ELSEVIER

Biophysical Chemistry 72 (1998) 201–210

Biophysical Chemistry

From bistability to oscillations in a model for the isocitrate dehydrogenase reaction

Gianluca M. Guidi, Albert Goldbeter*

Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C.P. 231, B-1050 Brussels, Belgium

Revision received 14 January 1998; accepted 13 February 1998

Abstract

Considered is a bienzymatic system consisting of isocitrate dehydrogenase (IDH, EC 1.1.1.42), which transforms $NADP^+$ into NADPH, and of diaphorase (DIA, EC 1.8.1.4), which catalyzes the reverse reaction. Experimental evidence as well as a theoretical model show the possibility of a coexistence between two stable steady states in this reaction system. The phenomenon originates from the regulatory properties of IDH. We extend the analysis of a theoretical model proposed for the IDH–DIA bienzymatic system and investigate the occurrence of different modes of bistability, with or without hysteresis, i.e. in the presence of two or only one limit point bounding the domain of multiple steady states. The analysis indicates that the two types of bistability may sometimes be observed sequentially as a given control parameter is progressively increased. We further obtain conditions in which sustained oscillations develop in the model. These results establish the isocitrate dehydrogenase reaction coupled to diaphorase as a suitable candidate for further experimental and theoretical studies of bistability and oscillations in biochemical systems.

Keywords: Bistability; Oscillations; Isocitrate dehydrogenase reaction

1. Introduction

The occurrence of sustained oscillations and the coexistence between two stable steady states – a phenomenon known as bistability – represent two of the most common modes of dynamic or functional (rather than structural) self-organization in regulated biochemical systems. Although several experimental examples of bistability [1–4] and oscillations are known in biochemistry (see Ref. [5] for a recent review of biochemical oscillations), the number of these examples remains reduced. It is therefore of interest to investigate possible new biochemical reactions capable of giving rise to these self-organization phenomena.

The experimental study of the kinetic properties of isocitrate dehydrogenase (IDH, EC 1.1.1.42) isolated from beef liver has shown that this enzyme reaction is subjected to a variety of positive and negative feedback regulations exerted by its substrate $NADP^+$ and its product NADPH [6–10]. Building on these results, Carlier [10] has obtained experimental evidence for bistable behavior in the IDH reaction when the latter is coupled to a second reaction, catalyzed by diaphor-

* Corresponding author. Tel.: +32 2 6505772; fax: +32 2 6505767; e-mail: agoldbet@ulb.ac.be

0304-3835/98/$19.00
PII S0301-4622(98)00134-3

ase (DIA, EC 1.8.1.4), which transforms NADPH back into $NADP^+$. A theoretical model for the IDH–DIA reaction system has been developed to account for these experimental observations [11]. Here we wish to present further results obtained by means of this model, both with regard to bistability and to the possible occurrence of sustained oscillations.

After recalling the model and its kinetic equations, we first show the occurrence of different modes of bistability, with or without hysteresis. While in the first case, the two stable branches of steady states are connected in two limit points by a branch of unstable steady states (which gives rise to a typical S- or N-shaped steady-state curve allowing hysteresis, as illustrated below in the inset of Fig. 6), the second type of bistability is encountered when one branch of stable steady states is no longer connected to the other stable branch. This situation, which occurs when one limit point vanishes or ceases to be physically accessible to the system, prevents hysteresis associated with reversible transitions between the two stable steady states and results in the possibility of irreversible transitions from one stable steady state to the other. Irreversible transitions have been observed in a number of biochemical models [11–18]. Apart from a few experimental studies [19–21], the phenomenon has not been extensively investigated, although it may well be of physiological significance.

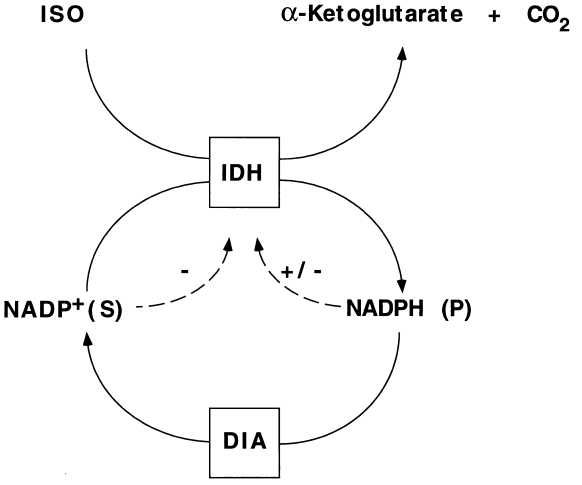

Fig. 1. The isocitrate dehydrogenase (IDH) reaction. The enzyme transforms tribasic isocitrate (ISO) and $NADP^+$ into α-ketoglutarate plus CO_2 and NADPH, respectively. Also indicated are the inhibition of IDH by $NADP^+$ as well as the inhibition and activation of this enzyme by NADPH. The system considered for bistability includes the reverse transformation of NADPH into $NADP^+$ catalyzed by the enzyme diaphorase (DIA).

Table 1

Experimental values of the constants for the IDH rate function [6–11].

k_0	2 s^{-1}
k_1	12.3 s^{-1}
K_m^I	17 μM
K_I	35 μM
K_A	0.25 μM
K_I'	7 μM
K_m^{III}	35 μM

In the last part of this work we show how the model for bistability in the IDH reaction can be extended to account for sustained oscillatory behavior. To this end, we incorporate into the model the variation of the second substrate–product pair, namely isocitrate and α-ketoglutarate. The results help to clarify the conditions in which oscillations might be observed experimentally in this reaction system and bring to light the link between bistability and oscillations.

2. Model for the isocitrate dehydrogenase reaction

The model considered for the transformation of $NADP^+$ into NADPH in the IDH reaction is shown in Fig. 1. The second substrate, isocitrate, can be considered as being in excess in the experiments [10,11], and its variation is not taken into account in the first stage. To demonstrate the occurrence of bistability, a second enzyme, diaphorase, was used to transform NADPH into $NADP^+$ [10,11]. The role of the second substrate of diaphorase, O_2, is also disregarded as it can be considered as remaining constant in the medium. The rate of the IDH reaction is governed by the function $f(S, P)$:

$$f(S,P) = k_0 \frac{E_I S}{K_m^I(1+P/K_I)+S}\left[1+\frac{k_1}{k_0}\frac{P}{K_A(1+S/K_I')+P}\right] \quad (1)$$

where P, S and E_I indicate the concentrations of NADPH, $NADP^+$ and IDH, respectively, and the constants are defined in Table 1.

The first term in the product accounts for the kinetic properties of the enzyme working with a minimum

turnover number, k_0, and includes the competitive inhibition of IDH by its product NADPH (P) with an inhibition constant K_I. The term between brackets describes the change in turnover number due to the activation by NADPH with an activation constant K_A, which leads to the maximum turnover $k_{max} = k_0 + k_1$. This term also includes the inhibition by the substrate, $NADP^+$ (S), characterized by the inhibition constant K_I'.

Diaphorase can be regarded as a Michaelian enzyme, showing a high affinity for its substrate NADPH. The kinetic expression describing the transformation of NADPH into $NADP^+$ by diaphorase is thus given by the function $g(P)$:

$$g(P) = \frac{k_{cat} E_{II} P}{K_m^{II} + P} \tag{2}$$

where $k_{cat} = 0.8\ s^{-1}$ and E_m indicates the diaphorase concentration while K_m^{II} denotes the Michaelis–Menten constant of the enzyme [8,10,11]. Experimentally, the value of K_m^{II} was not determined precisely, but was found to be less than 1 μM. In the following we have chosen $K_m^{II} = 0.01$ μM.

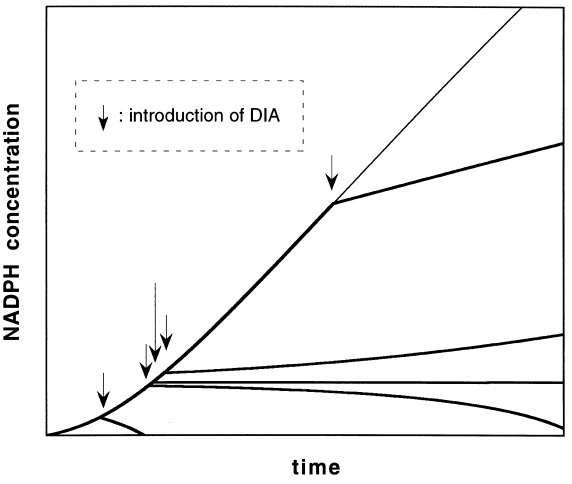

Fig. 2. Schematic representation of the experiment demonstrating bistability in the isocitrate dehydrogenase–diaphorase bienzymatic system [10,11]. The reaction is started in a medium containing $NADP^+$ and an excess of isocitrate, in the presence of IDH alone, which leads to the progressive accumulation of NADPH. The addition (vertical arrows) of the second enzyme, DIA, leads to the evolution towards a low or a high NADPH steady state, depending on the time of addition of diaphorase. When DIA is added at a critical time t^* (large arrow) separating these two evolutions, an intermediate, unstable steady-state level of NADPH is maintained during a prolonged amount of time which can be greater than 10 min [11].

The evolution of the system of two coupled enzyme reactions is governed by the following system of nonlinear kinetic equations:

$$\begin{aligned} \frac{dP}{dt} &= f(S,P) - g(P) \\ \frac{dS}{dt} &= -f(S,P) + g(P) \end{aligned} \tag{3}$$

where $f(S, P)$, given by Eq. (1), represents the transformation of $NADP^+$ into NADPH catalyzed by IDH, and $g(P)$, given by Eq. (2), pertains to the reverse transformation catalyzed by diaphorase. As the enzymes operate in a closed system with respect to $NADP^+$ and NADPH, the following conservation relation holds:

$$S + P = Z \tag{4}$$

where Z is the total, constant concentration of $NADP^+$ plus NADPH.

Eq. (4) allows us to reduce the two differential equations (Eq. (3)) to a single kinetic equation for P, while the second variable S is expressed as a function of P and of the total concentration Z:

$$\begin{aligned} S &= Z - P \\ \frac{dP}{dt} &= f(Z-P,P) - g(P). \end{aligned} \tag{5}$$

The system is at steady state when both enzymes operate at the same velocity so that the net production of NADPH and $NADP^+$ goes to zero:

$$f(Z-P,P) - g(P) = 0. \tag{6}$$

The NADPH concentration at steady state is given by the solutions to Eq. (6). Once Z is fixed, Eq. (6) reduces to a third-order polynomial equation in P [11] which admits one or three physically acceptable steady states, depending on parameter values.

3. Different modes of bistability, with or without hysteresis

The experiments which demonstrated the occurrence of bistability in the IDH–DIA reaction system are illustrated schematically in Fig. 2. In the presence of IDH alone, $NADP^+$ is transformed into NADPH in

the course of time, with a sigmoidal time evolution which reflects the activation of the enzyme by its product NADPH. If the second enzyme, diaphorase, is added at different times during this IDH-catalyzed accumulation of NADPH, the outcome can be either a decrease of NADPH down to a low level – this occurs when DIA is added early on (see first two arrows in Fig. 2) – or an increase up to a high level of NADPH – the latter evolution is observed when the same amount of DIA is added at a later stage (see last two arrows in Fig. 2). When the addition of DIA is made at a critical time value t^* [11], the current NADPH level is maintained constant over a long period of time (see large arrow in Fig. 2).

The analysis of the model for the IDH–DIA reaction system indicates that the behavior observed in the experiments schematized in Fig. 2 can be interpreted in terms of bistability [11]. The model indeed shows that for appropriate parameter values (see below), the bienzymatic system can evolve to either one of two stable steady states, separated by an unstable one. The theoretical results of Fig. 3 reproduce the effect of adding diaphorase at different times in a reaction medium containing only IDH initially. The inset in Fig. 3 shows that early addition of DIA leads to a low steady-state level of NADPH, while the addition after the critical time leads to the higher steady-state

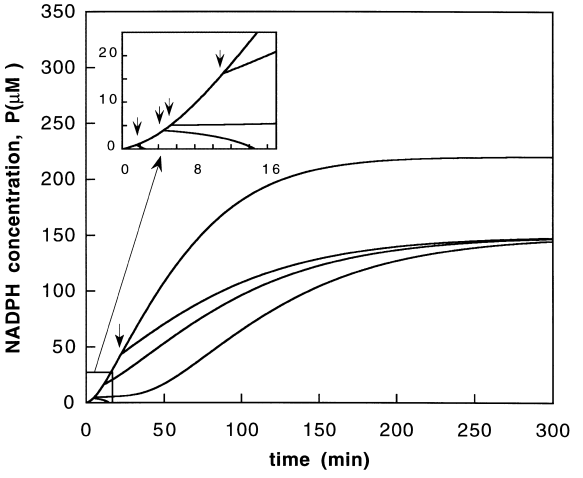

Fig. 3. Numerical simulations of the model reproducing the experimental observations on bistability shown schematically in Fig. 2. The inset represents an enlargement of the boxed domain, clarifying the system's behavior at early times. The results are obtained by numerical integration of (Eq. (3)) for the following parameter values: $Z = 220\ \mu M$, $E_I = 4$ nM, $E_{II} = 0.031\ \mu M$.

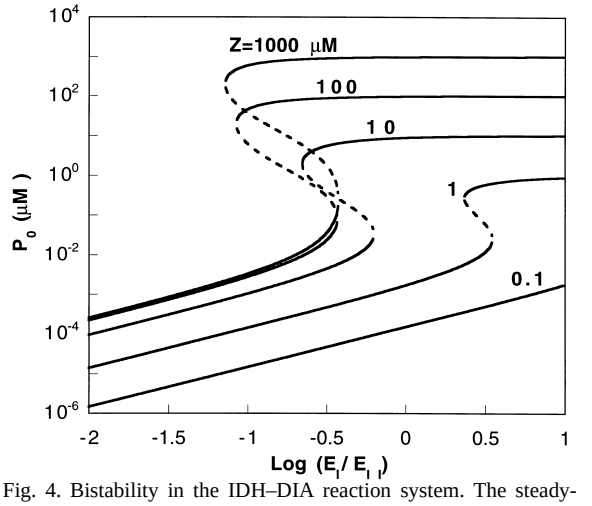

Fig. 4. Bistability in the IDH–DIA reaction system. The steady-state concentration of the product, NADPH (P_0), is plotted as a function of the logarithm of the ratio (E_I/E_{II}) of IDH versus DIA concentrations. The different curves are obtained for various values of the total amount of substrate, $Z = NADP^+ + NADPH$, as indicated on each curve. Bistability here is always accompanied by hysteresis. Parameter values are listed in Table 1. The results are obtained in this and similar subsequent figures by numerically solving the third-degree polynomial equation yielding the value of P_0 [11].

level of NADPH, as shown in the main part of Fig. 3. When DIA is added at the critical time t^* which separates the two types of evolution described above, an intermediate level of NADPH, corresponding to the unstable steady state, is maintained for some time, but eventually the system switches either to the lower stable state or to the upper stable state, as in the case illustrated in Fig. 3.

Because it is impossible to add DIA precisely at the critical time t^*, the initial NADPH concentration, at the time of addition of DIA, will be at a certain distance from the unstable state. The time spent by the system in the immediate vicinity of this state after the addition of DIA will depend both on this distance and on the occurrence of fluctuations. Experimental observations indicate, however, that this time may be relatively long as the system may remain for more than 10 min in the vicinity of the unstable state [11]. This result is recovered in the numerical simulations (see the inset to Fig. 3).

The model allows for a detailed analysis of the conditions in which bistability occurs in the IDH–DIA reaction system. Thus, the phenomenon of bistability is illustrated in Fig. 4 where the steady state

concentration of NADPH is plotted for different values of the total substrate concentration (Z) as a function of the ratio (E_I/E_{II}) of IDH and DIA concentrations. A single steady state is observed for a value of $Z = 0.1$ μM, but for larger values of Z in this figure two stable steady-state values of the product concentration P_0, separated by an unstable steady state, are observed in a range bounded by two critical values of the ratio (E_I/E_{II}). Each of these curves can be associated with a phenomenon of hysteresis: starting on the lower branch of stable steady states, as (E_I/E_{II}) increases, the value of P_0 suddenly increases up to the higher branch of stable steady states when the limit point (denoted LP2; see inset to Fig. 6 below) is reached; then, when (E_I/E_{II}) decreases, the value of P_0 progressively decreases until it drops abruptly as the system returns to the lower branch of stable steady states when the second limit point (denoted LP1) is passed.

Whereas bistability is always associated in this model with hysteresis as a function of the ratio (E_I/E_{II}), this is no more the case as a function of parameter Z. As shown in Fig. 5 where the value of P_0 is plotted as a function of Z for different values of (E_I/E_{II}), a single value of P_0 is obtained for high values of the

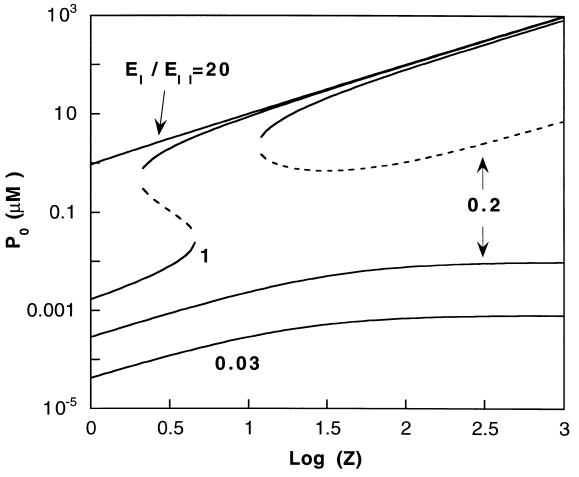

Fig. 5. Bistability with or without hysteresis. The steady-state concentration of the product, NADPH (P_0), is plotted as a function of the logarithm of the total amount of substrate, Z, for different values of the ratio (E_I/E_{II}) of IDH versus DIA concentrations, as indicated on each curve. For high and low values of (E_I/E_{II}), e.g. for (E_I/E_{II}) = 20 or 0.03, monostability occurs. Bistability with or without hysteresis is observed for (E_I/E_{II}) = 1 and 0.2, respectively. Parameter values are as in Fig. 4.

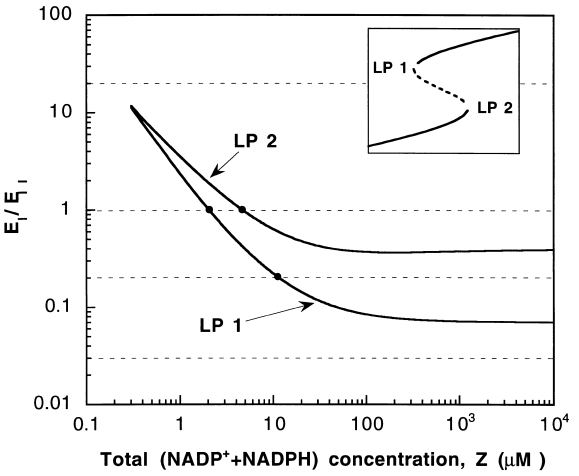

Fig. 6. Loci of the limit points LP1 and LP2 bounding the domain of bistability, as a function of the ratio (E_I/E_{II}) and of parameter Z. The two limit points are defined schematically in the inset. The dashed horizontal lines refer to the different values of the ratio (E_I/E_{II}) considered in Fig. 5. The loci have been determined numerically by means of the program AUTO [22].

ratio of enzyme concentrations. As (E_I/E_{II}) progressively decreases, bistability occurs, in a range bounded by two limit points corresponding to two critical values of Z. When (E_I/E_{II}) further decreases, the limit point LP2 corresponding to the higher critical value of Z vanishes by going to infinity: the system cannot switch from the lower to the upper branch of stable steady states as the value of Z is progressively increased, but the reverse transition from the upper to the lower branch is still possible upon decreasing Z. This situation is associated with an irreversible transition between two stable steady states. For lower values of (E_I/E_{II}), bistability disappears and the system can only reach a single steady state for a given value of Z.

To get a more precise view of when either hysteresis or irreversible transitions occur, it is useful to plot in the (E_I/E_{II}) versus Z parameter plane the loci of the limit points LP1 and LP2 which bound the region where multiple steady states are encountered. The loci, shown in Fig. 6, are determined numerically by means of the continuation program AUTO [22] applied to system (3). The dashed horizontal lines refer to the four values of (E_I/E_{II}) considered in Fig. 5. When there is no intersection of the horizontal line with the locus of LP1 or/and the locus of LP2, the system admits a single steady state, as for (E_I/

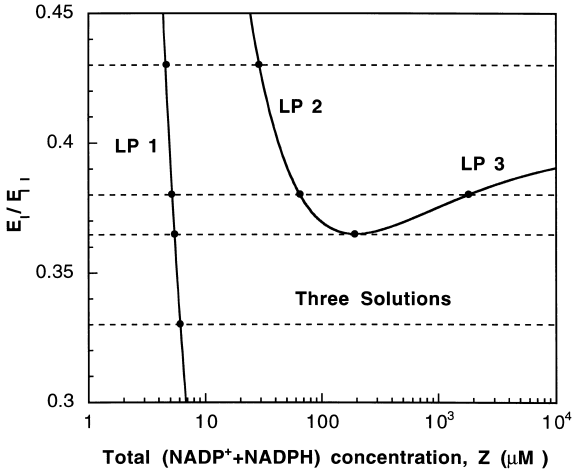

Fig. 7. Enlargement of a detail of Fig. 6, showing the existence of a third limit point, LP3, at large values of Z, in a narrow range of (E_I/E_{II}) values. The dashed horizontal lines refer to the four cases illustrated in Fig. 8.

E_{II}) = 0.03 or 20. When the ratio (E_I/E_{II}) is much less or much larger than unity, the unique steady state corresponds to a low and a large value of P_0, respectively. If the horizontal intersects with the locus of LP1 only, the situation is that of an irreversible transition, as illustrated in Fig. 5 for (E_I/E_{II}) = 0.2. Finally, when the horizontal intersects with both loci, hysteresis occurs, as shown in Fig. 5 for (E_I/E_{II}) = 1.

Shown in Fig. 7 is an enlargement of the part of Fig. 6 where (E_I/E_{II}) ranges from 0.3 to 0.45 (however, the scale of the ordinate is no longer logarithmic). It is clear that a third limit point, denoted LP3, exists in a limited range of (E_I/E_{II}) values. The consequence of the existence of LP3 is illustrated in Fig. 8 where the four panels relate to the values of (E_I/E_{II}) indicated by the dashed horizontal lines in Fig. 7. The situations corresponding to the upper and lower horizontal lines are similar to those encountered in Fig. 5, and illustrate the cases of hysteresis (upper left panel) or irreversible transition (lower right panel). However, when the horizontal line intersects with the three loci of LP1, LP2 and LP3, as for (E_I/E_{II}) = 0.38, a domain of bistability with hysteresis is followed, as Z increases, by a domain of bistability without hysteresis associated with an irreversible transition from the lower to the upper branch of stable steady states (upper right panel). The interest of this situation is that the system successively presents the two types of bistability as a single control parameter is continuously varied. The fourth case illustrated in Fig. 8 (lower left panel) corresponds to the intermediate situation in which LP2 and LP3 coalesce.

The various modes of bistability described above can occur as long as the second substrate, isocitrate, remains at a sufficient level. When the concentration of isocitrate is allowed to decrease progressively due to its consumption in the IDH reaction, bistability eventually disappears and is manifested only as a transient phenomenon, although the model predicts that the time during which bistability can be observed could be of the order of hours [11].

4. From bistability to oscillations

To observe sustained oscillations, it is necessary that the system be open to a constant influx of the second substrate, isocitrate (I). The model considered for periodic behavior in the IDH–DIA bienzymatic system is schematized in Fig. 9. We assume that isocitrate is injected at a constant rate v, and that the second product of IDH, α-ketoglutarate, is removed at a rate proportional to its concentration. Similar results are obtained when including an efflux of all variables, as would occur in an open reactor. The rate equation for IDH remains unchanged, except for the added Michaelian term describing the effect of the second substrate, I. The dynamics of the system is now governed by two coupled kinetic equations describing the time evolution of the isocitrate and NADPH concentrations:

$$\frac{dI}{dt} = v - f_1(Z-P,P,I)$$
$$\frac{dP}{dt} = f_1(Z-P,P,I) - g(P) \qquad (7)$$

where:

$$f_1(S,P,I) = k_0 \frac{E_I S}{K_m^I(1+P/K_I)+S}\left(\frac{I}{K_m^{III}+I}\right)\left[1+\frac{k_1}{k_0}\frac{P}{K_A(1+S/K_I')+P}\right] \qquad (8)$$

The experimental values of the parameters are listed in Table 1.

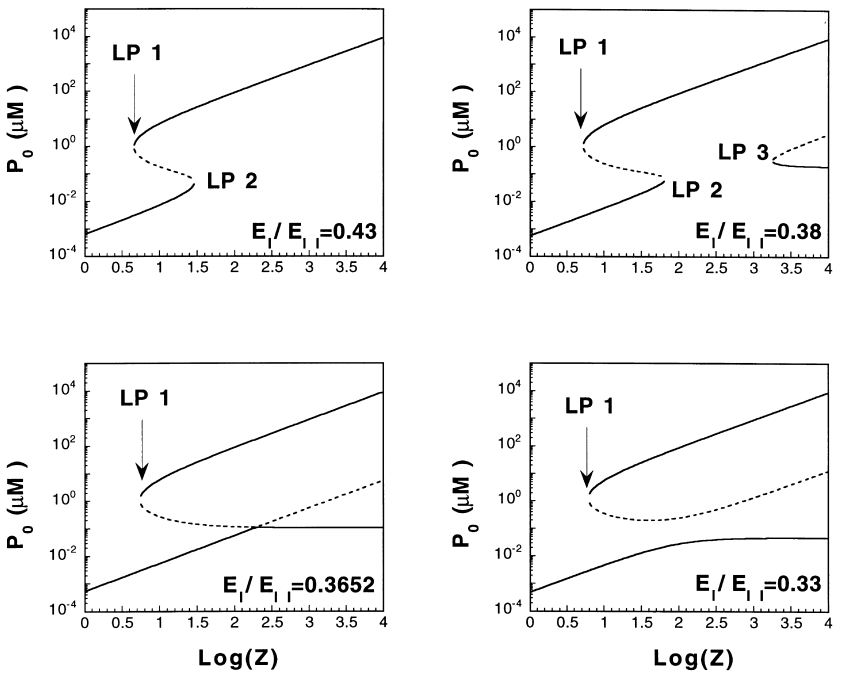

Fig. 8. Bistability with or without hysteresis can occur sequentially as a function of a control parameter. The steady state concentration of the product, NADPH (P_0), is plotted as a function of the logarithm of the total amount of substrate, Z, for different values of the ratio (E_I/E_{II}) of IDH versus DIA concentrations. Bistability with hysteresis is observed for (E_I/E_{II}) = 0.43 (top left panel). For (E_I/E_{II}) = 0.38 (top right panel), the phenomenon is followed by the occurrence of bistability without hysteresis. As (E_I/E_{II}) further decreases, the limit points LP2 and LP3 progressively come closer to each other until they merge when (E_I/E_{II}) = 0.3652 (lower left panel). At lower values, e.g. (E_I/E_{II}) = 0.33, only bistability without hysteresis subsists (lower right panel).

The system of Eq. (7) admits a single steady state, because of the assumption of a constant input of substrate I. Nevertheless, we shall see that the occurrence of oscillations in system (7) can be related to the occurrence of bistability in system (5) that pertains to conditions in which the concentration of isocitrate is kept constant.

Linear stability analysis of Eq. (7) yields the conditions in which sustained oscillations of the limit cycle type occur around an unstable steady state. Two examples of sustained oscillations generated by the model are shown in Fig. 10 (upper and middle panels, referring to situations denoted a and b, respectively). The thick gray curve in the lower panel yields the steady-state value of NADPH (P_0) as a function of the quantity η (which, as shown by Eq. (9), reduces to E_I when I is in excess) in the absence of influx of isocitrate, as determined in Section 3.

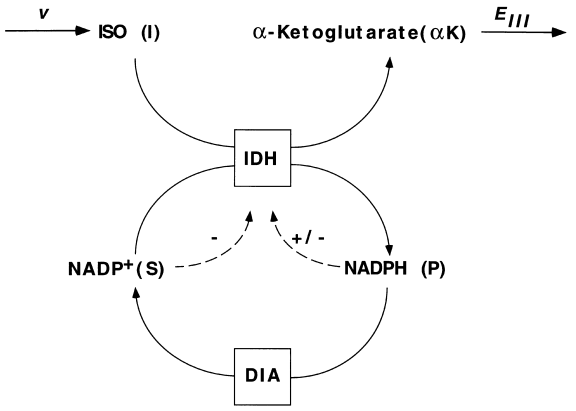

Fig. 9. Bienzymatic system considered for sustained oscillations. The model, involving isocitrate dehydrogenase and diaphorase, is similar to that considered for bistability in Fig. 1, but the second substrate, isocitrate, is injected at a constant rate, while the product α-ketoglutarate is removed in an auxiliary reaction catalyzed by some enzyme E_{III}.

$$\eta = \frac{E_I I}{K_m^{III} + I} \tag{9}$$

The solid or dashed black lines in this lower panel indicate the trajectory followed by the system in Fig. 10a,b, when η varies as a result of the periodic change in I. In the latter conditions, the trajectory follows more or less closely the two stable branches of the steady state curve and repetitively performs a hysteresis loop that translates into limit cycle behavior.

The parameter which triggers this repetitive movement is the isocitrate influx, v. On the lower branch of the steady state curve, I (and, hence, η) increases as a result of v which exceeds the rate of isocitrate consumption in the IDH reaction. When the limit point is reached, the concentration of NADPH abruptly increases as a result of the activation of IDH. The rate of this enzyme now exceeds the influx v, so that the level of isocitrate begins to decrease and the system follows the upper branch of the steady state curve towards the left, until the other limit point is reached. The system now returns to the lower branch of the S-shaped curve, and the increase of isocitrate resumes as the rate of IDH again becomes lower than the substrate input v.

The difference between Fig. 10a,b lies in the amount of IDH. Whereas the amount of DIA is equal to 0.1 μM in both cases, IDH = 0.1 μM in Fig. 10a and 0.5 μM in Fig. 10b. Because $NADP^+$ and isocitrate are consumed by IDH at a higher rate in Fig. 10b, the increase in NADPH and the concomitant decrease in η are more rapid, so that the jump to the upper branch of the S-shaped steady-state curve is no longer vertical as it is in Fig. 10a. Another consequence of the difference in IDH level is that the period of the oscillations is shorter in Fig. 10b: the phase in which NADPH decreases is indeed brief, in contrast to Fig. 10a in which a long plateau in NADPH is observed.

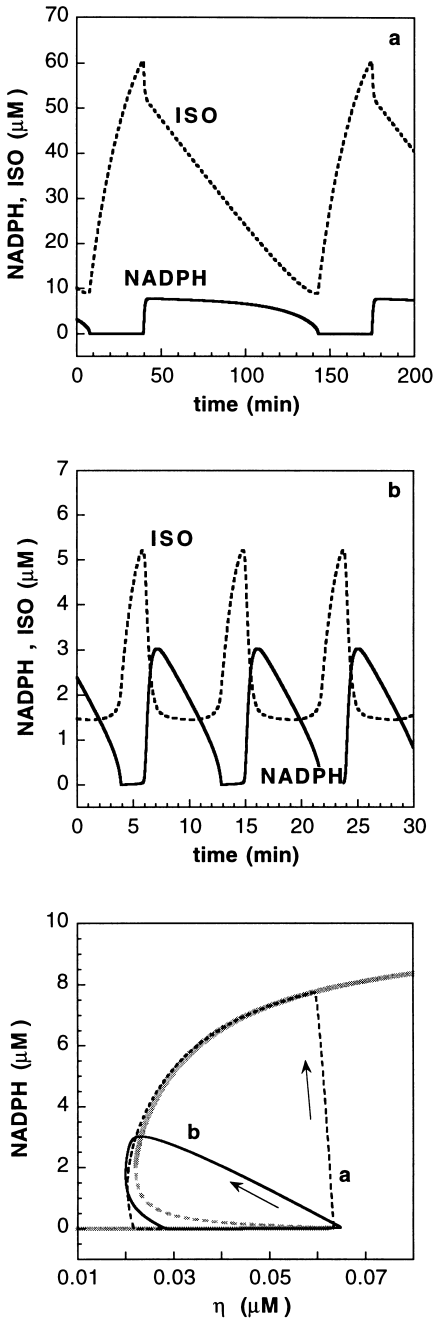

Fig. 10. Sustained oscillations in the IDH–DIA bienzymatic system. The curves are obtained by numerical integration of Eq. (7) for two values of the amount of IDH, E_I = 0.1 μM (a) and 0.5 μM (b), at a fixed value of E_{II} = 0.1 μM. Shown is the time evolution of the concentrations of isocitrate (ISO) and NADPH as a function of time (top and middle panels), as well as the corresponding evolution in the NADPH versus η plane (bottom panel). As indicated by Eq. (9), η is related to the variable amount of isocitrate and to the fixed concentration of IDH, E_I. The thick gray curve in the bottom panel yields the steady-state concentration of the product, NADPH (P_0), obtained from Eq. (6), as a function of η (which, as shown by Eq. (9), reduces to E_I when I is in excess) in the system without isocitrate influx (see Sections 2 and 3). Parameter values are: Z = 10 μM, v = 72 nM/s.

5. Discussion

The analysis of a model for the regulated isocitrate dehydrogenase reaction coupled to a reverse reaction catalyzed by diaphorase indicates that this bienzymatic system is a good candidate for further theoretical and experimental studies of bistability and oscillations in a biochemical context. The present results and a previous study [11] show, indeed, that various modes of bistable behavior can be observed in a model for this enzymatic reaction system. The occurrence of bistability in the model accounts for experimental observations [10,11] which demonstrate the coexistence between two stable steady states in this biochemical system.

Bistability in the model for the IDH–DIA reaction system is generally accompanied by hysteresis. However, the coexistence phenomenon can also occur in this system in the absence of hysteresis when one of the two limit points associated with the domain of bistability goes to infinity. An interesting feature of the present model is that it predicts the possibility of observing sequentially, as a function of the same control parameter, bistability with and without hysteresis (see Figs. 7 and 8).

Irreversible transitions between multiple steady states have been studied theoretically in a variety of models for chemical [23–25] and biochemical [11–18] reactions. Experimental evidence for the phenomenon has been obtained in several in vitro biochemical systems [19–21]. Also the sequential occurrence of bistability with and without hysteresis shown in Figs. 7 and 8 has been seen in a model for a cyclical bienzymatic system regulated by negative feedback in the form of substrate inhibition, in conditions where such a system is open to a flux of substrate and product [18].

The present analysis has been extended beyond the case of bistability, to uncover the conditions in which sustained oscillations may occur in the IDH–DIA bienzymatic reaction system. Our results indicate that limit cycle oscillations can occur as a result of IDH regulation when the system is open to a flux of isocitrate (Fig. 9). The limit cycle trajectory in the phase plane follows more or less closely the two limbs of the S-shaped steady state curve obtained in the absence of isocitrate influx, depending on the relative amounts of isocitrate dehydrogenase and diaphorase (Fig. 10). Together with other parameters such as the total substrate concentration and the rate of isocitrate injection, the ratio of the amounts of the two enzymes governs the period of the oscillations.

The question arises as to whether bistability and oscillations can both be observed in the IDH–DIA system as a function of a single control parameter. The answer reached in the present study is negative. Indeed, when isocitrate is kept constant and the system is governed by Eq. (5), only bistability can occur. Conversely, when isocitrate is injected at a constant rate and the system is governed by Eq. (7), only oscillations can be observed as the system then possesses a unique steady state. The possibility nevertheless remains that sustained oscillations and bistability might both occur in conditions of a fully open system in which an influx and an efflux of each biochemical species takes place.

The analysis of a model for the IDH–DIA coupled reactions accounts for the occurrence of bistability observed in this bienzymatic system [11]. The present results complement our previous theoretical analysis of bistability and further indicate that isocitrate dehydrogenase, coupled to a second enzyme such as diaphorase, may also provide a new example of oscillatory enzyme reaction.

Acknowledgements

This work was supported by the program ‘Actions de Recherche Concertée’ (ARC 94-99/180) launched by the Division of Scientific Research, Ministry of Science and Education, French Community of Belgium. We wish to thank Dr. Marie-France Carlier for stimulating discussions.

References

[1] H. Degn, Nature 217 (1968) 1047.
[2] A. Naparstek, J.L. Romette, J.P. Kernevez, D. Thomas, Nature 249 (1974) 490.
[3] K. Eschrich, W. Schellenberger, E. Hofmann, Arch. Biochem. Biophys. 205 (1980) 114.
[4] E. Simonet, C. Bourdillion, J.F. Hervagault, M. Gervais, J. Phys. Chem. 100 (1997) 19148.
[5] A. Goldbeter, Biochemical Oscillations and Cellular Rhythms, The Molecular Bases of Periodic and Chaotic Behaviour, Cambridge University Press, Cambridge, UK, 1996.

[6] M.F. Carlier, D. Pantaloni, Eur. J. Biochem. 37 (1973) 341.
[7] M.F. Carlier, D. Pantaloni, G. Branlant, J.F. Biellmann, FEBS Lett. 62 (1976) 236.
[8] M.F. Carlier, D. Pantaloni, Biochemistry 15 (1976) 1761.
[9] M.F. Carlier, D. Pantaloni, Biochimie 58 (1976) 27.
[10] M.F. Carlier, Thèse de Doctorat en Sciences, Université Paris-Sud, 1976.
[11] G.M. Guidi, M.F. Carlier, A. Goldbeter, Biophys. J. 74 (1998) 1229.
[12] A. Babloyantz, G. Nicolis, J. Theor. Biol. 34 (1972) 185.
[13] H.-S. Hahn, P.J. Ortoleva, J. Ross, J. Theor. Biol. 41 (1973) 503.
[14] T.A. Rapoport, R. Heinrich, BioSystems 7 (1975) 120.
[15] J.E. Lisman, Proc. Natl. Acad. Sci. USA 82 (1985) 3055.
[16] M. Kaufman, R. Thomas, J. Theor. Biol. 129 (1987) 141.
[17] J.F. Hervagault, S. Canu, J. Theor. Biol. 127 (1987) 439.
[18] J.F. Hervagault, A. Cimino, J. Theor. Biol. 140 (1989) 399.
[19] A. Cimino, J.F. Hervagault, FEBS Lett. 263 (1990) 199.
[20] J. Frenzel, W. Schellenberger, K. Eschrich, Biol. Chem. Hoppe–Seyler 376 (1995) 17.
[21] M.A. Coevoet, J.F. Hervagault, Biochem. Biophys. Res. Comm. 234 (1997) 162.
[22] E.J. Doedel, Congr. Num. 30 (1981) 265.
[23] B.F. Gray, J.H. Merkin, G.C. Wake, Math. Comput. Modelling 15 (1991) 25.
[24] M.A. Sadiq, J.H. Merkin, Math. Comput. Modelling 20 (1994) 27.
[25] G.M. Guidi, A. Goldbeter, J. Phys. Chem. A 101 (1997) 9367.