

pH oscillations in the hemin–hydrogen peroxide–sulfite reaction

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Oscillatory behaviour in the pH value has been observed during the oxidation of sulfite by hydrogen peroxide mediated by hemin, a well known enzyme model compound, in a continuous-flow stirred tank reactor. The dynamics of this reaction has been studied for a variety of flow rates of the reactants. As the flow rates increase, the oscillations evolve from relaxation oscillations to more complex shapes, displaying, among others, bursting behaviour. A reaction mechanism is proposed that involves the autocatalytic oxidation of HSO_3^- by H_2O_2 , while slow equilibria between different pH-dependent forms of hemin account for the feedback loop which gives rise to oscillatory dynamics. It is shown in experiments that no participation of CO_2 is required for oscillations to occur.

Introduction

Oscillatory dynamics are of great importance in biochemical and biophysical reaction systems.^{1,2} Over the last decades, numerous examples of oscillating biochemical reactions have been reported, most of which involve a vast number of components and rather complicated reaction pathways. The complexity of such mechanisms allows for a precise and fine tuned regulation of biological metabolism. However, the high degree of complexity hampers the fundamental understanding of the underlying mechanisms which lead to such nonlinear behaviour.

The increasing evidence for the importance of biochemical and biophysical oscillatory processes has brought new life to the study of simple biochemical oscillatory reactions. The most simple conceivable oscillators consist of reactions which comprise single enzymes and their substrates. So far, only a surprisingly scant number of such simple enzymatic systems are known, beyond any doubt, to oscillate. Simple biochemical oscillators include the well-studied peroxidase–oxidase reaction (see reviews, ref. 3–5), a catalase–ascorbate–oxygen system,⁶ and a biomimetic cytochrome P450 reaction system.⁷ However, the latter system is already more complex as it inherently involves transport across a phospholipid membrane.

In parallel to studies of oscillatory enzyme systems, considerable research efforts have been dedicated to the design and investigation of simple chemical reaction systems which show oscillations in the pH of the reaction medium. The first reaction showing this property was designed systematically a decade ago and involved hydrogen peroxide, sulfite and hexacyanoferrate.⁸ Mechanistically, this system is composed of two subsystems, an autocatalytic reaction, as realised in the pH-dependent oxidation of HSO_3^- by H_2O_2 , and a negative feedback which consumes

H^+ .^{8,9} Later, modifications in the nature of the feedback reactions gave rise to a family of pH oscillators;^{10–15} among these, it is important to mention a prototypical minimal system that involves HSO_3^- , H_2O_2 and hydrogen carbonate. It has recently been shown that even traces of hydrogen carbonate may be sufficient to establish an efficient negative feedback loop.^{10,13}

The dynamics observed in this family of pH oscillators are very rich. When investigated as an open system (*i.e.* operated in continuous-flow stirred tank reactors, CSTR), coexisting stationary states,¹⁶ as well as periodic^{9,10,13,15} and chaotic^{11,12} oscillations have been generated. The light-sensitivity of hexacyanoferrate has been exploited to manipulate the dynamics of this variant of the pH oscillator by applying suitable illumination.¹²

In biological systems, changes in the pH of the reaction medium play a prominent role, since many physiological parameters are affected by the pH value, *e.g.* the activity of enzymes or the permeability of membranes. Propagating waves of protons have also been observed in a cellular medium, as in the case of yeast cells during glycolysis¹⁷ and in neutrophil cells.¹⁸

We are interested in studying a minimal pH oscillator which involves an enzyme or an enzyme model compound. To this purpose, we chose hemin, which is known to be part of the active centres of many enzymes that carry a heme as prosthetic group. Thus, hemin plays the role of a model substrate for a variety of enzymes which possess such a structural feature. In the present article, we study the dynamics displayed by a reaction system which combines the autocatalytic step of the pH oscillators with a feedback loop which is provided by reactions of the enzyme model compound hemin.

Experimental

Reactant solutions were prepared daily from reagent grade H_2O_2 (Baker), hemin (Fluka), Na_2SO_3 (Merck) and H_2SO_4 (Riedel-de Haën) without further purification. Water produced by an ion-exchanger and a membrane filtration purifying system was used to prepare the solutions. All solutions were stored in the dark in a refrigerator (at 8 °C). Stock solutions were discarded 36 h after preparation.

The H_2O_2 solution was prepared by dilution of a 30% stock solution. Its H_2O_2 content was checked spectrophotometrically using three wavelengths in the range 210–250 nm.¹⁹ The 0.075 M sulfite solution was prepared by dissolving Na_2SO_3 in N_2 or argon-saturated aqueous solutions that contained 0.2 mM H_2SO_4 . Finally, hemin was dissolved in a 40 μM NaOH solution, since this enzyme model compound is insoluble in acidic or neutral aqueous medium. To obtain complete dissolution of the hemin crystals, solutions of hemin in alkaline medium were shaken for 12 h prior to use.

For experiments under argon atmosphere, the water was bubbled with argon for 30 min prior to the preparation of the stock solutions. During the preparation of these solutions and while transferring them to the gas-tight syringes, an argon stream was passed over the solutions. Bubbling was avoided in order to prevent the loss of any volatile compounds. During the experiments a stream of argon was supplied to the head volume of the CSTR to prevent the uptake of CO_2 from the atmosphere.

The reactant solutions were stored in gas-tight syringes (50 ml) that were placed in the thermostating block of the syringe pump. Immediately prior to the start of an experiment, the reactor was filled with 15 ml of each of the three reactant solutions. The experiment was subsequently started by activating the step-motor driven syringe pump, which supplied the reactants to the reactor. All experiments were carried out at 25.0 ± 0.3 °C.

The dynamics of the hemin system were studied in a water-jacketed glass CSTR with a liquid volume of 45 ml. The reactant solutions were supplied to the reactor through three Teflon tubes which enter the reactor through its lid. The ends of the tubes were immersed in the reaction solution in the CSTR. Excess liquid was removed from the reactor by a capillary connected to an aspirator. A glass pH electrode (Mettler-Toledo) was used to monitor the dynamics of the reaction. It is connected to a computer *via* an A/D converter and to a x,t -chart recorder. Data is stored on a computer for later analysis.

The dynamic behaviour of the hemin– H_2O_2 –sulfite reaction system was investigated at different flow rates of the reactants. The flow rate k_f , which is the reciprocal residence time τ of the reactants in the CSTR, is used as the bifurcation parameter in all CSTR experiments. Since the reactor

is fed by three reactant streams containing 50–100 μM hemin, 0.1 M H_2O_2 , and 0.075 M Na_2SO_3 , respectively, the reactor concentration of these reactants is one third that of the corresponding stock solutions.

Results

CSTRs allow a reaction system to settle on its asymptotic dynamic states. Therefore, the subsequent experiments were performed under CSTR operation mode. The flow rate of the reactants through the reactor was varied and the dynamic behaviour of the reaction system was monitored by a pH-sensitive electrode.

At low flow rates (*i.e.* $k_f < 7.0 \times 10^{-4} \text{ s}^{-1}$), the hemin- H_2O_2 -sulfite reaction settles onto an acidic stationary state, the pH of which lies below ~ 6.5 . When the flow rate exceeds the critical value of $k_{f, \text{crit}} = 7.0 \times 10^{-4} \text{ s}^{-1}$, the reaction system begins to show oscillatory behaviour (Fig. 1(a)). At flow rates slightly above $k_{f, \text{crit}}$, these periodic oscillations are sinusoidal. As the flow rates increase further, the amplitudes of the oscillations grow significantly (Fig. 2), and sharp peaks

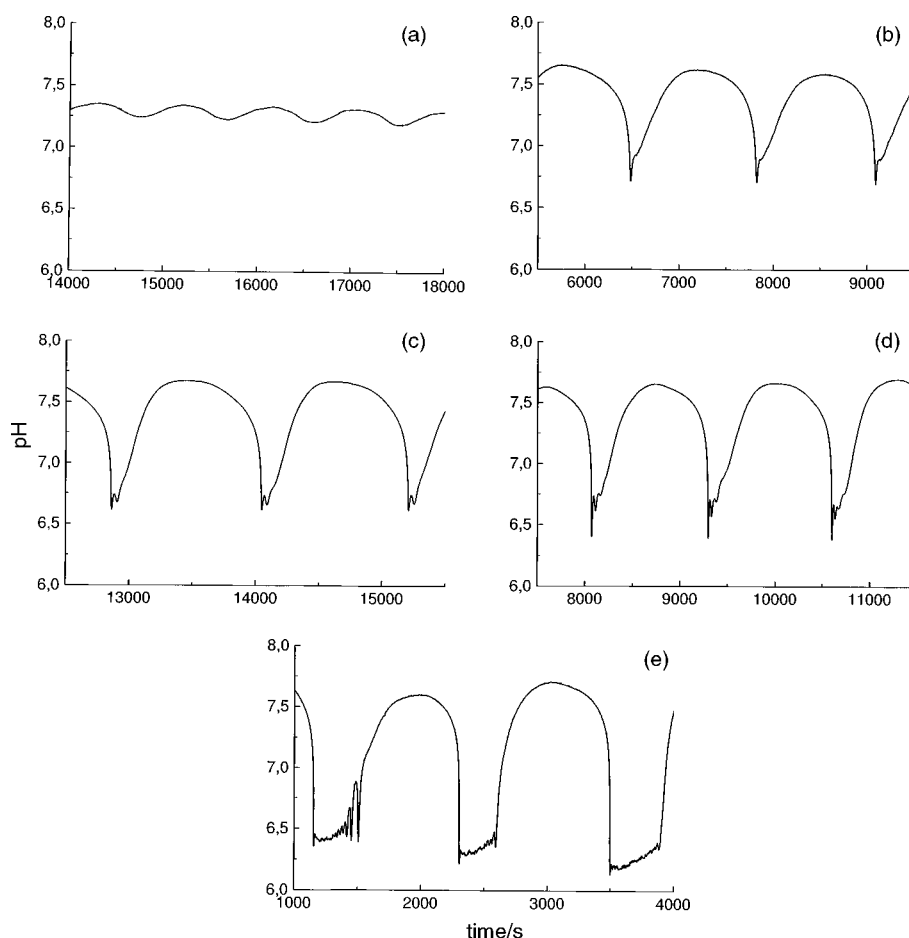


Fig. 1 Oscillations observed for increasing values of the flow rate k_f in the hemin- H_2O_2 - SO_3^{2-} system under atmosphere. (a) At $k_f = 7.4 \times 10^{-4} \text{ s}^{-1}$ the oscillations are sinusoidal and have low amplitudes. (b) At $k_f = 1.3 \times 10^{-3} \text{ s}^{-1}$ the oscillations still have a simple periodicity, however, the peaks in the acidic domain become sharper. (c) At $k_f = 1.5 \times 10^{-3} \text{ s}^{-1}$ a second minimum arises in the acidic region, thus yielding a bursting oscillation composed of one large-amplitude oscillation and one small-amplitude oscillation. (d) Bursting oscillations, consisting of one large-amplitude oscillation and two small-amplitude oscillations (at $k_f = 1.8 \times 10^{-3} \text{ s}^{-1}$) and (e) oscillations where bursting plays a prominent role, at $k_f = 2.2 \times 10^{-3} \text{ s}^{-1}$.

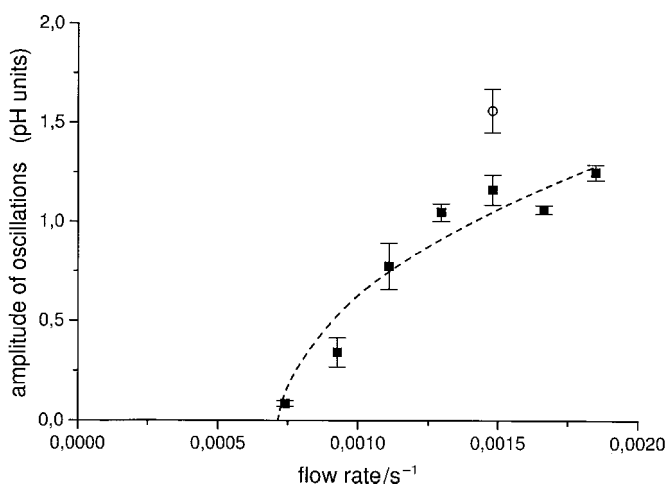


Fig. 2 Dependence of the amplitude of the oscillations on the flow rate k_f of reactants into the CSTR. Data obtained (■) under atmosphere, (○) under argon. The dashed line represents a fit of a square root dependence of the amplitude of oscillations A on the distance of the onset of oscillations to the data, according to $A = A_0(k_f - k_{f, \text{crit}})^{1/2}$, with $k_{f, \text{crit}} = 7.0 \times 10^{-4} \text{ s}^{-1}$ and $A_0 = 38$.

develop at low pH region of the oscillations (Fig. 1(b)). The growth rate of the amplitudes is well described by a square root dependence of the amplitude A from the difference between the actual flow rate k_f and the critical flow rate $k_{f, \text{crit}}$, i.e. $A = A_0(k_f - k_{f, \text{crit}})^{1/2}$. By contrast, the periods of the oscillations are much less affected by the rise in k_f , as seen in Fig. 3.

Upon further increases in the flow rate, the simple periodicity of the oscillations no longer persists. Instead, we observe the emergence of bursting oscillations at $k_f \sim 1.4 \times 10^{-3} \text{ s}^{-1}$. The oscillations develop a second minimum in the pH, which is characterised by a much lower amplitude than the original minimum (Fig. 1(c)). The initial transition occurs from “simple” periodic oscillations with a single periodicity to periodic oscillations whose oscillatory cycle comprises one large-amplitude oscillation and one small-amplitude oscillation (Fig. 1(c)). As the flow rate is further increased, the number of small-amplitude oscillations augments by one, resulting in oscillatory cycles composed of one large-amplitude oscillation and two small amplitude oscillations

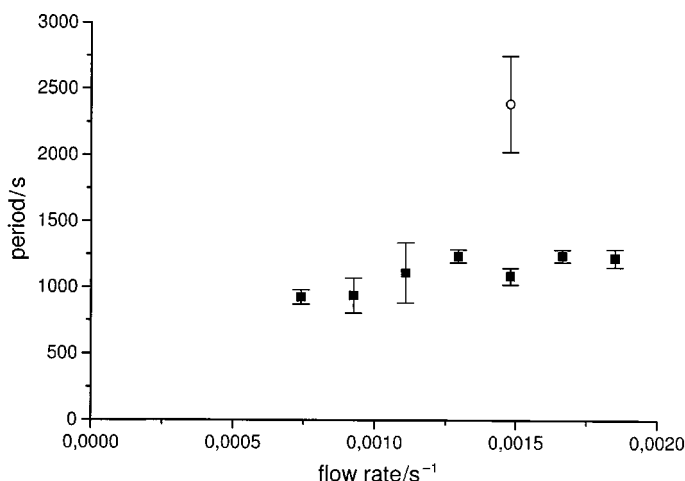


Fig. 3 Dependence of the period of the oscillations on the flow rate k_f of reactants into the CSTR. Data obtained (■) under atmosphere, (○) under argon.

(Fig. 1(d)). The number of such small bursts augments continuously as the flow rate increases. Such behaviour is known for mixed-mode oscillations which follow a so-called period-adding scenario.^{20–22} At high flow rates these small-amplitude bursts become a quite prominent feature. One observes oscillations that perform a long series of such spikes when the oscillation is in the acidic part of the oscillatory cycle (Fig. 1(e)). At high flow rates, the reaction system spends almost as much time in the acidic part of the oscillatory cycle as in its alkaline part. Finally, at even higher values of the flow rate (*i.e.* at $k_f > 3.3 \times 10^{-3} \text{ s}^{-1}$) the oscillations cease and give way to an alkaline stationary state whose pH is above ~ 7.6 .

The experiments described so far were conducted under aerobic conditions, *i.e.* the reactant solutions and the reactor were equilibrated with the atmosphere. However, Rábai and Hanazaki¹⁰ and Frerichs and Thompson¹³ reported that even trace amounts of hydrogen carbonate may generate a HCO_3^- -driven feedback loop and hence induce oscillatory behaviour. In order to assess the contribution of CO_2 and O_2 to the oscillatory dynamics of the hemin- H_2O_2 -sulfite reaction system, we repeated the CSTR experiments using an argon atmosphere instead of aerobic conditions. To eliminate traces of CO_2 and O_2 from participating in the reaction, all solutions were prepared under argon. The head space of the CSTR was flushed with argon throughout the course of the experiments.

As in the experiments under atmosphere, the reaction under argon also showed oscillatory dynamics, which comprised simple periodic as well as bursting oscillations (Fig. 4). However, for given values of the flow rates, the amplitudes of the oscillations were found to be larger than those obtained under aerobic conditions (Fig. 2). Similarly, the period of oscillations was observed to be almost twice as long under anaerobic conditions as the experiments run under aerobic conditions (Fig. 3). Due to the lengths of the oscillatory periods (*ca.* 40 min) only a few oscillations could be detected in the present reactor (of 45 ml volume). In addition, the dynamics depicted in Fig. 4 is seen to be still transient. At present, experiments are under way to determine the asymptotic dynamics of this system under argon.

Finally, we conducted a series of experiments to assess whether the hemin- H_2O_2 -sulfite reaction may also show oscillatory behaviour under batch conditions, *i.e.* in a reactor without any inflow or outflow of chemicals. Under batch conditions, we did not detect oscillations, however, by operating the reactor under fed-batch conditions, damped oscillations of the pH value could be observed. Damped oscillations (not shown) lasted for up to 30 min, before the reaction settled to a stationary state at around $\text{pH } 7.2 \pm 0.5$. It is worth noting that the window of reaction conditions leading to damped oscillations is narrow. If the reaction is performed under reaction conditions

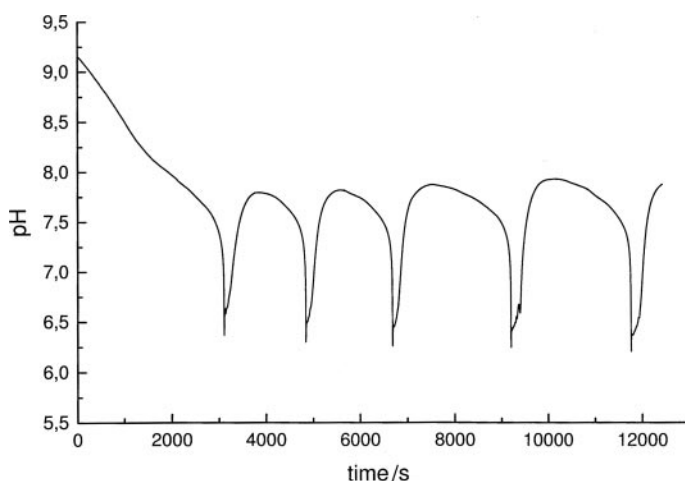


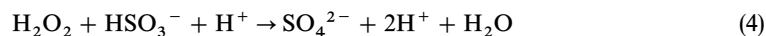
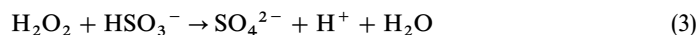
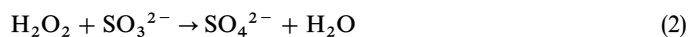
Fig. 4 Dynamical behaviour of the hemin- H_2O_2 - SO_3^{2-} system under argon atmosphere at $k_f = 1.5 \times 10^{-3} \text{ s}^{-1}$. Note that the shapes of the oscillations are similar to those shown in Fig. 1. However, the dynamics are still in the transient regime, due to the long duration of the period of the oscillations.

that do not lead to damped oscillations, the reaction settles in stationary states which are characterized either by a very high pH (9–10) or a very low pH (3–4), respectively.

Mechanism

The hemin oscillator belongs to the family of pH oscillators based on the oxidation of sulfite by H_2O_2 . These chemical oscillators share a common set of reactions that yield an autocatalytic production of H^+ , while they differ in the H^+ -consuming reactions that are involved in the feedback loop required for oscillatory dynamics.

The reactions involved in the autocatalytic oxidation of sulfite by H_2O_2 are well-established,^{8–15} and their rate constants have been reinvestigated recently in dependence on the temperature.¹⁶ The autocatalytic part of the mechanism comprises the protonation/deprotonation of SO_3^{2-} (eqn. (1)), and the reduction of H_2O_2 by SO_3^{2-} (eqn. (2)) and by HSO_3^- (eqn. (3) and (4)):

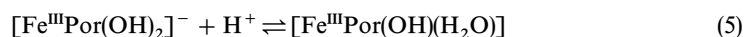


It is important to emphasise that the oxidation of HSO_3^- by H_2O_2 (eqn. (3) and (4)) is pH dependent and that it is autocatalytic in protons in an acidic medium (eqn. (4)).

The remaining task is to establish which reactions are involved in the negative feedback loop, *i.e.* which reactions slowly consume H^+ . In the following, we discuss which reactions may take over this function in the hemin– H_2O_2 – SO_3^{2-} system.

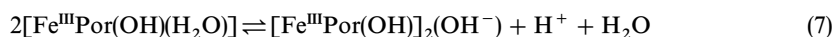
It has been shown recently that low concentrations of CO_2 and HCO_3^- may give rise to a negative feedback loop.^{10,13} To test whether these compounds are responsible for the occurrence of oscillations in the hemin oscillator, we performed experiments under argon atmosphere, which exclude the participation of CO_2 and HCO_3^- in the reaction. As in the experiments run in contact with air, we also observed oscillations under argon atmosphere conditions (Fig. 4). Therefore, we conclude that participation of CO_2 and HCO_3^- is not essential for the emergence of a feedback mechanism. In other words, other H^+ -consuming reactions provide the feedback steps of the hemin– H_2O_2 – SO_3^{2-} system.

A plausible source for the negative feedback is the acid–base behaviour of aqueous hemin solutions. In an aqueous medium hemin may coordinate either one of two water molecules or hydroxy groups to the iron atom. Which and how many of these ligands are coordinated is strongly dependent on the pH.^{23–26} In aqueous alkaline solutions, hemin shows acid–base equilibria between a porphyrin which coordinates with two hydroxy ligands $[\text{Fe}^{\text{III}}\text{Por}(\text{OH})_2]^-$, a porphyrin coordinating to one hydroxy and one aquo ligand $[\text{Fe}^{\text{III}}\text{Por}(\text{OH})(\text{H}_2\text{O})]$ and a porphyrin coordinating to two aquo ligands $[\text{Fe}^{\text{III}}\text{Por}(\text{H}_2\text{O})_2]^+$ (in this notation Fe and Por stand for the central iron atom and the porphyrin moiety of hemin, respectively):



The $\text{p}K_{\text{a}}$ value of equilibrium (5) has been determined as $\text{p}K_{\text{a}(5)} \sim 13$ while the $\text{p}K_{\text{a}}$ values reported for equilibrium (6) vary from 4.8²³ to ~ 6.5 .²⁴ Due to its high $\text{p}K_{\text{a}}$ value, equilibrium (5) can be disregarded when modelling the hemin oscillator.

In addition to equilibria (5) and (6), hemin is also known to form μ -oxo-dimers, which in turn are in H^+ -dependent equilibrium with monomer species. Of interest for the hemin oscillator is the equilibrium between the monomers $[\text{Fe}^{\text{III}}\text{Por}(\text{OH})(\text{H}_2\text{O})]$ and the corresponding μ -oxo-dimers:



for which $\text{p}K_{\text{a}}$ values of 7.1²⁵–7.24²⁶ have been determined. These equilibria show that, depending on the pH of the reaction medium, hemin may exchange ligands, thus releasing or consuming H^+ .

In our model, we include the autocatalytic reactions (1)–(4), as well as the acid–base equilibria (6) and (7) involving hemin species. The latter two acid–base equilibria form the feedback reactions in the hemin oscillator. This mechanism is able to model the essential dynamical features observed in the experiments.

Discussion

In the present paper we investigated oscillations of the pH value that occur during the hemin mediated oxidation of sulfite by hydrogen peroxide. Different types of oscillatory behaviour were detected as a function of the flow rates of the reactants through the CSTR. Oscillations begin at $k_f, \text{crit} = 7.0 \times 10^{-4} \text{ s}^{-1}$ and their amplitudes grow with increasing flow rates. The exponent of the increase in amplitude is found to be 0.5, in good agreement with predictions for supercritical Hopf bifurcations.²⁷ At flow rates exceeding $1.4 \times 10^{-3} \text{ s}^{-1}$ the simple relaxation oscillations give way to mixed-mode or bursting oscillations. The shape of these oscillations is reminiscent of those observed in other physiological systems,^{1,2,28} like in the oscillations of Ca^{2+} in hepatocytes stimulated by adenosine triphosphate (ATP).²⁹

Upon a continuous increase in the flow rate the number of bursting oscillations per oscillatory cycle successively increases by one burst. Thus, the complexity of the periodic (bursting) state as a function of k_f increases by following a so-called period-adding sequence. Bursting behaviour has previously been reported for other, inorganic, pH oscillators which also pertain to the family of pH oscillators involving the oxidation of SO_3^{2-} by H_2O_2 .^{30,31} However, to our knowledge, a period-adding sequence in response to changes in a bifurcation parameter has not yet been observed in a member of the SO_3^{2-} – H_2O_2 –family of pH oscillators.

Period-adding sequences have already been observed in an enzymatic reaction system, the peroxidase–oxidase (PO) reaction.^{20,21} The major difference between the period-adding sequences of the PO system and the hemin– SO_3^{2-} – H_2O_2 system is that, so far, chaotic dynamics have not been detected in the hemin system. However, in both reaction systems, the period-adding sequences allow for a fine tuning of the shape of the bursting oscillations by small changes in the bifurcation parameter. Given that the shape of bursting oscillations may be the “carrier” of physiological information, *e.g.* as known for Ca^{2+} oscillations,²⁹ the possibility to tune the shape of the oscillations may be of physiological relevance for these enzyme and enzyme model systems.

The active centres of the peroxidases, that support the oscillating PO reaction, contain a heme group which structurally and chemically is very similar to hemin. Thus, hemin is predestined to serve as a model compound for mimicking processes occurring at the reactive centre of the peroxidases and other heme-containing enzymes.³²

Interestingly, horseradish peroxidase has been found to catalyse the oxidation of sulfite by H_2O_2 .^{33–35} However, the peroxidase-catalysed reaction of SO_3^{2-} and H_2O_2 proceeds without production or consumption of H^+ by peroxidase. In addition, experiments with denaturated horseradish peroxidase, *i.e.* an enzyme whose protein part is irreversibly altered, do not support the oxidation of sulfite by H_2O_2 .³⁵ These findings indicate that there is a difference between the hemin– H_2O_2 –sulfite reaction system and the peroxidase-catalysed oxidation of sulfite by H_2O_2 : While the protein moiety of peroxidase is involved in the enzymatic catalysis, the hemin system neglects any contribution that does not stem directly from processes occurring at the hemo-porphyrin. Thus, hemin can serve as a model compound for processes that occur at the active centres of heme-carrying enzymes.

In the hemin– H_2O_2 –sulfite reaction system, hemin is actively involved in the feedback reactions which consume H^+ . Preliminary experiments performed under argon atmosphere (Fig. 4) indicate that the hemin system displays the same dynamic behaviour as under air (Fig. 1). Thus, it is concluded that neither CO_2 nor HCO_3^- are essential to induce oscillatory and bursting behaviour in the hemin system. However, the difference in period and amplitude of oscillations observed for identical experimental conditions, but for different compositions of the gas-phase, show that—albeit not essential to generate oscillations— CO_2 and HCO_3^- contribute to the dynamics of the hemin system under atmospheric conditions.

To conclude, the hemin– H_2O_2 –sulfite reaction system can be considered as a new member of the family of pH oscillators that have the reaction of H_2O_2 and sulfite reaction in common. In addition to its rich dynamics (which is presently under investigation), an attractive feature of the

hemin-H₂O₂-sulfite system is the biological relevance of hemin as an enzyme model compound which can mimic processes that take place at the heme groups of enzymes. In this context, an interesting function is that of a model for catalases. These enzymes promote the disproportionation of H₂O₂ and they are known to be involved in oscillatory reactions.⁶

Acknowledgements

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References

- 1 A. Goldbeter, *Biochemical Oscillations and Cellular Rhythms. The Molecular Bases of Periodic and Chaotic Behaviour*, Cambridge University Press, Cambridge, 1996.
- 2 L. Glass and M. C. Mackey, *From Clocks to Chaos. The Rhythms of Life*, Princeton University Press, Princeton, 1988.
- 3 R. Larter, L. F. Olsen, C. G. Steinmetz and T. Geest, in *Chaos in Chemistry and Biochemistry*, ed. R. J. Field and L. Györgyi, World Scientific, Singapore, 1993, pp. 175–224.
- 4 A. Scheeline, D. L. Olson, E. P. Williksen, G. A. Horras, M. L. Klein and R. Larter, *Chem. Rev.*, 1997, **97**, 739.
- 5 M. J. B. Hauser and L. F. Olsen, in *Transport and Structure—Their Competitive Roles in Biophysics and Chemistry*, ed. S. C. Müller, J. Parisi and W. Zimmermann, Springer, Heidelberg, 1999, pp. 252–272.
- 6 A. J. Davison, A. J. Kettle and D. J. Fatur, *J. Biol. Chem.*, 1986, **261**, 1193.
- 7 A. P. H. J. Schenning, J. H. Lutje Spelberg, M. C. P. F. Driessen, M. J. B. Hauser, M. C. Feiters and R. J. M. Nolte, *J. Am. Chem. Soc.*, 1995, **117**, 12655.
- 8 Gy. Rábai, K. Kustin and I. R. Epstein, *J. Am. Chem. Soc.*, 1989, **111**, 3870.
- 9 Gy. Rábai and I. Hanazaki, *J. Phys. Chem.*, 1994, **98**, 2592.
- 10 Gy. Rábai and I. Hanazaki, *J. Phys. Chem.*, 1996, **100**, 10615.
- 11 Gy. Rábai, *J. Phys. Chem. A*, 1997, **101**, 7085.
- 12 Gy. Rábai and I. Hanazaki, *J. Am. Chem. Soc.*, 1997, **119**, 1458.
- 13 G. A. Frerichs and R. C. Thompson, *J. Phys. Chem. A*, 1998, **102**, 8142.
- 14 V. Vanag, *J. Phys. Chem. A*, 1998, **102**, 601.
- 15 Gy. Rábai, N. Okazaki and I. Hanazaki, *J. Phys. Chem. A*, 1999, **103**, 7224.
- 16 I. Hanazaki, N. Ishibashi, H. Mori and Y. Tanimoto, *J. Phys. Chem. A*, 2000, **104**, 7695.
- 17 Th. Mair and S. C. Müller, *J. Biol. Chem.*, 1996, **271**, 627.
- 18 H. R. Petty, R. G. Worth and A. L. Kindzelskii, *Phys. Rev. Lett.*, 2000, **84**, 2754.
- 19 M. S. Morgan, P. F. van Trieste, S. M. Garlick, M. J. Mahon and A. L. Smith, *Anal. Chim. Acta*, 1988, **215**, 325.
- 20 M. J. B. Hauser and L. F. Olsen, *J. Chem. Soc., Faraday Trans.*, 1996, **92**, 2857.
- 21 M. J. B. Hauser, L. F. Olsen, T. V. Bronnikova and W. M. Schaffer, *J. Phys. Chem. B*, 1997, **101**, 5075.
- 22 K. P. Zeyer and F. W. Schneider, *J. Phys. Chem. A*, 1998, **102**, 9702.
- 23 O. S. Ksenzhek and S. A. Petrova, *Bioelectrochem. Bioenerg.*, 1978, **5**, 661.
- 24 C. Bartocci, F. Scandola, A. Ferri and V. Carassiti, *Inorg. Chim. Acta*, 1979, **37**, L473.
- 25 T. Uno, A. Takeda and S. Shimabayashi, *Inorg. Chem.*, 1995, **34**, 1599.
- 26 M. F. Zipplies, W. A. Lee and T. C. Bruice, *J. Am. Chem. Soc.*, 1986, **108**, 4433.
- 27 R. Seydel, *From Equilibrium to Chaos—Practical Bifurcation and Stability Analysis*, Elsevier, New York, 1988.
- 28 J. Keener and J. Sneyd, *Mathematical Physiology*, Springer, New York, 1998.
- 29 C. J. Dixon, N. M. Woods, K. S. R. Cuthbertson and P. H. Cobbold, *Biochem. J.*, 1990, **269**, 499.
- 30 Gy. Rábai and I. Hanazaki, *J. Phys. Chem. A*, 1999, **103**, 7268.
- 31 Gy. Rábai, A. Kaminaga and I. Hanazaki, *Chem. Commun.*, 1996, 2181.
- 32 Z. Genfa and P. K. Dasgupta, *Anal. Chem.*, 1992, **64**, 517.
- 33 T. Arais, K. Miyoshi and I. Yamazaki, *Biochemistry*, 1976, **15**, 3059.
- 34 C. Mottley, R. P. Mason, C. F. Chignell, K. Sivarajah and T. E. Eling, *J. Biol. Chem.*, 1982, **257**, 5050.
- 35 C. Mottley, T. B. Trice and R. P. Mason, *Methods Enzymol.*, 1982, **22**, 732.