Δ). Approximation made using one exponential (a) and by Eq. (6) (b).

Kinetics of change in PGHS peroxidase activity on preincubation with H_2O_2 under the same conditions biphasic showing a fast (<10 sec) loss of ~50% peroxidase activity followed by a slow loss of the remaining peroxidase activity of PGHS (Fig. 1).

The above-mentioned regularities are well described by the following simple scheme (Scheme 1) which depicts transformations of a protein molecule, where *a*, *b*, *c*, and *d* are PGHS intermediates formed by the action of hydrogen peroxidase.

The initial form of the enzyme (*a*) is converted into form *b* with the first-order rate constant k_0 (this is a fast (<10 sec) process of 50% peroxidase activity loss), then *b* is converted into *c* with the rate constant k_1 (this is a slower process of loss of the remaining peroxidase activity).

Since the kinetics of cyclooxygenase activity loss is not accompanied by changes with a typical duration <10 sec, it should be accepted that intermediate *b* in Scheme 1 retains 100% of the cyclooxygenase activity.

Scheme 1 can describe a typical lag period on the curve of PGHS cyclooxygenase activity loss on preincubation with H_2O_2 (Fig. 1) in the case when intermediate *c* has 100% cyclooxygenase activity.

$$a \xrightarrow{k_0} b \xrightarrow{k_1} c \xrightarrow{k_2} d$$

Kinetic model of loss of cyclooxygenase and peroxidase activities of PGHS on preincubation with hydrogen peroxide (designations are explained in the text)

Scheme 1

For Scheme 1, expressions for experimentally determined relative activities, that is, activities normalized to their values at time zero are as follows:

$$C_{\text{rel}}^{\text{CO}} = \frac{1}{a_0}(k_a^{CO}[a] + k_b^{CO}[b] + k_c^{CO}[c] + k_d^{CO}[d]), \quad (1)$$

$$C_{\text{rel}}^{\text{PO}} = \frac{1}{a_0}(k_a^{PO}[a] + k_b^{PO}[b] + k_c^{PO}[c] + k_d^{PO}[d]), \quad (2)$$

where $C_{\text{rel}}^{\text{PO}}$ and $C_{\text{rel}}^{\text{CO}}$ are relative peroxidase and cyclooxygenase activities of PGHS, respectively; k_{i}^{PO} and k_{i}^{CO} are apparent relative catalytic constants of peroxidase and cyclooxygenase reactions for corresponding intermediates; a_0 is total PGHS concentration.

A steady-state solution of differential equations describing Scheme 1, provided that $k_0 >> 6$ min^{-1}, gives the following expression for $t > 10$ sec:

$$[a] = 0, \quad (3)$$

$$[b] = a_0 e^{-k_1 t}, \quad (4)$$

$$[c] = a_0 \frac{k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right). \quad (5)$$

These expressions give equations for relative cyclooxygenase ($C_{\text{rel}}^{\text{CO}}$, Eq. (6)) and peroxidase ($C_{\text{rel}}^{\text{PO}}$, Eq. (7)) activities describing the experimental data (Fig. 1), provided that $k_{\text{a}}^{\text{CO}} = 1$, $k_{\text{b}}^{\text{CO}} = 1$, $k_{\text{c}}^{\text{CO}} = 1$, $k_{\text{d}}^{\text{CO}} = 0$; $k_{\text{a}}^{\text{PO}} = 1$, $k_{\text{b}}^{\text{PO}} = 0.5$, $k_{\text{c}}^{\text{PO}} = 0$, $k_{\text{d}}^{\text{PO}} = 0$:

$$C_{\text{rel}}^{\text{CO}} = \frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_2 - k_1}, \quad (6)$$

$$C_{\text{rel}}^{\text{PO}} = 0.5 \cdot e^{-k_1 t}. \quad (7)$$

To evaluate k_1 and k_2, experimental data (partly presented in Fig. 1) were approximated by nonlinear regression according to Eqs. (6) and (7). Results are presented in Table 1.

Equation (6) is symmetric about k_1 and k_2. In the simplest Scheme 1, it is provided that k_1 constants in Eqs. (6) and (7) account for the same process (loss of peroxidase activity). That is why k_1 from Eq. (6) was assigned a value of one of two experimentally determined values so that it was close to the k_1 value from approximation of independent experiments according to Eq. (7). As follows from Table 1, Scheme 1 using two parameters (k_1 and k_2) describes the processes of PGHS cyclooxygenase and peroxidase activity loss on preincubation with hydrogen peroxide well.

Table 1. Kinetic parameters of PGHS inactivation caused by preincubation with H_2O_2 (Scheme 1, Eqs. (6) and (7)). Experimental data (Fig. 1) were approximated by nonlinear regression according to Eqs. (6) and (7)

Detected activity	k_1, min^{-1}	k_2, min^{-1}
Cyclooxygenase (in the presence of ferrocyanide as electron donor)	1.5	7.2
Cyclooxygenase (in the absence of electron donor)	1.4	4.1
Peroxidase (TMPD as electron donor)	2.0	–
Peroxidase (ABTS as electron donor)	3.2	–

Loss of peroxidase and cyclooxygenase activities of PGHS during reactions catalyzed by it. Since PGHS does not undergo inactivation in the free state, inactivation processes occur during reactions, so it obviously proceeds via intermediates [9-11]. The simplest suggestion is that loss of both activities during cyclooxygenase reaction occurs as a result of one process and loss of both activities during peroxidase reaction also occurs as a result of one process (however, the former and the latter processes may be not the same). In this case, plots of changes of both activities during a certain reaction should be completely symbatic. However, as shown in Figs. 3 and 4 (these are Figs. 2 and 3 from [3]), runs of the relative cyclooxygenase and peroxidase activities in some cases differ significantly. Consequently, cyclooxygenase and peroxidase activity losses are different processes. In principle, these processes may proceed via various intermediates; however, as a first approximation, let us suggest that these processes proceed via the same intermediates (during the same incubation).

Let us define enzyme activity (A) as capacity for catalyzing a reaction which proceeds on incubation and enzyme property (B) as capacity for catalyzing an alternative reaction. If we consider cyclooxygenase activity as catalytic activity, then peroxidase activity will be an enzyme property, and vice versa. Let us consider the simplest scheme – enzyme inactivation during reaction catalyzed by it (Scheme 2). The cycle presented in Scheme 2 shows qualitative state of free enzyme form and other intermediates. Intermediates X_0... X_{i}... possess catalytic activity (A) as well as property (B). The cycle (X_0... X_{i}... X_0) corresponds to one catalytic cycle of reaction. It is suggested that inactivation proceeds only via intermediate X_{i} by conversion of this intermediate into a catalytically

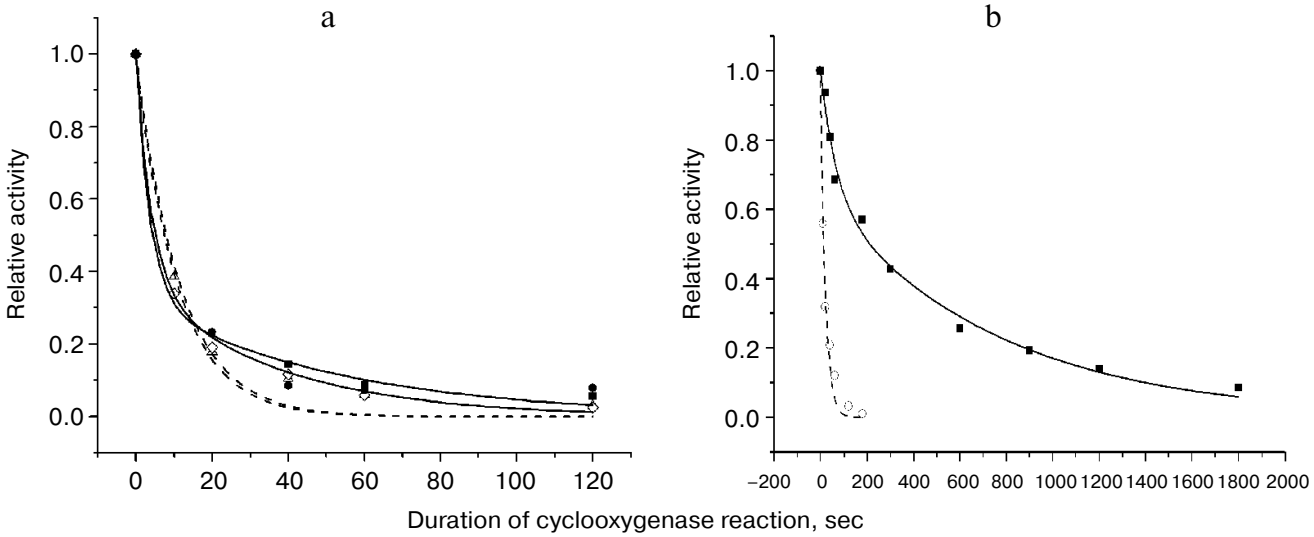


Fig. 3. a) Relative peroxidase activity (B_{rel}) (squares, circles) and relative cyclooxygenase activity (A_{rel}) (triangles, rhombuses) of solubilized PGHS preparation versus duration of cyclooxygenase reaction. Squares: 18 μg/ml PGHS, 150 μM AA, O_2. Peroxidase activity was determined after addition of 1.5 mM H_2O_2 and 60 μM TMPD into the reaction mixture. Circles: 9 μg/ml PGHS, 150 μM AA, O_2. Peroxidase activity was determined after addition of 1.5 mM H_2O_2 and 750 μM 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS). Triangles: 45 μg/ml PGHS, 150 μM AA, O_2. Rhombuses: 75 μg/ml PGHS, 150 μM AA, O_2. Solid lines are plotted by approximation of data (squares, circles) using Eq. (19), dashed lines (triangles, rhombuses) – using Eq. (20). b) Relative peroxidase activity (B_{rel}) (squares) and relative cyclooxygenase activity (A_{rel}) (circles) of solubilized enzyme preparation versus duration of cyclooxygenase reaction in the presence of electron donor ABTS. Squares: 9 μg/ml PGHS, 150 μM AA, 750 μM ABTS, O_2. Peroxidase activity was determined after addition of H_2O_2 into the reaction mixture. Circles: 15 μg/ml PGHS, 150 μM AA, 750 μM ABTS, O_2. The solid line is plotted by approximation of data using Eq. (19), dashed line – using Eq. (20).

inactive intermediate X^* with the rate constant λ_1. Loss of enzyme property occurs via conversion of the same intermediate X_i into an intermediate Y_i with the rate constant λ_2. Having lost the property, intermediate Y_i retains catalytic activity, that is, capacity for participating in a cycle ($Y_0 \ldots Y_i \ldots Y_0$). Catalytically inactive enzyme form X^* possesses property (B) and looses it as a result of conversion into X^{**} with rate constant λ_3. Catalytically active intermediate Y_i looses catalytic activity as a result of conversion into intermediate form X^{**} devoid of the property as well as catalytic activity with the rate constant λ_4. Forms X^* and X^{**} do not possess catalytic activity. We suggest that the above-mentioned property is equal for intermediates X_i and X^*, but Y_i and X^{**} are devoid of it. Scheme 2 allows description of the processes of peroxidase and cyclooxygenase inactivation during both cyclooxygenase and peroxidase reactions.

Let us designate the total quantity of catalytically active intermediates in Scheme 2 as X and Y:

$$\sum_i [X_i] = X\,,\ \sum_i [Y_i] = Y\,, \qquad (8)$$

Then the material balance equation will be:

$$E_0 = X + Y + [X^*] + [X^{**}], \qquad (9)$$

where E_0 is the total enzyme concentration.

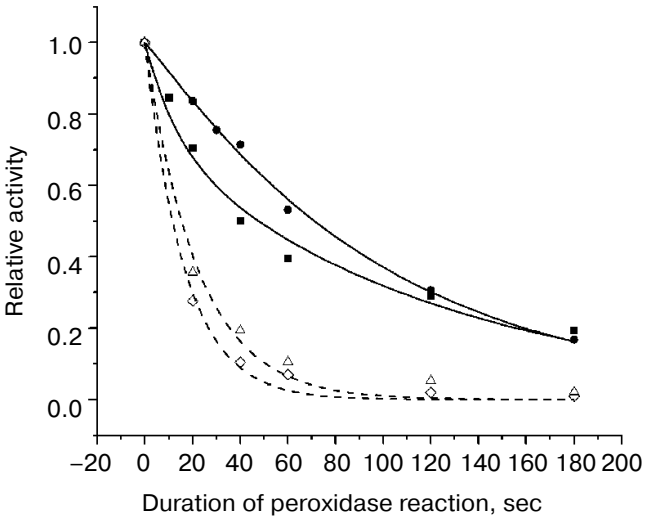
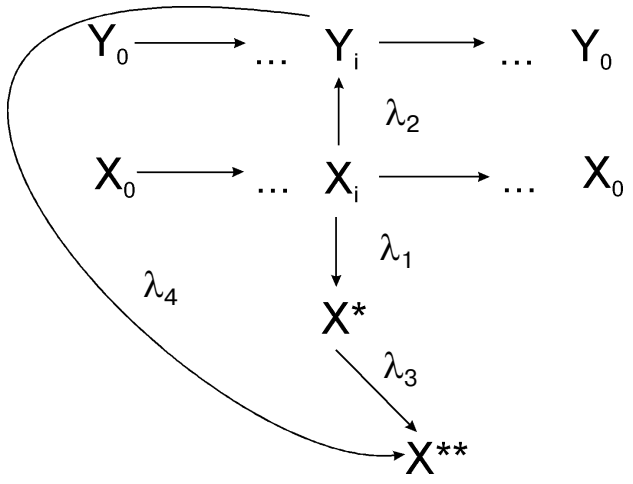


Fig. 4. Relative cyclooxygenase activity (B_{rel}) of purified (squares, circles) and relative peroxidase activity (A_{rel}) of solubilized PGHS preparation (triangles, rhombuses) versus peroxidase reaction time. Squares: 5.3 μg/ml PGHS, 2 μM hemin, 60 μM TMPD, 1.5 mM H_2O_2. Cyclooxygenase activity was determined after addition of 150 μM AA into the reaction mixture. Circles: 5 μg/ml PGHS, 2 μM hemin, 1.5 mM potassium ferrocyanide, 1.5 mM H_2O_2. Cyclooxygenase activity was determined after addition of 150 μM AA. Rhombuses: 18 μg/ml PGHS, 60 μM TMPD, 1.5 mM H_2O_2. Triangles: 216 μg/ml PGHS, 1.5 mM potassium ferrocyanide, 1.5 mM H_2O_2. Solid lines are plotted by approximation of data using Eq. (19), dashed lines – using Eq. (20).



Kinetic model of PGHS cyclooxygenase and peroxidase inactivation during reaction (designations are explained in the text)

Scheme 2

We suggest that all X intermediates and all Y intermediates are in a steady state and as the first approximation, possess equal catalytic properties (the rate constants for X intermediates are equal to the corresponding rate constants for Y intermediates). Then $[X_i]/X$ and $[Y_i]/Y$ ratios for intermediates subject to inactivation are equal and determined by concentrations of substrates:

$$\frac{[X_i]}{X} = \frac{[Y_i]}{Y} = \alpha \,. \tag{10}$$

At time zero all the enzyme is in form X. Solution of the differential equations corresponding to this scheme is as follows:

$$X = E_0 e^{-\alpha(\lambda_1+\lambda_2)t}, \tag{11}$$

$$[X^*] = E_0 \frac{\alpha\lambda_1}{\alpha(\lambda_1+\lambda_2)-\lambda_3}\left(e^{-\lambda_3 t} - e^{-\alpha(\lambda_1+\lambda_2)t}\right), \tag{12}$$

$$Y = E_0 \frac{\alpha\lambda_2}{\alpha(\lambda_1+\lambda_2)-\alpha\lambda_4}\left(e^{-\alpha\lambda_4 t} - e^{-\alpha(\lambda_1+\lambda_2)t}\right), \tag{13}$$

$$A = A_X X + A_Y Y + A_{X^*}[X^*] + A_{X^{**}}[X^{**}] \,, \tag{14}$$

$$B = B_X X + B_Y Y + B_{X^*}[X^*] + B_{X^{**}}[X^{**}], \tag{15}$$

where A is enzyme activity, B is its property, A_X, A_Y, A_{X^*}, and $A_{X^{**}}$ are specific activities of X, Y, X^*, and X^{**} forms, respectively, and B_X, B_Y, B_{X^*}, and $B_{X^{**}}$ are specific properties of X, Y, X^*, and X^{**} forms, respectively. Accounting for the above-mentioned assumptions, Eqs. (14) and (15) are transformed into (16) and (17):

$$A = A_X(X + Y) \,, \tag{16}$$

$$B = B_X(X + [X^*]), \tag{17}$$

As quantitative characteristics of activity and property, it is convenient to use relative activity (A_{rel}) and relative property (B_{rel}), that is, experimental values normalized to their values at time zero. Then substituting Eqs. (11)-(13) into Eqs. (16) and (17), we obtain the following equations for A_{rel} and B_{rel}:

$$A_{rel} = \frac{\lambda_1 - \lambda_4}{(\lambda_1+\lambda_2)-\lambda_4} e^{-\alpha(\lambda_1+\lambda_2)t} + \frac{\lambda_2}{(\lambda_1+\lambda_2)-\lambda_4} e^{-\alpha\lambda_4 t}, \tag{18}$$

$$B_{rel} = \frac{\alpha\lambda_1}{\alpha(\lambda_1+\lambda_2)-\lambda_3} e^{-\lambda_3 t} + \frac{\alpha\lambda_2 - \lambda_3}{\alpha(\lambda_1+\lambda_2)-\lambda_3} e^{-\alpha(\lambda_1+\lambda_2)t}. \tag{19}$$

The equation for A_{rel} is a sum of two exponentials. Since activity loss during catalysis of like reaction is a single exponential process (Figs. 3 and 4) (for cyclooxygenase as well as peroxidase reaction), it is natural to suggest that $\lambda_1 = \lambda_4$. It is obvious, because λ_1 and λ_4 describe activity loss of X_i and Y_i forms possessing equal catalytic properties. Then change of A_{rel} with time is described by Eq. (20):

$$A_{rel} = e^{-\alpha\lambda_1 t}. \tag{20}$$

If the condition $\alpha\lambda_2 \cong \lambda_3$ is satisfied for corresponding α value for a certain substrate concentrations, then B_{rel} will be revealed in the experiment as one exponential:

$$B_{rel} = e^{-\alpha\lambda_3 t}. \tag{21}$$

These formulae are obtained in a general case. Only three independent constants completely describing the suggested scheme are included in Eqs. (19) and (20). To evaluate their values, experimental data (Figs. 3 and 4) were approximated by nonlinear regression according to these formulae. As shown in the above-mentioned figures, the data are described well by the derived equations.

Let us consider enzyme inactivation during the cyclooxygenase reaction. In this case, cyclooxygenase activity acts as catalytic activity (approximation by Eq. (20)), whereas peroxidase activity acts as a property (approximation by Eq. (19)). For data presented in Fig. 3a, the obtained constants are given in Table 2, and for data presented in Fig. 3b – in Table 3.

Now let us consider enzyme inactivation during the peroxidase reaction. In this case, peroxidase activity acts as catalytic activity (approximation by Eq. (20)), whereas cyclooxygenase activity acts as an enzyme property (approximation by Eq. (19)) (Fig. 4). The obtained constants are given in Table 4.

Table 2. Kinetic parameters of PGHS inactivation during the cyclooxygenase reaction in the absence of electron donor (Scheme 2, Eqs. (19) and (20)). Experimental data (Fig. 3a) were approximated by nonlinear regression according to Eqs. (19) and (20)

Experimental data	$\alpha(\lambda_1 + \lambda_2)$, min^{-1}	$\alpha\lambda_2$, min^{-1}	λ_3, min^{-1}	$\alpha\lambda_1$, min^{-1}
Enzyme property (peroxidase activity)	16.5	11.5	1.2	–
Catalytic (cyclooxygenase) activity	–	–	–	5

Table 3. Kinetic parameters of PGHS inactivation during the cyclooxygenase reaction in the presence of electron donor (Scheme 2, Eqs. (19) and (20)). Experimental data (Fig. 3b) were approximated by nonlinear regression according to Eqs. (19) and (20)

Experimental data	$\alpha(\lambda_1 + \lambda_2)$, min^{-1}	$\alpha\lambda_2$, min^{-1}	λ_3, min^{-1}	$\alpha\lambda_1$, min^{-1}
Enzyme property (peroxidase activity)	3.74	0.74	0.11	–
Catalytic (cyclooxygenase) activity	–	–	–	3

Table 4. Kinetic parameters of PGHS inactivation during the peroxidase reaction for $K_3[Fe(CN)_6]$ and TMPD as electron donors (Scheme 2, Eqs. (19) and (20)). Experimental data (Fig. 4) were approximated by nonlinear regression according to Eqs. (19) and (20)

Experimental data	$\alpha(\lambda_1 + \lambda_2)$, min^{-1}	$\alpha\lambda_2$, min^{-1}	λ_3, min^{-1}	$\alpha\lambda_1$, min^{-1}
Enzyme property (cyclooxygenase activity) ($K_3[Fe(CN)_6]$ as electron donor)	3.2	0.5	0.62	–
Catalytic (peroxidase) activity ($K_3[Fe(CN)_6]$ as electron donor)	–	–	–	2.7
Enzyme property (cyclooxygenase activity) (TMPD as electron donor)	4.8	1.62	0.51	–
Catalytic (peroxidase) activity (TMPD as electron donor)	–	–	–	3.2

Constant values $\alpha\lambda_1$ were obtained by approximation of the experimental data (Figs. 3 and 4, dashed lines) according to Eq. (20). The other constants were obtained by fixing $\alpha\lambda_1$ value on approximation of data (Figs. 3 and 4, solid lines) by Eq. (19). Using only three parameters, Eqs. (19) and (20) completely describe the various processes (activity loss during like reaction and activity loss during alternative reaction).

DISCUSSION

Earlier it was reported that on preincubation with peroxides, their nature does not effect the rate constant of PGHS peroxidase inactivation, which is on average 24 min^{-1} at 24°C [4]. These data partly coincide with our results. Our experiments of PGHS preincubation with hydrogen peroxide and *tert*-butylperoxide also showed that the rate constant of peroxidase inactivation does not depend on the nature of the peroxide, but we obtained the rate constant value less than in [4] – 4-6 min^{-1}.

It is reported [12] that after complete loss of PGHS cyclooxygenase reaction more than 60% of peroxidase activity is retained in the presence of electron donor (compared with control in which arachidonic acid was absent). We have found that loss of peroxidase activity under the cyclooxygenase reaction conditions depends on the presence or absence of electron donor. If the

cyclooxygenase reaction is performed without electron donor (the latter is added only for activity detection at the end of the experiment), peroxidase and cyclooxygenase inactivation rate constants will numerically coincide (Fig. 3a). If the cyclooxygenase reaction is performed in the presence of electron donor, 70% of the peroxidase activity is retained after complete loss of cyclooxygenase activity (Fig. 3b); this corresponds with data reported in [12].

It is reported [13] that only holoenzyme is inactivated during the cyclooxygenase reaction. This indicates that the products of the cyclooxygenase reaction do not cause inactivation of the enzyme molecule. However, facts presented in [14] indicate that byproducts of arachidonic acid oxidation may cause PGHS inactivation; these byproducts are present in solution during the cyclooxygenase reaction and may covalently bind to the enzyme molecule. Analogously, it is suggested in [15] that the products of nonenzymatic decomposition of prostaglandin H_2 may interact with PGHS, thus inactivating it.

However, kinetic considerations indicate that the products of enzymatic reaction cannot be the only reason for suppression of PGHS activity: as can be easily shown, for mechanisms in which interaction between enzyme and reaction product is an origin of inactivation, the maximal yield of reaction product is directly proportional to a square root of the total enzyme concentration. In the case of PGHS, the maximal yield of cyclooxygenase and peroxidase reactions linearly depends on the total enzyme concentration [9, 16] and consequently – nonlinearly on square root of the total enzyme concentration. This indicates that bimolecular interaction with the reaction product cannot be an origin of enzyme inactivation during cyclooxygenase and peroxidase reactions.

It was suggested in [1], that loss of cyclooxygenase and peroxidase activities is caused by destruction of the heme group. However, in this case loss of both activities to an equal degree should be observed in all the experiments. This is not found experimentally, consequently, the suggestion is wrong.

The simple kinetic models of enzyme inactivation in the course of reaction suggested in this work explain such data as the presence of peroxidase activity after complete loss of cyclooxygenase activity ([12] and Fig. 3b) and also the presence of cyclooxygenase activity after complete loss of peroxidase activity (Fig. 4). Any one of the earlier suggested models [2, 4-8] cannot explain these facts. Elucidation of the molecular mechanisms causing the kinetic phenomena described by Scheme 2 will be the next step in studying the phenomenon of PGHS inactivation during reactions catalyzed by it.

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