A Dynamic Enzymatic Switch

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Received: February 22, 1998; In Final Form: April 21, 1998

The kinetics of the peroxidase-catalyzed oxidation of iodide was found to be strongly nonlinear under certain conditions. At slightly acidic pH, the reaction behaves like an enzymatic "iodine clock", i.e., the reaction is switched on after a well-defined time depending on the initial concentrations of the reactants. This behavior is studied experimentally and is shown to represent a true dynamic enzymatic switch. Numerical simulations of a mechanistic model strengthen this conclusion.

Introduction

The complex biochemical network as it is known today comprises thousands of enzymes and substrates, partly identified, the majority probably unknown. The structure of this network is determined by the way the components interact with each other. There are many different types of inhibition and activation. Moreover, pathways can be unidirectional, reversible, branched, or cyclic, to name but a few of the existing possibilities. Because of this interwoven structure, some scientists have compared the biochemical network to a neural network. In a more mathematical context the similarity to a computer was emphasized and the term "(bio)chemical computer" was created. The idea that the biochemical network responds "intelligently" and flexibly to all kinds of information is appealing and plausible considering the success of these structures. The theoretical aspects of these biochemical and chemical computers have recently attracted much interest.[1−4] Biochemical reactions should play the role of switching devices in such computers. Indeed, chemical and enzymatic switches have been described theoretically[4−6] and experimentally.[7]

Early theoretically described switching devices used the nomenclature introduced for switches in electronics.[1,5] Thus, very simple chemical and biochemical reaction systems have been proposed which could function as monoflops, flipflops, etc. One of the simplest of such possible switches is a monoflop. A monoflop (monostable multivibrator) is defined as a device which switches between a stable and a metastable state. When perturbed, it is able to switch from the stable to the metastable state. After a certain time, which depends on the properties of the switch, it returns to the stable steady state by itself.[8]

Recent theoretical work focused on the properties of more complex biochemical reaction systems, particularly multienzyme systems in which futile cycles of substrates take part.[3,4,6] Enzymatic futile cycles of substrates are frequently found in the biochemical network and provide remarkable features for computational processes. Implications for glycolysis have been suggested[9] and verified experimentally.[10]

Despite the accumulating literature on theoretical predictions of biochemical switching devices, hardly any experimental in vitro systems provide evidence for dynamic systems working as switches. In contrast to the theoretically predicted systems, most of the experimentally verified enzymatic switches work via structural modifications which turn a function on or off.[7,11,12] One well-known example is the phosphorylation and dephosphorylation of enzymes which change their activity. In a *dynamic* switch, however, the switching event is accomplished purely by the mechanism and kinetics of the respective reactions.

According to the above-mentioned theoretical work, there should be enzymatic switches which consist of only a few reactions. Knowledge of such switches is crucial for the understanding of biochemical regulation mechanisms in the complex network. In preliminary studies, we found the kinetics of the peroxidase-catalyzed oxidation of iodide to be strongly nonlinear in a broad range of the respective parameters.[13] We examined this behavior and studied the underlying reaction mechanism.

The enzymatic oxidation of halides plays an important role in the iodination of thyroglobulin[14] and probably in microbicidal and cytotoxic functions.[15,16] Because this reaction occurs in vivo, its ability to display various dynamic behavior is of great importance. The mechanism of the peroxidase-catalyzed oxidation of halides has been studied with various peroxidases. It has been suggested that the oxidation of iodide succeeds via a two-electron step and mainly involves the native peroxidase (ferriperoxidase) and the so-called compound I (EO), an enzymatic intermediate.[17] Most of the other peroxidase-catalyzed reactions involve compound II as well.[18−20] The enzymatic intermediates differ in the oxidation state of the iron in the heme of the peroxidase. Even though there is some evidence that hypoiodous acid (HOI) is formed in the course of the enzymatic oxidation of iodide, it is still a matter of controversy whether HOI or only an enzyme bound form EOI is formed.[21,22] The reaction has a pH-optimum below pH 4 and otherwise depends on the individual conditions.[23,24] If the pH is increased, hydrogen peroxide is partially pseudocatalatically decomposed.[25] It has been suggested that the latter behavior arises from the reaction of hydrogen peroxide with HOI or the equivalent EOI to form oxygen and iodide.[26] The following mechanism has been proposed by Shah et al.:[22]

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S1089-5639(98)01236-5 CCC: $15.00
Published on Web 06/24/1998

$$E + H_2O_2 \rightarrow EO + H_2O \quad (1)$$

$$EO + I^- \rightarrow EOI \quad (2)$$

$$EOI + H^+ \rightarrow E + HOI \quad (3)$$

$$HOI + I^- \rightarrow I_2 + OH^- \quad (4)$$

$$I_2 + I^- \rightleftharpoons I^-_3 \quad (5)$$

$$HOI + H_2O_2 \rightarrow O_2 + I^- + H_2O + H^+ \quad (6)$$

We found that the reaction behaves like an enzymatic iodine clock (the reaction starts only after a well-defined time, producing iodine) at only slightly acidic pH values and also represents a true enzymatic switch which acts in a monoflop-like fashion. As far as we know, this is the first autonomous one-enzyme switch discovered so far.

We used a system of ordinary differential equations (ODEs) to simulate the reaction on the basis of known reaction steps. We show that several feedback loops are crucial factors in the reaction mechanism. Our simulations fit the experimental results very well. The simulations support the view of the system as an enzymatic switch.

Materials and Methods

Horseradish peroxidase (170 U/mg, RZ 1,9) was purchased from Sigma. The experiments were performed in a macrocuvette placed in a Perkin-Elmer double-beam spectrophotometer (Lambda 15). A 4 mL sample containing sodium acetate or sodium phosphate buffer at different concentrations and pH values, and starch (soluble starch from Merck), hydrogen peroxide from Sigma (a 100 mM stock solution was freshly prepared daily), potassium iodide (Merck), and horseradish peroxidase in different concentrations was stirred with a velocity of 900 ± 10 rpm. The temperature was kept constant at 25 °C. The concentration of the starch−iodine complex was measured as the absorbance at 600 nm. In addition, the oxygen concentration was determined by an oxygen electrode placed into a stirred 1.25 mL sample during several experiments. Simulations were performed by numerical integration of ODEs on a PC using Dynamical Software Professional (Dynamical Systems Inc., USA).

Results

We studied the kinetics of the peroxidase-catalyzed oxidation of iodide at different pH values. We found strong nonlinearities which show a significantly higher degree of nonlinearity compared to simple saturation kinetics at almost all studied values of pH for certain parameters.[13] At low values (pH 5−5.5), the kinetics showed an overshooting behavior (Figure 1). In this parameter range, the reaction started very fast, reaching a maximum iodine concentration after a few seconds. After this initial burst, the iodine concentration decreased more slowly to the final steady state. Depending on the intial conditions, this decrease was either rather steady or had the nonuniform shape shown in Figure 1.

The time until the steady state was reached depended on the initial conditions within the respective parameter range. When we used the initial conditions of the experiment shown in Figure 1 as reference, increasing hydrogen peroxide concentration led to an increasing time until the steady state was reached, whereas increasing the enzyme or the iodide concentration had the opposite effect. Performing the experiments without the addition of starch and following the iodine concentration as absorbance at 460 nm gave us similar results. However, because of the low extinction coefficient of iodine and interference with the absorbance of the enzyme at this wavelength (which changes with time as well), the resulting kinetics had a somewhat less pronounced maximum.

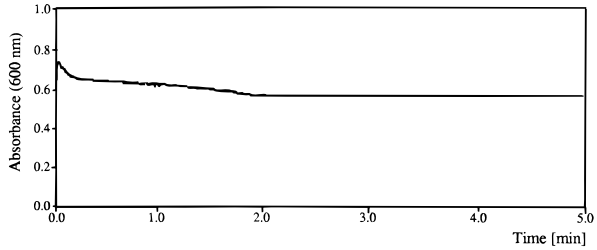

Figure 1. Iodine concentration in the course of an experiment with initial concentrations in the reaction of 0.2 mM hydrogen peroxide, 0.2 mM KI, 0.15 mg/mL starch, and 2.6 μM enzyme in 100 mM sodium acetate buffer, pH 5.1. Because of the high initial velocity of the reaction and the short delay between the start of the reaction (addition of enzyme) and the start of the recording, the initial steep increase in iodine from zero is only partly seen.

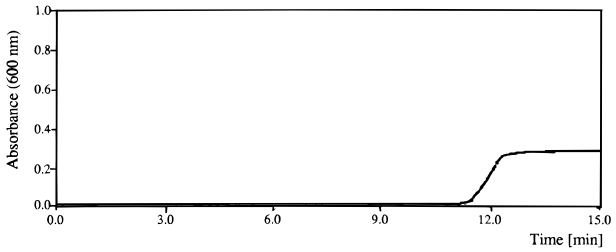

Figure 2. Iodine concentration in the course of an experiment with initial concentrations in the reaction of 1 mM hydrogen peroxide, 0.1 mM KI, 0.15 mg/mL starch, and 2 μM enzyme in 100 mM sodium phosphate buffer, pH 6.5.

The kinetics at slightly acidic pH values (pH 6−6.5) showed a completely different behavior. At certain parameter values, the reaction behaved like an enzymatic iodine clock, i.e., the reaction started only after a well-defined time (Figure 2). Initially no iodine production was observed. After a very reproducible lag period the reaction started and the reaction rate accelerated to a maximum reaction rate which was maintained until the steady state was reached. In the studied parameter regime in which the clock behavior was observed, this time depended in a roughly linear manner on the hydrogen peroxide concentration. Doubling the hydrogen peroxide concentration approximately doubled the lag time. Increasing the concentrations of enzyme or iodide had the opposite (decrease of lag time) effect, but the dependence was not linear. In addition, increasing iodide concentrations soon changed the resulting kinetics from the timefuse-like behavior to a stepwise increase in the iodine concentration (Figure 3) such that fast iodine production was observed right from the start of the reaction until a certain level was reached, and this level was maintained until a more gentle rise led to the final concentration of iodine. Again, the experiments performed without starch led to similar results, but the resulting kinetics showed somewhat smaller slopes at the start of iodine production and, because of the above-mentioned effects and the relatively low resulting iodine concentration, the kinetics were harder to follow.

We repeated some of the experiments at pH 6.5 with the same initial concentrations in a 1.25 mL sample and used an inserted oxygen electrode to follow the oxygen concentration. We observed a linear increase in the oxygen concentration from

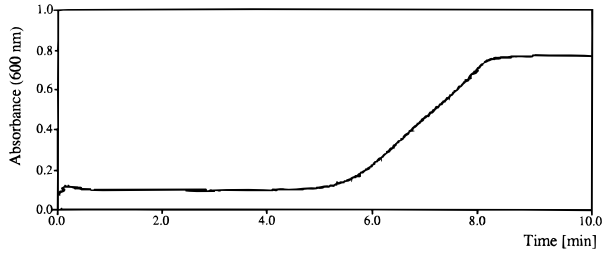

Figure 3. Iodine concentration in the course of an experiment with initial concentrations in the reaction of 1 mM hydrogen peroxide, 0.2 mM KI, 0.15 mg/mL starch, and 2 μM enzyme in 100 mM sodium phosphate buffer, pH 6.5.

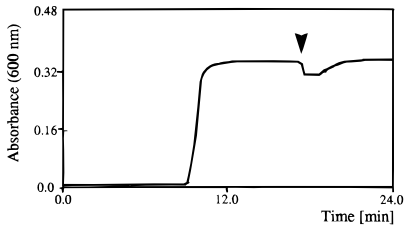

Figure 4. Small perturbation of the steady state of the iodine concentration. Initial concentrations in the 4 mL sample were 1 mM hydrogen peroxide, 0.1 mM KI, 0.15 mg/mL starch, and 2 μM enzyme in 100 mM sodium phosphate buffer, pH 6.5. At the time indicated by the arrow, 20 μL of a 100 mM hydrogen peroxide solution was added. The system returned from the metastable state to the steady state spontaneously.

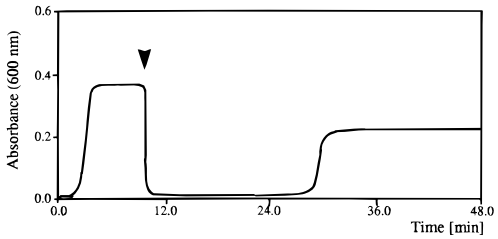

Figure 5. Strong perturbation of the steady state of the iodine concentration. Initial concentrations in the 4 mL sample were 0.5 mM hydrogen peroxide, 0.1 mM KI, 0.15 mg/mL starch, 2 μM enzyme in 100 mM sodium phosphate buffer, pH 6.5. At the time indicated by the arrow, 45 μL of a 100 mM hydrogen peroxide solution was added. After switching to a metastable state, the system returned spontaneously to the stable steady state.

time zero until a maximum concentration, followed by a slow decline which was probably due to the escape of oxygen into the air. The maximum concentration of oxygen was reached at the time or shortly after the production of iodine began in the respective experiments.

To examine the switching behavior at pH 6.5 further, we added increasing amounts of hydrogen peroxide to the system after the steady state was reached. The iodine concentration dropped immediately to a lower level and after a certain time which depended again on the amount of hydrogen peroxide added, the system spontaneously returned to the steady state (Figures 4 and 5). In the experiment shown in Figure 5, the concentration of iodine in the steady state after the perturbation was not the same as before. Very high concentrations of hydrogen peroxide decreased the iodine concentration irreversibly. In this case, even when additional iodide was added, the system remained in the zero-iodine state, indicating that the enzyme was inactivated.

To investigate the underlying mechanism which led to this highly nonlinear behavior, we studied the possible mechanisms with respective simulations. A mechanism was proposed for

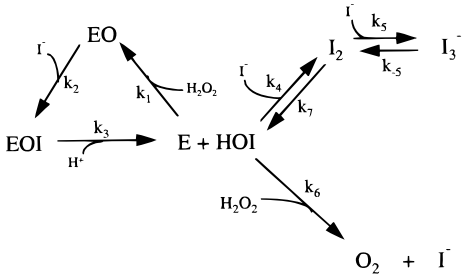

Figure 6. Schematic presentation of the model used for the simulations.

the peroxidase-catalyzed oxidation of iodide.[22] Integrating the respective ODEs, we tried to simulate the observed behavior, but we did not succeed with the above-described reactions (1−6). According to our experiments at pH 5.2 and according to the literature,[27] the production of iodine is reversible and so we added the reverse of reaction (4) to our mechanism:

$$I_2 + OH^- \rightarrow HOI + I^- \qquad (7)$$

The resulting set of ODEs was:

$$\frac{d[H_2O_2]}{dt} = -k_1 \times [H_2O_2] \times [E] - k_6 \times [H_2O_2] \times [HOI]$$

$$\frac{d[E]}{dt} = -k_1 \times [H_2O_2] \times [E] + k_3 \times [EOI]$$

$$\frac{d[EO]}{dt} = k_1 \times [H_2O_2] \times [E] - k_2 \times [EO] \times [I^-]$$

$$\frac{d[EOI]}{dt} = k_2 \times [EO] \times [I^-] - k_3 \times [EOI]$$

$$\frac{d[I^-]}{dt} = -k_2 \times [EO] \times [I^-] - k_4 \times [HOI] \times [I^-] + k_6 \times [H_2O_2] \times [HOI] + k_7 \times [I_2] - k_5 \times [I^-] \times [I_2] + k_{-5} \times [I_3^-]$$

$$\frac{d[HOI]}{dt} = k_3 \times [EOI] - k_4 \times [HOI] \times [I^-] - k_6 \times [H_2O_2] \times [HOI] + k_7 \times [I_2]$$

$$\frac{d[I_2]}{dt} = k_4 \times [HOI] \times [I^-] - k_7 \times [I_2] - k_5 \times [I^-] \times [I_2] + k_{-5} \times [I_3^-]$$

$$\frac{d[I_3^-]}{dt} = k_5 \times [I^-] \times [I_2] - k_{-5} \times [I_3^-]$$

$$\frac{d[O_2]}{dt} = k_6 \times [H_2O_2] \times [HOI] - p \times [O_2]$$

where k_1-k_7 represent the rate constants and p represents the rate of oxygen escaping from the solution. The rate constant k_3 includes the proton concentration. Figure 6 shows a schematic representation of the model.

With this system, it was possible to simulate the respective experiments (Figure 7) using known rate constants from the literature and others fitted by the simulations (Table 1). The rate constant p for the escape of oxygen from the reaction is high compared to normal rates of diffusion. However, it was

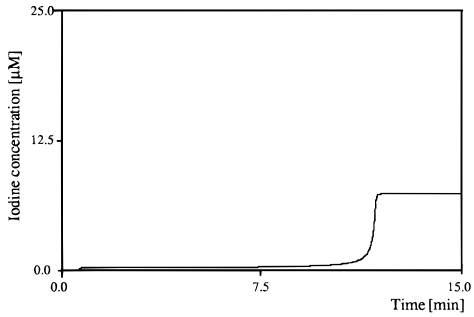

Figure 7. Simulation of the experiment shown in Figure 2. Rate constants are as shown in Table 1 and parameters (initial concentrations) are according to Figure 2.

TABLE 1: Rate Constants Used for the Simulation of the Experimentally Observed Behavior by Integrating the Set of ODEs (1−9)

constant	pH 6.5	source	pH 5.2	source
k_1	1.8×10^7 M^{-1} s^{-1}	ref 35	1.8×10^7 M^{-1} s^{-1}	ref 35
k_2	1.3×10^4 M^{-1} s^{-1}	ref 24	4.5×10^5 M^{-1} s^{-1}	fitted
k_3	5×10^2 s^{-1}	fitted (incl. [H$^+$])	1.7×10^4 s^{-1}	fitted (incl. [H$^+$])
k_4	1×10^6 M^{-1} s^{-1}	ref 36	2×10^7 M^{-1} s^{-1}	ref 36
k_6	1.5×10^{10} M^{-1} s^{-1}	fast K_{eq} [29]	1.5×10^{10} M^{-1} s^{-1}	fast K_{eq} [29]
k_{-5}	2×10^4 s^{-1}	fast K_{eq} [29]	2×10^4 s^{-1}	fast K_{eq} [29]
k_6	8.3×10^6 M^{-1} s^{-1}	fitted	2.5×10^3 M^{-1} s^{-1}	fitted
k_7	1.3×10^7 s^{-1}	fitted	6.7×10^7 s^{-1}	fitted
p	0.2 s^{-1}	fitted	0.2 s^{-1}	fitted

observed that if the reaction was left unstirred, small bubbles of gas were built during the reaction; it is possible that besides diffusion of oxygen in and out of the solution, bubbles of oxygen also escaped from the reaction because of the high initial rate of the formation of oxygen. The respective parameters (initial concentrations) were in accordance with the real experiments. The production of the iodine−starch complex was not included in the model. It was assumed that it is a fast and reversible reaction and thus has no significant influence on the shape of the resulting kinetics. This is in accordance with the literature.[28] Iodine species which are of minor importance under these conditions were also not included, because species with very low production rates or with very high decomposition rates such as IO^- [29] and H_2OI^+ [27] did not significantly change the result of the respective simulations. The formation of IO^- [29] was also neglected. The addition of such an unbranched reaction (which does not exhibit a feedback on the reaction) to one of the branches of the model did not qualitatively change the resulting simulations either.

Variation of the individual rate constants had in most cases a large influence on the shape of the resulting kinetics. We define the kinetic behavior which is characterized by a start of the reaction after a well-defined time and an acceleration of the reaction rate after the start as timefuse behavior. Crucial factors for the occurrence of this behavior are a high rate of reaction (7) and the relation between rate constants k_4 and k_6. Furthermore, the overall velocity of the enzymatic reactions 1−3 had a strong influence on the time which was needed until the reaction reached the steady state. Changing initial parameters (concentrations) according to the experiments described above lead to the results shown in Table 2 which fit the experimental data well. Simulating the behavior of the oxygen concentration in the course of a reaction at pH 6.5 led to the same steady increase in concentration up to a maximum and then a slow decline as observed in the real experiments.

TABLE 2: Time Until the Steady State Was Reached in Some Experiments (T_{exp}) at pH 6.5 and in the Respective Simulations (T_{sim})[a]

intial concn	T_{exp} (min)	T_{sim} (min)	behavior
2 μM E, 1 mM H_2O_2, 0.1 mM KI	12.4	11.8	timefuse
2 μM E, 1.25 mM H_2O_2, 0.1 mM KI	15.7	14.6	timefuse
2 μM E, 0.75 mM H_2O_2, 0.1 mM KI	8.8	8.9	timefuse
2 μM E, 0.5 mM H_2O_2, 0.1 mM KI	6.2	6	timefuse
2 μM E, 0.25 mM H_2O_2, 0.1 mM KI	3.3	3.0	timefuse
2 μM E, 0.5 mM H_2O_2, 0.25 mM KI	3.2	5.0	stepping
2 μM E, 0.5 mM H_2O_2, 0.2 mM KI	3.8	5.3	stepping
2 μM E, 0.5 mM H_2O_2, 0.125 mM KI	5.1	5.6	timefuse
4 μM E, 0.5 mM H_2O_2, 0.1 mM KI	2.8	3.0	stepping
1.5 μM E, 0.5 mM H_2O_2, 0.1 mM KI	2.7	7.8	timefuse

[a] The qualitative behavior was always identical in the simulations compared to the experiments except that the stepping behavior was less pronounced (i.e., the height of the first step was smaller).

We also tried to simulate the behavior with different mechanisms, because the mechanism of the reaction is still a point of discussion. Because it is still debated whether HOI is an intermediate of the reaction or EOI is the only intermediate, we removed HOI from the model mechanism, such that reactions 4 and 6 proceeded with EOI as a substrate. We obtained a model with which it was very hard to get timefuse-like behavior. Only with very unrealistic parameters (a very high k_6/k_4 ratio or extremely high k_7) was it possible to observe such behavior. Under these conditions hardly any iodine would be produced and the amount would be barely detectable in an experiment.

Furthermore, because iodide and hydrogen peroxide are able to react with each other in a purely inorganic reaction, we studied this reaction, experimentally and theoretically, in order to evaluate the importance of the enzyme as catalyst for the observed strongly nonlinear behavior.

$$H_2O_2 + I^- + H^+ \rightarrow HOI + H_2O \qquad (8)$$

Because the reaction is strongly favored at low pH, high concentrations of the reactants are needed to initiate the reaction at pH 6.5. No iodine production was observed with the low concentrations used in the enzymatic experiments even after hours had elapsed. If higher concentrations were chosen, simple saturation kinetics was observed, no matter what substrate concentrations were used. Modeling of this inorganic reaction reproduced these phenomena. Reactions 4−8 led to a system which showed no timefuse behavior or anything similar irrespective of which rate constants were chosen. Including an additional artificial major intermediate *X* between the substrates and HOI led to a system which easily produced the timefuse-like behavior.

To verify the possible mechanisms further, rate constants of pH-dependent reactions were changed according to the proton concentration at pH 5.2. Additionally, the rate constants of reactions 2 and 3 had to be increased, but because they represent enzymatic reactions, they were not simply adjusted to the different proton concentration, but were fitted by simulations. Rate constant k_6 was also fitted by simulations. The resulting system simulated the experiments made at pH 5.2 very well (Figure 8). The influence of the respective parameters is shown in Table 3 and is also in good agreement with experimental results.

Discussion

The enzymatic oxidation of iodide is strongly nonlinear under certain conditions. We observed overshooting behavior at pH

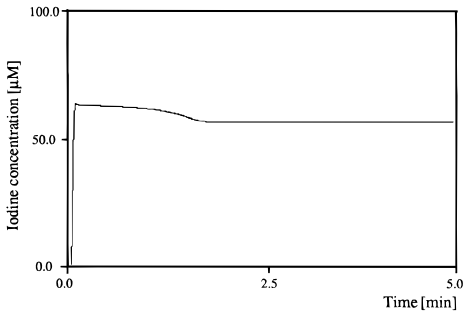

Figure 8. Simulation of the experiment shown in Figure 1. Rate constants are as shown in Table 1 and parameters (initial concentrations) are according to Figure 1.

TABLE 3: Time until the Steady State Was Reached in Some Experiments (T_{exp}) at pH 5.2 and in the Respective Simulations (T_{sim})[a]

initial concn	T_{exp} (min)	T_{sim} min)	behavior
2.6 μM E, 0.2 mM H_2O_2, 0.2 mM KI	2	1.8	overshoot
2.6 μM E, 0.275 mM H_2O_2, 0.2 mM KI	2.4	2.3	overshoot
2.6 μM E, 0.5 mM H_2O_2, 0.2 mM KI	3.7	3.8	overshoot
0.65 μM E, 0.2 mM H_2O_2, 0.2 mM KI	5	4.1	overshoot
2.6 μM E, 0.2 mM H_2O_2, 0.1 mM KI	4.2	2.6	overshoot
2.6 μM E, 0.2 mM H_2O_2, 0.3 mM KI	1.1	1.4	overshoot

[a] The qualitative behavior was always identical in the simulations compared to the experiments.

5.2 and timefuse behavior at pH 6.5. The latter behavior represents a unique enzymatic iodine clock, because the time until the iodine production starts is well defined and reproducible. Iodine clock reactions are known from chemistry.[30,31] Clock reactions stand out with their unusual behavior because at least one of the participating reactions starts only after a well-defined time. So far, several variants of the original iodine clock have been discovered, all of them obeying a similar mechanism and with no enzymatic reactions involved. In our case, a simple biochemical system consisting of one enzyme, two substrates, and buffer leads to the observed clock behavior via an obviously different mechanism.

Furthermore, the system displays switching behavior. We studied the enzymatic switch further by perturbing the system with hydrogen peroxide during the steady state. The system responded by switching to a metastable state in a monoflop-like fashion. This response is typical for true switching behavior.[1,5] However, this new enzymatic switch is not a perfect monoflop, because the metastable state is a function of the perturbation. Furthermore, when a rather large amount of hydrogen peroxide was added, the system returned to a steady state with a somewhat lower concentration of iodine than before. This is probably partly due to the dilution caused by the added volume. Additional IO_3^- could be formed, so that some amount of iodine/iodide is lost.

We examined the underlying mechanism by simulations integrating the respective ODEs. We found that it was not possible to simulate the experiments at pH 6.5 without reaction 7, the reverse reaction of reaction 4. This reaction seems to be crucial for the switching behavior and its reaction rate had to be rather high. Another crucial factor is the ratio of reaction rate constants k_6 to k_4. At pH 6.5 and at rather high hydrogen peroxide concentrations, the irreversible reaction 6 (gas production) is favored. While reaction 6 proceeds, iodide is recycled. So, on the one hand there is a feedback loop via reaction 6 to the start of reaction 1, and on the other hand in respect to reaction 4 and to the overall production of iodine, hydrogen peroxide displays classical substrate inhibition. The recyling of iodide makes it possible that the rate of reaction 4 stays constant for constant HOI in the course of the reaction, but the rate of reaction 6 decreases with the constantly decreasing hydrogen peroxide concentration. At some point, the rate of reaction 6 is comparatively low and HOI starts to accumulate—reaction 4 is switched on.

Adjusting rate constants which are influenced by the proton concentration, we also successfully simulated the experimental results at pH 5.2 (Figure 8). At pH 5.2 reaction 4 is favored in comparison to reaction 6, because reaction 4 is faster at pH 5.2 and reaction 6 is slower. The fact that it was possible to simulate different types of behavior with one model is strong evidence that the mechanism employed agrees in most aspects with the proposed model.

We studied alternative mechanisms for our system. We found it difficult to get plausible results with a mechanism which prceeds only via EOI and not HOI. We suggest a possible reason for this observation: EOI is rigidly controlled and is not able to accumulate easily in a sufficient manner because of the limited amount of enzyme in the experiment. We conclude that HOI, not only EOI or EO, is produced as an intermediate at this pH.

To examine the importance of the enzyme for the observed behavior, we studied the inorganic oxidation of iodide experimentally and theoretically. We could not observe any behavior in the respective experiments at pH 6.5 which resembled the findings in the enzymatic experiments. Simulating this reaction with various rate constants did not succeed either. Inclusion of an artificial major intermediate into the mechanism for the inorganic reaction made it possible to produce timefuse behavior. We conclude that at least one major intermediate between the reactants and the branching point, i.e., the point where the feedback loops originate, is necessary to produce timefuse behavior in such a system. This is obviously fulfilled in the enzymatic reaction. This finding is in accordance to the reported observation that in some cases the ability of a system to produce highly nonlinear behavior by means of feedback loops is strongly dependent on the presence of intermediates in the respective loops.[32−34]

To summarize, we found nonliner kinetic behavior in the peroxidase-catalyzed oxidation of iodide which represents an enzymatic iodine clock and a dynamic enzymatic switch. We were able to simulate the respective experiments and conclude that the following factors are crucial for these unique properties: (1) A high rate constant k_7, (2) A ratio of k_4 to k_6, favoring reaction 6 at pH 6.5 if hydrogen peroxide concentration is high, (3) The presence of feedback reaction 6 recycling iodide, (4) The occurrence of HOI, (5) The existence of at least one major intermediate between the substrates and the branching point HOI.

We clearly demonstrated that autonomous enzymatic switches in simple reaction systems exist in vitro. The simulation of such strongly nonlinear behavior helps to determine the mechanism of the respective reaction, because the resulting kinetics is strongly dependent on the individual steps and the way these steps interact with each other. The studied reaction plays an important role in vivo and because the observed behavior depends mainly on the pH of the reaction (which is in the physiological range) and on concentration ratios of the reactants, the behavior might well play a role in vivo. Excess hydrogen peroxide can be discarded without affecting the concentration of iodide. An important role in the regulation of hydrogen peroxide, a known second messenger, is possible. According

to our results, excess hydrogen peroxide could be destroyed via the pseudocatalatic path while the limited iodide concentration stays constant until a certain level of hydrogen peroxide concentration is reached.

Acknowledgment. We would like to thank the DFG and the Fonds der Chemischen Industrie for financial support.

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