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Finding complex oscillatory phenomena in biochemical systems

An empirical approach

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Starting with a model for a product-activated enzymatic reaction proposed for glycolytic oscillations, we show how more complex oscillatory phenomena may develop when the basic model is modified by addition of product recycling into substrate or by coupling in parallel or in series two autocatalytic enzyme reactions. Among the new modes of behavior are the coexistence between two stable types of oscillations (birhythmicity), bursting, and aperiodic oscillations (chaos). On the basis of these results, we outline an empirical method for finding complex oscillatory phenomena in autonomous biochemical systems, not subjected to forcing by a periodic input. This procedure relies on finding in parameter space two domains of instability of the steady state and bringing them close to each other until they merge. Complex phenomena occur in or near the region where the two domains overlap. The method applies to the search for birhythmicity, bursting and chaos in a model for the cAMP signalling system of *Dictyostelium discoideum* amoebae.

1. Introduction

Because of the details known about their underlying mechanism, oscillatory phenomena which occur in enzymatic systems [1–3] provide useful models for other, less known biological rhythms. The theoretical analysis of biochemical oscillations thus serves a dual purpose: besides accounting for the occurrence of metabolic periodicities and throwing light on their physiological significance, models for oscillatory enzyme reactions may be used for predicting new kinds of dynamic phenomena and for analyzing the transition from simple to complex oscillatory behavior. To illustrate the latter approach, this paper presents a comparative survey of the results obtained in the theoretical analysis of a class of biochemical models whose regulation leads to increasingly complex modes of oscillatory behavior.

Starting with the prototype model of a product-activated allosteric enzyme proposed for glycolytic oscillations, we show how modifications of this simple, two-variable model may lead to the coexistence of two simultaneously stable types of oscillations. Extension of the model to a multiply regulated biochemical system further shows how other complex oscillatory phenomena such as bursting and aperiodic oscillations (chaos) may occur. These phenomena are widespread in biological systems [4].

Complex phenomena often occur for a restricted set of parameter values; finding their domain of existence may therefore be a difficult task. Conventional stability analysis, indeed, does not

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permit one to predict the type of dynamic behavior which occurs when a unique steady state becomes unstable. On the basis of our modeling studies, we outline an empirical strategy for finding birhythmicity, bursting and chaos in parameter space. This approach successfully applies to the search for complex oscillatory phenomena in a model for the cyclic AMP (cAMP) signalling system of *Dictyostelium discoideum* amoebae.

2. From simple to complex patterns of oscillatory behavior

The two-variable, product-activated enzymatic system schematized in fig. 1a has been studied [5–7] as a model for the phosphofructokinase reaction which produces glycolytic oscillations in yeast with a period of several minutes [1]. Key factors in the molecular mechanism of these oscillations are the allosteric nature of phosphofructokinase and the positive feedback exerted by a reaction product.

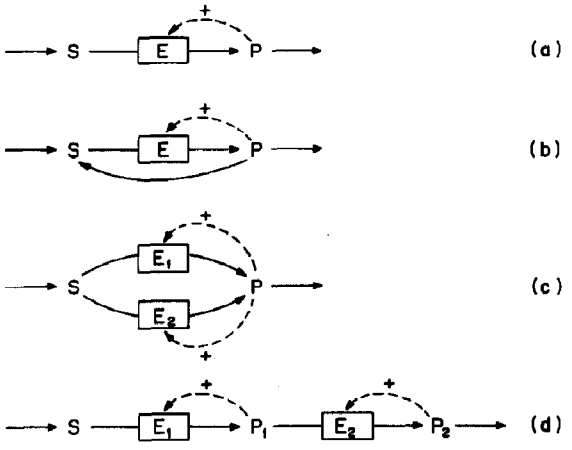

Fig. 1. Models of enzymatic reactions producing simple and complex oscillatory phenomena. In the basic model (a) proposed for glycolytic oscillations, substrate S is transformed into product P in a reaction catalyzed by an allosteric enzyme E; the enzyme is activated by the reaction product (dashed line). In panel b, this process is supplemented by recycling of product into substrate. The coupling of two reactions of type a either in parallel or in series is considered in panels c and d, respectively. In each model the substrate is supplied at a constant rate and the final product is removed at a rate proportional to its concentration. All models admit simple limit cycle oscillations. Coexistence between a stable steady state and a stable limit cycle (hard excitation) or between two stable limit cycles (birhythmicity) occurs for models b–d. Bursting, chaos, and trirhythmicity occur in model d.

The only modes of dynamic behavior admitted by the model of fig. 1a are the evolution towards either a stable steady state or a regime of sustained oscillations. These oscillations correspond to a stable limit cycle in the phase plane formed by the substrate and product concentrations. Evolution to the limit cycle occurs beyond critical values of the control parameters, when the unique steady state admitted by the system becomes unstable [5–7].

Building on this simple model for glycolytic oscillations, we have analyzed a class of models related to it, but not directly based on experimental observations, in order to determine conditions for the occurrence of complex oscillatory phenomena. Our first approach was to analyze the interplay between two instability-generating mechanisms in the three-variable model of fig. 1d; thereafter we returned to simpler two-variable models such as those of fig. 1b and c. We shall begin with the latter, in order to proceed with progressively increasing complexity.

When a recycling of product into substrate is added to the model of the product-activated enzyme (fig. 1b), the system becomes capable of two distinct types of bistability [8]. In addition to the modes of behavior found in the model of fig. 1a, the system may indeed exhibit a coexistence between a stable steady state and a stable limit cycle, a situation which leads to *hard excitation* [9], or a coexistence between two simultaneously stable periodic regimes.

In the latter situation of *birhythmicity* [10], two stable limit cycles are found in the phase plane for the same set of parameter values; they are separated by an unstable cycle. Each of the two stable limit cycles possesses its own basin of attraction, so that the system may switch back and forth between them upon appropriate perturbation [8]. Owing to the nested nature of the two stable limit cycles which enclose the unique unstable steady state, the smallest cycle possesses a smaller basin of attraction. It is therefore easier to pass from the small-amplitude to the large-amplitude oscillations than to effect the reverse transition.

Birhythmicity has not yet been observed in biochemical systems, but it has been demonstrated in chemical oscillatory reactions [11]. Observations on oscillations in the heart [12,13] suggest the occurrence of the phenomenon in cardiac tissue. Given the interest and possible physiological significance of birhythmicity, we have investigated its occurrence in another two-variable system related to the basic model of fig. 1a. The model considered (fig. 1c) is that of two autocatalytic isozymes that catalyze the same reaction with different kinetic properties. This situation amounts to the coupling in parallel of two oscillatory enzyme reactions. The analysis of this model indicates that as in the model of fig. 1b, hard excitation and birhythmicity may occur (Y.X. Li and A. Goldbeter, manuscript in preparation). In both models, the two phenomena arise when a single domain of instability in parameter space has just been broken into two instability domains separated by a region in which the steady state is stable.

The coupling in series of two product-activated enzyme reactions (fig. 1d) further enlarges the repertoire of complex oscillatory phenomena. In this three-variable model, indeed, the various modes of dynamic behavior described for the models of fig. 1a–c are recovered, but additional phenomena that cannot be observed in two-variable systems may occur [10]. First, complex periodic oscillations in the form of bursting arise. This phenomenon, which is commonly observed in neurobiology, as exemplified by the bursting R15 neuron of *Aplysia* [14], consists in bursts of high-frequency oscillations separated by a quiescent phase; this pattern is repeated in a periodic manner. The second novel phenomenon is that of irregular, aperiodic oscillations. That this behavior represents deterministic chaos [15] is indicated by the sensitivity of the system's trajectories with respect to initial conditions, and by the sequence of period-doubling bifurcations leading to aperiodic oscillatory behavior [10].

To analyze bursting, we have resorted to a variety of complementary methods including the use of one-dimensional maps [16]. This has permitted us to understand the passage of a bursting pattern with n spikes per period to a pattern with $n+1$ spikes. The analysis shows that these simple patterns are separated by more complex patterns of bursting in which sequences of n, j, k... spikes are repeated periodically. That such a result may bear on known examples of bursting is indicated by the fact that the piecewise linear, one-dimensional map obtained for the model of fig. 1d presents a striking resemblance to that obtained in a model for bursting in pancreatic β-cells [17].

A further use of the model for the multiply regulated system of fig. 1d is that it allows one to assess the relative occurrence of each of the modes of oscillatory behavior in parameter space [10]. The numerical study of the model in the v-k parameter space (v and k relate, respectively, to the constant input of substrate and to the rate constant for product removal) shows that in spite of the complex regulatory structure of the system, the most common behavior, besides the evolution to a stable steady state, remains that of simple periodic oscillations. The second most common mode of dynamic behavior is that of bursting. Two relatively smaller domains have been identified in which the system exhibits birhythmicity. Trirhythmicity occurs when these domains overlap. Finally, three distinct domains of chaos have been found; these domains are comparable in size with those of birhythmicity.

The models of fig. 1 are all based on positive feedback, which remains the most common source of oscillatory behavior in biochemical and other biological systems [2]. Similar modes of dynamic behavior occur in systems controlled by a combination of positive feedback and substrate inhibition [18].

3. Complex oscillatory phenomena in a model for cAMP signalling in *Dictyostelium*

The above results were established for relatively abstract models obtained by progressively increasing the complexity of the model proposed for glycolytic oscillations. How these results relate to cell biology can be discussed by using the cAMP signalling system of *Dictyostelium* amoebae as an example.

After starvation, *D. discoideum* amoebae aggregate by a chemotactic response to cAMP pulses

emitted with a periodicity close to 10 min by cells which behave as aggregation centers (for reviews, see refs. 19 and 20). Other cells respond chemotactically to these signals and relay them towards the periphery of the aggregation field. Theoretical models suggest [21] that the origin of cAMP oscillations lies in the positive feedback exerted by cAMP on its own synthesis. Experiments indicate [19,20] that extracellular cAMP binds to a cell surface receptor and thereby activates adenylate cyclase which transforms intracellular ATP into cAMP. The latter metabolite is transported into the extracellular medium and hydrolyzed by phosphodiesterase. Much as in the models of fig. 1, the positive feedback loop present in cAMP synthesis (fig. 2) gives rise to an instability of the steady state, and to the subsequent evolution of the system towards a limit cycle corresponding to sustained oscillations of cAMP.

Extracellular cAMP induces phosphorylation of the cAMP receptor [22,23]; such modification is likely accompanied by receptor desensitization. The analysis of a model for cAMP signalling based on this process (fig. 2) accounts for a large number of experimental observations on cAMP relay and oscillations, as well as on the response of the signalling system to stepwise increases in extracellular cAMP [24,25]. Of relevance to the present discussion is the finding of complex oscillatory phenomena in this model for cAMP signalling. Thus, bursting occurs, in the form of a periodically repeated pattern in which several peaks of cAMP are followed by a phase of reduced cAMP synthesis [26]. For closely related parameter values, the system admits a coexistence between small- and large-amplitude oscillations [27,28]. Near this region of birhythmicity, aperiodic oscillations of cAMP are obtained [26]. These oscillations arise through a sequence of period-doubling bifurcations, which suggests that they represent deterministic chaos [29].

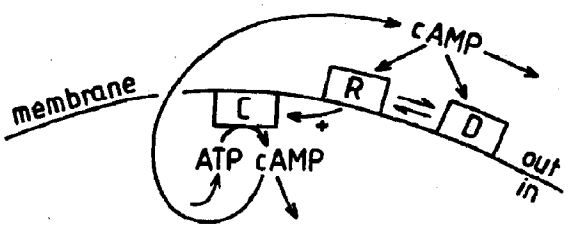

Fig. 2. Model for the cAMP signalling system governing aggregation and differentiation in *Dictyostelium discoideum* amoebae. Extracellular cAMP binds to the active state (R) of the receptor and thereby activates adenylate cyclase (C) which transforms intracellular ATP into cAMP. Binding of cAMP induces the transition to the desensitized receptor state (D). Arrows indicate synthesis of ATP, transport of cAMP into the extracellular medium, and cAMP hydrolysis by phosphodiesterase. The model accounts for relay and periodic oscillations of cAMP; birhythmicity, bursting and chaos also occur in parameter space in or near regions where two initially distinct instability domains overlap.

The origin of complex oscillatory phenomena in cAMP signalling will be addressed below. The question arises as to whether there exists any experimental evidence for bursting, birhythmicity or chaos in *Dictyostelium*. Birhythmicity has not been observed in cAMP signalling, but such a phenomenon has to be demonstrated by means of perturbation experiments which have not yet been conducted. As regards bursting, 'doublet' and 'triplet' waves observed in the course of aggregation on agar [30] might represent patterns of bursting in which two or three peaks of cAMP are followed by a quiescent phase over a period.

Evidence for chaotic behavior may be more conclusive. Experiments carried out by Durston [31] on pacemaker mutants of *Dictyostelium* might indeed be interpreted in terms of chaos. By measuring the time interval between successive waves of amoebae aggregating on agar, Durston showed that this interval is centered around a sharp peak in the wild type whereas it is highly irregular in the mutant Fr17. The mode of intercellular communication in this mutant was referred to as 'aperiodic signalling'. Later studies in suspensions of Fr17 cells provided preliminary evidence for 'erratic' oscillations of cAMP [32]. Further studies are needed to determine whether the irregular synthesis of cAMP in Fr17 is governed by deterministic chaos. The theoretical analysis definitely suggests such a possibility and predicts that if the oscillations in Fr17 are truly chaotic, addition of phosphodiesterase should change the behavior of the mutant from chaotic to periodic [26,29].

4. Finding domains of complex oscillatory phenomena in parameter space: an empirical approach

From the comparative analysis of the models of fig. 1a–d, we may reach conclusions which can be used to delineate a strategy for finding complex oscillatory phenomena in parameter space. Our discussion only bears on autonomous systems, and therefore does not address the situation of forcing an oscillator by a periodic input. Chaos and multiple periodic regimes readily arise in the latter conditions, as exemplified by studies of glycolytic oscillations subjected to a periodic input of substrate [33,34].

In the model of fig. 1d, two endogenous oscillators are coupled in series. In the case of periodic forcing of an oscillatory system, one of the oscillators is exogenous, and the other endogenous. One major difference, however, is that the amplitude and frequency of the periodic input can be controlled at will in the case of forcing; moreover, the two oscillators are always active in that case. This explains the relative ease of finding complex behavior in periodically forced oscillatory systems. Quasi-periodic oscillations on a torus are often observed in such systems but have not been found in the model of fig. 1d.

All the models of fig. 1 share the property that limit cycle oscillations occur around an unstable steady state in some domain of parameter space. The models of fig. 1b–d sometimes possess two distinct domains of instability in parameter space, as schematized in fig. 3a for two arbitrary parameters λ_1 and λ_2 (in practice, instability domains are determined by linear stability analysis around the steady-state solution(s) admitted by the kinetic equations). Hard excitation and birhythmicity occur in these models when the two domains of instability of the steady state are sufficiently close to each other (fig. 3b). Then, indeed, if the branch of periodic regimes defined by the limit cycle observed in one of these domains extends outside the boundaries of the domain into the second region of instability, two different phenomena occur. First, a stable steady state coexists with a stable limit cycle between the two instability domains. On the other hand, birhythmicity will arise in the part of the second instability domain where the branch of periodic regimes originating from the first domain is present and coexists with the

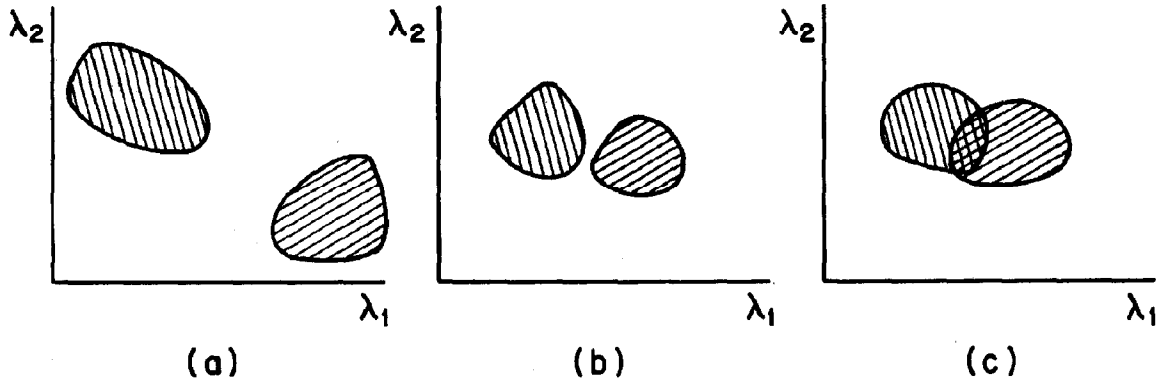

Fig. 3. Empirical approach for discovery of complex oscillatory phenomena in parameter space. The method applies when linear stability analysis around a steady state reveals the existence of two distinct domains of instability (hatched regions) in a plane formed by two parameters, λ_1 and λ_2 (a). If the steady state is unique, limit cycle oscillations around this unstable state occur in both domains. (b) The two oscillatory domains are brought close to each other by appropriate change in some other parameter(s) λ_k ($k = 3, 4...$) of the system. Birhythmicity and hard excitation may occur under these conditions when the limit cycle in one domain occurs through a subcritical Hopf bifurcation (see text). (c) Upon further change in λ_k the two domains merge. Complex oscillatory phenomena such as bursting or chaos are likely to occur in the region where the two oscillatory domains overlap. This empirical approach applies to autonomous systems which admit a single steady state, but may also bear on systems admitting multiple steady states (see, e.g., ref. 18). The existence of multiple instability domains is favored by the coupling between distinct endogenous oscillatory mechanisms.

newly formed limit cycle. This situation implies that the limit cycle in the first domain of oscillations, at the border that is closest from the second instability region, originates through a subcritical Hopf bifurcation. Such is the origin of birhythmicity in the models of fig. 1b [8] and d [10].

The presence of two distinct domains in which the unique steady state is unstable may either reflect the presence of two instability-generating mechanisms or the separation of a single instability domain, due to a single instability-generating mechanism, into two parts, owing to the presence of another reaction or regulatory process. The latter situation is exemplified by the model of fig. 1b, whereas the former occurs in the models of fig. 1c and d.

If a third parameter is changed so that two domains of instability due to two distinct instability-generating mechanisms merge, as schematized in fig. 3c, two different oscillatory mechanisms are likely to be active at the same time and their interplay may give rise to complex phenomena such as bursting or chaos. A prerequisite for such forms of behavior is that the system comprises at least three variables, but such a condition will generally hold if the kinetics contain two distinct oscillatory mechanisms (an exception is provided by the model of fig. 1c). In support of this conjecture, aperiodic oscillations (chaos) and complex periodic oscillations (bursting) occur in the model of fig. 1d precisely in the region where two initially distinct instability domains overlap [10]. One way in which this intuitive approach might gain more solid foundations could be provided by the study of simpler models based on normal forms [35].

That the empirical approach for finding bursting and chaos actually works is shown by the fact that it readily permitted us to find both phenomena in the model for cAMP signalling described in section 3. We were at first surprised to observe bursting and chaos in this model, since it contains a single positive feedback loop (see fig. 2). The existence of two distinct domains of instability in some parameter planes suggested, however, that more than one instability-generating mechanism might be present despite the uniqueness of the destabilizing, autocatalytic process. Two oscillatory mechanisms, coupled in parallel, can indeed be recognized in the model of fig. 2. These mechanisms share the positive feedback exerted by cAMP on its own synthesis, and differ in the process that limits autocatalysis. In one case, the limiting process is substrate depletion, due to the assumption of a constant input of ATP, and in the second this role is played by receptor desensitization. It is in the region where the two instability domains overlap, i.e., when the two limiting effects acquire comparable importance, that birhythmicity, bursting or chaos were found by numerical simulations [26]. What seems more difficult to predict a priori is which one of these complex phenomena will occur in this domain.

Once these phenomena are found in parameter space, more elaborate methods of analysis may be used. One such method, proposed by Rinzel [36] for the analysis of bursting, combines bifurcation diagrams and phase plane analysis in systems evolving on two different time scales (see ref. 36 for a recent account). Such a method has been applied to elucidate the origin of bursting and birhythmicity in the multiply regulated system of fig. 1d as well as in the model for cAMP signalling in *Dictyostelium* [16,28].

Given the absence of any general, straightforward procedure, it appears that the empirical approach described here could prove useful in the search for complex phenomena, in theoretical as well as experimental studies of oscillations in chemical and biological systems.

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