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ONSET OF BIRHYTHMICITY IN A REGULATED BIOCHEMICAL SYSTEM

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We analyze the onset of multiple oscillatory regimes in a two-variable biochemical model previously proposed for glycolytic oscillations. The model, based on the activation of an allosteric enzyme by a reaction product, is modified by introduction of recycling of product into the substrate. This modification creates the conditions for birhythmicity in which two stable oscillatory regimes coexist under the same conditions. The detailed route by which birhythmicity develops from a single oscillatory regime is elucidated by means of bifurcation diagrams. It is shown that birhythmicity provides added sensitivity to the oscillatory system as the same type of perturbation may produce a switch from one periodic regime to the other and back, when applied at the appropriate phase of each of the two oscillations.

1. Introduction

A conspicuous property of many nonlinear systems is their capability of reaching two different stable steady states under a given set of experimental conditions [1]. Several biochemical systems exhibit such a phenomenon of bistability [2–4]. It has become increasingly clear that bistability possesses a dynamic counterpart, birhythmicity [5], in which a given system evolves into either one of two stable oscillatory regimes depending on the initial conditions. Birhythmicity has not yet been observed in biochemical systems. A theoretical study of a three-variable model representing a sequence of two autocatalytic enzyme reactions showed that the phenomenon can arise from the coupling between two instability-generating mechanisms; complex periodic oscillations as well as aperiodic oscillations (chaos) were also observed for closely related parameter values [5]. These findings were recently corroborated by an experimental study of a chlorite-bromate-iodide system in which the coupling between two autocatalytic chemical reactions was found to produce similar types of behavior [6].

The mechanism for the onset of birhythmicity remains unclear. A detailed study of the phenomenon calls for a simple two-variable model, since the dynamic behavior of two-variable systems is amenable to phase-plane analysis [7]. Such a model is investigated in the present paper. It is based on a biochemical model previously proposed for glycolytic oscillations. This model, which in its early version admits a single periodic regime, is modified so as to admit two coexisting oscillatory modes. By means of bifurcation diagrams, we elucidate the route by which birhythmicity develops from a single oscillatory regime. In addition, we demonstrate how the system may reversibly switch between the two periodic regimes in response to the same type of perturbation, i.e., addi-

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tion of substrate or product. Birhythmicity provides added sensitivity as the switch from one regime to the other closely depends on the phase of the oscillations at which the perturbation is applied.

2. Model and kinetic equations

Glycolytic oscillations occur in yeast and muscle under conditions of constant substrate input, as a result of the activation of phosphofructokinase by a reaction product [8–10]. The original phosphofructokinase model [11,12] is that of an allosteric enzyme transforming a substrate (α) into a product (γ), the product being a positive effector of the enzyme. The dynamic behavior of this model in the phase plane (α, γ) has been analyzed extensively [13,14]. The results of the phase-plane analysis are summarized in fig. 1.

Of utmost importance for oscillations is the S-shaped nature of the nullcline $(d\gamma/dt) = 0$ for appropriate values of the parameters. The steady state (α_0, γ_0) is the intersection of this curve with the nullcline $(d\alpha/dt) = 0$. Whenever the slope $(d\alpha/d\gamma)$ at steady state on the sigmoid nullcline is sufficiently negative, the steady state is unstable and the system evolves towards a stable limit cycle as depicted in fig. 1. The substrate nullcline can be shifted towards the right by increasing input v, without changing the product nullcline. Therefore, upon increasing v from a low initial value, the system in fig. 1 enters a domain of oscillations in A and leaves it in B. The existence of a single instability domain bounded by two critical values of v agrees with the observation that glycolytic oscillations occur in yeast extracts when the substrate input is between 20 and 160 mM/h [8].

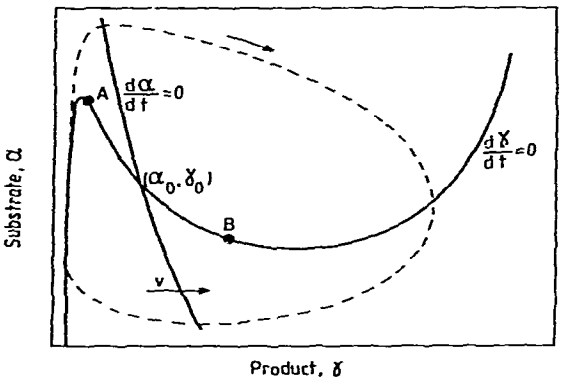

Fig. 1. Schematic phase-plane diagram for the product-activated enzyme reaction of fig. 2 in the absence of product recycling. The system is governed by eqs. 1 with $\sigma_i = 0$. As indicated by the arrow, the nullcline $(d\alpha/dt) = 0$ can be moved to the right by increasing v. The unique steady state (α_0, γ_0) is unstable whenever it lies between points A and B on the nullcline $(d\gamma/dt) = 0$; thus an increase in v can bring the system from A to B across the instability domain. The dashed line indicates the stable limit cycle enclosing the unstable steady state (redrawn from ref. 14).

In order to create the conditions for birhythmicity, we have modified the phosphofructokinase model by incorporating recycling of product into the substrate (fig. 2). Such a process takes place in the lower part of glycolysis where ADP, the product of phosphofructokinase, is recycled into the substrate, ATP. Introduction of product recycling does not increase the number of variables of the system which is now governed by the two kinetic equations:

$$\frac{d\alpha}{dt} = v + \frac{\sigma_i \gamma^n}{K^n + \gamma^n} - \sigma_M \phi(\alpha, \gamma)$$

$$\frac{d\gamma}{dt} = q\sigma_M \phi(\alpha, \gamma) - k_s \gamma - \frac{q\sigma_i \gamma^n}{K^n + \gamma^n} \qquad (1)$$

with

$$\phi(\alpha, \gamma) = \frac{\alpha(1+\alpha)(1+\gamma)^2}{L + (1+\alpha)^2(1+\gamma)^2}.$$

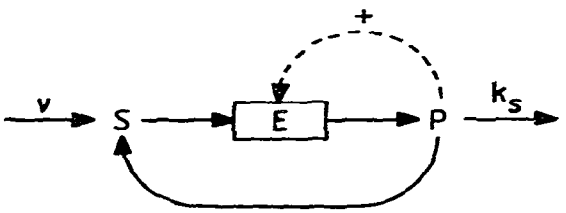

Fig. 2. Model of a product-activated enzyme reaction with recycling of product into substrate. This model admits two stable oscillatory regimes for appropriate values of the parameters.

The function ϕ is the rate of reaction, divided by the maximum rate, for a dimeric allosteric enzyme activated by its product [11,12] when the enzyme obeys the concerted model of Monod et al. [15] with exclusive binding of substrate to the R state (considering a larger number of enzyme subunits and nonexclusive binding of substrate to both T and R states would not affect the qualitative behavior of the model [14]). The parameter v relates to the constant input of substrate divided by the Michaelis constant K_S, whereas k_s relates to the removal of product. The new term in the equations, $\sigma_i\gamma^n/(K^n+\gamma^n)$, refers to recycling of product γ into substrate α. We assume that this reaction is catalyzed by a cooperative enzyme obeying the Hill equation with a Hill coefficient of n (we have obtained birhythmicity in the present model only with Hill numbers equal to or larger than 3).

Concentrations α and γ denote the substrate and product concentrations divided, respectively, by K_S and by the dissociation constant K_P of the product for the regulatory site of the activated enzyme (E_1); $q = K_S/K_P$; $\sigma_M = V_M/K_S$ and $\sigma_i = V_i/K_S$ are the normalized maximum rates of E_1 and of the recycling enzyme, respectively; $K = K_m/K_P$ where K_m is the Michaelis constant of the recycling enzyme.

The main features of the present model are two-fold. As in its previous version, we consider an allosteric enzyme subject to positive feedback. In addition, due to recycling of the product – a process which creates a futile cycle –, the substrate is produced from two parallel inputs, namely, one which is constant, and the other which depends on the operation of the system. Although the above hypotheses derive from the glycolytic pathway, we would like to stress that the primary goal of the present work is not to propose an extended model for glycolytic oscillations, but rather to analyze in a simple two-variable model how multiple stable oscillations arise from regulatory processes in biochemical systems.

3. Onset of birhythmicity

The effect of the recycling of product into substrate is governed by two parameters, K and σ_i. The first relates to the threshold of the recycling function: as long as $\gamma < K$, recycling is negligible, whereas it reaches its half-maximum value when $\gamma = K$. The second parameter, σ_i, measures the magnitude of product recycling as compared to the constant input v.

In fig. 3 are shown successive bifurcation diagrams as a function of parameter v, for values of σ_i increasing from 0 to 2. As explained in the figure legend, these diagrams are obtained by a combination of linear stability analysis and computer simulations. In each diagram the ordinate gives the steady-state value of the substrate concentration, α_0, whereas the presence of a periodic regime is represented by the maximum amplitude α_M of the substrate in the course of the oscillations. Both for the steady state and for periodic regimes, a solid line denotes stability whereas a dashed line refers to an unstable (periodic or steady) state. Since from eqs. 1 the steady-state value of the product concentration is simply $\gamma_0 = qv/k_s$, we have varied v for given values of q and k_s so that the abscissa measures at the same time the substrate input v and the steady-state level of product. As a consequence, the steady-state curve in fig. 3 coincides with the sigmoid nullcline $(d\gamma/dt) = 0$ (see fig. 1).

For $\sigma_i = 0$ (fig. 3a), we recover the situation obtained previously [11–14]. When the substrate input v reaches a critical value, the steady state becomes unstable as a result of the positive feedback exerted by the product on the enzyme. This corresponds to the supercritical bifurcation of a stable limit cycle whose amplitude successively rises, passes through a maximum, and declines as v increases. In the following, we shall refer to this stable periodic regime as limit cycle 1 (LC1). The oscillations disappear when the substrate input passes a second critical value. In the absence of product recycling, we thus observe a single domain of instability of the steady state as a function of v and, hence, a single domain of oscillations.

In fig. 3b, we observe that for a small value of σ_i, the recycling of product into substrate has as main effect the enlarging of the domain of oscillations. Note also the small deformations in the α_0 and α_M curves around the value of v corresponding to $\gamma_0 = 10$, which is the value taken for the threshold constant K. Although it renders the slope

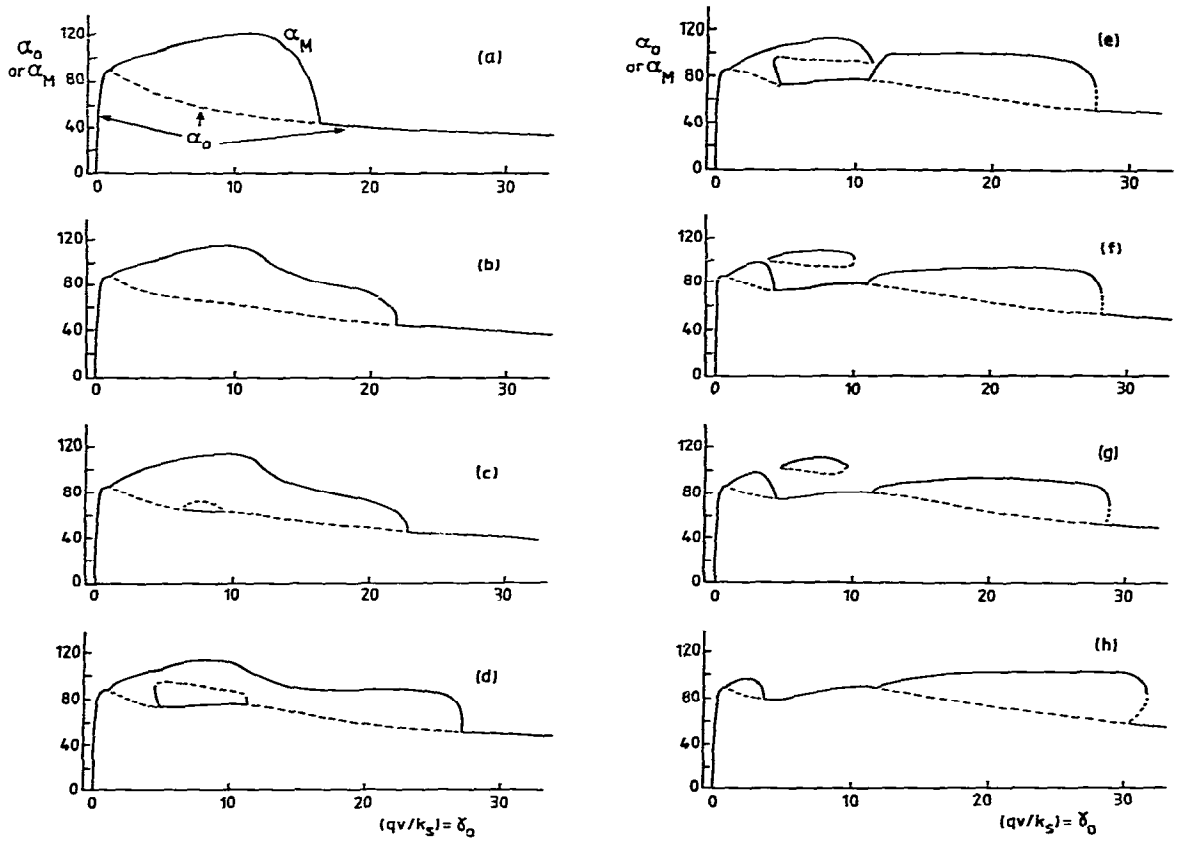

Fig. 3. Effect of product recycling on the appearance of birhythmicity. Shown are a series of bifurcation diagrams as a function of v for different values of the maximum recycling rate σ_1 (in s^{-1}): (a) 0, (b) 0.5, (c) 0.6, (d) 1.2, (e) 1.3, (f) 1.4, (g) 1.5, (h) 2. As indicated in panel a, each diagram shows the steady-state level of substrate α_0 (lower curve), as well as the maximum amplitude α_M of substrate oscillations (upper curves). Stable and unstable regimes are represented by solid and dashed lines, respectively. The stability properties of the steady state are determined by linear stability analysis [1,7], whereas numerical integration of eqs. 1 yields the stable and unstable periodic regimes. Parameter values are $L = 5 \times 10^6$, $\sigma_M = 10\ s^{-1}$, $K = 10$, $n = 4$, $q = 1$, $k_s = 0.06\ s^{-1}$ [11,12].

$(d\alpha/d\gamma)$ less negative, the 'bump' in the sigmoid nullcline is not sufficient to stabilize the steady state. For a slightly larger value of σ_1, however, a small domain of stability appears near $\gamma_0 = 10$ (fig. 3c). As expected in a two-variable system where two stable solutions have to be separated by an unstable orbit [1,7], the stable steady state in this domain is enclosed by an unstable limit cycle of small amplitude. This gives rise to a phenomenon of hard excitation, i.e., coexistence of a stable steady state with a stable oscillation, since the stable limit cycle LC1 persists over the whole range of v values.

For still higher σ_1 values (fig. 3d), LC1 still exists in a single domain which is even enlarged. The region of stable steady state embedded in it is also larger,but on its left we observe the supercritical bifurcation of a second stable limit cycle (LC2).

The latter cycle rapidly turns around to merge with the unstable limit cycle which was already present in fig. 3c. The amplitude of the unstable periodic regime is larger and approaches that of the stable oscillation, whereas the depression in the amplitude of LC1 deepens. We have just witnessed the onset of birhythmicity in system 1. Indeed, there now exists a small domain of v values for which two stable limit cycles, LC1 and LC2, coexist under the same conditions.

For $\sigma_i \simeq 1.3\ s^{-1}$, the large-amplitude limit cycle of fig. 3a–d breaks up. It is captured by the unstable limit cycle on one side, whereas on the other it joins the steady state (fig. 3e). Here we observe birhythmicity at the two ends of the median domain of stable steady state.

In fig. 3f, the stable branch of LC2 merges with the left portion of LC1. This produces an isola where a stable limit cycle coexists with an unstable cycle. The isola becomes smaller as σ_i increases (fig. 3g), and finally disappears by coalescence of its stable and unstable branches. Two distinct oscillatory domains are then separated by a range of stable steady state (fig. 3h).

A stability diagram in the σ_i-v parameter plane (fig. 4) completes the picture provided by the bifurcation diagrams. The different behavioral domains are shown, and the sequence of fig. 3a–h can be recovered by making horizontal cuts for the appropriate values of σ_i. This diagram shows that birhythmicity occurs here at the boundaries of a domain of hard excitation which separates two domains of stable oscillations. These results corroborate and explain those found in the study of birhythmicity in a more complex model for two autocatalytic enzyme reactions coupled in series [5].

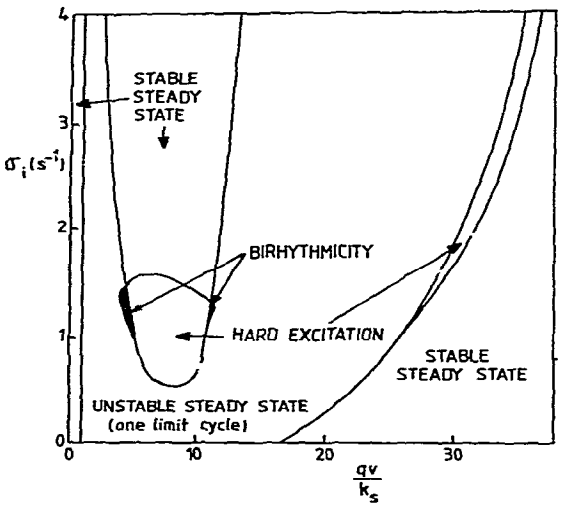

Fig. 4. Behavioral domains as a function of the constant input of substrate v and of the maximum rate of product recycling σ_i. Other parameter values are as in fig. 3. As in the latter figure, the diagram is established by combination of linear stability analysis and numerical simulations.

4. Switching between two stable periodic regimes

A typical phase-plane representation of birhythmicity is given in fig. 5. Shown here are the two stable limit cycles of fig. 3e for $qv/k_s \simeq 4.25$. They are separated by an unstable periodic trajectory. As expected in a two-variable system, the three limit cycles are nested as they enclose the unstable steady state.

How the switching from one stable periodic regime to the other can be realized is indicated by the arrows in fig. 5. The same type of perturbation – here, the addition of a moderate amount of substrate – can be utilized to pass from the small-amplitude to the large-amplitude oscillations, and vice versa, provided the addition is made at the appropriate phase of each of the two limit cycles. Switching from the smaller to the larger limit cycle can thus be achieved by perturbing the system as the substrate reaches its maximum in the course of small-amplitude oscillations (fig. 6a). The reverse transition occurs when the perturbation is made as the substrate and the product come close to their respective minima in the course of large-amplitude oscillations (fig. 6b).

From fig. 5 it is clear that a suprathreshold quantity of substrate has to be added in both cases to effect a switch between periodic regimes. The threshold depends on the phase and is given by the position of the unstable limit cycle which behaves as a separatrix between the two stable oscillatory

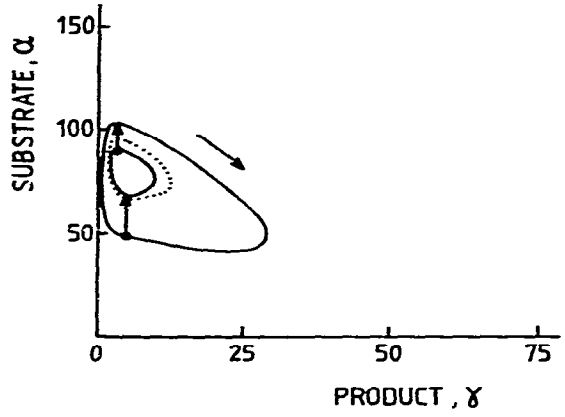

Fig. 5. Birhythmicity. The behavior of the model is represented in the phase plane (α, γ) for the situation considered in fig. 3e, for $r = 0.255\ s^{-1}$, i.e., $q v/k_s = 4.25$. The two stable limit cycles (solid lines) are separated by an unstable periodic trajectory (dashed line). The vertical arrows indicate how the system may switch from one stable cycle to the other upon substrate addition.

regimes. The switch from the small to the large limit cycle is easier to achieve than the reverse transition. Any perturbation of the small oscillations that brings the system across the separatrix will indeed lead to the larger limit cycle. For the reverse transition, the perturbation has to be adjusted in a more precise manner with respect to its amplitude and to the phase so as to bring the system into the basin of attraction of the small limit cycle. Consideration of fig. 5 further shows that the switch between the two oscillatory regimes can also be achieved by addition of suprathreshold amounts of product at appropriate phases.

5. Discussion

We have shown that an autocatalytic enzyme reaction exhibiting periodic behavior becomes capable of birhythmicity, i.e., oscillating in two different stable regimes under the same conditions, once it is coupled to a second process such as product recycling into substrate. Birhythmicity has previously been obtained in more complex biochemical models comprising at least three variables [5,16,17], and the phenomenon has been observed experimentally in a chemical system comprising several autocatalytic reactions [6]. An advantage of the present model is that it comprises only two variables. Therefore, it allows one to comprehend fully the onset of birhythmicity.

The series of bifurcation diagrams of fig. 3 shows how the phenomenon develops as product recycling gradually rises in importance. It occurs

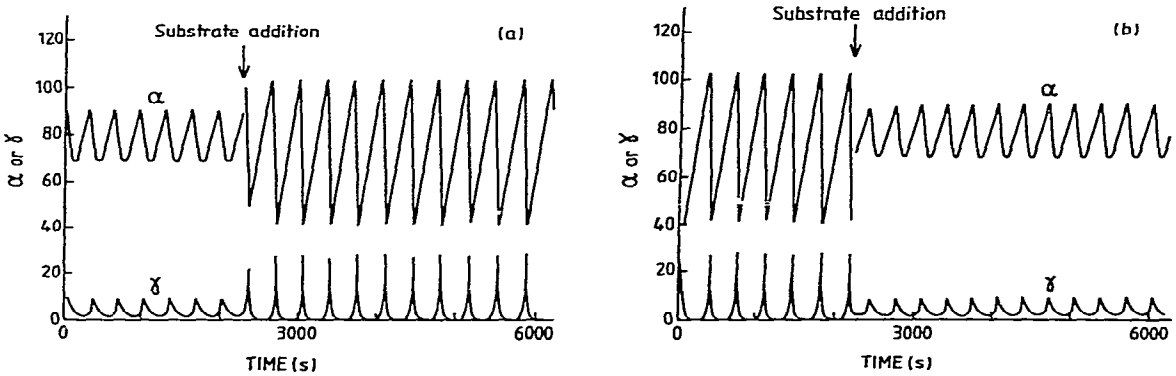

Fig. 6. Switching between two stable oscillatory regimes. The passage from the small-amplitude to the large-amplitude oscillations of fig. 5 is achieved in panel a by setting $\alpha = 100$ in $t = 2300$ s (initial conditions are $\alpha = 83.9$, $\gamma = 3.2$). The reverse transition is triggered in panel b by setting $\alpha = 70$ in $t = 2220$ s (initial conditions are $\alpha = 102.9$, $\gamma = 2.6$).

through the bifurcation of a small-amplitude limit cycle in the domain of existence of a large-amplitude periodic solution. The important point is that the passage from a single to two distinct instability domains creates the conditions for coexistence of two stable periodic regimes. The present route to birhythmicity could be generic given that a similar scenario for the onset of multiple oscillations has been observed in a three-variable system [5]. There, however, birhythmicity also occurred in another parameter domain by formation of a hysteresis loop between two stable periodic regimes; such a process has not been observed in the present model for the parameter values considered.

To obtain birhythmicity, we have modified a model for a product-activated allosteric enzyme, in which the substrate is supplied at a constant rate, into a model in which two inputs of substrate are coupled in parallel. The additional input, which originates from recycling of the reaction product, transforms the system into a futile cycle. Numerous futile cycles exist in cellular metabolism. The possibility therefore exists that futile cycles comprising an autocatalytic reaction may give rise to multiple stable oscillations. The conditions for the occurrence of a single oscillatory regime in an open futile cycle in the absence of autocatalysis have recently been investigated [18].

Erle et al. [19] have pointed out that coupling two sink reactions in parallel in a model for a product-activated enzyme may give rise to adjacent instability domains in parameter space. The conditions for birhythmicity outlined above could, in principle, be met in such an instance. Erle [20] gave a mathematical proof that multiple stable oscillations can indeed arise when the expression for the sink reaction(s) is modified in an appropriate way. The occurrence of multiple oscillations as a result of a biochemically reasonable modification of the sink reaction remains, however, to be illustrated explicitly by an example.

Two parameters govern the importance of product recycling versus constant input of substrate in the present model. We have mainly analyzed the effect of the magnitude of product recycling, as measured by the maximum rate σ_i. It is clear that parameter K is also important for the onset of birhythmicity. This parameter measures the threshold product concentration beyond which recycling becomes significant. If the threshold were to lie outside the oscillatory domain of fig. 3a, the mechanism for the onset of birhythmicity described in fig. 3b–h could not operate. Birhythmicity therefore requires a delicate balance between multiple regulatory processes. This is one of the reasons why it occurs in a parameter domain which is small compared to that of a single periodic regime. A similar conclusion has been reached in a previous theoretical analysis [5], and in the experiments on the chlorite-bromate-iodide system [6]. Aperiodic oscillations (chaos), when they occur, are also found in a small parameter domain [5]. As the latter phenomenon requires the interaction of at least three variables, it cannot be observed in the present model. In this sense the conditions for chaos are more stringent than those for birhythmicity.

The results on the switching between two stable periodic regimes illustrate a new type of sensitivity to external perturbations. Indeed, depending on their timing – i.e., on the particular phase of the oscillations – similar perturbations in the form of addition of a sufficient amount of substrate (or product) will elicit a shift from the first stable periodic regime to the second, or vice versa. Such behavior could be of physiological significance in that the same external signal may alternatively switch the behavior of a cellular system from one type of oscillation to another, and may thereby affect reversibly the frequency and the amplitude of a cellular periodic process. This phenomenon could also play a role in more complex systems such as the brain where multiple regulatory interactions between neurones are likely to create the conditions for multiple stable oscillations.

With respect to the effect of perturbations on the dynamic behavior, birhythmicity provides more plasticity than bistability. A switch between two coexisting stable steady states may require addition of a chemical species to pass from one state to the other, and removal of this species to operate the reverse transition for a given set of conditions. This is to be contrasted with birhythmicity where the transition between the two stable periodic regimes can be produced in both directions by a single stimulus owing to the existence of an additional

degree of freedom, namely, the phase at which the perturbation is applied.

The importance of the timing of the perturbation in the switching between periodic regimes can be related to Winfree's work [21] on the suppression of rhythmic behavior by critical perturbations. There, a system admitting a single limit cycle was perturbed. The perturbation was delivered at the appropriate phase with the appropriate magnitude so as to bring the oscillatory system into the vicinity of its singular point.

Whereas the present model can mainly be considered as a two-variable prototype for multiple oscillations, it raises the possibility that birhythmicity might occur in the glycolytic pathway under conditions of constant input of substrate. Whether or not this is the case could be investigated in more complex glycolytic models that would include more realistic kinetics for the recycling of product, constancy of the adenylate pool, as well as the recently discovered regulation of phosphofructokinase by fructose 2,6-bisphosphate [22]. Models based on the latter regulation have already been considered [23], although no search for birhythmicity was attempted in these studies.

An experimental test of birhythmicity could be performed in yeast extracts undergoing glycolytic oscillations. Previous studies on the phase shifts induced by pulses of ADP or ATP were indicative of the existence of a single oscillatory regime [8,9]. In these studies, the hexose substrate was injected at a constant rate. As product recycling in glycolysis takes place between ADP and ATP, a situation closer to that considered in the present model would be to inject both the hexose and ATP at a constant rate. Perturbation of the system by ATP or ADP pulses for different substrate injection rates would show whether the transition between two stable periodic regimes can be induced in these conditions.

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